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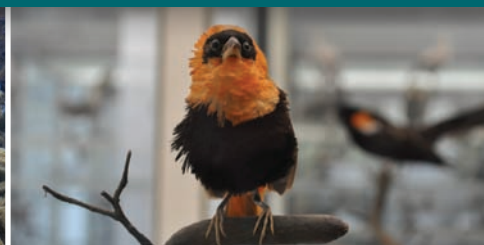
Environmental Aspects
of Zoonotic Diseases

Robert Armon and Uta Cheruti

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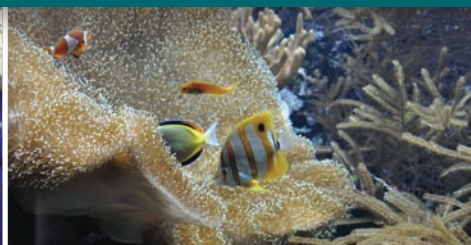
Environmental Aspects of Zoonotic Diseases

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of Zoonotic Diseases

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Preface

"We are a cluster of molecules trying to comprehend themselves"

During my "Sabbatical" year at ESR (Environmental Science and Research Institute) in Christchurch, New Zealand, I was startled by the small population of this country (~4.4 millions) and the huge sheep population (~40 millions) both inhabiting the two islands of this country. While discussing different environmental issues with my colleague and host, Dr. Marion Savill, I wondered if such a massive ratio of domestic animals to humans does not reflect itself in water and environmental pollution and consequently in high numbers of zoonoses. Further verifications, revealed that indeed New Zealand has a high prevalence of Campylobacteriosis, a consequence of this country's vast numbers of free-grazing sheep. A brief look at a dataset published by the from Ministry of Agriculture and Forestry (NZ), according to reports for the year 2007, the country domestic animal inventory was (in millions): ~6 dairy cattle, 1.4 deer, 38.5 sheep, 0.4 pigs, 0.1 goats, 0.066 horses and 19.8 poultry. While preparing myself for the one year visit, I gained knowledge of some facts about this remote country such as, its area size is close to that of the UK and even larger (Table I). As a consequence, I decided to link some numerical data for comparison. The ratio of animals per person (animal density) in New Zealand is 6.25 higher than in the UK (in spite of there being almost twice as many animals in the UK!) and zoonoses are ~6.1 higher. These two countries have two important and comparable parameters that help in such an assessment: area size and ethnical similarity. Recently, Kabore *et al.* (2010) evaluated the association of potential zoonotic gastroenteritis in children (e.g., giardiasis, salmonellosis and campylobacteriosis) with environmental risk factors (livestock densities and drinking water quality) in rural Quebec (Canada). Using statistical tools (negative binomial regression models accounting for overdispersion and adjusted for clustering) the authors found that all three zoonoses were significantly correlated to animals density. In addition Giardiasis incidence was also positively associated with poor drinking water quality (without a statistically significant association). Snel *et al.* (2009) using a simple linear regression model, analyzed ten years of giardiasis cases in New Zealand (rural and urban cases) to find whether an association between disease rates and animal density exists, at the territorial authority level. Their results did not reveal a significant correlation between giardiasis and farm animal density at this level. Their explanation

of the nonsignificant results was that giardiasis distribution is largely linked to anthroponotic (human) reservoirs and much less to zoonotic sources in rural environments and to contributions from overseas travel. It should be borne in mind that giardiasis is mainly a waterborne disease and zoonotic to a much smaller degree. Nonetheless, the same authors (Snell *et al.*, 2009), using the same simple linear regression model, analyzed ten years of cryptosporidiosis cases in New Zealand (rural and urban cases) to find whether an association between disease rates and animal density exists, at the territorial authority level. This time they reported a significant correlation between farm animal density and cryptosporidiosis, with much higher rates in rural areas. In this case, the results supported the fact that the *Cryptosporidium* parasite is mainly zoonotic (transmitted from farm animals to humans) and animal density is indeed a significant risk factor as already pointed out by the Canadian study and our number manipulations!

Table I. Some data related to domestic animals, country area, population and zoonoses in two countries (UK and New Zealand) (based on 2007 data from both countries).

Animal	New Zealand ($\times 10^7$)	UK ($\times 10^7$)
Cattle	6	5.5
Deer	1.4	0.002
Sheep	38.46	14.2
Pigs	0.36	3.6
Goats	0.11	0.069
Horses	0.06	0.2
Poultry	19.8	125
Total	**66.19**	**148.57**
Population	4.4×10^7	62×10^7
Country area size	268,021 km^2	243,610 km^2
Ratio-animals/human	15	2.4
Ratio-animals/area (No./km)	2470	6098
Reported zoonoses (year)	518.100 (2009)	84.989 (2009)
Zoonoses ratio between NZ/UK	6.096	
Animals/human ratio between NZ/UK	6.25	

In light of these basic data, we raised an important question that had troubled us for a long time: OK, the natural situation is as it is but how do we, human kind, by our "continuous development prerogative", and unpreventable natural disasters (e.g., earthquakes, floods, draughts, global warming, tsunami, etc.) jointly impact zoonoses? The present book is a result of over 2,000 scientific publications collected, sorted and critically explored to accomplish the interesting and stimulating depiction of the various relationships between environment, anthropogenic environmental intervention and zoonoses. We are very grateful to the meticulous and comprehensible book "Zoonoses- Infectious diseases transmissible from animals to humans" (3rd edition) edited by Krauss *et al.* (2003) that helped us to navigate systematically our way through such a huge mass of zoonotic pathogens.

We would like also to thank our families and students for their indulgent patience and to apologize for the unjustified absence when they needed us as a consequence of our passion for this fascinating topic.

Robert Armon and Uta Cheruti,
Technion, Haifa, 2011

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Brief CV of the authors

Prof. Armon was born in Bucharest, Romania. He studied Biology (B.Sc.) and Environmental virology (M.Sc.) at Hebrew University of Jerusalem, Israel. He earned his D.Sc. in Environmental virology from the Faculty of Biotechnology and Food Engineering (Technion, Haifa, Israel). Post-Doctoral studies followed at University of Rhode Island, USA (one year) and Institut Armand-Frappier, University of Québec, Canada (two years). Since 1994, is a faculty member of Civil & Environmental Engineering Faculty at Technion (Israel Institute of Technology, Haifa, Israel). Prof. Armon is teaching Environmental Microbiology and carries research on related issues in this area. He published over 84 scientific publications in peer-review international journals, wrote different chapters in several books and is a member of various international scientific organizations (IWA, HRWM, ISEI, ASM, etc.).

Robert Armon

Dr. Cheruti was born in Gelsenkirchen, Germany. She completed her undergraduate degree in Biology at the Technical University RWTH Aachen and her M.Sc. in Immunology and Parasitology at Tel-Aviv University, Israel. She completed her Ph.D. (*Dr. rer. nat.*) in Immunology at the Johannes Gutenberg University in Mainz, Germany. After one year Postdoctoral research at Israel Oceanographic & Limnological Research Institute, Israel, she is at present in charge with all biological laboratories (undergraduate and graduate) and advanced research at the Civil & Environmental Engineering Faculty at Technion (Israel Institute of Technology, Haifa, Israel).

Uta Cheruti

Acknowledgements

*"*Tanto sa ciascuno, quanto opera*"
[Ones knowledge is built upon his industriousness]

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*Bartolommeo Aquarone (1857) "Vita di Fra Jeronimo Savonarola". LIBRO SECONDO
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Chapter 1

Introduction

Chapter 1.1

Human population and socio-economic distribution

"*How Much Land Does a Man Need?*"

Count Lyev Nikolayevich Tolstoy (1828–1910)

It is a factious reality that all the creatures upon this globe have to live together in harmony. In Genesis 7:15-17 (Old Testament) the description of man and animal contact is very much emphasized : "*They had with them every wild animal according to its kind, all livestock according to their kinds, every creature that moves along the ground according to its kind and every bird according to its kind, everything with wings.[15] Pairs of all creatures that have the breath of life in them came to Noah and entered the ark.[16] The animals going in were male and female of every living thing, as God had commanded Noah. Then the LORD shut him in*." We doubt if the zoonotic concept was clear at that time, but the story describes the close relationship between humans and other "God created" creatures. The only problem, to evolve into a serious one, was the humans themselves.

Since the start of the 20th century, human population has more than tripled its size from ~1.8 to ~6.9 billion (Figure 1.1.1) (Steck, 2010). According to United Nations database, there are several scenarios on of the human population prospect, some based on status-quo: women's productivity does not change [birth rate remains 2.82 children/woman (constant progression, red line)] and therefore the population will continue to expand to ~13 billion in 2050; the more optimistic ones are based on the hypothesis that the birth rate will drop from 2.82 to 2.15 children/woman, including a life expectancy increase from 65 to 76 years [blue lines: high and low estimation dotted lines and medium continuous line] to range from 8 to 11 billion (9 billion medium) in 2050 (Figure 1.1.2). There are many reasons for this huge increment, but perhaps the most egregious one is the advanced medical treatment (scientific revolution) that followed the industrial revolution. Wars, famine, natural catastrophes, economic scrambles, etc. did not affect the global population's exponential growth as medicine did. If we want to understand this phenomenon, perhaps the most perceptive step is to glance at the records of the deceased registered throughout the centuries by the Catholic Church in Europe of the Middle Ages. An average human life of 40 years was the upper limit while people reaching the advanced ages of 50 and 60 were considered to be the village elders! These days, a retired person in his late 60's is still considered "young". In nature, the animal population is largely controlled by food availability, territory and epizootics which are in harmonious balance with environmental factors. Interestingly, we (the humans) are competent to

control these populations for our own benefit, for instance animals' domestication in large numbers. However, who controls our own population? There are two main factors that might control it: our progressive awareness and environmental capacity. Of the awareness part, in spite of cultural "bottle necks" (illiteracy, diverse cultural and religious norms), there is a general endorsement that our population cannot grow continuously at the same rate and has to slow down or enter a "zero" growth kinetic. Of the environmental capacity part there is "no need" for direct awareness, as it blows itself up in our face. Increasing industrial capacity to its limits created a new problem of intense pollution that in turn affected the population's well-being and its environment. It is not within our scope to analyze different models as we are neither demographers nor population scientists, but the common sense of microbial growth can be in large applied to the human population based solely on reproduction, though the time scale is different. Immigration or migration cannot be considered as they do not change the population size but only its composition. In terms of socio-economic composition, it can be in general stated that the "North" is rich and "South" is poor. Indeed, large areas of the South include more developing countries than the "North" that harbors more developed countries. There is no need to say that migration/immigration from the "South" to the "North" is a continuous process, with some positive aspects related to population growth (e.g., education merged with socio-economic progress tend to impact birth rate).
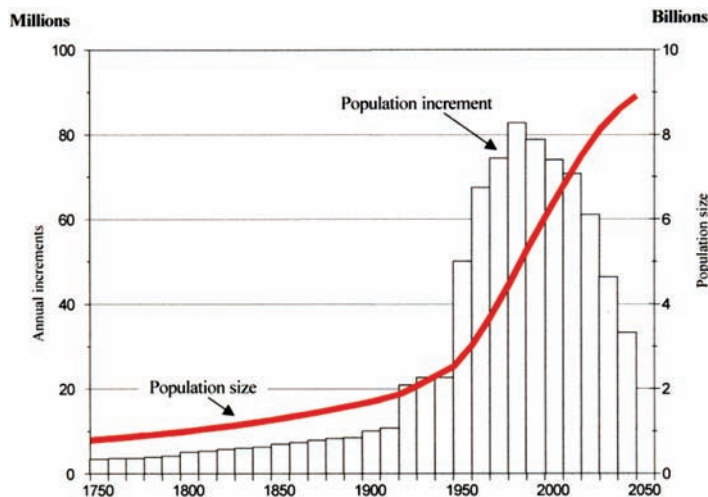
Figure 1.1.1. Historical world population growth from 1750 to 2050. (Source: United Nations Population Division)

Another interesting trend in human population is the continuous relocation from rural to urban areas in developing countries (Figure 1.1.3). The trend is linked to economic aspects such as rapid industrial development in urban areas and decline in agricultural activities that are less profitable and demand long and tiresome labor. According to 2005 FAOSTAT (Food and Agricultural Organization statistical databases), at that time the world population of 6.4 billion was divided in 49% urban and 51% rural populations. As a result, the socio-economic division of urban population between high and low/middle income countries, from 1975 up to 2015, shows a steady linear increase in low/middle income countries (from 1.6 billion to 3.9 billion) with a minor increase in high income countries (from 0.5 to 0.8 billion).

En milliards

Trois hypothèses pour l'évolution de la population mondiale :

Selon le bureau de la population des Nations unies, la plus vraisemblable est l'hypothèse moyenne : pour les périodes 1995-2000 et 2045-2050, elle prend en compte d'une part, la réduction du nombre d'enfants par femme (de 2,82 à 2,15), et d'autre part, une amélioration de l'espérance de vie à la naissance (de 65 à 76 ans). Le taux d'accroissement naturel tomberait ainsi de 1,35% en 1995-2000 à 0,47% en 2045-2050.

Progression constante :

La ligne bleue représente ce que serait l'évolution démographique si le taux de fertilité actuel (2,82 enfants par femme) restait le même jusqu'en 2050.

Sources : *The World at Six Billion* (octobre 1999) et *World Population Prospects: The 2006 Revision* (février 2007), Nations unies, département des affaires économiques et sociales, division de la population, New York.

Figure 1.1.2. World population evolution. (2009). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 10:36, April 20, 2011 from http://maps.grida.no/go/graphic/world-population-evolution, credit to *Philippe Rekacewicz (Le Monde diplomatique)*

Urban and rural population in less developed regions (billions)
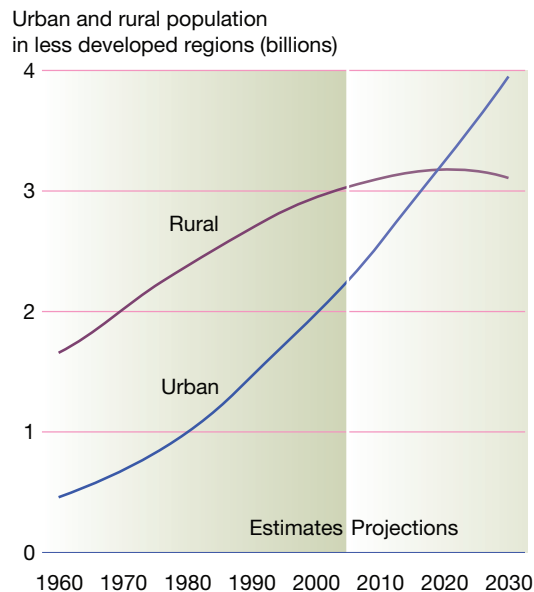
Figure 1.1.3. Trends in urban and rural populations, less developed regions, 1960-2030 (estimates and projections) . (2009). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 21:34, May 4, 2011 from http://maps.grida.no/go/graphic/trends-in-urban-and-rural-populations-less-developed-regions-1960-2030-estimates-and-projections. credit to *Hugo Ahlenius, Nordpil*

Population in thousand million

Sources: Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat, *World Population Prospects: The 2004 Revision*; Global Footprint Network, 2005.
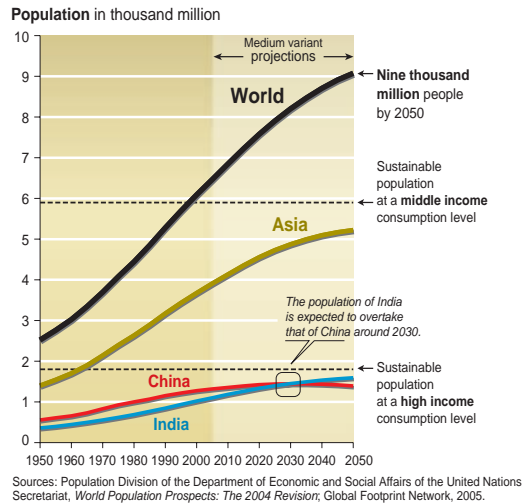
Figure 1.1.4. World Population. (2006). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 21:28, May 4, 2011 from http://maps.grida.no/go/graphic/world_population, credit to *Emmanuelle Bournay* (Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat, World Population Prospects: The 2004 Revision; Global Footprint Network, 2005)
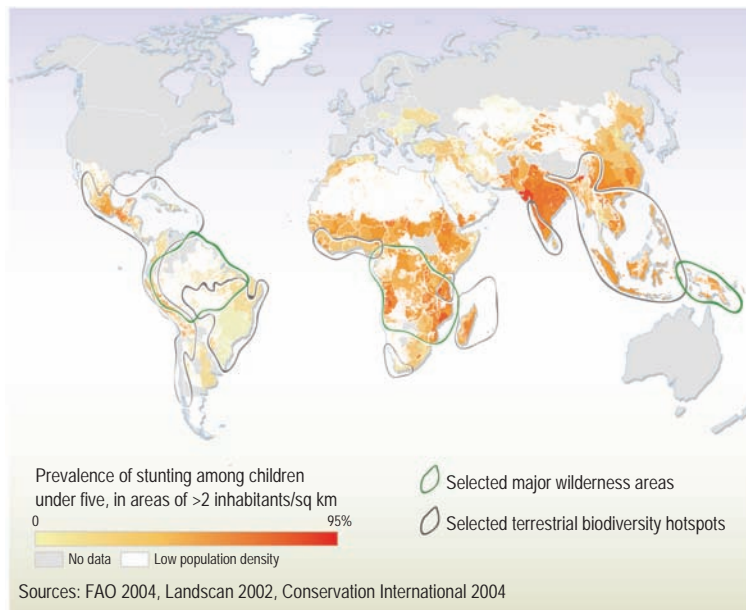
Figure 1.1.5. Global poverty-biodiversity map. (2004). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 21:34, May 4, 2011 from http://maps.grida.no/go/graphic/global-poverty-biodiversity-map, credit to Hugo Ahlenius, UNEP/GRID-Arendal (Landscan, FAO, Conservation International)

In relation to environmental sustainability (i.e., how many people can be supported at a certain consumption level) it seems that the growing world population will reach a point (by 2050) that ~2 billion people will be expected to be sustained at a high income consumption level, while ~4 billion at middle income consumption level and ~3 billion at a low income consumption level, of a total of ~9 billion people (Figure 1.1.4). The predicted 2050 low income population (~3 billion people), in other words very poor populations, will reside largely in the geographic areas with high biodiversity (Figure 1.1.5). Consequently, without much choice, this population will unsustainably extract resources threatening biodiversity and their own health. Health problems expressed as stunted growth in children are also very apparent in these areas where poverty and high population density coincide. Poverty (or low income) is also an indicator of economic development however, low incomes can be also found in highly developed countries (e.g., USA, Russia, etc.) although masked by overall calculations for the entire population. The high income countries consume and also produce more waste but due to advanced technology and lower population density they will be able to sustain themselves. In contrast, the new members of the "middle income countries club" such as China and Indonesia will have deal with intense economic growth versus resources depletion and energy use that may become a "bottle-neck" as a result of their highly dense population! (Figure 1.1.6).
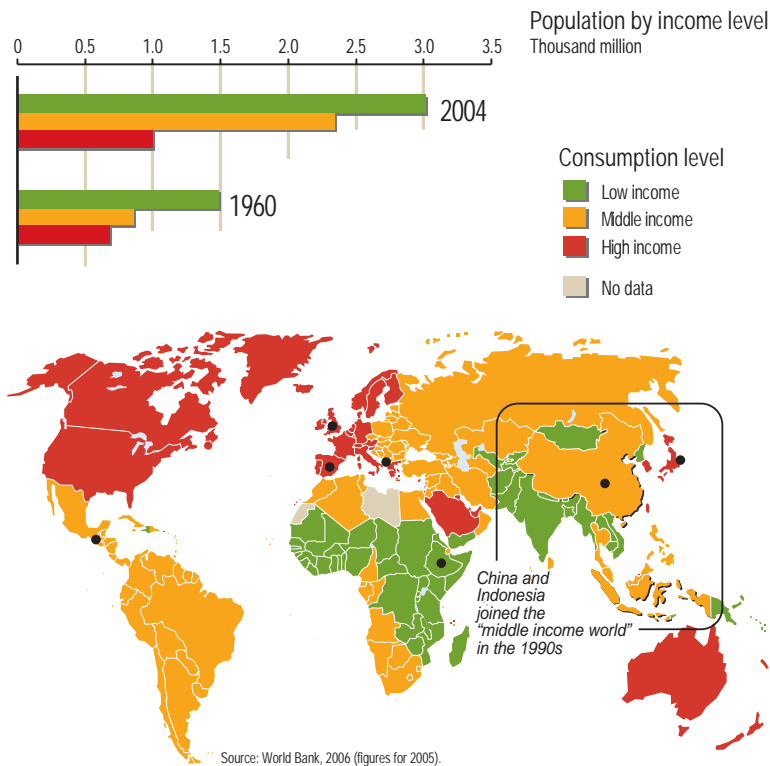
Figure 1.1.6. Population by income level. (2006). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 21:38, May 4, 2011 from http://maps.grida.no/go/graphic/population-by-income-level, credit to Emmanuelle Bournay [World Bank, 2006 (figures for 2005)]

POVERTY AND CHOLERA IN KWAZULU-NATAL
JANUARY 2001

Localisation of cholera

- Core zone of illness
- Direction of illness
- District reporting of cholera cases (January 2001)

Household poverty

- Very High
- High
- Medium
- Low
- Very Low

0   100 km

Sources: Statistics South Africa
(www.statssa.gov.za), World Bank and
South African Department of Health.
Adapted by UNEP/GRID-Arendal
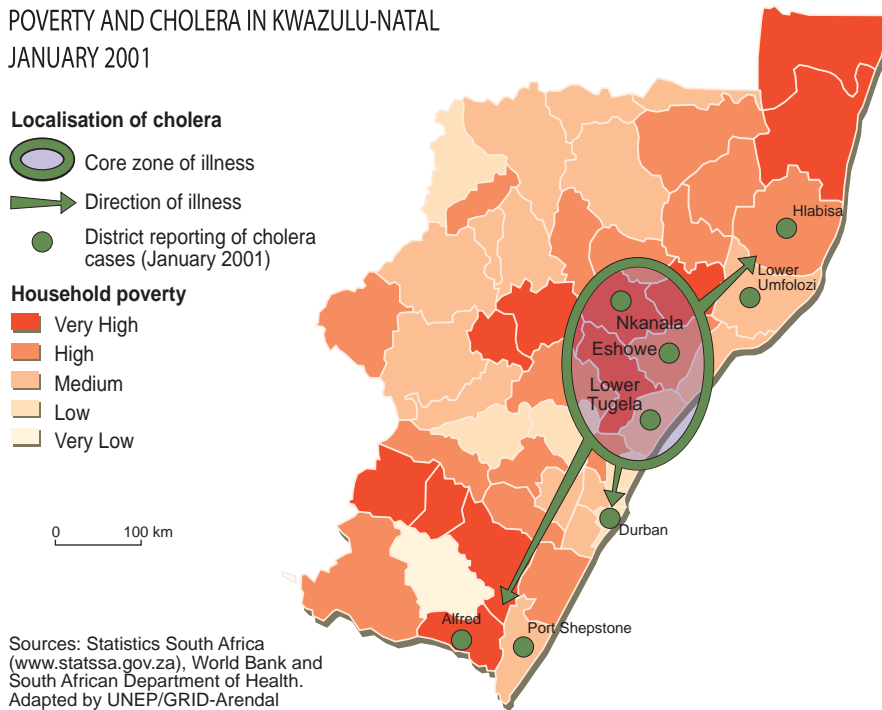
Figure 1.1.7. Socioeconomic status and cholera spread in South-Africa (Kwazulu-Natal January 2001) (2002). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 12:37, January 20, 2011 from http://maps.grida.no/go/graphic/poverty-and-cholera-in-kwazulu-natal-january-2001, credit to Philippe Rekacewicz, UNEP/GRID-Arendal (Statistics South Africa (www.statssa.gov.za), World Bank and South African Department of Health. Adapted by UNEP/GRID-Arendal)

In summary, one of the major global holdups is population growth beyond the sustainability of our planet, coupled with other phenomena also related to population density: climate change, waste production and pollution, biodiversity decline, etc. (Dasgupta, 1995).

We wonder if even one individual among the global big poor population would understand the following equation (representing Gini coefficient-inequality measure) (Milanovic, 2002):

$$(1) \qquad Gini = W_i + B_i + L_i = \sum_{i=1}^{n} Gini_i p_i \pi_i + \frac{1}{\mu} \sum_{i=1}^{n} \sum_{j>1}^{n} (y_j - y_i) p_i p_j + L_i$$

Without much knowledge of economics and mathematics, this poor population certainly understands the everyday burden of food and water supply, health care and lack of reliable education and living conditions. All these parameters are directly linked to the spread of infectious diseases as shown in Figure 1.1.7. Diseases caused by various pathogenic organisms (universally called infectious diseases) described along this book, are significantly correlated to socio-economic level of large populations. Figure 1.1.7 shows a classical link between poverty, lack of safe drinking water and the spread of cholera in one of the poorest areas of the world (Kwazulu-Natal, South-Africa).
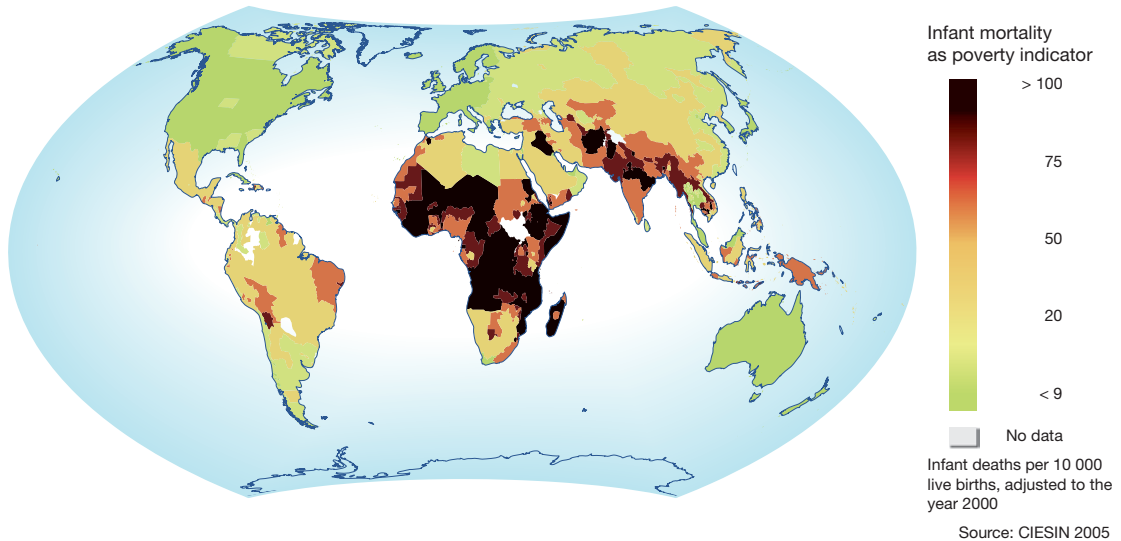
Figure 1.1.8. World poverty distribution. (2008). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 16:48, May 7, 2011 from http://maps.grida.no/go/graphic/world-poverty-distribution, credit to Hugo Ahlenius, UNEP/GRID-Arendal (Center for International Earth Science Information Network (CIESIN), Columbia University. 2005. Global subnational infant mortality rates. Available at: http://www.ciesin.columbia. edu/povmap/ds_global.html (Accessed April 28, 2008)
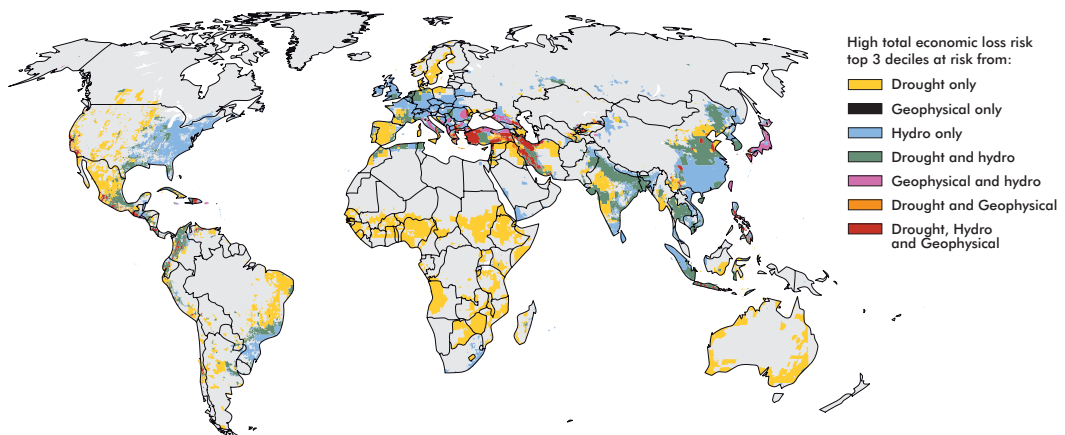
Figure 1.1.9. Natural hazard hotspots, by risk type. (2007). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 16:53, May 7, 2011 from http://maps.grida.no/go/graphic/natural-hazard-hotspots-by-risk-type, credit to Hugo Ahlenius, UNEP/GRID-Arendal (Center for Hazards & Risk Research. 2005. Natural Disaster Hotspots - A Global Risk Analysis: Global Multihazard Frequency and Distribution. http://www.ldeo. columbia.edu/chrr/research/hotspots/coredata.html (Accessed June 1, 2007)

75% of all poor people still live in rural areas, where they are heavily dependent on natural resources such as, soil, water, forests and fisheries (Figure 1.1.8). Figure 1.1.8 also represents poverty expressed by the infant death rate, an obvious measure of deprived health care and malnutrition in these areas. Many developing countries own these natural resources and therefore are still able to support a potential minimal wealth for poor people and their communities. However, as many of these natural resources are renewable they need to be properly managed in order to support these population in the long term. Poverty alleviation can occur through improved natural resource management but also by birth control in order to reduce the stress of the human population on natural resources for a sustainable development. Another interesting point related to poverty is the link to global hot spot disasters (droughts, floods, earthquakes, etc.) (Figure 1.1.9). With climate change, the frequencies of certain natural hazards are expected to increase. Figure 1.1.9 represents a graphical overview of an analysis of hazard exposures and historical vulnerability some natural hazards, together with population distribution and economy. Obviously, poor populations will suffer the most from these hazards and their ability to overcome such disasters is close to nil.

Diseases caused by various pathogenic organisms (universally called infectious diseases) described in this book are significantly correlated to socio-economic level of large populations.

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Chapter 1.2

Climate

"Climate is what we expect, weather is what we get."

Mark Twain (1835–1910)

It is not within the scope of this brief chapter to solve the debate whether climate changes are a consequence of human activity or just a natural succinct phenomenon that occurs periodically for millions of years (see ice age). The balance between entering energy (sun radiation) and leaving energy (energy reflected by earth), where the atmosphere plays a crucial role as a thermal isolator, are the main components that impact earth's climate (Figure 1.2.1). Our contribution to greenhouse gases is continuous through industrial development, increasing ground and air-traffic and incineration.

Natural climate forcings (forcing is a destabilizing factor of the above system) are mainly natural (e.g., the sun's brightness, small variations in Eart's orbit and axis rotations and volcanic eruptions) but also manmade (e.g., aerosol production, CO_2 and greenhouse gases emission and deforestation) (Kirilenko and Sedjo, 2007) (Figure 1.2.2). Nevertheless, over a century global warming is an undisputed scientific fact, expressing itself in rapid mountain glacier retreat and a corresponding sea level rise (Berger *et al.*, 2010) (Figure 1.2.3).

Warming has its impact on major ecological and biological systems including a large variety of parasites, their vectors and the final hosts. Varou *et al.* (2007), reviewing the tick-borne viral disease Crimean-Congo hemorrhagic fever (CCHF), reported that climate changes may affect CCHF epidemiology through influencing survival and reproduction of *Hyalomma* ticks and their hosts. For example, mild winters preceded outbreaks of this virus in Turkey and Kosovo, while interruption of agricultural activities by the reintroduction of cattle and sheep, a decrease in hare hunting and conversion of floodplains into agricultural land have been associated with CCHF outbreaks in Russia, Bulgaria, former Yugoslavia and Turkey. A different view on another tick-borne disease (tick-borne encephalitis-TBE virus) has been published by Randolph (2010). The author suggested that climate change alone could not explain the increase pattern and upsurge of the TBE virus due to its uniformity (e.g., in Baltic countries) and other factors seem to be more relevant to the phenomenon. Among these factors, Randolph (2010) suggested: "1) agricultural reforms resulting in changed land cover and land use, and an increased reliance on subsistence farming; 2) reduction in the use of pesticides, and also in the emission of atmospheric

pollution as industries collapsed and 3) increased unemployment and poverty, but also wealth and leisure time in other sectors of the population, as market forces took hold".
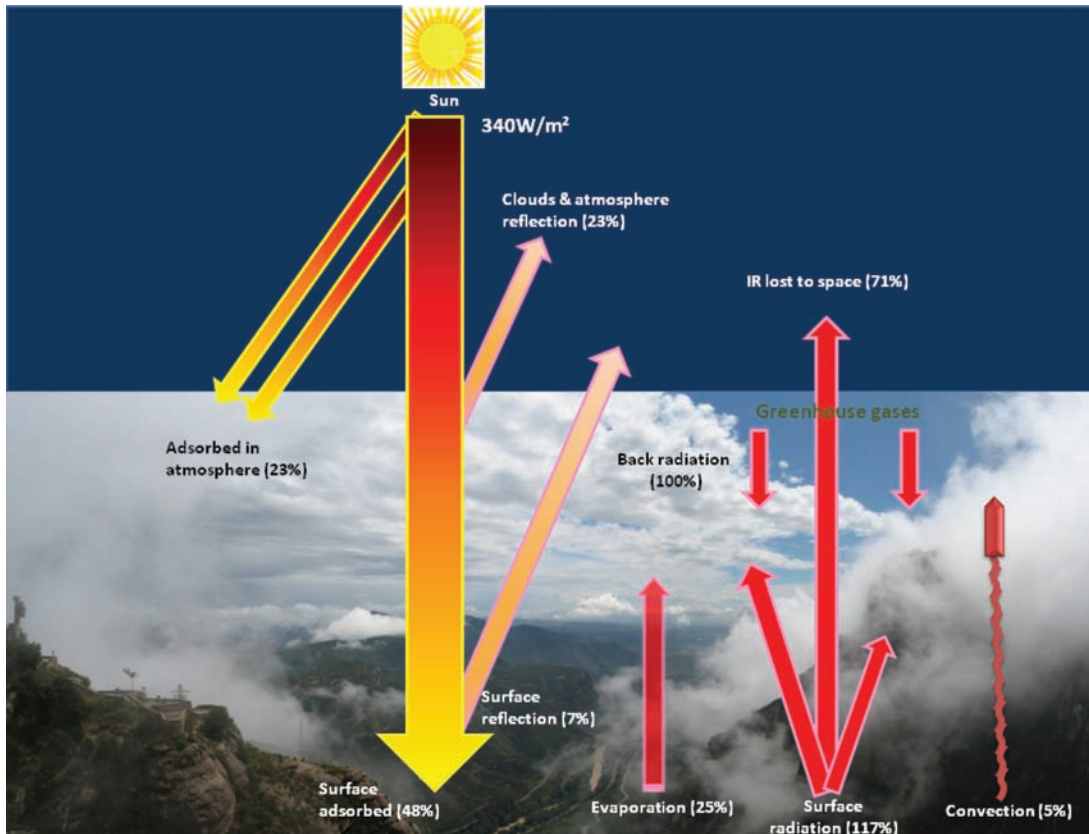
Figure 1.2.1 Radiation balance between sun and earth. (Adapted from NASA site http://earthobservatory. nasa.gov/Features/EnergyBalance/page1.php)

In Portugal, Leishmaniases, a zoonosis endemic in the Mediterranean basin, has shown a significant increase in cases number and a shift from pediatric to adult cases (mainly immunocompromised, HIV/AIDS). The authors suggested that climate amelioration may increase Leishmaniasis' arthropod vector, the phlebotomine sand flies, densities and activity favoring higher incidences (Campino and Maia, 2010). In Belgium, Nephropathia epidemica (NE), an emerging rodent-borne viral disease had shown a sharp increase in incidences for more than a decade (Clement *et al.*, 2009). The authors suggested that bank voles might be responsible for this hantavirus through their known cyclic population peaks impacted by climate warming. In a recent study from Germany, an outbreak of Leptospirosis was recorded among strawberry harvesters (Desai *et al.*, 2009). Leptospirosis is primarily known to be present in tropical countries with connection to agricultural exposures, and much less in temperate countries (such as Germany). The authors suspected that direct contact of humans (with hand lesions) with water, soil and infected voles may cause this disease. In addition, a preceding warm winter contributed to vole' population growth; hence the reemergence of Leptospirosis.
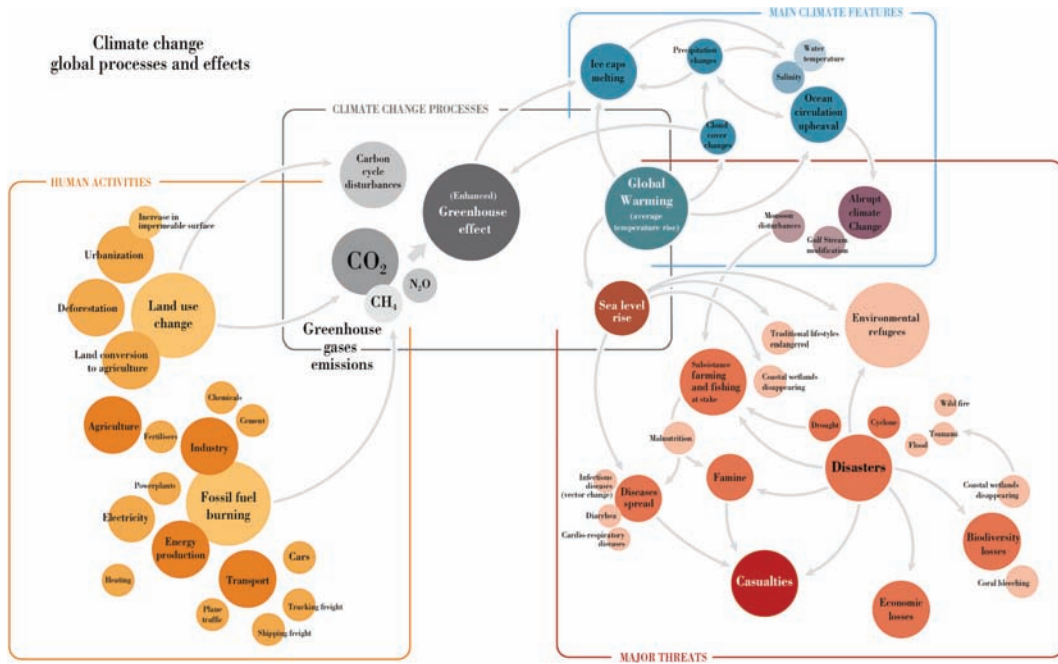
Figure 1.2.2. Climate change global processes and effects. (2009). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 23:10, April 26, 2011 from http://maps.grida.no/go/graphic/climate-change-global-processes-and-effects1, credit to UNEP/GRID-Arendal
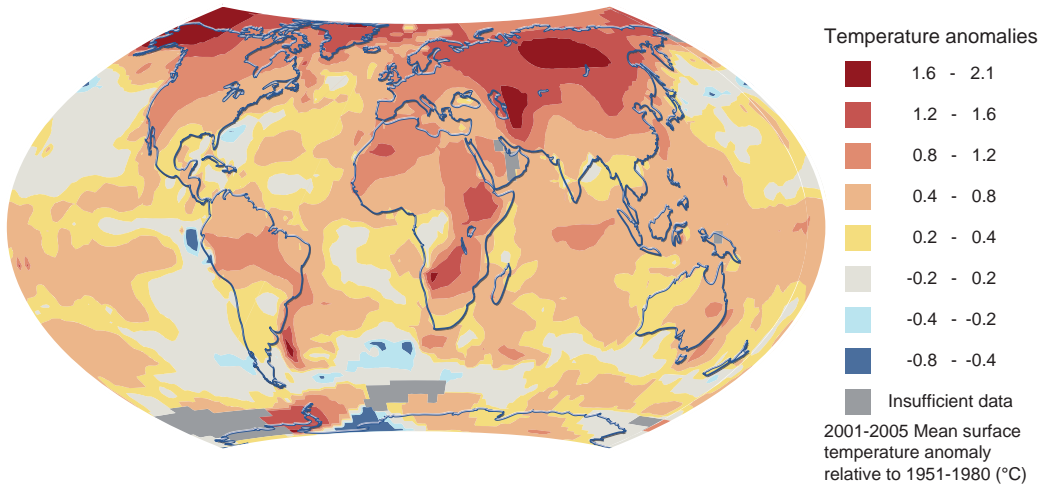
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Finally, the best examples of zoonotic disease spread as a partial result of climate changes are cholera and malaria. Cholera had almost disappeared, remaining endemic mainly in India, Pakistan and China; nevertheless, since 1960 it has reemerged worldwide in the last five decades (Figure 1.2.4). As this disease is connected to water and poverty conditions (see chapter 1.4 and chapter 1.1), increasing floods due to climate changes between dry periods (e.g., El Niño and its consequences in South America and other regions) support the emergence of cholera. The second best example is malaria (*Plasmodium falciparum*) that is connected to one of the major climate forcings: CO_2 atmospheric rise. The malaria parasite is transmitted by mosquito' vectors to humans and mosquitoes in turn are impacted by CO_2 concentrations that according to different models will increase in certain geographic areas, therefore increasing the vectors' natural distribution (Figure 1.2.5). Among the endless reports on reemerging zoonotic diseases connected to so many factors, including climate change, it should be emphasized also that warming (the main trend in climatology) could affect the various pathogens in a different way (bacteria will react differently from viruses or parasites or helminthes) and therefore it is not an easy task to conclude in general terms, and some reservations should be considered before major conclusions (Randolph, 2010).
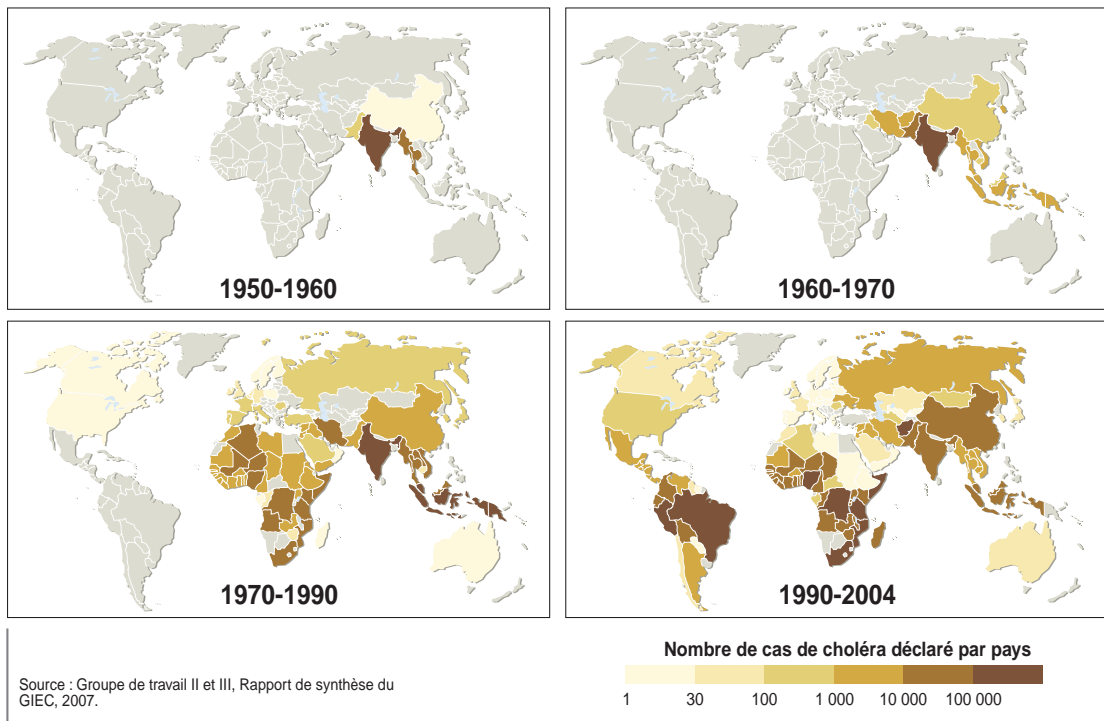
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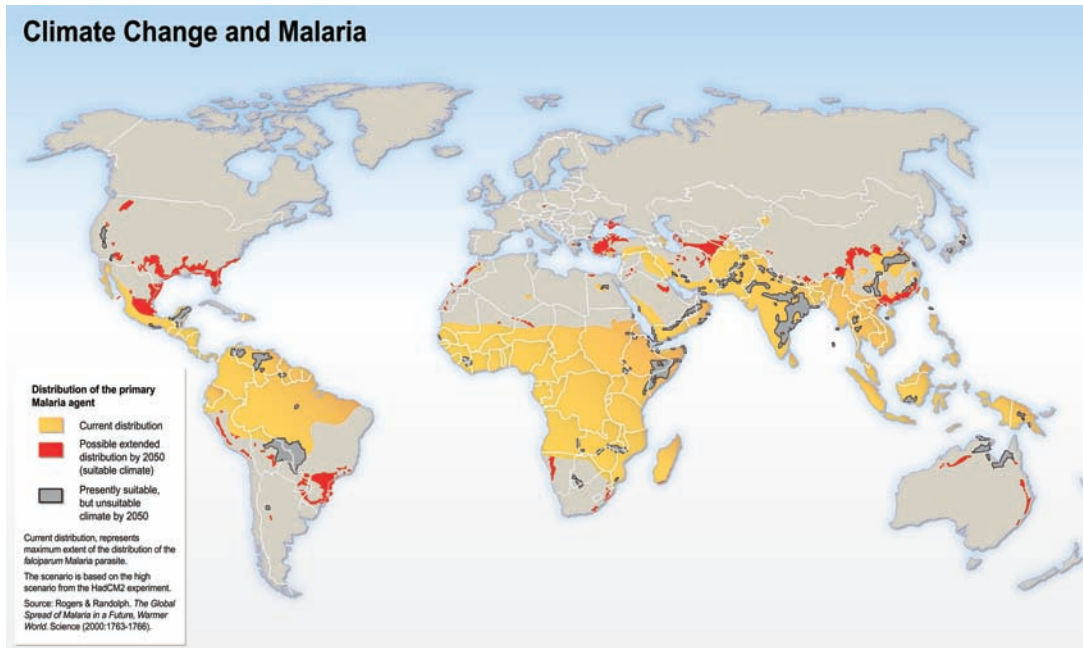
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Chapter 1.3

Geographic variability

"*One of my talking points will be the accident of geography.*"

Janis Joplin (1943-1970)

Geographic variability should be looked at in relation to continents and much less at the country level. Most of the globe's terrestrial area is mostly steppe like (shrubs and small trees) while only certain areas (in South America, Mid Africa, South and Southeast Asia and Northern parts of Canada, Scandinavia and Russia) are covered by evergreen and deciduous forests (Figure 1.3.1). Other areas are large deserts (Sahara and Serengeti in Africa, Arabian Peninsula, Gobi in Asia, Mojave and Sonoran deserts in North America and Patagonian desert in South America). Besides the North and South Poles, with their extreme climate and desert -like characteristics due to lack of available water, most global geographic areas are inhabitable by animals and humans. As zoonoses are linked to the presence of both factors, animals and humans, geographic variability (forests, rivers, lakes, etc.) may impact the spread of pathogenic agents and their vectors. For example, ~240 million people (mostly from the low-economic level) depend on forested areas for their living, where in certain countries >8% of the GDP is based on the forestry industry. Deforestation is one of the most important ecological factors that impact the greenhouse effect. According to FAO (Food and Agricultural Organization), deforestation causes 25% of greenhouse gas emissions when combined with industrial pollution from developed countries. It should be also remembered that forests are also complex ecosystems that contribute to biological diversity and the, hydrological cycle and are a source of new plant species unknown to us that may help in the future. The reader will find in this book many examples of zoonoses' reemergence through geographic changes, such as dam construction, flooding of fertile soil and even the construction of highways, which alter the geographic variability and pose new challenges for different organisms. Biological systems are highly adjustable and in short periods of time are able to adjust to new geographic areas posing a real threat to human health. These systems are often so complex that it takes many years of research to really understand their way of action, mostly too late.
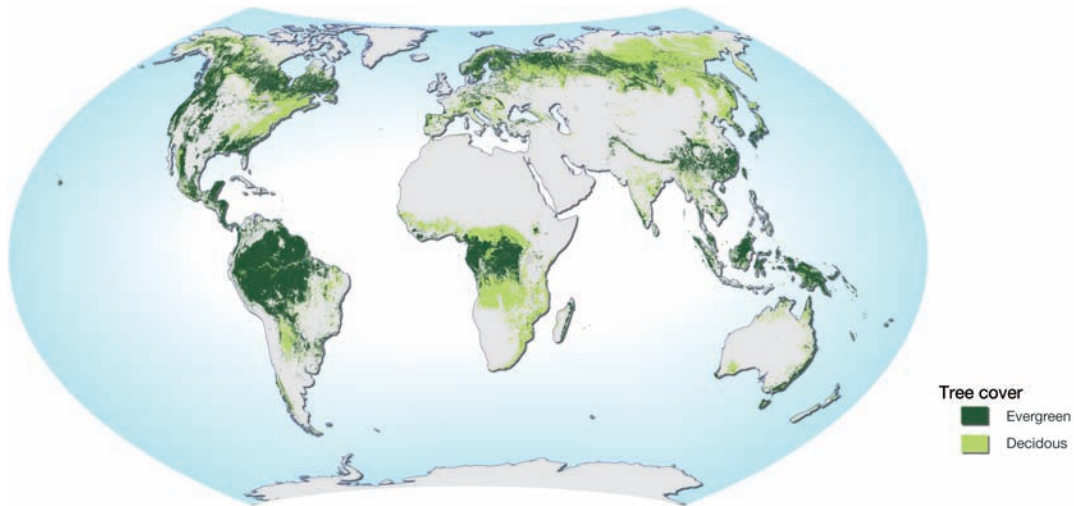
Source: EC Joint Research Centre 2003

Figure 1.3.1. World map of forest distribution (Natural resources - forests). (2008). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 21:45, May 4, 2011 from http://maps.grida.no/go/graphic/world-map-of-forest-distribution-natural-resources-forests, credit to Hugo Ahlenius, UNEP/GRID-Arendal (European Commission, Joint Research Centre. 2003. Global Land Cover 2000 database. http://www-gem.jrc.it/glc2000 (Accessed July 15, 2007)

Chapter 1.4

Water and wastewater

"*Water is life's mater and matrix, mother and medium. There is no life without water*"
Albert Szent-Gyorgyi (1937 Nobel Prize in Medicine).

It can be stated with high confidence that water is the most important component of life. Water also provides the best direct contact milieu between humans and animals, making infectious disease transfer possible.

Geographically, there is a certain injustice in the distribution of water around the globe! There are countries in which rain may reach annual precipitations of 13,299 mm while in others only <200 mm. The huge gap between these two extremities indicates the difficulties that certain countries face when water resources are over or under the global average. Potable water is a necessity without which life is not possible. However, mostly in Africa, the share of the population that has access to improved drinking water ranges from <40 to 55% (>1 billion people), and therefore water is a source of continuous contamination with pathogenic agents (Figure 1.4.1). A second look at Figure 1.4.1 reveals that most of the same countries that suffer from poverty and infectious diseases (Figure 1.1.8 and Figure 1.2.5) have also less access to safe drinking water. Global fresh water withdrawal can be generally divided in three categories according to primary use: agricultural, industrial and domestic sectors (Figure 1.4.2). Figure 1.4.2 compares water use according to the above sectors at the national level. Indeed, developed countries predominantly but not exclusively use more fresh water for industry and developing countries for the agricultural sector. The agricultural sector is more spendthrift the domestic and industrial sectors, and therefore more problematic in countries with low water resources (e.g., Middle East countries). The solution to these problems would come from advanced agricultural technology (e.g., drip irrigation combined with extensive wastewater treatment).

Wastewater, which is the other facet of water, has also a great potential for water scarcity remediation. However, according to its nature, wastewater has to be well treated before reuse, constituting one of the major hazards in spreading infectious diseases around the globe. Wastewater problems differ according to various global regions and their economic development: for example, in Africa it impacts public health, in Europe it causes eutrophication through input of nutrients into the coastal waters reducing productivity and creating anoxic dead zones and in Asia ecosystem deterioration through river basins' pollution (Figure 1.4.3).
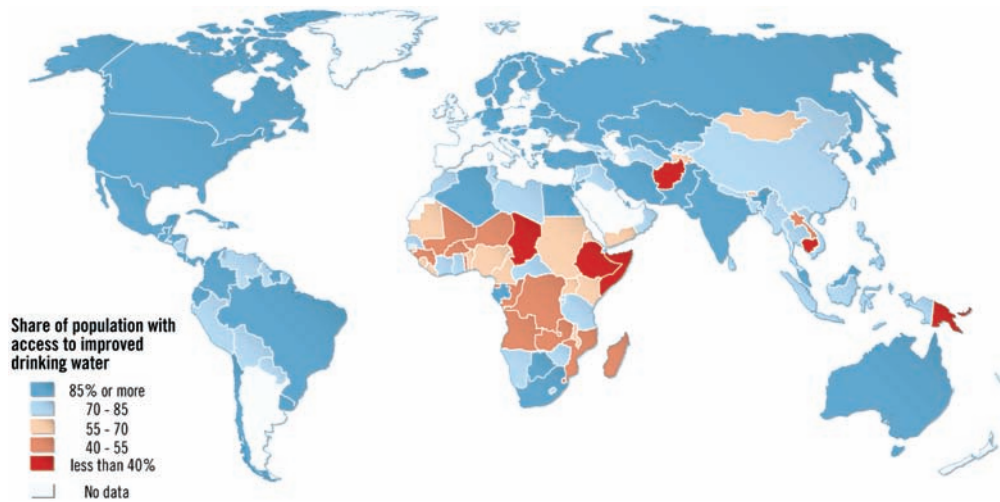
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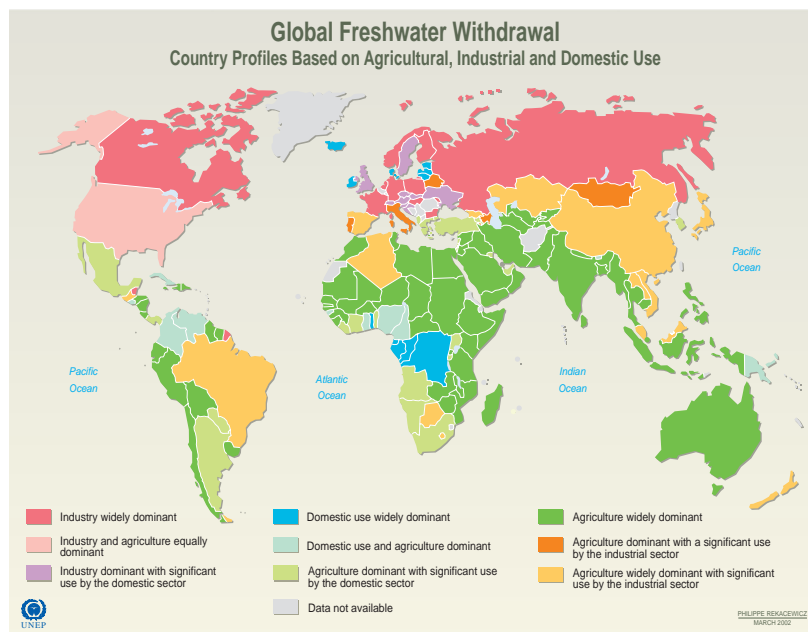
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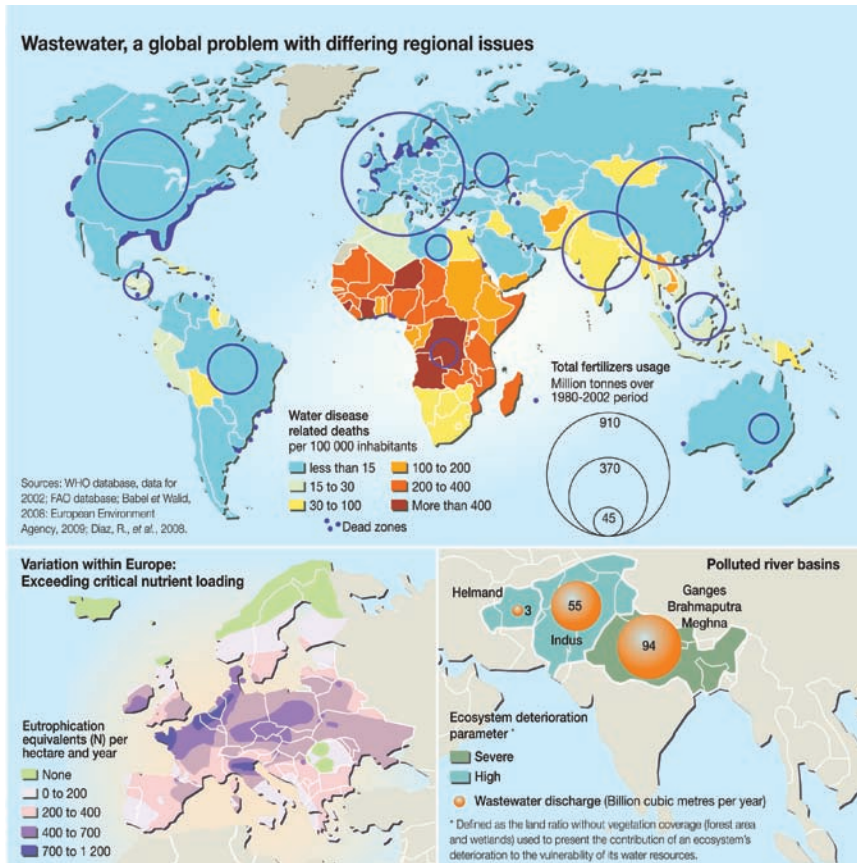
Figure 1.4.3. Wastewater, a global problem with differing regional issues. (2010 March). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 16:15, May 7, 2011 from http://maps.grida.no/go/graphic/wastewater-a-global-problem-with-differing-regional-issues, credit to UNEP/GRID-Arendal (WHO database, data for 2002; FAO database; Babel et Walid, 2008: European Environment Agency, 2009; Diaz, R., et al., 2008)

According to the author's , more than 30 years' experience in environmental microbiology, wastewater has been one of the major bottlenecks that has affected rivers, lakes and other water bodies through contamination with many infectious agents. However, in the 21st century, with the development of advanced treatments (e.g., membrane technology, advanced oxidation processes and molecular methods) it is possible to treat wastewater and produce excellent water quality, safe for drinking and other uses (Haarhoff and Van der Merwe, 1996). The only impasse is the economic price requisite imposed on many poor countries in order to achieve this goal.

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Chapter 1.5
Global animal trade

"*Of all the animals, man is the only one that is cruel. He is the only one that inflicts pain
for the pleasure of doing it*"

Mark Twain (1835–1910)

The livestock, wildlife and animal products global trade is massive and multifaceted. There are no general predetermined rules among country members of the World Trade Organization (WTO) to control animals' movement and by and large it is based on bilateral agreements. However, member countries of WTO are bound to a sanitary and phytosanitary agreement (http://www.wto.org/english/tratop_e/sps_e/ spsagr_e.htm) concerning food safety and animal/plant health regulations based on a good veterinary infrastructure. Wild and domestic animals are the main milieu in which a large variety of zoonotic agents are seeded and disseminated. While domestic animals can be and are controlled by veterinarians, wild animals are completely out of human control when trade is illegal. Feral animals are subjected to disease through a variety of vectors: arthropods, direct contact, predation and exposure to human environments (refuse, wastewater, carcasses, slaughterhouses, etc.). According to early 1990s' estimates, TRAFFIC evaluated the value of legal wildlife products imported globally to be around USD160 billion (http:// www.traffic.org/trade). Among the imported wildlife are: ~40 000 live primates, four million live birds, 640 000 live reptiles and 350 million live tropical fish. In Asia, which is one of the major global wild animal trade centers, it is estimated that several billion direct and indirect contacts among wildlife, humans and domestic animals occur each year (Karesh *et al.*, 2005). As a result new diseases affecting both humans and animals continue to emerge, such as severe acute respiratory syndrome (SARS) and highly pathogenic avian influenza (e.g., H5N1) (discussed later in this book) or the detection of *Pseudamphistomum truncatum* (an opisthorchid fluke parasite) in the English otter linked with the introduction of two freshwater fishes functioning as intermediate hosts for this fluke, colonizing many rivers in southern UK (Pinder and Gozlan, 2003). However, relevant legislation for international wildlife trade relates to three main areas: animal health, animal welfare and the international movement of endangered species. The issue of endangered species is probably the only positive aspect of this huge trade in wild animals, contributing to the rescue of wild animals otherwise eternally exterminated under environmental pollution and human continuous pressure.

On the other hand, the trade volume of domestic animals (e.g., cattle, pigs, sheep and poultry) is huge with several countries importing millions of dollars's worth (cattle and pigs in the USA, pigs and chicken in Belgium, pigs in the UK, etc.) (Fèvre *et al.*, 2006). When dealing with such enormous numbers of domestic animals it cannot be excluded that some accidental infections may happen affecting human and animal health, in spite of regular and rigorous veterinary inspections.

The problems related to the risk of contracting zoonotic infections and outbreaks in urban populations have been described. Such a risk is related to the widely-accepted practice of keeping pets and wild animals in city flats and the absence of knowledge in the general population about animals' parasites and measures of individual prophylaxis of zoonosis. Large numbers of stray dogs and the habitation of wild animal species in territories of cities represent additional threats. A conclusion about the need for the inclusion of prophylactic and anti-epidemic measures in programs of city improvement has been reached.

Pets' movement is also an important issue related to zoonoses. One of the best examples is leishmaniasis (*Leishmania infantum*) outbreaks in dogs (the main reservoir host) in the USA as a result of military personnel returning home with their pets from Mediterranean area. Other diseases, such as heartworm (*Dirofilaria immitis*), babesiosis (*Babesia canis*), ehrlichiosis (*Ehrlichia canis*), echinococcosis (*Echinococcus granulosus* and *E. multilocularis*), rabies, bovine tuberculosis, *Trypanosoma brucei rhodesiense*, foot and mouth disease (FMD) and Rift Valley fever are all likely to be introduced through domestic pets and their movement (Chevalier *et al.*, 2004; Fèvre *et al.*, 2006).

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Chapter 1.6

Environmental pollution

"The significant problems we face cannot be solved at the same level of thinking we were at when we created them."

Albert Einstein (1879–1955)

Environmental pollution may be defined as "irresponsible discharge or disposal of toxic waste by humans based on the anticipation that Mother Nature will take care of it". Whether intentional or unintentional, environmental pollution by humans is a fact (see Figure 1.2.2). However, in the last decades, some remediation emerged following scientific community warning and political awareness. Besides the environment, pollution is a multi-edged sword that may affect also humans, animals, pathogens and their vectors (Chu *et al.*, 2008). Briefly, pollution sources can be chemical (from various industrial activities), biological (from waste and agricultural activity) and radioactive (from domestic and military industries). The chemical pollution emitted by different industries is huge (most of chemical pollution originates from developed countries) affecting human and animal health in the short- and long-term based on production processes and practices (Figures 1.6.1 and 1.6.2).

There are three environments affected significantly by human activity and pollution: soil, water and air. It can be stated that soil pollution is more pervasive than water and air (due to its relative volume and also its structure). Figure 1.6.3 represents different human activities (industrial and municipal waste) in some European countries (developed and developing). Plants are the primary organisms that accumulate contaminants that are further consumed by animals and humans. Ingestion, inhalation and direct contact with such polluted soil may also impact their health.

Contaminated soil does contribute substantially to groundwater pollution depending on the local geology affecting many areas: potable and bathing waters, different household uses, agriculture and industry (Figure 1.6.4).

It is clear that water contamination with a large variety of chemicals and biological components has a direct impact on our health. However, some long-term effects are also noticeable, such as alteration of global nutrient cycles. Johnson et al. (2010) evaluated the impact of nitrogen and phosphorus eutrophication on human and wildlife health. According to these authors there are several postulated mechanisms that link eutrophication to several pathogen transmission modes (direct horizontal and

indirect vector-borne or complex life cycle): 1) host abundance and distribution; 2) pathogen virulence alteration and 3) changes in host susceptibility. These links seem to occur more frequently in tropical and subtropical regions that are the most vulnerable ones. Indeed, oligotrophic limestone-based wetlands that were "polluted" by phosphorous-enriched runoff caused replacement of rush vegetation with dense cattail. This vegetation shift had important consequences on the larval mosquito community: from *Anopheles albimanus* to *Anopheles vestitipennis* (each preferring a typical habitat, respectively) which is a superior vector of the parasite *Plasmodium* to humans (Grieco *et al.*, 2006). Another excellent example is nutrient input in mesocosms containing snails, larval amphibians and parasites (Johnson *et al.*, 2007). Via eutrophication of such mesocosms, enhanced growth of algae occurred that affected positively the growth and reproduction of snail intermediary hosts (susceptible to parasitic miracidia). In addition, infected host snails from the high nutrient condition did produce, on average, twice as many parasites as did snails from a low nutrient environment.
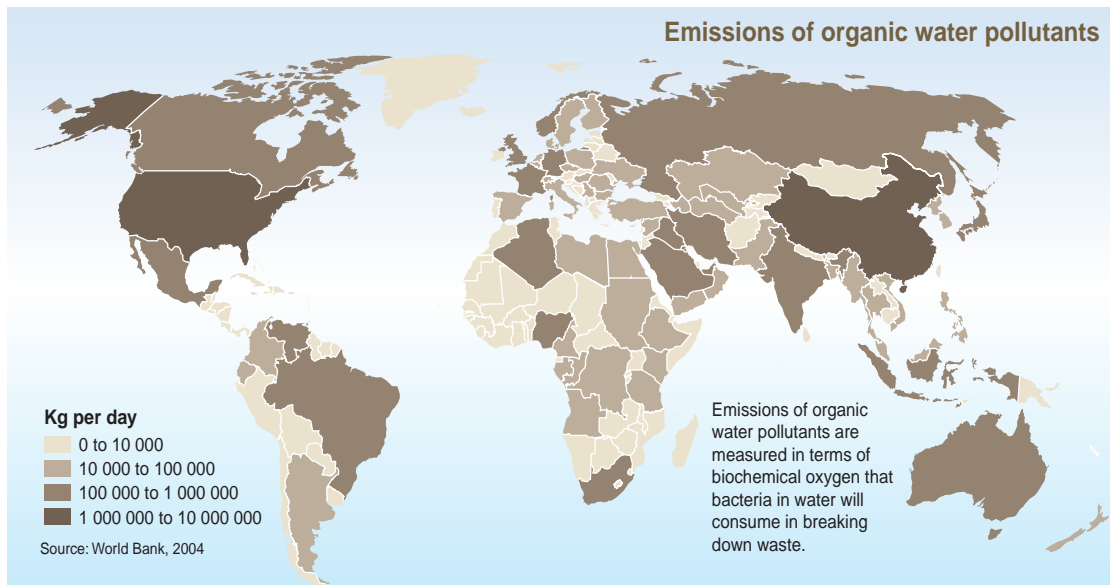
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A nice example of environmental pollution in urban areas that affected animals and potentially humans was described by Scialfa *et al.* (2010). These authors reported on the presence of *Leptospira interrogans* in a highly dense rat' population (*Rattus norvegicus*) in a suburban area of Buenos Aires. The high density of the rat' reservoir is directly connected to environmental pollution (refuse, waste, etc.) and poverty.

Finally, Fayer *et al.* (2004) linked land to sea environment through wastewater discharge into estuaries and coastal waters. Encysted zoonotic protozoan parasites present in raw sewage (e.g., *Giardia*, *Cryptosporidium* and *Toxoplasma*) contaminate bathing beaches, infect a wide range of marine animal hosts (sometimes with fatal results in some populations) and are concentrated through filtration by shellfish to be further consumed raw by humans and marine mammals.
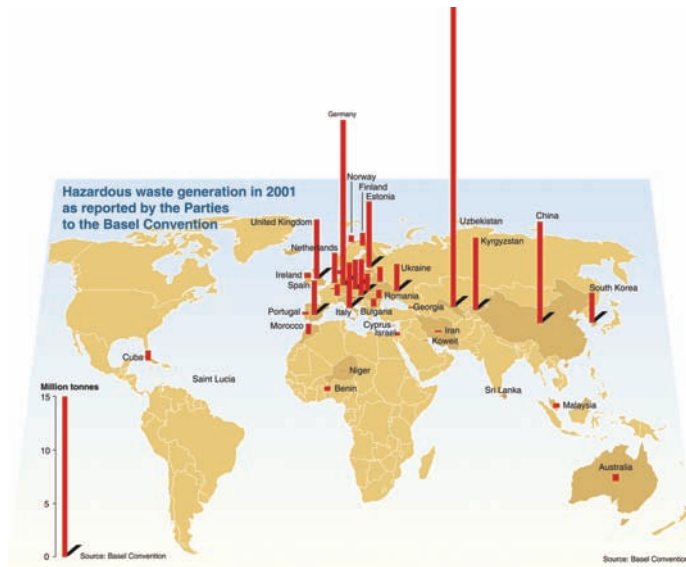
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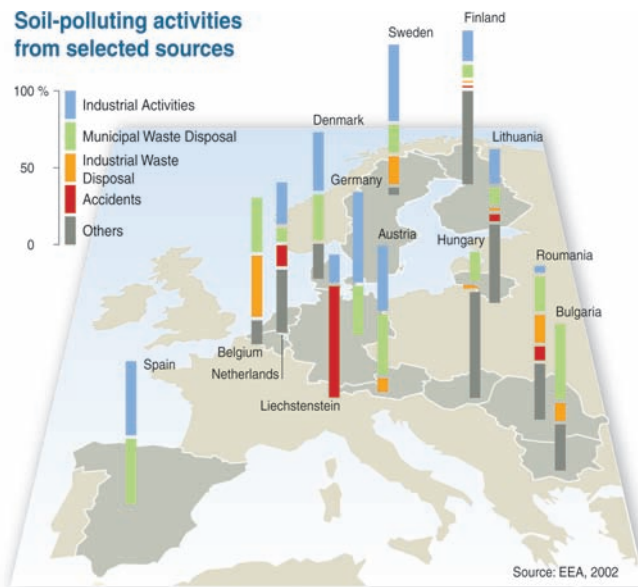
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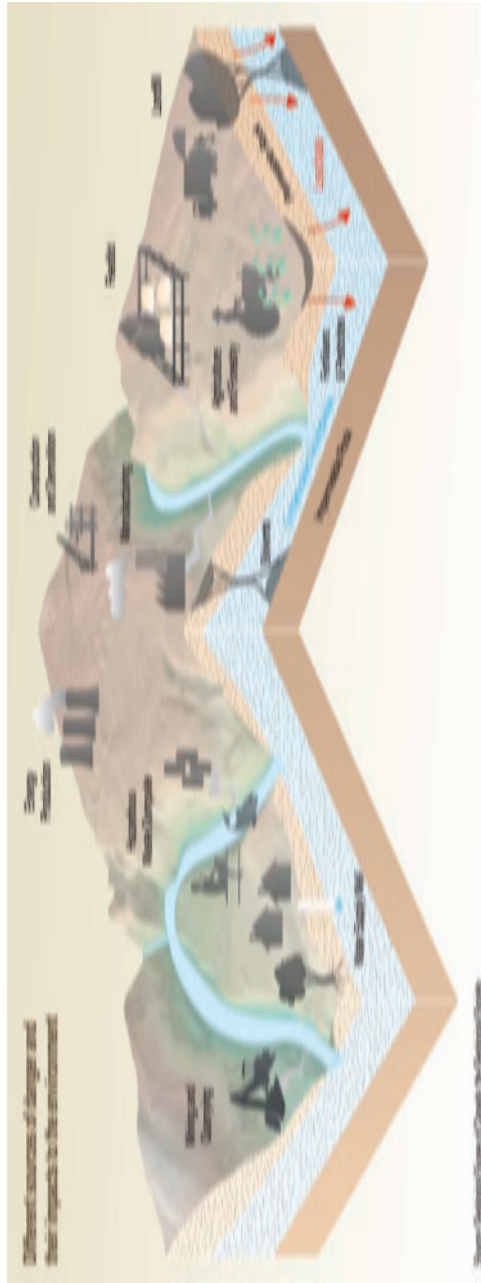
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Chapter 2

Bacterial Zoonoses

Chapter 2.1

Anthrax

[*BACILLUS ANTHRACIS*]

Bacillus anthracis is a gram-positive, facultative anaerobic, rode-shaped, spore-forming bacterium, able to synthesize a protein capsule (D-glutamate), and is the sole pathogenic bacterium to carry its own adenylyl cyclase virulence factor (edema factor). *B. anthracis*' spores are highly resistant and capable of surviving over decades or centuries in soil and of resisting extreme temperatures, harsh chemical treatment and low-nutrient environments. The actual infectious dose required to cause human disease via cutaneous contact is 10^4 to 10^7 spores (experimentally performed on shaved and abraded skin of DBA/2 mice, Hahn *et al.*, 2005), via inhalation is considered to be between 8×10^3 to 1.5×10^4 (Seidel and Cotta, 2002) while via ingestion is still unknown, although experimental evidence suggests that it is approximately $> 10^8$ spores (Beatty *et al.*, 2003). The disease can represent itself in several forms dependent on the infectious routes: 1. *Respiratory*: flu-like symptoms for several days, followed by severe respiratory collapse (fatality ranges from 45 to 92%); 2. *Gastrointestinal* (through ingestion) by hematemesis (blood vomiting), severe diarrhea, acute inflammation of the intestinal tract, loss of appetite and lesions along the gastrointestinal (GI) track (fatality ranges from 25 to 60 %) and 3. *Cutaneous* by appearance of skin lesions that eventually forms an ulcer with a black center (~20% fatality if not treated).

2.1.1 *B. ANTHRACIS* AND ENVIRONMENT

2.1.1.1 Water and soil

Antrax is an acute infection of cattle, sheep and other grazing animals transmitted zoonotically to humans or by direct human-human contact. In 1875, Robert Koch was the first to identify and to show the pathogen transmission routes (Koch, 1876). There are three forms of the disease according to these routes: gastrointestinal, pulmonary and cutaneous. *Bacillus anthracis* [phylum: Firmicutes] has a worldwide distribution. As already mentioned, *B. anthracis*' spores are highly resistant to environmental conditions and can prevail for decades (Willis, 2004), therefore historically being considered as the ultimate biological weapon. Recently, Lewerin *et al.* (2010) reported on an outbreak of anthrax that occurred in a beef cattle herd in the southern part of Sweden, 27 years after the last outbreak. Following an

on-site/laboratory combined investigation, the authors concluded that the contamination source was roughage (in spite of a negative laboratory analysis). The roughage contained soil and dust from a field that had been flooded in the previous year and had dried up in the following year as a result of a very dry summer. According to local history, it was reported that in the early 1900-s during anthrax outbreaks animal carcasses were dumped in the flooding river; therefore it is very possible that long-standing anthrax spores remained in this area. A closely related picture was described in Lake Rukwa Valley, Tanzania, where an epidemic of 239 human cases occurred (predominantly of males between 15 and 35 years old) (Webber, 1985). The infective source was meat from animals dying of anthrax, however no intestinal cases were reported. Interestingly, the epidemic was seasonal, reaching a peak towards the end of the dry period when cattle were close grazing, and declined rapidly once rains commenced and grass began to grow.

In another episode, Fox *et al.* (1977) described an epizootic anthrax event in Falls County, Texas during June and July, which resulted in the death of 236 animals (228 cattle, 5 horses, 2 mules, and 1 pig) on 48 premises without any cases of human illness. According to these authors, anthrax spores were apparently precipitated by drought, and infection appeared to be the result of intrinsic ingestion of contaminated soil and grass. Buravtseva *et al.* (2003) reviewing anthrax epidemiological data following floods in the Southern Federal District regions of Russia, reported that no epidemic complications were noted in the affected territories during the year of 2002.

In simulation experiments of arid environment (sand) contaminated with bacterial spores, common wind erosion preventive materials (such as Surtac and Soiltac) were topically applied to prevent airborne spores' dispersion (Lloyd *et al.*, 2006). Surtac is a solution comprised of a carbohydrate polysaccharide blend, surfactants, mordants and water while Soiltac is a polymer-based emulsion; both are biodegradable materials. Both Surtac and Soiltac were effective dust palliatives reducing the spores' spread due to the solidification of the top surface forming a solid crust.

In 1993, a remote area of Canada (west of Great Slave Lake in the Northwest Territories) were bisons, mooses and black bears are free-ranging, was hit by anthrax resulting in 172 bisons, 3 mooses and 3 black bear carcasses (Gates *et al.*, 1995). The outbreak occurred after a prolonged period of dryness between April and mid-July of 1993 which followed several successive years of bisons' habitat flooding. The authors provided the "spore concentration hypothesis" as the most conservative explanation for the occurrence of anthrax under those observed conditions (Figure 2.1.1). One of the major characteristics of *B. anthracis*'s spores is their hydrophobicity (Doyle *et al.*, 1984; Leishman *et al.*, 2010) although in food such as milk and orange juice they revealed moderate hydrophobicity when experimentally extracted by a method using bacterial/microbial adherence to hydrocarbons (BETH/METH) (Leishman *et al.*, 2010; Rosenberg, 1984). Basically, this theory is based on the hydrophobic characteristics of *B. anthracis* spores that float on the water's surface during flood and then concentrate on growing plants during drier periods, favoring the infection of grazing animals (Anonymous, b). In this study, scavenging originating from carcasses was prevented by the application of formaldehyde.

Mongoh *et al.* (2008) analyzed statistically an outbreak of anthrax in North Dakota, during the year 2005, that affected a large variety of mammal species such as: cattle, bison, horses, sheep, elk, deer, pigs, and llamas. In this study, the presence of very wet conditions prior to the outbreak and dry conditions during it were statistically significant for predicted occurrence of anthrax. For example, the mean precipitation during the period was 3.05" with a median of 2.50″. Before the outbreak, the period between mid-May and June recorded a mean of about 7.10" of precipitation (wet conditions) while between the end of June and July, when most cases were reported, the region recorded a mean precipitation of only 1.90″ (dry conditions), therefore indicating that weather conditions do play a significant role in anthrax outbreaks. In another study, Lindeque and Turnbull (1994) analyzed anthrax mortality records of elephants and

plain ungulates in Etosha National Park, Namibia. The seasonal peak among the elephants was November (the end of the dry season) while among ungulates it was March (towards the end of the rainy season). In their study, *B. anthracis* was detected in 3.3% of water and 3.0% of soil samples collected at different times of the year from 23 sites, not associated with known cases of anthrax. Higher rates (26.0%) were found in waterholes samples at the time of an outbreak in elephants. Based on these results it is clear that climate plays a major role in the natural infection cycle of anthrax (Table 1). Scavengers' importance was confirmed by feces collection from vulture, jackal and hyena from the vicinity of confirmed anthrax carcasses, yielding $>$50% positive samples of *B. anthracis* spores (some with substantial large numbers) compared to none found in feces unconnected to known anthrax carcasses. Despite typical *B. anthracis* levels of $>10^7$ FU/ml in sick animals' blood, spore levels in carcass contaminated soil ranged from undetectable to $10–10^3$ CFU/ml. Importantly, after experimental monitoring the authors reported a rapid loss of spores' viability in soil and water in relation to their types. In addition, the extent of *B. anthracis* survival and sporulation in water has been shown to be related to its initial concentration.
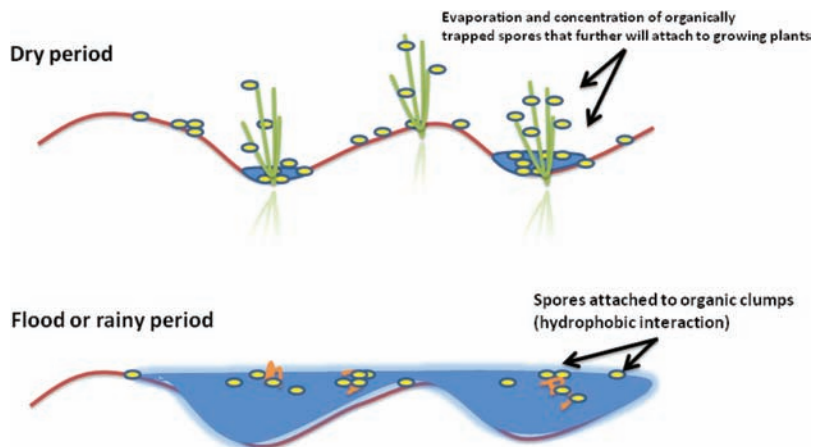
Figure 2.1.1. *B. anthracis* spores "concentration theory" during dry and wet weather (adapted from Dragon and Rennier, 1995)

The frequently cited Van Ness (1971) made the following observations regarding *B. anthracis* ecology: 1) spores persist in incubator areas where characteristically low-lying depressions or rocklands with standing water devitalized of plant life exist or dried-up water courses or hillside seeps where organic matter has accumulated during floods or runoff and 2) infections were observed only in association with limestone soils or affected by calcareous soils rich in nutrients (Hugh-Jones 1975).

Nevertheless, other means of contamination had been documented such as: spores shedding through feces and urine of infected animals (Choquette, 1970); various insects such as mosquitoes and tabanids (deer, horse, and stable flies) that have been implicated in the transmission of spores from animal to animal (Choquette, 1970; Krishna and Mohiyudeen, 1958; McKendrick 1980; Turell and Knudson, 1987) from animal to vegetation (De Vos, 1990; Choquette,1970; Ebedes 1976; Krishna and Mohiyudeen, 1958; Sen and Minett, 1944) by spore contaminated soil adhering to the coats of wallowing animals (bison) (Ebedes, 1976; Whitford, 1979); or the adherence of blood and viscera to the fur and feathers of scavengers from infected carcasses (Ebedes, 1976).

Table 2.1.1 Selected global anthrax cases linked to soil, water and climate factors and their intersection.

Site and cases number	Year	Environmental factors (rainfall)
Ascension Parish, Louisiana, USA 23/75 livestock premises	1971	A "dry period" followed by a "wet period" (PI)[a]
Falls County, Texas, USA 18/48 livestock premises	1974	A "dry period" not followed by a "wet period"
Goulburn Valley, Victoria, Australia 83 premises	1997	The weather conditions preceding and during the epizootic approximated those of a drought (avg. 29°C)
Pembina, Grand Forks, Nelson, Traill, and Steele counties, North Dakota, USA 24/31 livestock premises	2000	July-August (average rainfall 3″–4″) in the area of Red River
Kinney, Val Verde, Uvalde, Edwards, and Real counties, Texas, USA 71 premises (1637 dead animals) 11 cases laboratory confirmed	2001	June-September 8″-32″ annual rain
Etosha National Park, Namibia 2,935 cases	1964–1992	The peak incidence of anthrax in plains ungulates occurred in March, at the end of the rainy season. The peak incidence of anthrax in elephants occurred in November, near the end of the dry season and the beginning of the wet season.
Northern Canada, 1,098 dead bisons	1963–1992	Hot, dry summer months of June through September, preceded by a wet spring. High ambient temperatures.

a- Possibly increased risk (PI)

Table 2.1.1 (references)
1. Fox, M.D., Kaufmann, A.F., Zendel, S.A., Kolb, R.C., Songy, C.G. Jr. *et al.* (1973) Anthrax in Louisiana, 1971: epizootiologic study. *J.*
2. Fox, M.D., Boyce, J.M., Kaufmann, A.F., Young, J.B. & Whitford, H.W. (1977) An epizootiologic study of anthrax in Falls County, Texas
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4. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services [USDA APHIS, VS]. Epizootiology http://www.aphis.usda.gov/vs/ceah/cei/taf/emerginganimalhealthissues_files/anthrax.pdf.Accessed 1st of August 2010.
5. Lindeque, P.M. & Turnbull, P.C.B. (1994) Ecology and epidemiology of anthrax in the Etosha National Park, Namibia. *Onderstepoort J.*
6. Gainer, R.S. & Saunders, J.R. (1989) Aspects of the epidemiology of anthrax in Wood Buffalo National Park environs. *Can. Vet. J.* **30**,
7. Dragon, D.C., Elkin, B.T., Nishi, J.S. & Ellsworth, T. R. (1999) A review of anthrax in Canada and implications for research on the disease
8. Dragon, D.C. & Rennie, R.P. (1995) The ecology of anthrax spores: tough but not invincible. *Can. Vet. J.* **36**, 295–301.

Soil	Management factors (pastures, water sources, supplement feed, others)	Reference
Loam and clay (alkaline pH) (PI)Calhoun-Olivier (acidic pH)		1
Loam (PI) Clay Sand Top soil (0–17mm) acidic to mildly acidic (PI)	Surface water drainage. (PI)Water permeability 15–500mm/hour. (PI)	2
Poorly drained swamps containing alluvial soils. The soil in the anthrax-affected region had been disturbed recently due to improvements in the irrigation system and reconstruction of channels and drainage systems.	The geographical location of the epizootic was historically a major route for movement of cattle and sheep from southern Victoria to northern Victoria.	3
	Horse mortality (12.16%), Cattle (85.7 %), Sheep (none).	4
	Cervids, bovids, equids and camelids (wide range species) white tailed deer, red deer, fallow deer, elk, antelope, gazelle, water buffalo, beef cow, goat, sheep, bison, horse, mule, donkey, and llama	
The persistence of spores in karstveld soils was higher than in other soil types.	Elephants and ungulates (Burchell's zebra, blue wildebeest, springbok) *B. anthracis* was recovered from approximately 50 percent of the fecal specimens from nearby scavengers (vulture, jackal and hyena). Only 3.3 % of water and 3% of soil samples were positive. Hot, wet season (1.4% positive samples) cold, dry season (5.7% positive samples) and hot, dry season (3.5% positive samples). The concentration of spores in the soil adjacent to carcasses (5.0×10^4 maximum) compared to blood specimens from carcasses (7.0×10^9 maximum). *B. anthracis* was highly susceptible to ultraviolet radiation and to solar radiation after as few as 4 hours of exposure during laboratory experiments *B. anthracis* was able to multiply and sporulate in spring water and water from gravel-pits during laboratory experiments, if the waters were contaminated with low dilutions (i.e., high concentrations) of blood.	5
Flooded soil	Intense mating activity, high densities of insects, and high densities of bison.	6,7,8

Am. Vet. Med. Assoc. **163**, 446–451.
J. Am. Vet. Med. Assoc. **170**, 327–333.
on an anthrax outbreak in Australia in 1997. 3rd International Conference on Anthrax, University of Plymouth, 7–10

and ecology of anthrax [online]. Available at:

Vet. Res. **61**, 71–83.
953–956.
in northern bison. 3rd International Conference on Anthrax, University of Plymouth, 7–10 September, 1998. *J. Med. Microbiol.* **87**, 208–213.

Furthermore, water and wind seem also to play a major contribution in spores' dispersion from decomposing carcasses and other infected material that can be easily lifted under very dry conditions (Choquette, 1970; Ebedes, 1976; Heath and Brewitt, 1982). The 1979 Sverdlovsk (now Yekaterinburg) incident is a classical example of accidental contamination of large areas with *B. anthracis* spores originating from a biological warfare facility. A strain of *B. anthracis* produced as a bio-warfare arsenal in the Military Compound 19, near Sverdlovsk, a highly infective strain ("Anthrax 836") was accidentally released from the facility exhaust pipes that lacked the required filter. Before remounting the missing filter, the accidental released cloud of *B. anthracis* spores had spread south on the northerly wind that prevailed shortly before the outbreak. According to epidemiological data, most victims worked or lived in a narrow zone extending from the military facility to the southern city limit. Farther south, livestock died of anthrax along the zone's extended axis. Following an investigation after the event, it was undoubtedly concluded that aerosol of anthrax pathogen that escaped from the military facility caused this outbreak (Meselson *et al.*, 1994). Fortunately, "only" 64 casualties were recorded; however, if the wind direction had been from south to north (toward Sverdlovsk city) a much higher numbers of casualties would been recorded at the time.

Dragon and Rennie (1995) in an excellent review, based on reported outbreaks summarized, the main characteristics of environmental conditions that favor *B. anthracis* spores epizootic infections. A summary of these factors are schematically presented in Figure 2.1.2, where some have been scientifically demonstrated while others are more speculative though they cannot be ruled out.
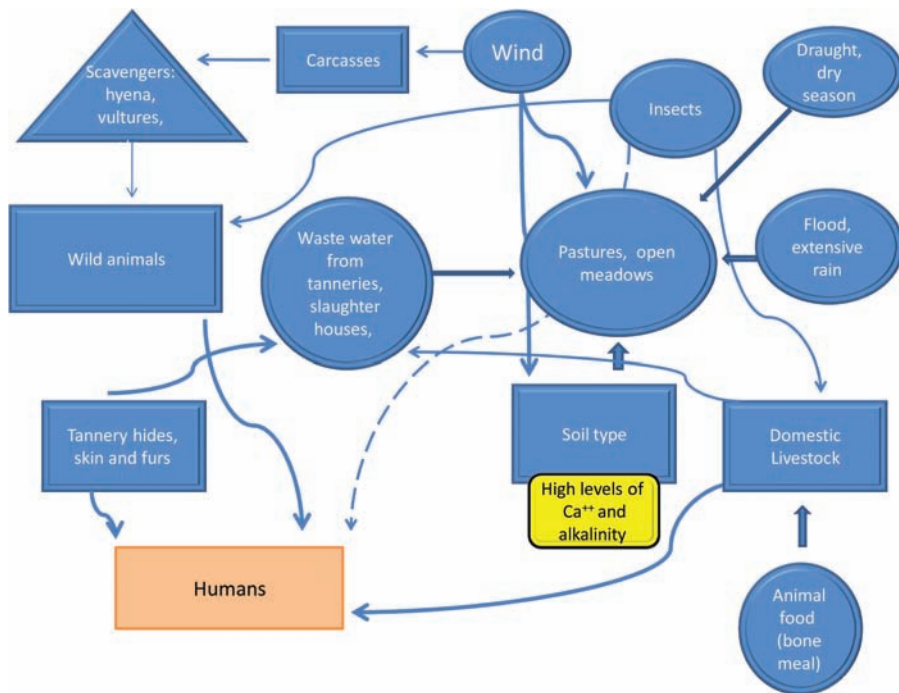
Figure 2.1.2. Environmental factors affecting human infection with *B. anthracis*

As a final point, in order to clarify the whole conundrum picture, it is clear that environment is a very complex matrix in which many biotic and abiotic factors are involved and closely related. Schuch and Fischetti (2009) in their extensive and outstanding study, demonstrated the important role of *B. anthracis*'s bacteriophages during its environmental phase through pseudolysogeny, that provides alternatives to sporulation involving the activation of soil-survival and endosymbiotic capabilities in earthworms gut (biofilm formation, etc.) (Figure 2.1.3). Birds do feed on earthworms; therefore while not being directly infected they can spread it through continents by their annual migratory cycle (Anonymous, 2010). It would be interesting to look for *B. anthracis* spores shed by migratory birds at their regular stop-over sites.
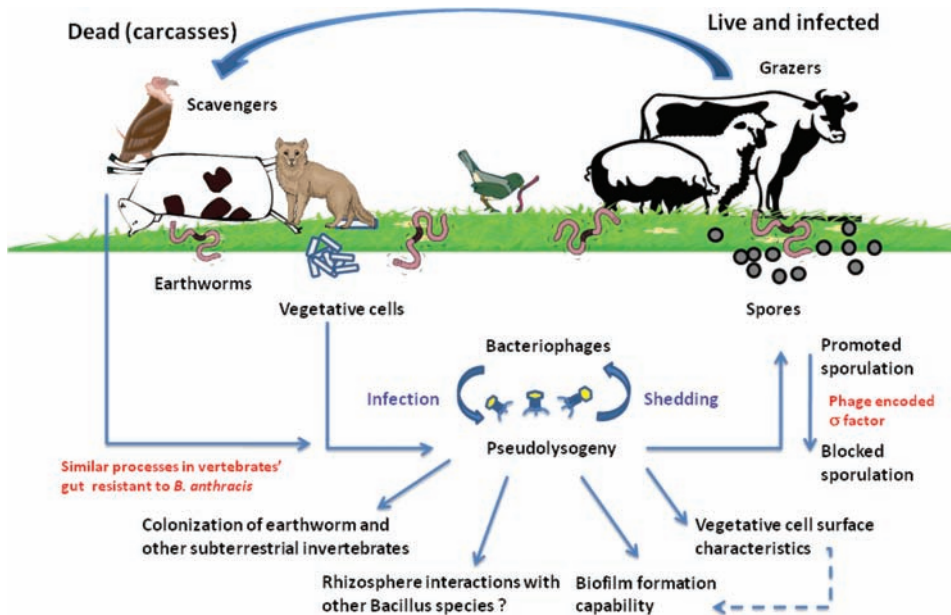
Figure 2.1.3. Biotic factors involved in *B. anthracis* spores dispersion (adapted from Schuch and Fischetti, 2009)

2.1.1.2 Conclusions and remarks

The scientific debate on *B. anthracis* spores ecology and their infection routes continues. Of their fierce environmental resistance there is no doubt: *B. anthracis* spores subsist for decades (Wilson and Russell, 1964; Willis, 2004) in spite of some reports questioning this fact (Turnbull *et al.*, 1985) and even at extreme pressure of 600 Atm (Certes, 1884). The published literature shows that regardless of regular herd vaccination, sporadic epizoonotic events occur around the globe. After more than 100 years of research, spores detection methodology is still inadequate to identify accurately their presence in the environment, especially from soil (Marston *et al.*, 2008) and only a recently published report presented an antibiotic supplemented medium that supports *B. anthracis* growth (with a selectivity of 96.8 to100%) inhibiting others *Bacillus* species that are commonly present and interfer in soil environment (Luna *et al.*, 2009).

Molecular methods (PCR, DNA probes, etc.) should solve the detection problems as described by Cheun *et al.* (2003) that used nested and real-time PCR to detect *B. anthracis* spores seeded in soil samples.

It should be pointed out at this point in time, that these methods still do not differentiate between viable and dead/injured spores while direct cultivation does.

According to the literature, the main source of *B. anthracis* spores still remains affected animals and their carcasses where soil plays a major role. Per se, water flow, winds, wildlife and human activity (such as soil transfer and agricultural activity) can turn out to be additional factors in spreading the spores (Seidel and Cotta, 2002; Metz, 1980; Masner, 1938; Lutz, 1913; Cabello and Cabello, 2008). Regulations on meadow use for domestic growth of herds as well soil transfer should include seasonal testing, while heavy rain and floods should be a warning in any country -side where anthrax is endemic. Anthropogenic activities such as deforestation, irrigation, urbanization and industrial activity can impact wildlife that is a potential carrier of the disease, bringing it closer to humans, causing reemergence of this old disease. Finally, biological warfare should be abandoned and countries violating international lows should be chastised by the international community.

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Chapter 2.2

Bartonelloses (including cat scratch)

[*BARTONELLA SPP.*]

Bartonelloses can be found under several names (Carrion's, cat scratch, trench fever, bacillary angiomatosis and peliosis hepatis disease) that historically were reported along the years since the first described case by Carrión (in Latin the name means carcass!) in 1885 (Leonard, 1991; Scultz, 2010). *Bartonella* is a genus attributed to alpha subgroup 2 of phylum proteobacteria. Cells are gram-negative, aerobic, coccobacillary (pleomorphic), flagellated, motile and facultative intracellular organisms (Peters and Wigand, 1955). So far, the genus comprises 23 species identified thus far, but the following main species involved in zoonosis are: *Bartonella bacilliformis*, *B. henselae, B. quintana, B. elizabethae B. grahamii, B. vinsoni B. washonsis, B. clarridgiae and B. rochalimae* (Piemont and Heller, 1999; Guptill, 2010). Bartonella is now known to be strongly linked to various arthropod vectors (sand fly, mite, tick, lice, flea) and to wild and domestic reservoirs (including pet animals) such as: felids, canids, rodents, cattle, fishes, birds, horses, etc. summarized in Figure 2.2.1 (Podsiadly *et al.*, 2002; Chomel *et al.*, 2006; Chomel *et al.*, 2006a; Holmberg *et al.*, 2003; Guptill, 2010; Bermond *et al.*, 2002; Jones *et al.*, 2009; Breitschwerdt, 2008). Co-infection with several species was also reported that might well derive from different arthropods' bite or, so far unconfirmed, single arthropod species carrying several *Bartonella* spp. (Vissotto de Paiva Diniz *et al.*, 2009; Welc-Faleciak, 2009). The pathogen is now known to cause human diseases such as: bacillary angiomatosis, cat-scratch disease, chronic bacteraemia and lymphadenopathy, meningoencephalitis, stellar retinitis, myelitis, granulomatous, hepatitis, endocarditis, osteomyelitis and peliosis hepatitis (Jacomo *et al.*, 2002). The main clinical symptoms are high fever, chills, anorexia, malaise, lymphadenitis with purulent secretions, meningitis and paralysis (fatality rate 40 to 85% if untreated) (Maguiña and Gotuzzo, 2000).

2.2.1 *BARTONELLA* AND ENVIRONMENT

2.2.1.1 Insects

It is now clear that a variety of blood feeding (hematophagous) insects are the main vectors of *Bartonella*, while a variety of rodents and other mammals operate as reservoirs. Feliciangeli (2004) studied the natural

breeding places of sandflies. Sandflies ecotopes occupied by immature phlebotomines are typically organically rich moist soils, such as the rain forest floor, contaminated soil of animal shelters and earthen floor of human habitations. The addition of global warming to these conditions, which has been shown to favor insects' reproduction and their populations swell, might explain to some extent the new outbreaks of bartonelloses in remote sites that supply these conditions (Frazier *et al.*, 2006). An important contribution to the major role of arthropods in bartonelloses transmission was shown by Bown *et al.* (2004) in bank voles. Inoculation of bank voles with *B. taylorii* in an arthropod-free environment was not transmitted either horizontally or vertically, providing the clear evidence of the essential role of fleas (in this case) in *Bartonella* transmission among these rodents.
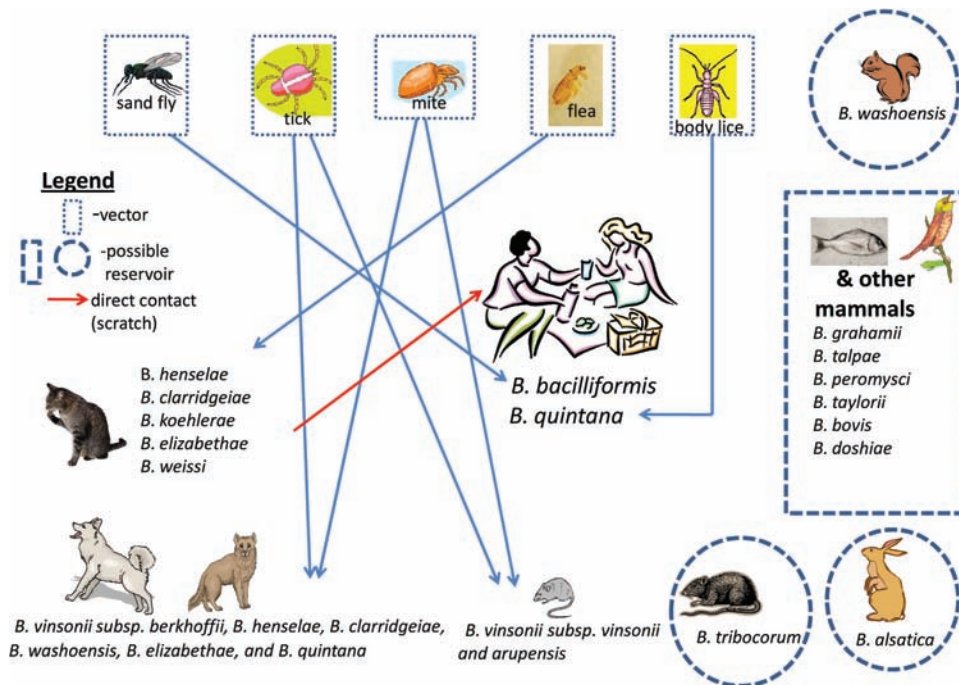
Figure 2.2.1. *Bartonella* spp. hosts, vectors and transmission

Ellis *et al.*, (1999) conducted a case-study of an acute outbreak of bartonellosis in the Urubamba Valley (Peru) that was previously unknown as an endemic area. These authors found from odds-ratio analysis that the case-patients group reported sand fly bites more frequently than individuals of neighboring households. However, sand flies (*Lutzomyia peruensis*) captured during this study did not show increased harborage of *Bartonella* and the truly positive, harbored *B. bacilliformis* and in one case *B. grahamii* only. It should be mentioned that no rodent survey was performed assuming that *B. bacilliformis* is a human pathogens not yet found in rodents. However, it cannot be ruled out that rodents do carry this species.

Another outbreak of *B. bacilliformis* in Ecuador was reported by Cooper *et al.* (1997). According to the authors' analysis, all case houses were located in isolated areas at the forest margin with presence of dead rodents in case houses only. They suggested that human bartonellosis is a zoonosis with a natural rodent

reservoir and that migrant humans infected in this way may become a temporary reservoir host in populated areas. Evidences of interspecies transmission and possible infection have been shown with *B. bacilliformis* in experimental animals by inoculation of susceptible animals including monkeys, dogs, donkeys, rabbits and guinea-pigs (Herrer, 1990). These authors also reported "the appearance of an epidemic of a rapidly fatal chicken illness characterized by fever, diarrhoea, and dyspnoea". The few avian survivors developed blood-containing 'verrugas' which were most noticeable on the head area. The avian epidemic spread slowly between communities, and most case households (63%) reported its appearance before the development of disease in human cases.

2.2.1.2 *Bartonella* in pets

It can be stated with a high degree of certainty that globally the most common pet animals are dogs and cats. Since their early domestication, they have been one of the main sources of zoonotic diseases (Chomel *et al.*, 2006). However, additional pet animals have been shown to harbor *Bartonella* spp. Inoue *et al.* (2009) looked for the prevalence of *Bartonella* spp. in 546 small mammals (28 species) that had been imported as pets into Japan from Asia, North America, Europe, and the Middle and Near East. The animals examined carried four zoonotic *Bartonella* spp. that cause human endocarditis and neuroretinitis and six novel *Bartonella* spp. at a relative high prevalence (26.0%), presenting a marked risk of human infection with this pathogen.

2.2.1.3 Weather and global warming

Beldomenico *et al.* (2005) studied *Bartonella vinsonii* subsp. *berkhoffii* in the coyote population of Northern California along with two other pathogens. Applying univariate analyses, these authors concluded that *Bartonella* seropositivity was associated with higher precipitation and proximity to the coast (where higher precipitation occurs). Arthropods activity of flea, tick, mosquito and sandfly is closely associated with high humidity and warm temperature, explaining the ecology of *Bartonella* in such geographical areas. It should be mentioned that excavations, deforestation, etc. can disrupt rodents' regular habitats, setting off interchange environments and exposure of hosts to new vectors.

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Chapter 2.3

Borreliosis

[*BORRELIA BURGDORFERI*]

Genus Borrelia are gram-negative, non-spore forming, aerobic to microaerophilic, extracellular pathogenic spirochete containing 36 known species among which *Borrelia burgdorferi, B. recurrentis, B. afzelii*, *B. anserina*, *B. garinii*, *B. hermsii*, *B. valaisiana* and *B. vincentii* are the best known; all are transmitted by ticks and body lice by means of reservoirs such as rodents or other mammals (Figure 2.3.1) (Samuels and Radolf, 2010; Anderson *et al.*, 1985; Hanincová *et al.*, 2003; Gil *et al.*, 2005). In humans the disease is called Lyme borreliosis (named after the town Lyme in Connecticut, USA where it was first reported) (caused primarily by *Borrelia burgdorfii*) or relapsing fever (caused primarily by *Borrelia recurrentis*). The etiological factor was identified in the 70s by Willy Burgdorfer to be a tick-borne spirochetal bacterium (Burgdorf *et al.*, 1982). The symptoms of the diseases are mainly dermatological (erythema), rheumatological (arthritic episodes), neurological (chronic lymphocytic meningitis) and cardiological (myocarditis, pericarditis, etc.). At present *Borrelia* bacteria have been detected globally, in a variety of ticks and animals (Table 2.3.1.).

2.3.1 *BORRELIA* AND ENVIRONMENT

2.3.1.1 *Borrelia* and birds

The ability of birds to carry ticks over a large geographical area is undisputable (Reed *et al.*, 2003). There are many birds species (i.e. passerine birds that belong to the order *Passeriformes*, which includes more than half of all bird species), mainly ground foraging birds that are infested by ectoparasites, holding them on body sites that are difficult to preen (ear, eye and head) (Dubska *et al.*, 2009). This way, birds are able to transfer ticks and establish new endemic foci of boreliosis all over the globe, through their migratory activity (Figure 2.3.2). The reservoir question has still to be clarified as not all birds are capable of transmitting the infectious agent to tick vectors (Mather *et al.*, 1989).

Ground animals, including humans, seem to be the major reservoir of *Borrelia* (Figure 2.3.3). A surveillance study in Wisconsin and Northern Illinois, USA, showed that dogs' seroprevalence was between 0 to 40%. Spatial pattern was significantly correlated with human incidences of Lyme disease's

and with an abundance of the tick vector (*Ixodes scapularis*). Among surveyed dogs, seropositivity was definitely associated with increased tick exposure, time spent outdoors and living in forested and urban areas as well on sandy, fertile soils (Guerra *et al.*, 2001).
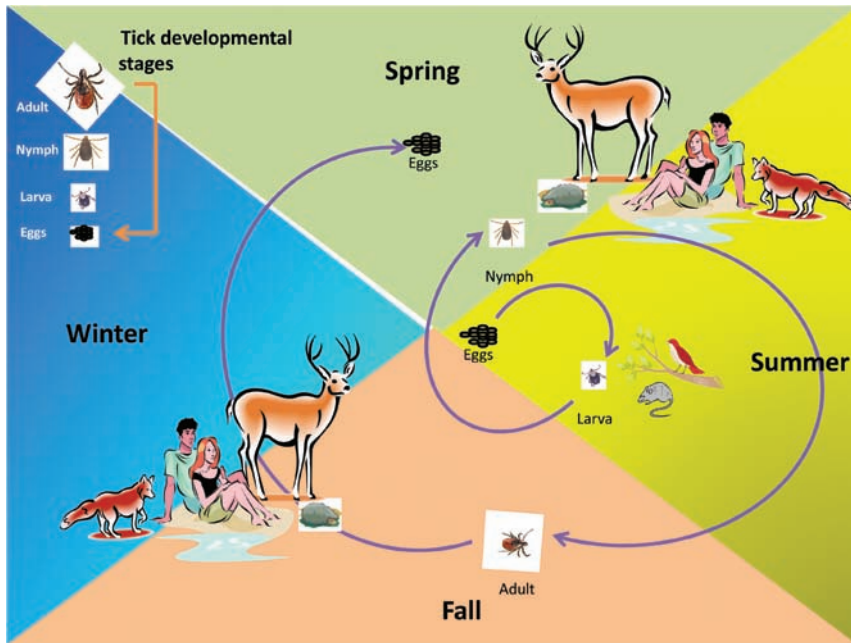
Figure 2.3.1. *Borrelia burgdorfii* infection cycle and environmental aspects (with permission, adapted and modified from CDC, Atlanta, GA, USA)
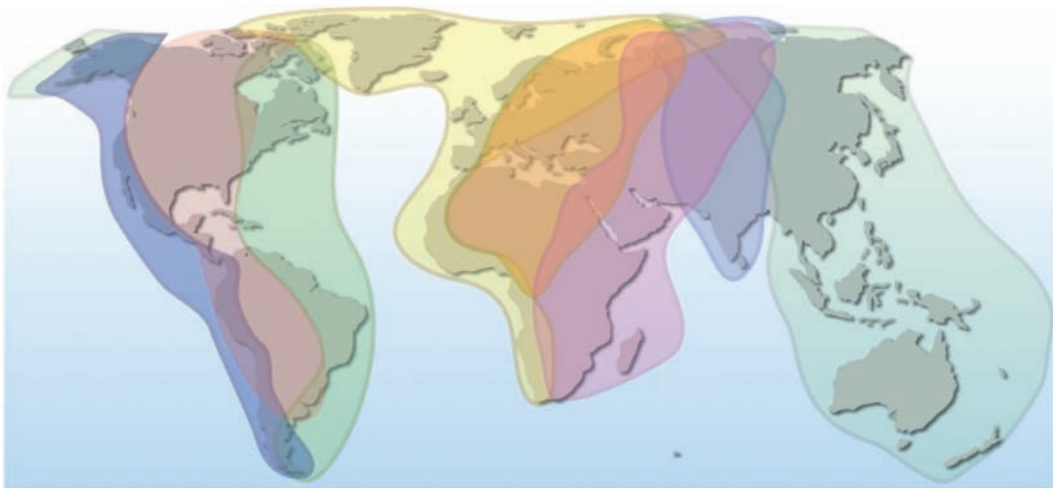
Figure 2.3.2. Global geographic areas covered by migratory birds. [With permission from http://maps.grida. no/go/graphic/major-global-bird-migration-routes-to-the-arctic (*Hugo Ahlenius, UNEP/GRID-Arendal*)]

Table 2.3.1 Worldwide geographical distribution of ticks as vectors of Borrelosis.

Tick spp.	Geographical site	Animal involved	Borellia sp.	Reference
Ixodes scapularis	North America	Canines, felines, and equines	*B. burgdorferi* (s.l.)[a], *B. afzelii, B. garinii, B. lusitaniae, B. spielmanii,* and *B. valaisiana.*	Crowder et al. (2010); Morshed et al. (2006)
Ixodes pacificus	North America	Western gray squirrels	*B. burgdorferi* and new strains	Crowder et al. (2010); Girard et al. (2009)
Ixodes ricinus	Europe	Various animals	*B. burgdorferi* (s.l.)	Piesman and Schwan (2010)
Ixodes persulcatus	Asia	Small rodents and imported reptiles	*B. afzelii, B. garinii*	Takada et al. (2001)
Ixodes ricinus *Alectorobius sonraai*	Africa (Tunisia, Algeria, Morocco, Mali)	Rodents, Humans and possible other animals	*B. lusitaniae B. crocidurae, B. garinii* and *B. burgdorferi*	Marjolet et al. (1995); Younsi et al. (2001)
Rhipicephalus (Boophilus) microplus -cattle tick	South America	Cattle	*B. theileri* and *B. lonestari*	Yparraguire et al. (2007)

[a] s.l. *senso lato*
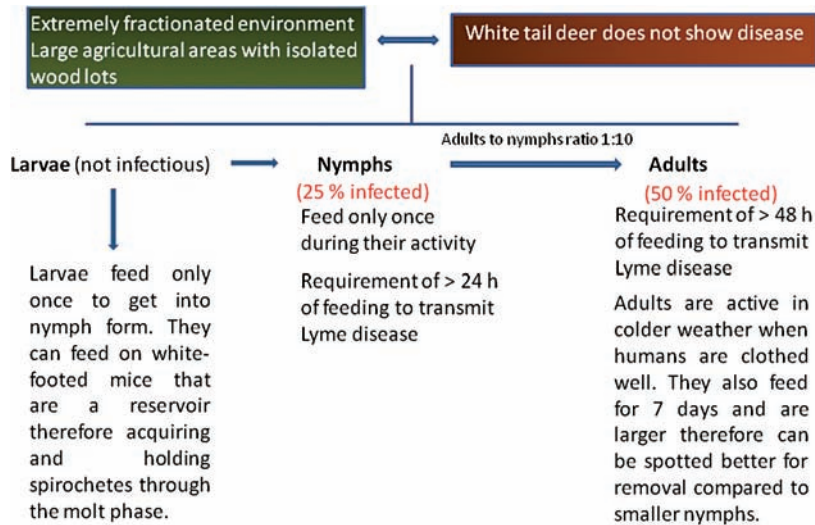
Figure 2.3.3. Environmental ground factors involved in the Lyme disease spread in USA (after Fish, 1995)

2.3.1.2 *Borrelia* and forest

As shown before, ticks the key vector of Lyme disease bacterium, reside mainly on the ground in humid, bushed and forested areas. A study based on the hypothesis that small forest patches (<2 ha) have a higher density of infected nymphal blacklegged ticks (a tick well known as the primary risk factor for Lyme disease) than larger patches (2–8 ha), was carried out in Dutchess County of southeastern New York state, USA (Allan *et al.*, 2003). The results showed a significant exponential decline in the prevalence of nymphal density and a significant linear decline in nymphal infection with increasing patch area. Therefore, as a result of forest patch size reduction, a dramatic increase in infected nymphs' density was observed (consequently escalating Lyme disease risk). It should be also mentioned that small forest patches are predominantly prone to high densities of different local rodents (in this particular case, whitefooted mouse) and consequently higher densities of infected nymphal blacklegged ticks.

2.3.1.3 Other environmental factors

Soils moistened by rain favor multiplication of ticks, therefore increasing the vector arsenal (Medlock *et al.*, 2008). Interestingly, in relation to rain, the relative scarceness of disease reports from Africa, where drought conditions subsist (mainly in the northern part of the continent), should be noted. Deforestation for agricultural purposes also increases the reservoir potential through the proliferation of various rodents in these areas, as already mentioned above. CO_2 elevated concentrations and the consequent expansion of warm climatic zones directly and indirectly impact the tick and animal life cycle. Finally increased trade and travel may spread vectors worldwide (Sutherst, 2004).

Deer fencing and modification of wildlife behavior in the suburban residential area (bird and animal supplemental feeding) encourage other ticks carriers such as white-footed mice, raccoons and squirrels to draw in closer contact with humans (Fish, 1995). Glass *et al.* (1995) reported similar observations using GIS system and adjusted analyses, that residence in forested areas and specific soils (loamy) was associated with an elevated risk of getting Lyme disease. Estrada-Pena (2003) using a model approach

based on recorded distribution of *Ixodes scapularis* in USA, looked for habitat changes of this tick for a period of 18 years (1982–2000). Based on discriminant analysis, the author found that the increased winter temperatures and expanded vitality of geographical vegetation (as a result of higher rainfall) resulted in the habitat switching from unsuitable to suitable for these ticks.

A more detailed report was performed in a temperate deciduous broad-leaved forest in South Moravia (Czech Republic), which looked for increased activity of local ticks under various environmental variables (Hubalek *et al.*, 2003). Although rainfall did not correlate well, air and soil morning temperature and relative humidity were reported to be closely correlated to tick activity. Burgess *et al.* (1993) showed infection with *Borrelia burgdorferi* in cows, rodents and birds in non-Lyme endemic counties. Interestingly, they also reported pathogen vertical transmission of *B. burgdorfii* in rodents (transplacental transmission in *M. musculus* and *P. leucopus*). New strains of the bacteria that cause Lyme disease have been found in British Columbia, according to a recently published study on ticks collected from birds across Canada (Scott *et al.*, 2001). These researchers found that the genetic makeup of three bacteria differed from previously known strains, with difference of opinions among the authors: one suggested that the novel strains did not show up (Lyme disease patients were test negative) while the others disagreed based on small genetic differences among the strains, not enough to discard the current testing for Lyme disease. In spite of this controversy, it is clear that new pathogen species are evolving continuously, while birds and ticks are excellent incubators beside humans.

In summary, forest (intensive agriculture and forest fragmentation), rain, tick population, reservoirs (rodents and other wild animals such as deer and birds), decline in pesticide use, social problems (homelessness and leisure time), increased temperatures and humidity, soil type and frequent contact with infected animals are all important factors of Borreliosis occurrence.

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Chapter 2.4

Brucelloses

[*BRUCELLA* SPP.]

Brucellosis can be found under different names: Bang's disease, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever and rock fever or undulant fever. The disease was first reported by Sir David Bruce in 1884 who identified the bacterium isolated from a sick British soldier positioned in Malta. *Brucella* bacterium is a gram-negative, non-spore forming rod, non-motile, facultative intracellular pathogen transmitted through ingestion of raw milk, cheese and meat or direct contact with an infected animal's secretions. At the moment, the genus *Brucella* contains 10 species, each with slightly different host specificity (Table 2.4.1). Transmission from human to human is rare but it may occur due to *Brucella*'s low infective dose (10–100 CFU) (Heymann and David, 2008), mainly in contact with mucous areas or skin lesions. The disease is characterized by acute undulating fever, headache, night sweats, fatigue, anorexia and complications such as arthritis, osteomyelitis and spondylitis and life persistence in chronic infection.

2.4.1 *BRUCELLA* AND ENVIRONMENT

Brucella have been isolated from a large variety of feral or domestic animals spread worldwide (Figure 2.4.1). Their association with human zoonoses is obvious and only vaccination of domestic herds can reduce the pathogen's dispersal. Another aspect, related to brucellosis transmission seen in high density animal herds, is winter elks' feed lines in Wyoming, USA (Maichak *et al.*, 2009). These authors concluded that: "1) reduction of elk density and time attending feed grounds, particularly on feed lines; and 2) protection of scavengers on and adjacent to feed grounds would likely reduce intraspecific transmission risk of brucellosis". Similar conclusions in relation to elks' density were supported by results in the Greater Yellowstone ecosystem, revealing a significant seropositivity rise from 0–7% in 1991/2 to 8–20% in 2006/7 respectively, as complemented well by limited hunter access to private lands (Cross *et al.*, 2010).

As an intracellular pathogen, *Brucella* would not be expected to survive extensively in environment. However, there is enough evidences revealing that this pathogen spreads through a large variety of

right

Table 2.4.1 *Brucella* spp. and their reciprocal relations between various animals and humans.

Brucella spp.	Biovar/Serovar	Year of discovery/report	Infected animal	Transmission to other animals	Human infection and high risk	Reference
B. melitensis	1–3	1893 and 1920	Goat, sheep	Cattle, dog	Yes, farmers, dairy workers, shepherds	Wilkinson (1993)
B. abortus	1–6,9	1901and 1920	Cattle	Goat, bison, elk, squirrel	Yes, farmers, ranchers, dairy workers, hikers-travelers	Beja-Pereira et al. (2009); Nol et al., (2009)
B. suis	1–5	1929	Pig, feral swine,	Hares, rodents, reindeer, caribou	Yes, workers in swine slaughterhouses, hunters	Anonymous (2009); Cvetnic et al. (2009); Munoz et al. (2010); Liamkin et al. (1983); Meng et al. (2009)
B. ovis	none[a]	1956	Sheep	Rams, red deer	No	Ridler et al. (2000)
B. neotomae	none	1957	Desert wood rat		No	Stoenner and Lackman (1957)
B.pinnipedialis	none	2001 and 2007	Seal	Porpoise	No	Prenger-Berninghoff et al. (2008); Foster et al. (2007)
B. microti	none	2007 and 2008	Vole		No, isolated also from soil	Scholtz et al. (2008b); Scholtz et al. (2008a)
B. inopinata	none	2008 and 2010	Human (breast implant)		Yes	Scholtz et al.(2010) Tiller et al. (2010)
B. canis	none	1968	Canids	Humans	Yes, dog owners, veterinarians	Brower et al. (2007)
B. ceti	none	2001 and 2007	Cetaceans (whale, dolphins, etc.)		No	Neimanis et al. (2008); Foster et al. (2007)
Serpositivity for Brucella not identified at species level				Caribou, grizzly bears, wolves, foxes		Zarnke et al. (2006); Martino et al. (2004)

animals (including humans) and one species, *B. microti*, isolated primarily from a vole, was also isolated from soil samples (Scholtz *et al.* 2008a). How this species survives in soil is still a puzzle; however these authors supplied attractive supporting explanations for their results: 1) *B. microti* untypically is a fast growing bacterium with increased metabolic activity, therefore organic, moist soil can support their growth and 2) whole genome comparison of *B. microti* and *B. suis* with other soil bacteria such as *Agrobacterium* and *Rhizobium* spp. exhibited fundamental similarities (Paulsen *et al.*, 2002). Whether soil organisms such as nematodes are the reservoir of this pathogen remains to be clarified, however some proofs exist as already shown with *B. anthracis*'s spores fate in soil (Schuch and Fischetti, 2009).
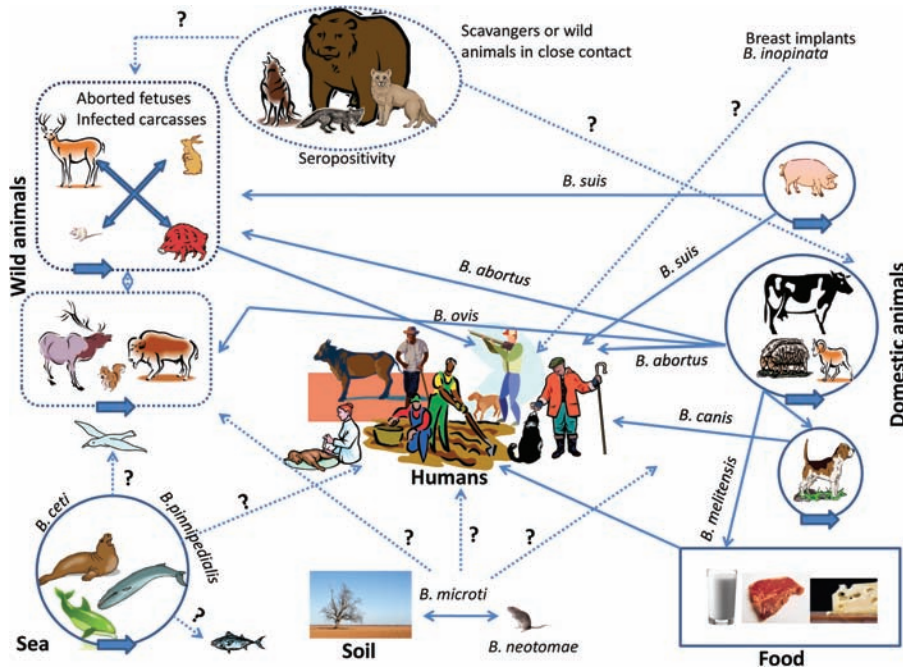
Figure 2.4.1. *Brucella* spp. cycles of infection among various animals and humans (full arrows-experimentally proved; dotted arrows- potentially unproved)

Interconnected with the soil ecosystem, Swartz *et al.* (2007) reported a "spectacular" reaction of *Brucella abortus* to the blue spectrum of natural light by means of a significant change in the reproduction and infectivity rate. This phenomenon was further explained by *Brucella* photosensing proteins (light-oxygen-voltage and histidine kinase proteins, "LOV-HK") involved in gene expression, that when knocked out impaired bacterium growth rate and infectivity significantly. This behavior fits the environmental phase of the pathogen excreted cells (in soil, exposed meat, milk, secretions or expelled placenta) exposed to blue light that activate the photosensing system in order to infect a new host successfully.

Another environmental important factor that acts as a *Brucella* reservoir is the wild boar (Munoz *et al.*, 2010; Meng *et al.*, 2009). It should be remembered here that this feral animal, like his domestic relative, feeds also on meat (carnivorous) in addition to vegetation in contrast to cattle, sheep and other ungulates.

Among the vast amount of reports on animals as *Brucella* carriers, reports on birds and fishes are scarce, though some are related to such potential interaction. Januszewski *et al.* (2001) showed that *B. abortus* strain

RB51 (vaccine) can multiply in squirrel and raven without clinical signs. A recent study of infected fish (Nile catfish) with *Brucella melitensis* was reported (El-Tras *et al.* 2010) suggesting that the source of infection was probably wastewater. Bogomolni *et al.* (2008) substantiated these results by surveying marine birds and mammals for amplicons to sequences from *Brucella* spp., among other pathogens, in the Northwest Atlantic Ocean. Their findings indicated that marine mammals and birds in this geographical location are reservoirs for potentially zoonotic pathogens that may be transmitted to beachgoers, fishermen and wildlife health personnel. They also speculated that zoonotic pathogens found in marine vertebrates were possibly acquired via contamination of coastal waters by sewage, run-off and agricultural or medical waste.

Moreover, risk factors closely related to *Brucella* infection were reported in many studies to be: hunting, consumption of raw meat and milk (foodborne), occupational or recreational (airborne), linked to travel and even to bioterrorism (Valderas & Roop, 2006).

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Chapter 2.5

Campylobacterioses

[*CAMPYLOBACTER JEJUNI*]

The genus *Campylobacter* comprises gram-negative spirals, microaerophilic, motile, non-spore forming and oxidase-positive bacteria. The following species belong to *Campylobacter* genus: *C. coli*, *C. concisus*, *C. curvus*, *C. fetus, C. gracilis*, *C. helveticus*, *C. hominis*, *C. hyointestinalis*, *C. insulaenigrae*, *C. jejuni*, *C. lanienae*, *C. lari*, *C. mucosalis*, *C. rectus*, *C. showae*, *C. sputorum* and *C. upsaliensis*. *C. jejuni* and *C. coli* are the main causes of bacterial foodborne disease in many developed countries. *C. fetus* is the main cause of spontaneous abortions in cattle and sheep, but also an opportunistic pathogen in humans. Clinical symptoms of Campylobacteriosis or as once called "cholera infantum" or "summer complaint" (Condran and Murphy, 2008; Samie *et al.*, 2007) are inflammatory diarrhea or dysentery syndrome, cramps, fever, and pain (occasionally with blood). The disease is usually self-limiting and in most cases, symptomatic treatment by reposition of liquid and electrolyte replacement is sufficient in human infections. The common routes of transmission are fecal-oral, ingestion of contaminated food or water and consumption of raw meat.

As already mentioned, *Campylobacter* is a pathogen related to animal food origin, especially poultry and other birds (e.g., seagulls excrete *C. lari*) (Figure 2.5.1). During the past decades *Campylobacter* has been shown to be responsible for enteritis in humans and animals. The natural habitats of most *Campylobacter* species are the intestines of birds and other warm-blooded animals such as: cattle, calves, sheep, dogs, cats, hamsters, guinea pigs, mice and different zoo animals. Farm pigs are the main carriers excreting *C. coli* (Levin, 2007). These organisms may enter the environment, including drinking water, through the feces of different mammals, birds or infected humans (Table 2.5.1). *Campylobacter* had been shown to survive in aqueous environment for several weeks (at∼4°C) and possibly enter the human food chain through the animal slaughtering process. *Campylobacter* is sensitive to heat, acidic pH, food preservatives, some heavy metals and irradiation but has increasing resistance to antibiotics due to extensive exposure following farmers over utilization (Baserilashi *et al.*, 2006). Milk, mushrooms, hamburger, pork, shellfish and eggs are vehicles of *Campylobacter*, however most *Campylobacter* enteritis is acquired by the handling and consumption of poultry. Although, many methods and media have been developed for the

detection of the *Campylobacter* from various samples, universally accepted methods and media are not yet available.
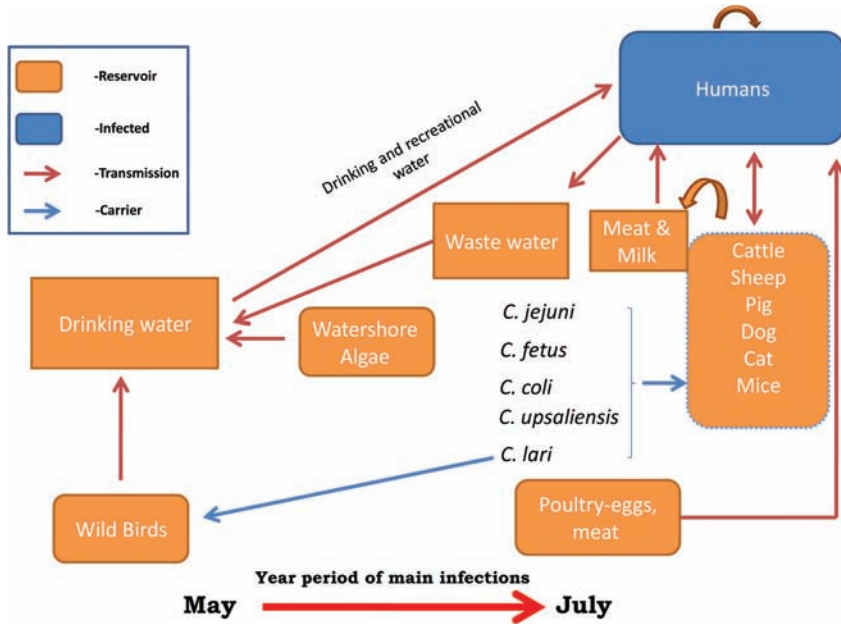
Figure 2.5.1. *Campylobacter* spp. infection cycles
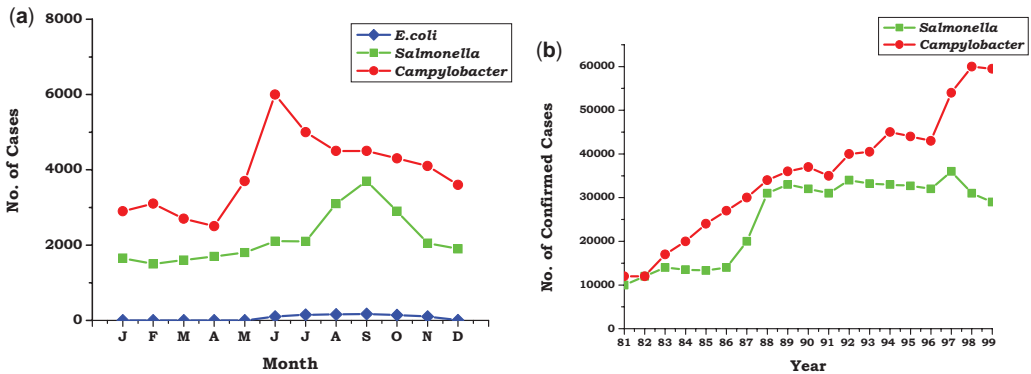
Figure 2.5.2. Number of confirmed cases of *Campylobacter*, *Salmonella* and enteropathogenic *E. coli* in UK and Wales related to season and years (1981–1999). **A**) distribution by month; **B**) distribution by year [adapted with permission from Elsevier Ltd., K. Jones. (2001) The *Campylobacter* conundrum. *Trends in Microbiology*, 9, 365–366.]

Table 2.5.1 *Campylobacter* spp. isolation from different environments.

Isolation site	Country	Method	Lowest	Highest	Strains	Seasonal trend	Reference
Ribble, Calder and Hodder rivers	UK	Glass microfibre filtration method and a most probable number (MPN) method	<10 CFU/100 ml	> 10–230 CFU/100 ml	C. jejuni, C. coli, C. laridis	High-late autumn and winterLow-spring and summer	Bolton et al.
Urban Wastewater treatment and composting	France	Quantitative real-time PCR and culture method	10–200 target genes/ml (October)	10^6 target genes/ml (April)	C. jejuni	Dry weather	Wéry et al., (2008)
Cattle, sheep, birds and other wildlife feces and water	Cheshire, UK	Culture method and single reaction PCR	% of samples testing positive 11 (C. jejuni in cattle feces) 21 (C. coli in cattle and birds feces), 0 (C. lari in sheep feces), 7 (C. hyointestinalis in water, sheep and wildlife feces)	% of samples testing positive 36 (C. jejuni in cattle feces) 21 (C.coli in sheep feces),7 (C. lari in birds feces), 7 (C. hyointestinalis in cattle feces)	C. jejuni, C. coli, C. lari, and C. hyointestinalis	Lower numbers during rainy weather	Brown et al., 2004
Contaminated urban flood water	Utrecht, The Hague The Netherlands	MPN	2.3×10^3	2.4×10^4	Campylobacter sp.	Heavy rain	ten Veldhuis et al., 2010
Soil, poultry manure, irrigation water, and freshly harvested vegetables	Malaysia	MPN-PCR	0 MPN/g	1100 MPN/g	Campylobacter spp.	Dry weather	Chai et al., 2009
Beach sand	UK	Culture method and serotyping	31% prevalence	66% prevalence	C. jejuni, C. coli and C. lari	Rainy weather	Bolton et al., 1999
Organic pigs, paddock environment and crow-birds and rats	University research farm in Taastrup, Denmark	Culture method, colony-blot hybridization, real-time PCR, serotyping and pulse-field gel electrophorsis (PFGE)	10^3 CFU/g	10^7 CFU/g	C. jejuni and C. coli	Dry weather	Jensen et al., 2006

2.5.1 *CAMPYLOBACTER* AND ENVIRONMENT

Campylobacter is sensitive to dry conditions, and therefore high relative humidity and/or precipitations contribute to its environmental survival. Thomas *et al.* (2006) used case-crossover methodology to examine the impact of high weather events (extreme rainfall and spring snowmelt) on waterborne diseases outbreaks in Canada over a time span of 26 years (1975 to 2001). Their results based on dichotomized percentiles imply that the contributing factors such as warmer temperatures and extreme rainfall are important in the spread of waterborne disease outbreaks in Canada with implications for water management and public health initiatives.

Hartnack *et al.* (2009) showed that thermophilic *Campylobacter* spp. display seasonality in poultry and humans by means of an explorative time series analysis (in a 3 year survey in Lower Saxony, Germany). Noticeably, these authors found that the human campylobacterioses peak preceded the peak in broiler prevalence rather than occurring subsequently. Significant cross-correlations between monthly temperature and prevalence in broilers as well as between human incidence, monthly temperature, rainfall and wind force were identified. These results are also supported by Patrick *et al.* (2004) who examined the effects of temperature, precipitation, relative humidity, and hours of sunlight on *Campylobacter* incidence in humans and broiler flocks in Denmark, by using lag dependence functions, locally fitted linear models, and cross validation methods. According to their results, climatic factors explained a larger proportion of the variance in *Campylobacter* prevalence in broiler flocks than in humans. The authors also drew attention to Scandinavian countries where the amount of sunlight coupled with warmer temperatures during the summer months greatly affects human behavior (encouraging outdoor activities, such as picnicking and swimming) that may increase exposure to *Campylobacter* from environment and natural water sources (lakes and rivers). In addition, they speculated that increased exposure to UV radiation (sunlight) may interfere with the immune system, increasing susceptibility to infectious diseases (Norval, 2001).

Another study presented the interaction of *Cladophora glomerata*, a filamentous, green alga that grows on rocks and other hard surfaces in nearshore water, with a variety of enteric bacteria including *Campylobacter* by providing nutrients and possible regrowth potential (Ishii *et al.*, 2006; Verhougstrate *et al.*, 2010). The authors suggested that part of *Cladophora* mats, as a result of wind and wave action, can detach and release these attached microorganisms to surrounding waters, consequently affecting water quality.

Rainwater harvest in open reservoirs and containers was also shown to harbor *Campylobacter* and other enteric bacteria with possible regrowth potential (Albrechtsen, 2006; Schets *et al.*, 2010). It should be mentioned that birds that carry *Campylobacter* and excrete it in large numbers are the main source of roof contamination (Dast *et al.*, 2010).

Jones (2001) raised an interesting epidemiological question on the growing trend in *Campylobacter* infections levels (from 1981 to 1999 in Wales and UK) in comparison with other enteropathogens such as *Salmonella* and enteropathogenic *E. coli* that decreased in the same period (Figure 2.5.2 A,B). According to this author's opinion, the seasonality of human infections is related to two factors: 1) variability in *Campylobacter* population sizes in livestock and poultry and 2) variability in environmental loading (see Figure 2.5.2 A). The multi-annual increase in *Campylobacter* infections versus *Salmonella* decrease (see Figure 2.5.2 B) may be explained by the above assumptions and by slaughter industry procedures that contaminate the carcasses in spite of the fact that *Campylobacter* does not multiply outside the host cells, while *Salmonella* does. Interestingly, the peak of *Campylobacter* infections takes place in spring and early summer while *Salmonella* has its peak in late summer, related to higher temperature and regrowth potential of the later in contaminated food. As *Campylobacter* does

not multiply outside the host, the most essential factor in its contamination potential is the environmental load found in close proximity to food preparation!

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Chapter 2.6

Capnocytophaga infections

[*CAPNOCYTOPHAGA* SPP.]

Capnocytophaga is a facultative anaerobic gram-negative bacteria with fusiform shapes (but also existent as curved filaments, coccoid and spindle forms) that belongs to Flavobacteriaceae order/genus. *C. canimorsus* and *C. cynodegmi* species were isolated from the oral cavity of dogs, cats and rabbits while species such as *C. granulose*, *C. haemolytica*, *C. ochracea*, *C. sputigena* and *C. gingivalis* are normal inhabitants of the human oral cavity in association with periodontitis. *C. canimorsus* and *C. cynodegmi* species are involved in human severe infections such as: endocarditis, peritonitis, osteomyelitis and meningitis through animal bites and scratch wounds.

2.6.1 *CAPNOCYTOPHAGA* AND ENVIRONMENT

Suzuki *et al.* (2010) carried out a prevalence study on dogs and cats for the presence of *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* in Japan using a sensitive specific PCR method to distinguish between the two species. *Capnocytophaga canimorsus* prevalence in dogs was 74% and 57% in cats, while *Capnocytophaga cynodegmi* prevalence in dogs was 86% and 84% in cats. The high prevalence in both species indicates that this bacterium is wide spread among pet as well wild canines and felines. It should be pointed out that both animal reservoirs can transmit this pathogen to other animals (e.g. rabbit and possibly others) through bites or scratches (van Duijkeren *et al.*, 2006). With the increasing pet populations (dogs and cats) it is feasible to predict that more infections with *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* will be reported. The close encounter between dogs, cats and human in small habitats "as human best friend" presents a real environmental factor in the distribution of these pathogens.

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Chapter 2.7

Chlamydial infections

[*CHLAMYDOPHILA* SPP.]

Chlamydiales are a unique order of intracellular bacterial pathogens that cause a significant disease of birds and other animals, including humans. Chlamydiae are gram-negative, nonmotile, lacking energy producing metabolic reactions, therefore an obligate intracellular bacterium that utilizes host ATP (energy parasites). The disease transmitted from infected birds to humans is called psittacosis/ornithosis and is characterized by fever, chills, headaches, photophobia, interstitial pneumonia and myalgia. Transmission from other animals (mainly mammals) can cause: conjunctivitis, pneumonia, orchitis, endocarditis, glomerulonephritis and abortion. In Australia, new species were isolated from a variety of mammals, such as gliders, possums, bilbies, bandicoots and potoroos, revealing the wide distribution of this pathogen (Bodetti *et al.*, 2003; Horn, 2008). The host range of the phylum *Chlamydiae* encompasses a large variety of animals including vertebrates: mammals, marsupials, amphibians, birds, reptiles, fish, and invertebrates, such as insects, crustaceans, mollusks and protozoa (Horn, 2008; Amann *et al.*, 1997).

2.7.1 *CHLAMYDIA* AND ENVIRONMENT

A recent publication on Chlamydiaceae-like bacterium isolated from the feces of sea birds from the Bering Sea shows that *Chlamydia* has a worldwide distribution with high biodiversity (Christerson *et al.* 2010). Sea birds and fish, both shown to carry *Chlamydia*-related bacteria represent a possible interesting cross-contamination with this pathogen/symbiont and its relation to human disease from the anthropocentric point of view.

Feral pigeon populations in Europe that increased significantly in the last decades through pigeon feeding and uncontrolled reproduction is of a major concern in terms of spreading *C. psittaci* pathogen . The study by Magnino *et al.* (2009) performed on feral pigeons from eleven European countries, showed a *C. psittaci* seropositivity value ranging from 19.4% to 95.6% inculpating these birds as a real hazard to humans who can contract psittacosis through direct or indirect contact.

Other environmental resources found to contain *Chlamydia* species are: fountains, ponds (clear and muddy) garden pots and water treatment plants, where they are detected mostly in amoeba and mixed co-culture (Corsaro and Venditti, 2006; Corsaro and Venditti, 2009; Corsaro *et al.* 2009).

Among insects, flies are possibly the ubiquitous vectors due to their flying capabilities and human/animals proximity. Flies that use feces as breeding media, were shown to transmit *Chlamydia trachomatis* (a human pathogen that causes trachoma, urethritis and lymphogranuloma venereum) (Emerson *et al.*, 2001) and their direct contact with humans, food and excreta cannot dismiss the potential infection with other Chlamydial genera members, next to direct contact with carrier animals (Figure 2.7.1). Fruit bats were reported to excrete another family of Chlamydiales named Waddliaceae (*Waddlia malaysiensis* sp.) in their urine (Chua *et al.*, 2005). Fruit bats are common inhabitants of tree-covered urban areas with increased activity during summer nights, dropping large amounts of excreta on cars and buildings with which humans have direct contact.
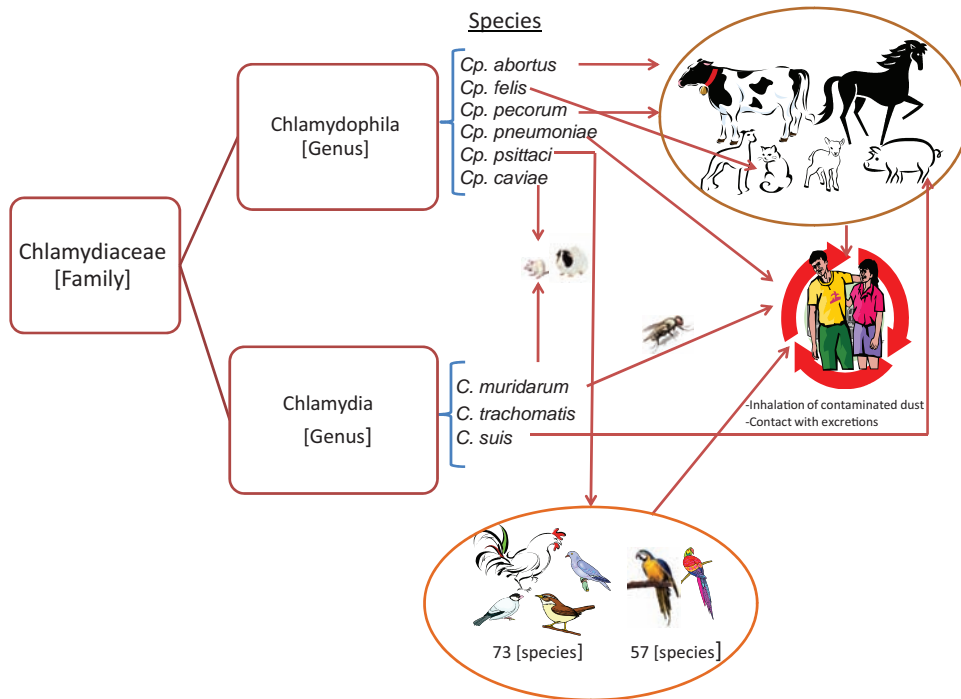
Figure 2.7.1. Chlamydiaceae in different animals and their human infection routes

Finally, Zhu and Wang (2008) reported the isolation and identification of a *Chlamydia* sp. from the alien Hawaiian marine sponge *Suberites zeteki* as a part of its associated microbial consortia.

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Chapter 2.8

Ehrlichioses

[*EHRLICHIA* SPP.]

Ehrlichia is a rickettsial bacterium of the Ehrlichiaceae family. The genus is a gram-negative intracytoplasmic obligate pathogen. The main reservoirs are mammals (dog, ruminants, horses, peridomestic animals, etc.) (Dumler and Bakken, 1995; Eremeeva *et al.*, 2009) but fish and horse nematodes were also identified as such (Wen *et al.*, 1996; Atwill *et al.*, 1996). Transmission occurs through a variety of infected ticks carried by different mammals and also birds (Björersdorff *et al.*, 2001; Parola and Roult, 2001). Several species can cause infection (Ehrlichiosis) in humans (Figure 2.8.1). The disease symptoms are: malaise, fever, headache, skin rash and in severe cases dyspenia, renal failure and CNS symptoms.

2.8.1 *EHRLICHIA* AND ENVIRONMENT

From the entomological point of view, *Ehrlichia* has a sylvanic zoonotic cycle but is also able to spread in urban areas where domestic animals for instance dogs and cats live in close proximity to humans (Comer *et al.*, 2001). Worldwide modern urban areas are highly populated due to migration from rural areas and foreign countries based on economic factors, increased mobility and continuous development. As a result, urban zoonoses increase due to homelessness, the decline of inner-city neighbourhoods, the increase in immonosuppressed individuals and additional complex factors (Olano and Walker, 2002). The reasons for the increase in these urban zoonoses are multifaceted. However, urban areas, due to their focal nature, also provide a better strategic control or prevention through physician awareness and public health surveillance support, in contrast to sylvanic or agricultural rural areas, where wildlife is almost uncontrollable.

In addition, tick species that were not classified as zoonotic vectors have been recently recognized as such (e.g., *Amblyomma americanum*) (Childs and Paddock, 2003) and subclinical infected companion animals could provide a reservoir for human tick-transmitted infectious agents, such as *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, the *Ehrlichia phagocytophila* group and *Rickettsia conorii* (Shaw *et al.*, 2001).
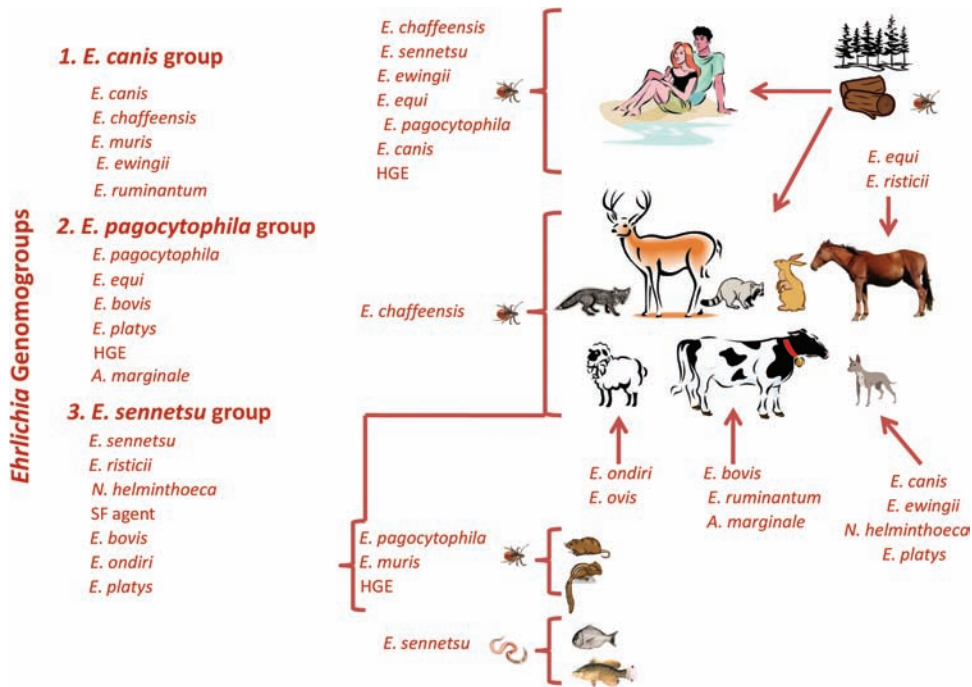
Figure 2.8.1. *Ehrlichia* spp. distribution in various animals and tick transmission

Fritz *et al.* (2005) reported two cases of Ehrlichosis in California during a period of two years (1997–1999). The residence area was densely populated by *Ixodes pacificus* ticks with over 85% of the study participants reporting tick contact in the preceding 12 months. However, the prevalence of *Ehrlichia* and *Anaplasma* in the tick population was very low, explaining the low disease frequency among humans. In South Korea, a serological survey was conducted designed to evaluate the presence of four canine pathogens among them two Ehrlichiae genera, *Anaplasma phagocytophilum* and *Ehrlichia canis*, on rural hunting and urban shelter dogs (Lim *et al.*, 2010). Dogs used for wild boar and pheasant hunting revealed a prevalence of 18.8% for *A. phagocytophilum* and 6.1% for *E. canis* while all urban stray dogs (a total of 692 dogs) were seronegative for both pathogens. This study indicates that rural hunting dogs are more exposed to vector-borne diseases.

To strengthen these results, in Brazil, dogs kept in apartments were compared with dogs with yard access for the presence of ectoparasites and hemoparasites. Not surprisingly, indoor dogs harbor ectoparasites (three tick types) at a lower prevalence than yard access dogs (2–12% compared to 14–35% respectively). Consequently, indoor dogs did not reveal hemoparasites at all and yard access dogs tested positive for *Ehrlichia canis* (16%) and another protozoan pathogen *Babesia canis* (2%) (Soares *et al.*, 2006).

The emergence of new canine tick-transmitted diseases results from several factors: expansion of tick range into urban and semi-urban areas, movement of infected dogs into non-endemic areas and the introduction of novel molecular techniques for diagnosis and pathogen identification (Shaw *et al.*, 2001).

Manangan *et al.* (2007) used a logistic regression model to compare the influence of physical environment, land cover composition, and landscape heterogeneity on distributions of *A. phagocytophilum* and *E. chaffeensis* in white-tailed deer, at multiple spatial extents in the Mississippi alluvial valley. The

Mississippi alluvial valley faces frequent flooding, and though it was historically covered by bottomland forests, it is presently dominated by agriculture, while the remaining forests are highly fragmented. Using white-tailed deer seropositivity results for these two pathogens (from 1981 to 2005), deer density, elevation, land cover, normalized difference vegetation index (NDVI), hydrology, and soil moisture, they found that the best fitting model was obtained for *E. chaffeensis* (90.5% sensitivity) compared to *A. phagocytophilum* (only 68% sensitivity). The lower fitting model for *Anaplasma phagocytophilum* was speculated to be a result of differences in the natural histories of tick vectors of this pathogen.

In a more extensive study, covering the area of southeastern United States, Wimberly *et al.* (2008) compared several spatial modeling approaches to predict the geographic distribution of *Ehrlichia chaffeensis and Anaplasma phagocytophilum* based on extended environmental modeling, such as logistic regression combined with spatial autocorrelation (pathogen tendency to cluster in space) and heterogeneity (environmental spatial variation). The results showed that the model prediction of *E. chaffeensis* based only on spatial autocorrelation had the best fit (Figure 2.8.2), while for *A. pagocytophilum* with its more complex zoonotic cycle and weak spatial pattern the combined model (spatial autocorrelation and heterogeneity) had the best fit (Figure 2.8.3).

The complexity in modeling *Ehrlichia chaffeensis* infection risk based on its prevalence in tick population was investigated by a deterministic model (prevalence in tick populations with spatial considerations) (Gaff and Schaefer, 2010). The complex nonlinear system interactions showed that areas that would be endemic in isolation may or may not sustain the disease depending on the surrounding habitat such as: control efforts shown to be far more effective when applied in wooded habitats than in neighboring grassy habitats and that additional increase in habitat fragmentation play a major role in predicting the endemicity of an HME (human monocytic ehrlichosis) outbreak.

Sinski (1999) in a review on vector-borne diseases in Poland pointed to the environmental crucial role of changes in farming systems in the Mazury Lakes district in which a great land area had been left under grassland and pasture favoring susceptible rodent species to become competent reservoirs. This phenomenon was observed in the whole of Europe alongside intensified agriculture.

Forest workers are expected to be at risk for Ehrlichosis. Blood samples of 4,368 forestry workers in the State of Baden-Wuertternberg (B-W), southwestern Germany, were tested for seroconversion against *Ehrlichia* spp. (genogroup *E. phagocytophila*) and other tick-borne pathogens (*Borrelia burgdorferi* sensu lato, TBE-virus) in parallel to the collection of 12,327 ticks (*Ixodes ricinus*) and analysis by PCR and genotyping (RNA and DNA) for the prevalence rate of these pathogens in this geographical area (Oehme *et al.*, 2002). The authors stated that the 5 to 16% prevalence of *Ehrlichia* spp. antibodies and the 2.6 to 3.1% tick prevalence suggests "that ehrlichiosis plays a role as a tick-borne disease in Germany, but as long as no clinical data are available, this will remain unclear".

Contact with forest parts, especially sitting and considerable contact with wood parts, results in a greater acquisition of nymphs, in spite of protective wear (hiking boots, hiking sandals, or running shoes) (Lane *et al.*, 2004). In a study conducted in southern Scotland (during 1996–1999) in three habitats for presence of the tick *Ixodes ricinus* as a vector of *Ehrlichia phagocytophila* infection, coniferous woods provided the highest risk of attachment by ticks, possibly due to ticks concentration on the grassy rides rather than other factors for example host or climatic (Walker *et al.*, 2001).

Direct contact with animals is the highest risk factor to contract Ehrlichiosis. Among such predisposed groups are: veterinarians, slaughterhouse and zoo workers, farmers and hunters. Deutz *et al.* (2003) used seroconvertion test to evaluate human exposure to several zoonotic agents among them *Ehrlichia*, compared hunters vs. other high risk occupational groups in south-east Austria. High seroprevalence (15%) was found in hunters compared to control (minor animal contact) and other high risk groups (veterinarians, farmers and slaughterhouse workers).
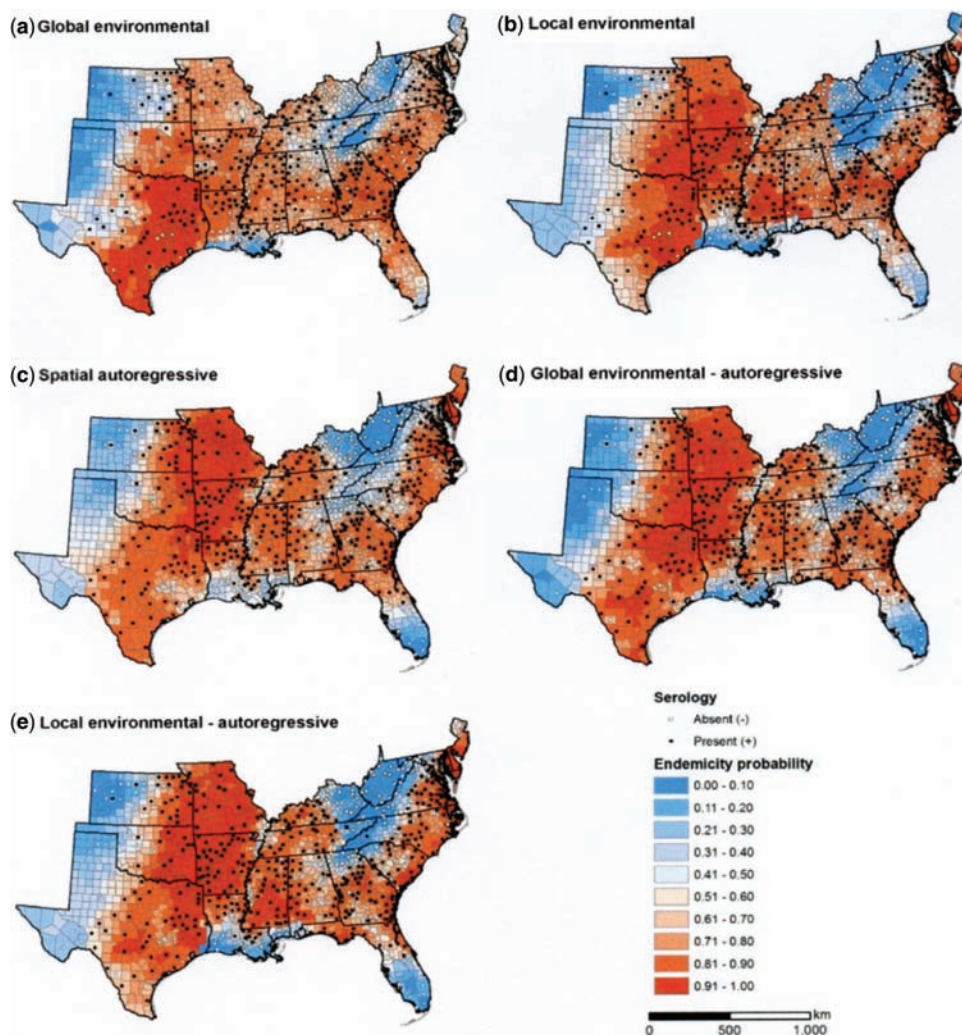
Figure 2.8.2. Predicted endemicity probabilities for *Ehrlichia chaffeensis* in the southeastern United States obtained from five Bayesian hierarchical models (Wimberly *et al.* (2008), with permission from International Journal of Health Geographics)

An interesting environmental aspect related to tick eradication by chemical means was outlined by Eisler *et al.*, (2003). The authors emphasized the importance of anti-ticks and tsetse pyrethroid insecticide use to prevent cattle loss (between US$ 4–5 billions/year) in Africa. However, beside its positive effect there are several risk factors that from the environmental point of view are significant: 1. uncontrolled application of pyrethoids can lead to acaricide resistance in tick populations (Bruce and Wilson, 1998); 2. insecticides can decimate invertebrate fauna responsible for cattle dung breakdown and subsequent incorporation in soil (Dantas-Torres, 2008) and 3. reduction in ticks' numbers attaching to cattle can also lead, paradoxically, to an increase in susceptibility to tick-borne diseases (Mahoney and Ross, 1972; Norval, 1992).
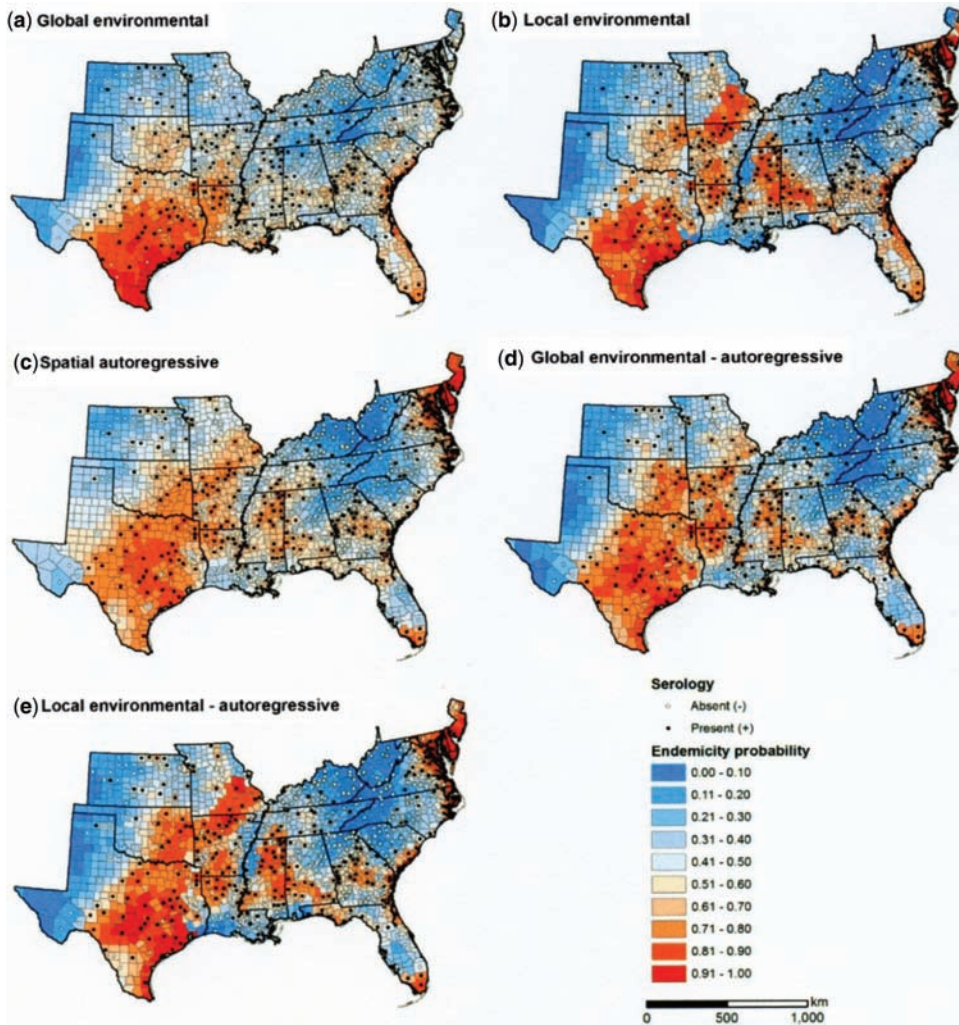
Figure 2.8.3. Predicted endemicity probabilities for *Anaplasma phagocytophilum* in the southeastern United States obtained from five Bayesian hierarchical models (Wimberly *et al.* (2008), with permission from International Journal of Health Geographics)

Gray *et al.* (2009) in a review article presented some evidences of climate impact on zoonotic tick-borne diseases affecting vector biology and disease transmission. The geographic extension of the vector tick *Ixodes ricinus* (and also *Dermacentor reticulates*) and increased prevalence of tick-borne encephalitis in Sweden could be partially related to climate change too (Figure 2.8.4). Warmer winters and hotter summers in this region may increase the seasonal activity of these vectors. The authors suggested that under a climate-warming scenario, ticks species establishment in more northern areas as permanent populations may be enhanced, with consequences on human health.
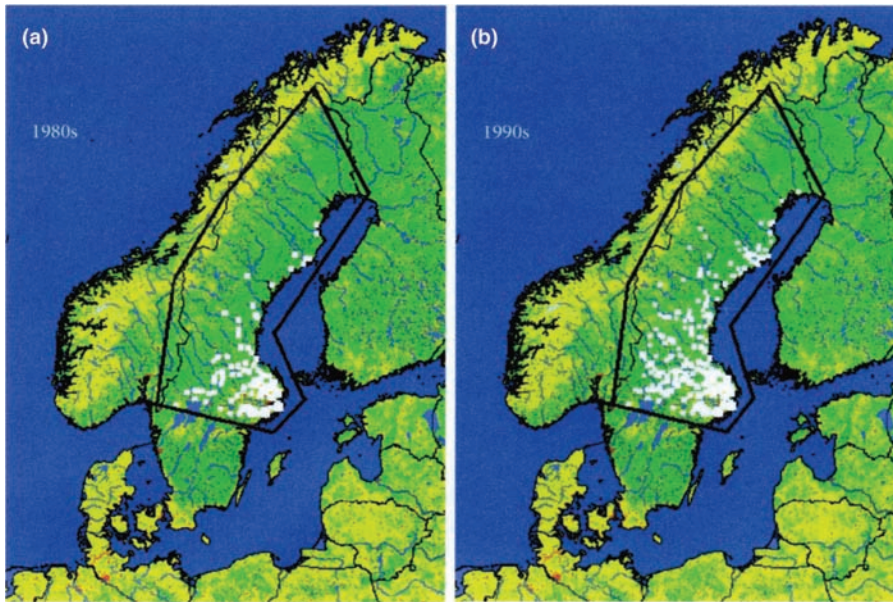
Figure 2.8.4. Tick distribution (white squares) in northern and central Sweden during two periods: (a) before 1980 and (b) in 1994 – 5. Black line frame represents the area studied. [with permission from Lindgren *et al.*, 2000, *Environ. Health Perspect.*]

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Chapter 2.9

Enterohemorrhagic
Escherichia coli infections

[ENTEROHEMORRHAGIC *ESHERICHIA COLI* (EHEC)]

Escherichia coli is a gram-negative, rod shaped, facultative anaerobic, motile (when flagella are present), non-sporulating bacterium and generally a benign commensal of warm blooded organisms including humans. However, some strains are highly virulent and cause mainly enteric diseases in humans and animals (Table 2.9.1).

As enterohemorrhagic *E. coli* is the most identified zoonotic pathogen in humans, this chapter will be dedicated to its characteristics and environmental link. Enterohemorrhagic *Escherichia coli* (EHEC) strains are highly infective *E. coli* (infective dose 10^1–10^2 CFU) capable of producing potent cytotoxins (Shiga toxins, expressed by Stx-s genes) or verotoxins and are able to cause A/E lesions (A/E: attachment-and-effacement) in the cecum and colon. The pathogen is transmitted through contaminated meat, milk and its products and contact with cattle feces (Whipp *et al.*, 1994). Direct contact with infected animals such as cattle and horses are also means of transmission. EHEC isolation was also reported from sheep, goats, water buffalos, and deer (Caprioli *et al.*, 2005). The disease presents itself as severe gastrointestinal diseases and hemorrhagic colitis characterized by watery diarrhea, abdominal pain, fever and bloody stool and sometimes with life-threatening post-infectious syndromes, such as hemolytic-uremic syndrome (HUS) or thrombotic-thrombocystopenic purpura (TTP)(Tschape and Fruth, 2001).

2.9.1 ENTEROHEMORRHAGIC *ESHERICHIA COLI* (EHEC) AND ENVIRONMENT

Farm animals, such as cattle, sheep, goats and potentially pigs are carriers and excretors of EHEC (Caprioli *et al.*, 2005; Rios *et al.*, 1999). Direct contact with animals or feed and their uncooked or partially cooked meat is the main transmission route to humans (Levine *et al.* 1993). Currently there are additional recognized environmental sources of infection and contamination: a) biotic (e.g., flies, rodents, birds and wildlife) (Miko *et al.*, 2009; Caprioli *et al.*, 2005) and b) abiotic factors (e.g., pasture and water) (Ogden *et al.*, 2002; Thran *et al.*, 2001; Ogden *et al.*, 2001) (Figure 2.9.1).

Table 2.9.1 Classification of human pathogenic *E. coli*.

Group name	Host	Human disease	Animal disease	Zoonoses	E. coli serogroup (examples)
Enterotoxigenic *E. coli* (ETEC)	Humans, pigs, sheep, goats, cattle, dogs, and horses	Choleriform enteritis (without fever), traveler's diarrhea	Enteritis in newborn and young	−	*E. coli* O26:H32
Enteropathogenic *E. coli* (EPEC)	Humans, rabbits, dogs, cats and horses	Causative agent of diarrhea, enteritis in infants	Enteritis	?	*E. coli* O26:H11
Enteroinvasive *E. coli* (EIEC)	Humans (only)	Dysenterylike colitis		−	*E. coli* O28ac, O112ac, O124, O136, O143, O144, O152, and O164
Enterohemorrhagic *E. coli* (EHEC)	Humans, cattle, goats and other ungulates	Hemolytic-uremic syndrome, hemorrhagic colitis, thrombotic thrombocytopenic purpura	Hemorrhagic colitis	+	*E. coli* O157:[H7], O26: H11/NM
Enteroaggregative *E. coli* (EAEC)	Humans (only)	Chronic enteritis without fever	?	−	*E. coli* O64:H4
Diffusely adherent *E. coli* (DAEC)	Humans, calves and pigs	Enteritis and urinary track infection	?	−	*E. coli* F1845+
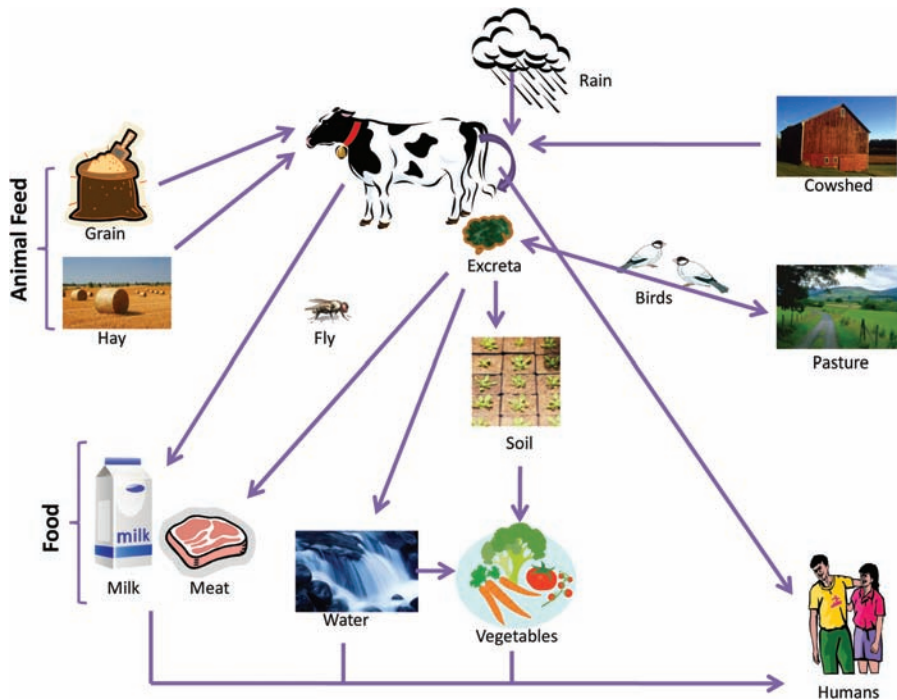
Figure 2.9.1. *E. coli* (EHEC) ecology and environmental aspects

A Swedish study performed on carrier calves that shed Enterohemorrhagic *Esherichia coli* (EHEC) revealed that those kept in a cowshed during the summer remained positive for four months after detection, while calves released to graze on pasture were pathogen free. The persistence of the pathogen in cowshed calves indicates a regular re-infection (Jonsson *et al.*, 2001).

Czajkowska *et al.*, (2008) looked at the survival of EHEC vs. environmental *E. coli* in wastewater and activated sludge of dairy sewage treatment plants during autumn-winter (temperature∼6°C) and spring-summer (temperature∼24°C). In activated sludge, *E. coli* O157:H7 bacteria were not detected (by direct plating method) after 21–28 days, and in dairy wastewater and activated sludges after 21–25 days. Environmental *E. coli* (non-pathogenic) survived longer: 35–42 days in activated sludge and 25–28 days in wastewater with activated sludges. However, these authors recommended based on sufficiently the extended persistence of EHEC in activated sludge, a thorough required treatment including disinfection prior to utilization of sludge as a soil fertilizer.

Wildlife meat from deer, wild boar, and hare was assessed as a potential carriers of *E. coli* (EHEC) by Miko *et al.* (2009). Based on genetic analysis, 32.8% of high-level virulence associated genes in humans were present in experimental isolates indicating that wild animals represent a reservoir for and a potential source of human pathogenic EHEC strains.

An additional way of dispersal and transmission was observed in beef slaughter by Stopforth *et al.* (2006). Preevisceration of carcasses washing prior to bung bagging during beef slaughter may allow pooling of cleanse water in the rectal area and consequent spread of potential pathogens. Surface sampling from the postwash bagging procedure revealed a 58.3% incidence rate of EHEC compared to 35 % from prewash bagging suggesting prewash as a much better practice for the control of pathogen

contamination, originating from the rectal area of carcasses. Ferianc *et al.* (2002) looked at *E. coli* isolates from the Danube and Moravia rivers (Slovakia) and compared these isolates to clinical ones (collected from urinary track disease). Molecular analysis of both river and clinical isolates revealed stx_2 gene carried on plasmid, a characteristic of enterohemorrhagic *E. coli* (EHEC) strains. The authors hinted at a possible health risk for bathers from direct contact with untreated river water. Finally, the Mediterranean fruit fly (*Ceratitis capitata*), a cosmopolitan pest of commercial and wild fruits, was artificially contaminated (through feed solution and enriched fecal material) with GFP (green fluorescence protein)-tagged *E. coli*. Flies inoculated with GFP-tagged *E. coli* harbored the bacteria for up to 7 days after initial contamination. Flies feeding on fruits were capable of transmitting the GFP tagged *E. coli* to intact apples, as were female flies that laid their eggs in fruit by puncturing the skin with their ovipositors and injecting batches of eggs into the wounds. Washing of contaminated apples with tap water did not eliminate the inoculated *E. coli*, revealing deeper internal inoculation of these bacteria by fruit fly into the fruit's internal tissues (Sela *et al.*, 2005).

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Chapter 2.10

Erysipeloid

[*ERYSIPELOTHRIX RHUSIOPATHIAE*]

Erysipelothrix rhusiopathiae is a rod shaped gram-positive, non-motile, non-spore forming, facultative aerobic bacterium that does not contain endotoxin. *Erysipelothrix rhusiopathiae* has a worldwide distribution in nature and can be found in soil and decomposing vegetation (Timofeeva *et al.*, 1978). It is primarily considered an animal pathogen, causing a disease known as erysipelas in animals and erysipeloid in humans. Pigs are most commonly affected, but cases have been reported in other animals such as birds (mainly in turkeys and ducks), sheep, fish, and reptiles (Brooke and Riley, 1999; Mutalib *et al.*, 1993; Mutalib *et al.* 1995; Anonymous, 1946). In pigs, the disease is known as "diamond skin disease." Less frequent animal sources of transmission to humans are: lambs, horses, cattle, dogs, mice, rats and fur-bearing animals (Timofeeva and Golovacheva, 1959; Popugalio *et al.*, 1983). The human disease called erysipeloid displays the following symptoms: inflammation with edema and hemorrhages on hand and fingers, itch without pus and fever. Occasional lymphangitis but rarely arthritis of joints and endocarditis may occur (Boo *et al.*, 2003).

2.10.1 ERYSIPELOID AND ENVIRONMENT

Erysipelothrix rhusiopathiae bacterium is ubiquitous and able to persist for a long period of time in the environment, especially where nitrogenous substances decompose (Klauder, 1938; Molin *et al.*, 1989). This characteristic may explain why Popugalio *et al.*, (1983) in a shoe factory found leather and casein glue contaminated with *E. rhusiopathiae*. Geographically, this pathogen can be found in terrestrial and marine locations while clinical cases have been reported worldwide (Japan, Russia, Australia, USA, Europe, etc.) (Wang *et al.*, 2010). The range of animals that are infected by and possibly transmitters of this bacterium includes vertebrates and invertebrates such as swine, sheep, cattle, horses, dogs, bears, kangaroos, reindeer, mice, rodents, seals, sea lions, cetaceans, mink, chipmunks, crustaceans, fresh and salt water fish, crocodiles, Caymans, stable flies, houseflies, ticks, mites, mouse, lice, turkeys, chickens, ducks, geese, guinea fowls, pigeons, sparrows, starlings, eagles, parrots, pheasants, peacocks, quail, parakeets, mud hens, canaries, finches, siskins, thrushes, blackbirds, turtledoves and white storks

(Figure 2.10.1) (Bricker and Saif, 1997; Reboli and Farrar, 1989; Oli'akova and Antoniuk, 1989; Wood, 1975; Grieco and Sheldon, 1970).
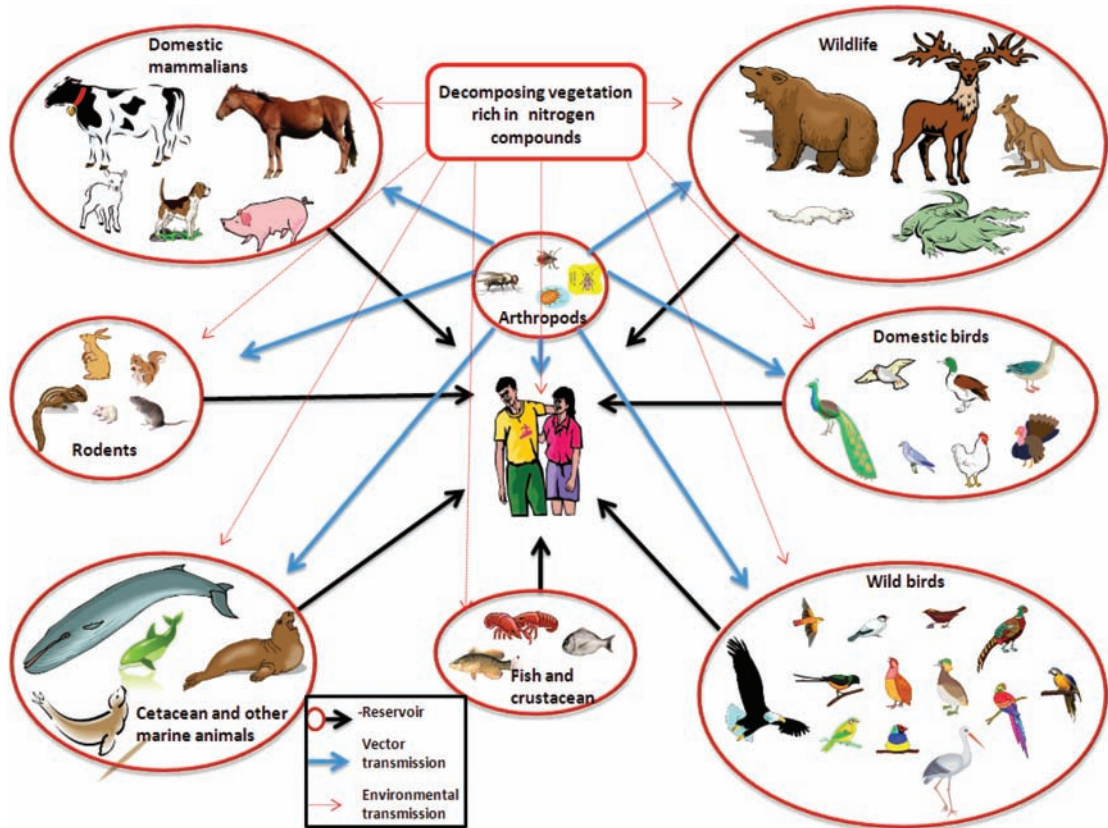
Figure 2.10.1. Ecology of *Erysipelothrix* spp. in biosphere related to human infection

Major transmission and infection cases were reported on persons working at meat, offal and bone processing plants where they had direct contact with infected animals (Savchenko *et al.*, 1984).

Human infections are mainly related to direct contact with swine and turkey through slaughterhouse and meat manufacture; however, in Western Australia, an erysipeloid-like infection referred to as "crayfish poisoning" occurred in lobster fishermen and handlers (Fidalgo *et al.* 2004), an incidence already reported in the 40's of the last century (Anonymous, 1946).

For three years (2007–2009), Bender *et al.* (2010) analyzed clinical and environmental isolates from affected pigs in Midwest United States. Environmental samples included: manure, feed, water lines, oral fluids, swab samples from wall, feed lines, air inlets, exhaust fans and nipple drinker. Except for feed line, all other environmental samples were positive for *Erysipelothrix* spp. in the range of 21.4 to 52.9%, feed and nipple drinkers having the highest percentage of positive samples.

A previous study demonstrated that this organism can survive for a maximum of 35 days in soil under diverse conditions of temperature, pH, moisture and organic content (Wood, 1973). Taking into account the continuous excretion of this pathogen by infected animals and high nitrogen content of decaying

vegetation, soil certainly can be considered as a realistic source of human infection, especially under poor hygienic conditions.

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Chapter 2.11

Glanders (Malleus or Farcy)

[*BURKHOLDERIA MALLEI*]

Burkholderia mallei is an aerobic, gram-negative rod, non-motile and non-fermentative bacterium, formerly named *Pseudomonas mallei*. The disease called Glanders, Malleus or Farcy can be acute and chronic and is characterized by skin lesions, multiple abscesses, respiratory tract necroses, pneumonia and sepsis. Predominant animals affected by this bacterium are: horses, donkeys, and mules, although other animals (e.g. sheep, goats, dogs, cats and zoo animals) may be infected through contact with ungulates or by consuming their meat (Alibasoglu *et al.*, 1986). Humans who come in contact with horses (e.g., veterinarians, horse dealers, farmers or horseback riders) or consume horse meat are at high risk (Fukuyo *et al.*, 2002; Wittig *et al.*, 2006). In stables, airborne infections are also feasible when infected secretions are dispersed as aerosols (Lever *et al.*, 2003; Whitlock *et al.*, 2007; Dvorak and Spickler, 2008).

2.11.1 *BURKHOLDERIA MALLEI* AND ENVIRONMENT

The genus Burkholderia comprises >40 different species isolated from a large variety of ecological niches (soil, water, plant rhizosphere, fungi, animals) as endophytes, endosymbionts and pathogens (El-Safey, 2005; Vandamme *et al.*, 2006) (Figure 2.11.1). Soil presence of *B. mallei* was reported in Ternate Island (Indonesia) revealing an isolation prevalence of 3.1% (Noura *et al.*, 2009) and in Jordan in crude petroleum oil contaminated soil where it had the capability of degrading diesel fuel (Saadoun, 2002). Many are known for their biological and metabolic properties involved in the biomineralization of a diverse variety of chemicals and as such are used in biotechnology. In a study conducted on bacterial isolates from Lake Mariut sediments (a fresh water lake located south of Alexandria, Egypt), isolated *B. mallei* was able to biodegrade or mineralize the phenoxy herbicide mecoprop (MCPP) under different conditions. Among the *Pseudomonas* spp., the highest mineralization efficiency was achieved by *P. mallei* (now *B. mallei*), while *P. pseudomallei* (now *B. pseudomallei*) revealed the highest biodegradation efficiency (El-Bestawy & Albrechtsen, 2007). Remarkable examples supporting these assumptions were recently published, revealing a variety of bacteria capable of biodegrading petroleum hydrocarbons species such as *B. mallei* and *B. pseudomallei* (described later) known as human and animal pathogens

(Malkawi *et al.*, 2009; Sahu *et al.*, 2008). In relation to petroleum products, Balogun and Fagade (2010) reported on emulsifying bacteria isolated from produce water with high chloride content (650 mg/L) obtained from an oil terminal in the Niger Delta, Nigeria. Among the isolated bacteria, *B. mallei* had the highest emulsification and de-emulsification indices (65 and 50% respectively).
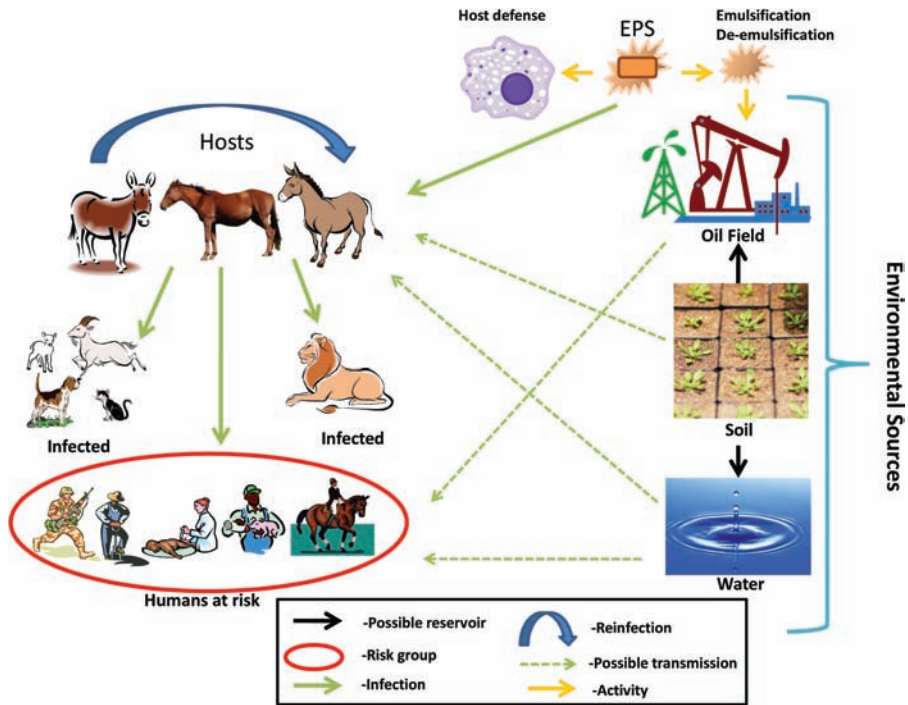
Figure 2.11.1. *Burkholderia mallei* infection routes and environmental sources

These characteristics are interesting as biosurfactant agents are substances that can break down the interfacial tension between polar and non-polar liquids in mixtures to form stable emulsions, however these bacteria can also de-emulsify due to the biodegradation properties of different hydrocarbons including their utilization of the excreted biosurfactants (Nadarajah *et al.*, 2002). The dual antagonistic activities can explain the bacterial pathogenic mechanism, by enhanced cell attachment and defense against host antibacterial activity (emulsification) and detachment and spread (de-emulsification). It would be interesting to find out whether these biosurfactants naturally excreted by *B. mallei* are similar or closely related to some virulence mechanisms expressed during infection, through its extracellular polysaccharide capsule (Dvorak and Spickler, 2008).

From the genetic point of view, *B. mallei* and its relatives reveal an interesting trend that facilitates their survival, infection and distribution in the ecological biosphere. Shaginyan and Chernukha (2003) in their review of *Burkholderia cepacia* complex based on comparative genomic molecular biology, hypothesized about a possible phylogenetic relations of *B. cepacia* complex with phytopathogens and human or animal pathogens species, such as *Burkholderia*, *Pseudomonas*, *Escherichia*, *B. mallei*, *B. pseudomallei*, *P. aeruginosa* and *E. coli*. The authors describe a possible role of alterations and

mutations in the genome of the complex *B. cepacia* bacteria (a genome having unusual properties, i.e., a big size and a considerable quantity of insertion sequences-IS) in creating the conditions for the "pulsing" evolution and "jerks" for a rapid change-over from saprophytism in soil environment to a pathogenic agent. Such a mechanism can explain the rapid and radical adaptation of microorganisms under new altering ecological niches. A similar trend was recently presented in a study on *B. mallei* and its genetic consistency in comparison with much more diverse species and the closely related: *B. pseudomallei*.

The *B. mallei* core genome shared by all these species is smaller compared to its relative *B. pseudomallei* while variable gene sets distributed across *B. mallei* strains are larger, revealing insertion sequence elements that contribute to its variable virulence when introduced into an animal host. Romero *et al.* (2006) working on *B. mallei* ATCC 23344, showed that this bacterium exhibits genome variability upon passage in mouse, horse and human patient by the accumulation of genome sequence variation at sequence repeats (SSRs) and other loci. The authors reported 12,000 simple sequence repeats found in this strain that may explain its phenotypic versatility.

Finally, the potential use of *B. mallei* as a biological weapon by armies and terrorists cannot be ignored based on historical experience (Larsen & Johnson, 2009). There is solid evidence that during the first and second world wars, both sites used *B. mallei* against their opponents including the second half of the last century (Martin *et al.*, 2007). Rose *et al.* (2005) challenged different potential bioterrorism agents (bacterial) also known as zoonotic pathogens, to chlorine inactivation. Among these pathogens, *B. mallei* revealed high susceptibility to chlorination at a low disinfectant concentration [Ct - 0.2 mg min/liter for 2 log reduction] compared to other highly pathogenic bacteria such as *Francisella tularensis* NY98 [Ct - 7.8 mg min/liter for 2 log reduction].

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Chapter 2.12

Leptospiroses

[*LEPTOSPIRA INTERROGANS*]

Leptospira interrogans is a motile, aerobic, gram-negative spiral shaped bacterium with hook or crooked shaped ends. The species *L. interogans* contains ~200 serovars that can be subgrouped in >20 serogroups. Animals, water, soil and infected humans are the main reservoirs for infection (Krawczyk, 2004; Bharti *et al.*, 2003). It affects humans and a wide range of animals, including mammals, birds, amphibians, and reptiles (Junge *et al.*, 2007; Niwetpathomwat *et al.*, 2006; Easterbrook *et al.*, 2007). In humans, the disease has a wide range of symptoms and has a biphasic behavior: the first phase is characterized by flu-like symptoms (fever, chills, myalgias, and headache) and sometimes even asymptomatic while the second phase (known as Weil's disease or as sometimes called Weil Syndrome) (Aydemir *et al.*, 2007) is much more severe presenting itself as meningitis, liver damage (ictohaemorrhagie causing jaundice), renal failure, cardiovascular problems and death (Suarez *et al.*, 1991).

2.12.1 LEPTOSPIRA AND ENVIRONMENT

There are several environmental key factors that increase Leptospirosis risk in animals and humans such as: a) rodent exposure; b) wildlife exposure; c) soil and water and d) management (Barwick *et al.*, 1997) (Figure 2.12.1). The link between *Leptospira* and water sources is very evident (Corwin *et al.*, 1990; Katz *et al.*, 1991; Aydemir *et al.*, 2007). Water can be directly contaminated by a large variety of animals or human excreta or indirectly by soil surface wash (floods, rainfall, run-off, etc.) (Manciuc *et al.*, 2007). Here is the place to mention a new report on *Leptospira interrogans* cell-binding activity *in vitro*, attributable to the attachment of proteoglycans (PGs) to a cell's glycosaminoglycan (GAG) chains, both shed by infected individuals in urine, making the transition from mammalian host to environment (soil and water) and vice versa possible (Breiner *et al.*, 2009). Numerous cases involving human contact with water contaminated sources have been reported comprising athletes, adventure tourists, military and swimmers (Jackson *et al.*, 1993: Boland *et al.*, 2004; Aoki *et al.*, 2001; Tunbridge *et al.*, 2001; Hadad *et al.*, 2006; Lettieri *et al.*, 2004; Stephan *et al.*, 2000). Canoe race participants and triathlon athletes were identified as having contracted *Leptospira* in the Liffey river, Dublin, Ireland. Investigation of both

cases revealed that direct contact and ingestion of contaminated water increase the risk of contracting Leptospiroidosis. In the canoe race case, abundant rainfall coupled with hydroelectric water release may have contributed to the outbreak (Boland *et al.*, 2004). Trueba *et al.* (2004) showed experimentally that *L. interrogans* serovar *canicola* can survive (based on motility test) for 110 days in distilled water (pH 7.2) and 347 days when incubated in a semi-solid medium (0.5% purified agarose in distilled water -pH 7.2), on the other hand when incubated in saline it survived only 21 days (Trueba *et al.*, 2002). Another study supports these findings, revealing that salinity critically impairs *Leptospira's* survival ability in sea water (Khairani-Bejo *et al.*, 2004). In this context, it should be mentioned that Gill *et al.* (1985) performing a *Leptospira* seroconversion survey among fish farm workers, reported that fish farming has a moderate risk for this pathogen; however in their case, no data were presented on water salinity (fresh or sea water), which may impact the pathogen's survival and explain their mostly negative results. In viscous semi-solid medium, *Leptospira* cells form aggregates indicating on the mechanisms by which this pathogen can survive for prolonged periods of time in natural aqueous environments as a part of its lifecycle. Ristow *et al.* (2008) published an additional potential survival mechanism for *Leptospira* based on biofilm formation "that is consistent with the life of saprophytic strains in water and may help pathogenic strains to survive in environmental habitats and to colonize the host". Pathogen transmission is also water-associated in cases of catastrophic events such as floods or hurricanes. Several studies showed that floods, through soil cover and wash, can transmit *Leptospira* to humans and animals especially when they live in close proximity to inundated areas (Anonymous, 2006; Vanasco *et al.*, 2002; Gaynor *et al.*, 2007). Sanders *et al.* (1999) found an increase in laboratory cases following a hurricane episode. Soil environment may also support the growth and survival of *Leptospira* especially if they are hygroscopic, euthrophic and close to neutral pH (Maksimenkova and Karpachevskii, 1985; Yang *et al.*, 1994). Wet soil is also a source of contamination for *Leptospira* (Yang *et al.*, 1994).
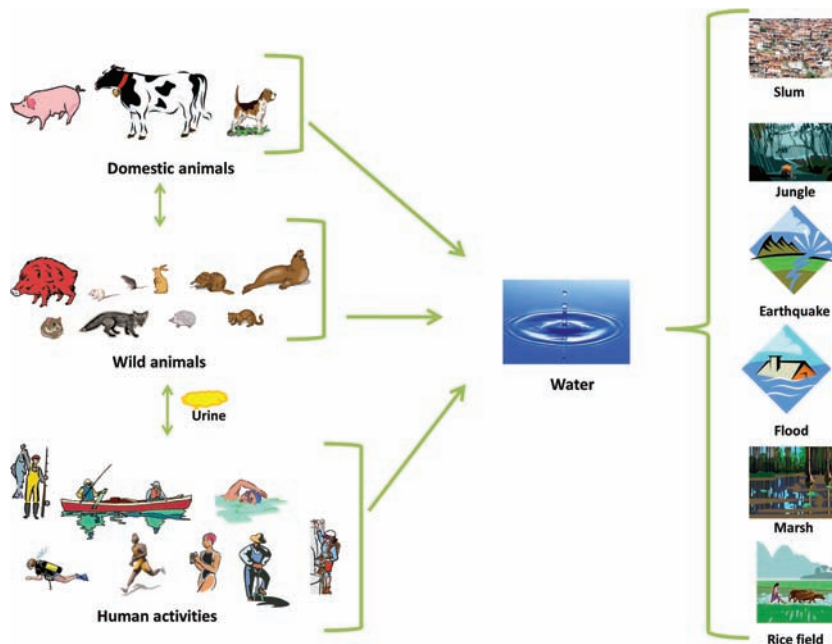
Figure 2.12.1. *Leptospira* spp. transmission and environmental sources

Sea shore, with its wet sandy composition is such an environment where many animals dwelling near freshwater estuaries (including wastewater spills) increases the potential for Leptospirosis transmission to humans and domestic animals. Cameron *et al.* (2008) studied pinnipeds population, using advanced molecular methods (PCR), for *Leptospira* infection as a potential source for epizootic, enzootic, and zoonotic spread of leptospirosis in a marine environment. According to these authors, it seems that human recreational activity, estuaries outfall, rodents and the close contact of infected animals are the main vectors for the spread of Leptospirosis among marine animals. Ganoza *et al.* (2006) surveyed urban and rural populations in the Peruvian Amazon for prevalence of pathogenic *Leptospira interrogans* serovar *icterohaemorrhagiae*. This pathogen was isolated only in samples from urban slum market areas, gutter water and human isolates but not in rural areas, suggesting a specific mode of transmission from rats to humans in dirty and densely populated urban areas. Similar results were obtained by Pereira and Andrade (1990) in a slum area of Rio de Janeiro, Brazil and by Scialfa *et al.* (2010) in Buenos Aires, Argentina. Human proximity to water sources was shown also to contribute to wildlife infection with *L. interrogans*. In the United States, two otter populations, one from an area of low human population density (Prince William Sound, Alaska) and a second one from one of high human population density (San Juan Islands, Washington State), were studied by indirect immunofluorescent antibody (IFA) test for seroprevalence against *L. interrogans* (Gaydos *et al.*, 2007). The absence of a seroprevalence shift in Alaska's otters advocates that life in proximity to excessive human density and its association with agricultural activities, domestic animals and rodent populations might enhance river otter exposure to *L. interrogans*.

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Chapter 2.13

Listeriosis

[*LISTERIA MONOCYTOGENES*]

Listeria monocytogenes is a gram-positive rod, facultative anaerobe and motile bacterium. It is perhaps the most virulent foodborne agent causing 20–30% fatality in infected people (Ramaswamy *et al.*, 2007). Infection by *L. monocytogenes* causes the disease listeriosis that manifests itself in humans as: septicemia, meningitis (or meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion. Beside infected humans, a large variety of animals can excrete the pathogen in feces, subsequently spreading it in soil, plants and surface water (Table 2.13.1).

2.13.1 LISTERIA AND ENVIRONMENT

Besides animals and humans, soil, water and vegetation all closely related to agricultural activities, are also carriers and reservoirs of *L. monocytogenes* (Moshtaghi *et al.*, 2003) (Figure 2.13.1). Lyautey *et al.* (2007) found a statistically significant link between the occurrence of *L. monocytogenes* and proximity to an upstream dairy farm and the degree of cropped land indicating *L. monocytogenes* pervasiveness in these catchments.

   Soil amended with fertilizers (primarily containing N and P) was shown to support and enhance the growth of *L. monocytogenes* in brown podzol soils in Russia (Sidorenko and Buzelova, 2008) while the presence of herbicides (e.g. simazine or picloram at 1–100 mg/L) in water samples enhanced the growth of *L. monocytogenes* while inhibiting *Pseudomonas* and *Aeromonas*, known as saprophytic pollution eliminators bacteria (Karaeva, 1988). Synthetic and mineral-oil based lubricants used in food industry were also shown to support *L. monocytogenes*' growth, survival and spread (Aarnislo *et al.*, 2007).

   In a study on fish farms in Finland tested for presence of *L. monocytogenes*, sea bottom soil samples were the ones that preserved the *L. monocytogenes* contamination for prolonged periods of time (Miettinen and Wirtanen, 2006) particularly at low temperatures (*L. monocytogenes* can grow at very low temperatures: $1.7 \pm 0.5°C$ and a broad range of pH values: 4.4 to 9.6) (Junttila *et al.*, 1988).

Table 2.13.1 Selected data on *L. monocytogenes* prevalence in various environments of different countries, revealing its worldwide distribution.

Country/Source	% of samples	Reference	Country/Source	% of samples	Reference
USA: Cow-calf operation cows	3.1	Mohammed et al. (2010)	**India**: Soil agricultural areas	17.5	Moshtaghi et al. (2003)
Calves	3.75		Soil animal-inhabited area	18	
Other animals feces	2.5		**Canada**: Surface waters	10	Lyautey et al. (2007)
Cut-grass	5.3		**USA**: urban environment	7.5	Sauders et al., (2006)
Soil	5.3		Natural environment	1.4	
Entry point at irrigation ditch	0		**Netherland**: Surface waters(canals, lakes, ditches, sewage	21	Dijkstra (1982)
Exit point from irrigation ditch	14.3		**Finland**: fish farms and surroundings (e.g., sea bottom soil)	18.4	Miettinen, H. & Wirtanen, G. (2006)
Water troughs	3.1		**Germany**: Soil surface	8.7–51.4	Weis and Seelinger (1975)
Water from ponds Feedlot Operation	6.5		Soil at 10 cm depth	3.2–33.3	
			Plants	9.7–44	
Cows	0.3		**Scotland, UK**: silage	2.5–44	Fenlon, (1985, 1999)
Soil	0.7		**Brazil**: urban river contaminated with domestic sewage and surface run-off from agricultural and cattle grazing lands	35–100	Ceballos et al. (2003)
Feed bunk	2.5				
Water trough	0.8				
Cow feed	1.7				
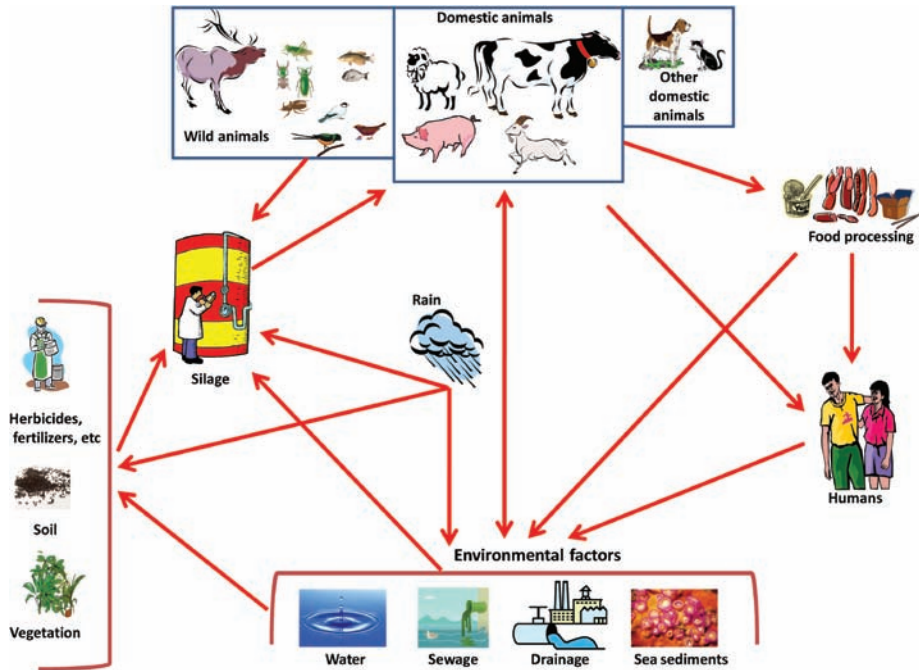
Figure 2.13.1. Environmental factors involved in the maintenance and infection of *L.monocytogenes*

Perhaps the major source of contamination attributed to its geographical widespread distribution is water resources with diverse qualities. Combarro *et al.* (1997) studying a sewage treatment plant's capability to remove *L. monocytogenes* found that the removal rate of 92% (in accordance with EEC regulations) was insufficient and levels of this pathogen increased in the downstream of an adjacent river. A sheep meningo-encephalithis was reported in Gippsland, Victoria, Australia caused by *L. monocytogenes*. This outbreak incident followed a period of continuous heavy rain and flooding of grazing pasture while the majority of affected flocks were located on poorly drained coastal sandy soil (Vandegraaff *et al.*, 1981).

Girardin *et al.* (2005) under field conditions studied *L. innocua* as a surrogate for the survival and transfer of *L. monocytogenes* in soil and on parsley leaves (as a result of rain or irrigation splash). Leaf contamination was expected as a result of rain and irrigation of fields amended with contaminated fertilizers (i.e., manure). At 20°C, the decline rates in soil were comparable for the two strains (*L. innocua* and *L. monocytogenes*) resulting in a decline of 7 log cycles in 90 days with a minute numbers on leaves. Improved survival was observed in winter with direct leaf contamination by spray (with bacterial low content soil) lacking internalization of *L. monocytogenes* in roots. Here it should be pointed out that a recent study revealed internalization of *Salmonella enterica* in leaves through open stomata (Kroupitski *et al.*, 2010) that may hint at possible internalization by *Listeria* too, through its motility, surface polysaccharides involved in adhesion to soil (Abu-Lail *et al.*, 2007) and persistence in poorly ripened silage of corn, grass, rye, oats and legumes (Ryser *et al.*, 1997; Driehuis and Oude Elferink, 2000). Regrowth capability of *L. monocytogenes* was also shown in extracts of dairy-based composts under different environmental conditions (Kim *et al.*, 2009).

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Chapter 2.14

Melioidosis

[*BURKHOLDERIA PSEUDOMALLEI*]

Burkholderia pseudomallei is a motile, aerobic, gram-negative rod shaped bacteria closely related to *B. mallei* (genus Burkholderia) that causes disease in animals, humans and even plants (Ketterer *et al.*, 1975; Lee *et al.*, 2010). The main disease in humans is melioidosis that manifests itself as abscesses with lymphangitis, high fever, malaise, acute septicemia, acute pulmonary infection and death.

2.14.1 *B. PSEUDOMALLEI* AND ENVIRONMENT

Burkholderia pseudomallei has been isolated from soil, water and food contaminated with animal excreta (Currie *et al.* 2001). The main characteristics that support its survival in hostile environments are: resistance to detergents/antiseptic compounds through its biodegradation capability, growth under nutrient deficiency, low pH, wide range of temperatures and dehydration, but susceptibility to UV irradiation (Dejsirilert *et al.*, 1991; Chen *et al.*, 2003; Inglis & Sagripanti, 2006). South-east Asia is one of the world endemic areas for *B. pseudomallei* and this fact is apparently linked to the rainy season (monsoons) and flooded rice fields that help transmit this pathogen as a result of direct contact with water or inhalation of aerosols supported by these climatic conditions (Wuthikanum *et al.*, 1995). Another described endemic area is northern Australia where clustered cases were associated with extreme weather events or environmental contamination (Cheng *et al.* 2006) (Figure 2.14.1). Water table movement, such as in cases of monsoonal rainy seasons, was speculated to have moved dormant or non-culturable state forms of this bacterium to the soil's surface, which in turn was converted into a more replicative and infectious form (Currie, 2008).

   B. pseudomallei's interaction with the protozoa *Acanthamoeba* sp. (in a similar fashion described for another human pathogen, *Legionella pneumophila*, by internalization through phagocytosis and chronic infection) and pathogenicity to plants and larval insects is believed to facilitate its environmental survival (Inglis *et al.*, 2000; Lee and Seleena, 1990). There are many factors that impact *B. pseudomallei's* distribution and persistence in the environment that include: a) physical factors (rainfall, flood, UV radiation, plowing/excavation, humidity and temperature) (Ketterer *et al.*, 1975; Inglis *et al.*, 2001) and

b) chemical (soil chemistry, pH, vegetation, fertilizers, genetical diversity, etc.) (Thomas *et al.*, 1979; Wuthiekanun *et al.*, 2009; Palasatien *et al.*, 2009). A recent study demonstrated experimentally that *B. pseudomallei* can survive salt stress through the alteration of secreted proteins (secretome) a process which may explain its environmental epidemiology (Pumirat *et al.*, 2009). Kaestli *et al.* (2009) surveyed the Darwin area in tropical Australia for soil presence of *B. pseudomallei* in two habitats: undisturbed and environmentally manipulated areas. Significant association at undisturbed sites was found to be linked to areas rich in grasses, and at environmentally disturbed sites the presence of livestock animals, lower soil pH and different combinations of soil texture and color were the significant factors. The close association to grass and livestock animals in both habitats is probably linked to *B. pseudomallei's* saprophytic characteristics and their excretion from infected animals (Figure 2.14.2).
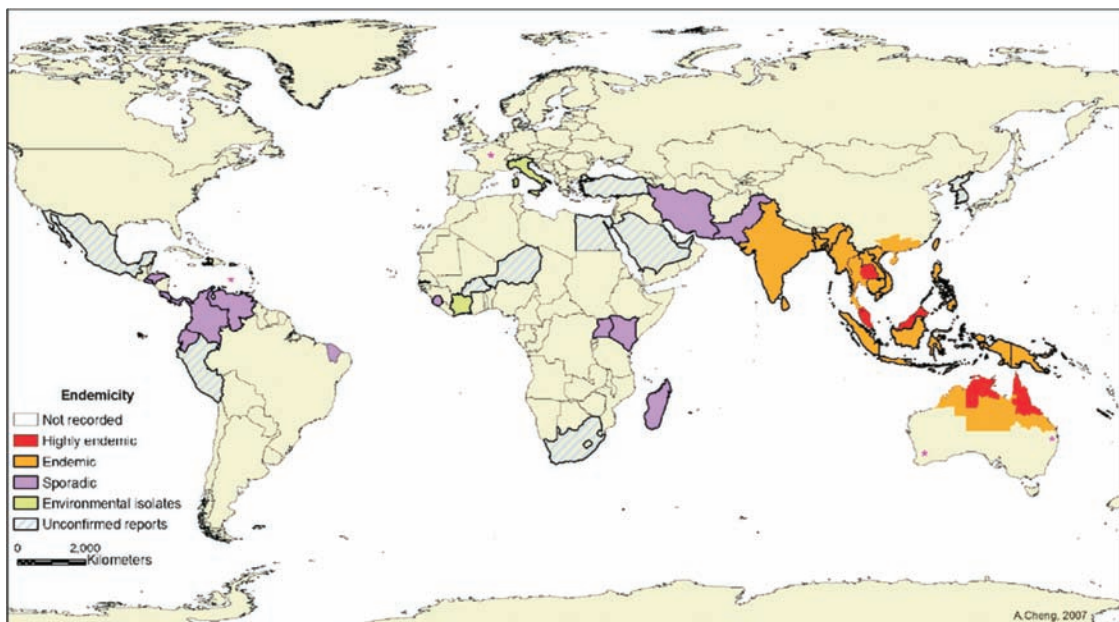
Figure 2.14.1. Global distribution of melioidosis (*Burkholderia pseudomallei*). (with permission from Currie, B. J., Dance, D.A.B. & Cheng, A.C. (2008), *Trans. R. Soc. Trop. Med. Hyg.* 102/S1, S1-S4.). *****-documented temperate outbreaks of melioidosis: France; southeast Queensland, Australia; and southwest Western Australia

B. pseudomallei's ability to survive and biodegrade extreme concentrations of phenol (1.5 g/l) in seven days was shown experimentally by Afzal *et al.* (2007) and in the context of other chemicals Gal *et al.* (2004) found a hand cleanse detergent as the source of human contamination with this pathogen which confirms its biodegradation capability.

As already highlighted, *B. pseudomallei* was also identified as a plant pathogen (Lee *et al.* 2009); nevertheless, this species, owing to a facultative intracellular lifestyle articulated by its presence in various eukaryotes, was demonstrated to be present in arbuscular mycorrhizal fungus (AMF) spores *in vitro* (Levy *et al.*, 2009). AMF is a worldwide phenomenon of the highly evolved mutualistic relationship (symbiosis) between fungi and vascular plant, and therefore *B. pseudomallei's* presence
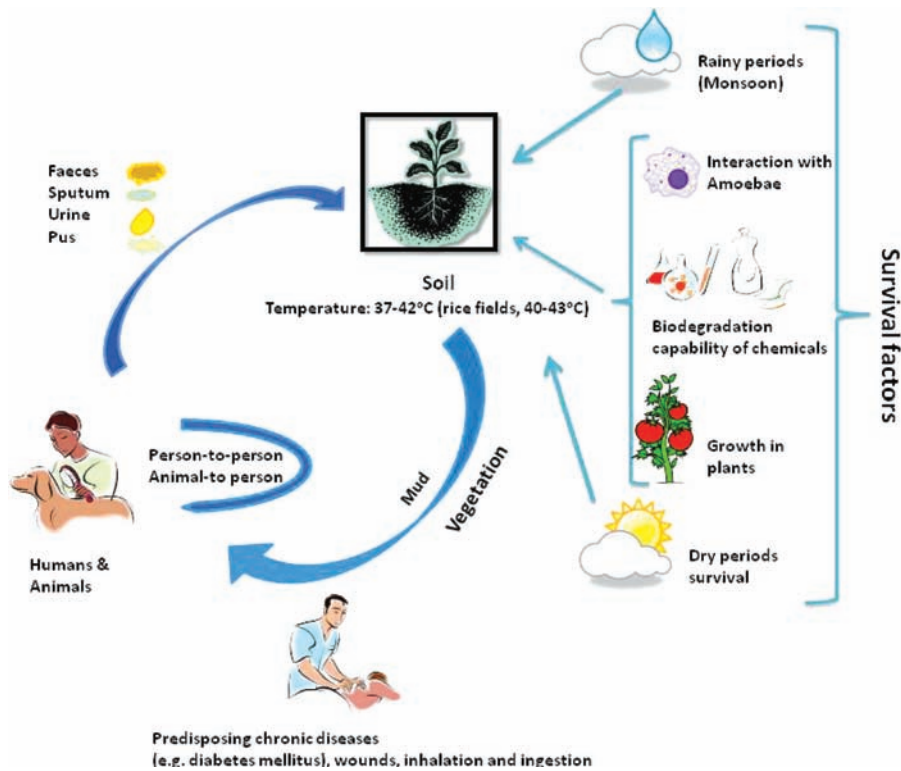
Figure 2.14.2. Melioidosis transmission cycle and environmental factors involved in *Burkholderia pseudomallei* survival

is very important in understanding the ecology and environmental aspects of plant-soil and animals interactions.

Another study showed an interesting biochemical divergence (utilization of L-arabinose as substrate, Ara^+ gene controlled) between some soil and clinical isolates of *B. pseudomallei* (clinical and some soil isolates were found to be Ara^-). Arabinose is known to be one of the major components of hemicellulose and pectin found in plants that may explain *B. pseudomallei's* pathogenicity toward plants (by carrying the Ara^+ gene) beside animals (Sirisinha *et al.*, 1998).

Wildlife animals were also shown to harbor *B. pseudomallei* as identified in a batch of feral cynomolgus monkeys (*Macaca fascicularis*) imported to Britain from the Philippines (Dance *et al.*, 1992).

Human migration as well imported work forces can also contribute to the spread of this disease as reported by Cahn *et al.* (2009) in relation to an agricultural worker from Thailand who was diagnosed with diabetes mellitus and melioidiosis in Israel. Finally, in the Northeast part of Thailand, Limmathurotsakul *et al.* (2010) performed a prospective cohort study on 2,243 patients identified with culture-confirmed melioidosis, admitted to a hospital in northeast Thailand between 1997 and 2006. These authors found that diabetes mellitus was the strongest and most statistically relevant risk factor responsible for the rise over time in the melioidosis prevalence in Thailand as an endemic area. The prevalence of diabetes in the Thai population of this area was 2.3% in 1991 and increased to 6.7% in 2004, with an average mortality rate of 42.6% from melioidosis over this study period.

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Chapter 2.15

Mycobacterial Infections

[*MYCOBACTERIUM TUBERCULOSIS*]

Mycobacterium tuberculosis complex includes: *M. africanum, M. bovis, M. bovis* BCG*, M. canettii, M. caprae, M. microti, M. pinnipedii*, and *M. tuberculosis* all involved in chronic tuberculosis in humans and animals (Vaerewijck *et al.*, 2005) (Figure 2.15.1). *M. tuberculosis* is a highly aerobic bacterium with high lipid content (mainly cell wall mycolic acid coating) which has made its Gram staining classification difficult, and therefore classical stainings such as acid-fast or Ziehl-Neelsen are used for identification. Due to their lack of an outer membrane, *Mycobacteria* are classified as acid-fast gram-positive bacteria. The disease is presented by subfebrile temperatures, lymphadenopathy, night sweat, cough (possibly with blood), fatigue, appetite loss/anorexia and erythema nodosum and in severe cases death (without treatment>50%). Extrapulmonary tuberculosis affects other organs: lymph nodes, genitourinary tract, pleura, meninges, skeletal system and peritoneum.

[*MYCOBACTERIUM MARINUM*]

Mycobacterium marinum was primarily isolated from a marine fish and only recently reported as an infectious source in humans causing a disease called "fish tank granuloma" (Aronson, 1926; Gray *et al.*, 1990). Dissemination from pet fish and immunocompromised granulomas was observed. In humans, it presents as granulomas, abscesses and in severe cases tenosynovitis, bursitis, arthritis and osteomyelitis.

[*MYCOBACTERIUM AVIUM* COMPLEX (MAC)]

Mycobacterium avium complex includes the following species: *M. avium*, *M. avium paratuberculosis*, *M. avium silvaticum*, *M. avium "hominissuis"* and *M. colombiense*. *M. avium* is the main agent of tuberculosis in birds and possibly connected to human infection too (Chiodini and Rossiter, 1996).

[*MYCOBACTERIUM ULCERANS*]

M. ulcerans is a slow-growing mycobacterium infecting the skin and subcutaneous tissues producing nodules/plaques and ulcerated lesions (Buruli ulcer) reported mostly in wetlands areas of tropical or subtropical countries (Uganda Buruli Group, 1971). It is the third most common mycobacteriosis after tuberculosis and leprosy in humans and animals (broad host range such as koalas, possums in Australia, lizards, amphibians, chick embryos, armadillos, rats, mice, captive alpaca and cattle) (Portaels *et al.*, 2001). Focal outbreaks were connected to flooding, human migrations and man-made topographic modifications such as dams and resorts. Deforestation and increased basic agricultural activities may have significantly contributed to the recent marked increases in incidences of *M. ulcerans* infections with rapid emergence, especially in West Africa.
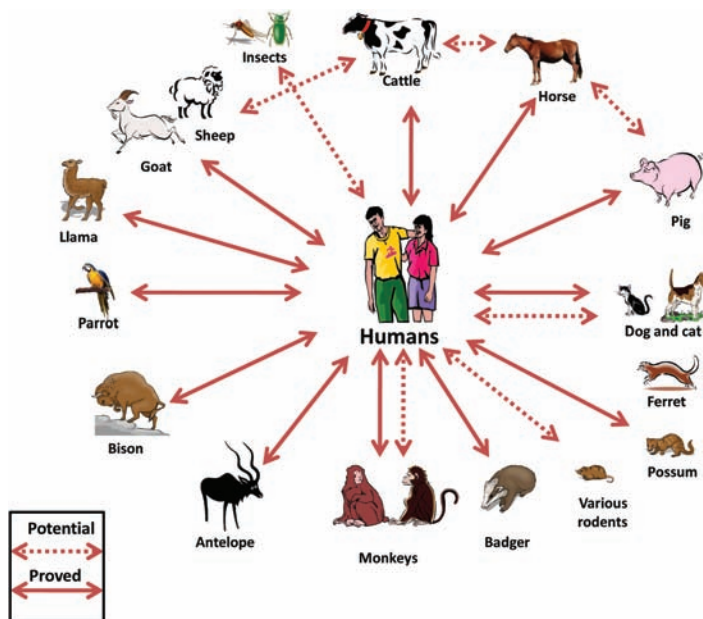
Figure 2.15.1. *Mycobacteria* spp. transmission from animals to humans

2.15.1 MYCOBACTERIUM AND ENVIRONMENT

Mycobacteria are known for their enhanced survival ability under adverse environmental conditions (dust and dried respiratory secretions). However, it is not clear what the exact mechanism that protects these pathogens in external environment of human and animal bodies is. It should be mentioned that there is a large group of *Mycabacteria* comprising saprophytic species (atypical/ non-tuberculous mycobacteria) that are mainly non-pathogenic or only occasionally pathogenic. There are many reports on the isolation of environmental *Mycobacteria* from soil, bogs, surface water, ground water and sea water as natural habitats (Norby *et al.*, 2007; Tsintzou *et al.*, 2000; LeChevallier *et al.*, 2000; Young *et al.*, 2005). However, till now there have been no reports of *M. tuberculosis* complex isolations from environmental sources but solely reports of infections through direct contact with infected domestic or wildlife animals

(including their excretions such as sputum, urine, milk and feces), consumption of uncooked milk or meat from tuberculous animals and inhalation of droplets when in contact with ill persons or animals (O'Reilly and Daborn, 1995). Historically, based on genetic evidence, the common ancestor of the causative agents of tuberculosis emerged 40,000 years in East Africa, an area from which the modern human population disseminated around the world, too. Between 10,000 and to 20,000 years later the pathogen split into two major lineages: one which infects humanoids and a second which spread from human to animals. In recent times (∼180 years ago), with overlapping human population expansion and industrial revolution, the human-associated pathogen lineages expanded (Wirth *et al.*, 2008). Some authors also indicated an additional direction that may impact these pathogens' persistence besides demographic expansion, especially in crowded cities (e.g., bacterial dormancy) (Shleeva *et al.*, 2010). The dormancy state of bacterial pathogens expresses itself by a drastic decrease of metabolic activity, enhanced resistance to harmful factors, and absence of cell division, all increasing its survival potential under unfavorable environmental conditions (Archuleta *et al.*, 2005). Several *in vitro* models, including prolonged hypoxic incubation in the post-stationary phase or under microaerophilic conditions after rifampicin treatment, demonstrated that viable cells of *M. tuberculosis* are capable of transition to a "non-culturable" state and that their colony forming capacity may be restored by special procedures (Sun and Zhang, 1999; Hu *et al.*, 2000). Whether these reports are correct or biased, the persistence of non-spore forming *M. tuberculosis* complex in less populated areas requires further research efforts.
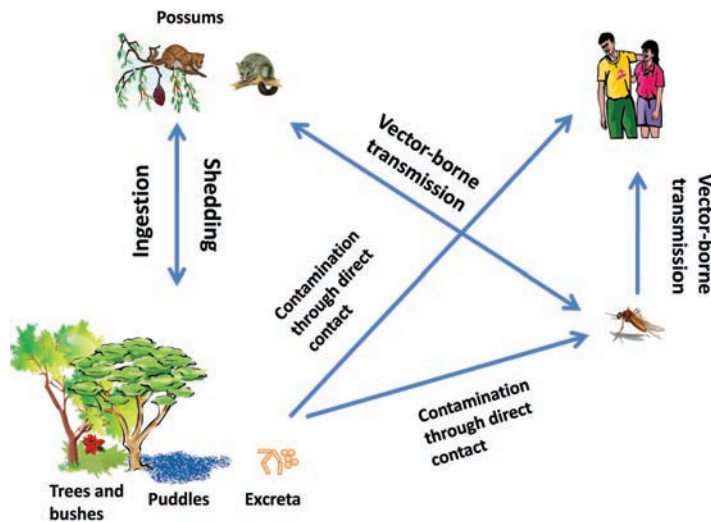
Figure 2.15.2. Suggested potential transmission routes of *M. ulcerans* shared by environment, possums, mosquitoes and humans

Finally, one should ask oneself whether this pathogen is present in the blood of infected animals there is any possible transfection through blood feeding insects such as mosquitoes, ticks, etc. Allen (1987) showed that fecal pellets (kept at room temperature) of an oriental cockroach that ingested heat-fixed sputum smears of viable *M. tuberculosis* intended for microscopic examination were positive for viable bacteria for up to 8 weeks. Fischer *et al.* (2003) infected experimentally nymphs of the same oriental cockroach (*Blatta orientalis*) with *M. avium* ssp. *paratuberculosis* and the avian tuberculosis *M. avium* ssp. *avium*. Both subspecies were isolated in excreta after 3 days and in homogenized bodies after 10 days. The authors

indicated a possible viable avian pathogen shedding and transmission, while infected orally. Kovalev (1983) discussed the role of ectoparasites (ticks) and wild birds as circulators, distributors and natural foci of *M. avium* tuberculosis. Furthermore, an aquatic insect predator (*Naucoris cimicoides*, of the order Hemiptera, genus Nepidae, commonly called waterscorpion) was shown to be infected by *M. ulcerans* through coelomic plasmatocytes that act as shuttle cells that deliver *M. ulcerans* to the salivary glands of these insects (Marsollier *et al.*, 2005). This is not a science fiction scenario as our cities surroundings are populated by many insects that have direct contact with humans and animals.

An interesting explanation of *M. ulcerans* transmission and reservoirs in Australian mammals such as possums (Fyfe *et al.*, 2010) was recently published. The authors through intensive study of fecal content of animals in endemic areas for *M. ulcerans*, found 41% positive samples compared to<1% in non-endemic areas. These findings indicate terrestrial mammals as additional reservoirs of *M. ulcerans* (Figure 2.15.2).

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Chapter 2.16

Pasteurelloses

[*PASTEURELLA MULTOCIDA*]

Pasteurella multocida is a non-motile gram-negative cocobacillus (pleomorphic) facultative anaerobe that forms capsules. *Pasteurella multocida* strains based on capsule antigens are divided into 5 serogroups (A, B, D, E and F) and later classified into 16 serotypes (1–16) based primarily on lipopolysaccharide antigens (Carter, 1955; Heddleston *et al.*, 1972). Other closely related species are: *P. dagmatis, P. canis, P. caballi, P. stomatis*, and *P. haemolytica. Pasteurella* can be found in wild and domestic animals (including pets and zoo animals) and even more exotic ones such as tortoises (Koch *et al.*, 1996; Snipes *et al.*, 1980).

Transmission from animals to humans is mainly through scratches or bites from carrier pets and other animals with which they come in contact (Garcia, 1997). The disease manifests itself as skin/subcutaneous septic phlegmon, edema, pain, fever, vomiting, headache, diarrhea, lymphangitis and rarely sepsis, pneumonia, meningitis and endocarditis.

2.16.1 *PASTEURELLA* AND ENVIRONMENT (FIGURE 2.16.1)

In humans, numerous reported cases revealed that *P. multocidas'* source is domestic or wild carrier animals (e.g., pets such as dogs and cats) based on a correlation between applied antibiotics in animal farms and these airborne bacteria's resistance to these specific antibiotics. According to an aerosol spread study, the authors concluded that a 250 to 500 m radius from source is an area of increased risk of infection mainly by dust as the bacteria carrier (Muller and Dinter, 1988). Water has been also shown to carry this pathogen. In an outbreak in Nebraska, besides carcasses of dead waterfowls, water samples from several wetlands were found positive for the presence of *P. multocida* (Price & Brand, 1984). In terms of time, Blanchong *et al.* (2006) suggested that *P. multocida*, in spite of its presence in wetland, was not isolated after 7 weeks since the initial outbreak, indicating that wetlands are unlikely to serve as a long-term reservoir for *P. multocida*. *P. multocida* was isolated even from dental unit waterlines, making direct contamination through open dental procedures a possible health risk (Göksay *et al.*, 2008). Lane *et al.* (1992) reported an outbreak of Pasteurollosis in cattle due to sewage and effluents flood irrigation of grass pastures following earlier excessive rain and cold weather. Bredy and Botzler (1989) tested experimentally the survival of *P. multocida* in water under various conditions: protein and sucrose content, pH, temperature, NaCl and

clay. *P. multocida* was found to survive the longest time period of >1 year under increased protein (175 mg/ml) and NaCl (>0.5%) presence. Other parameters such as clay, sucrose and pH did not play a significant role in the bacteria's survival. An interesting observation, supported by other studies, showed a rapid decline of this pathogen at low temperature (<3°C). Temperature dependent survival was in accordance with other reports that showed growth of this pathogen between 12 to 43°C (Wilson & Miles, 1975). In contrast, *P. multocida* inoculated in soil was shown to survive best at 3°C (Dimov, 1964). Winter is the main season of waterfowl infection and death, which is at odds with this pathogen's survival data at low temperatures. Some explanations for this discrepancy were presented based on a large quantity of excreted bacteria and high density of waterfowl in close proximity. However it should be remembered that the external temperature of these avians' skin is much higher than 3°C and the attached bacteria can survive for prolonged time periods. Another point that possibly explains this inconsistency is the divergence between soil, water and bacteria interactions. Bacteria are able to form biofilms on soil particles that increase their viability compared to the water free-state in which microorganisms are much more vulnerable (Armon *et al.*, 1997). However, the species *Pasteurella haemolytica* have been isolated from grass, water and straw bedding samples collected from two grazing fields in use by sheep, and from ewes affected with mastitis. *P. haemolytica* supported by colder, wetter weather had prolonged survival time in environment, data confirmed also by laboratory experiments with distilled water, phosphate-buffered saline, Todd-Hewitt broth and ewe's milk kept at 4°C (Burriel, 1997). Ytrehus *et al.* (2008) reported on a Musk ox's fatal epizootic pneumonia outbreak (caused by *P. multocida*), triggered by extraordinary weather conditions, characterized by high temperatures and humidity, a pattern directly linked to global warming. According to these authors, these unusual conditions seem to modify musk ox susceptibility to infection being the decisive factors in the disease outbreak.
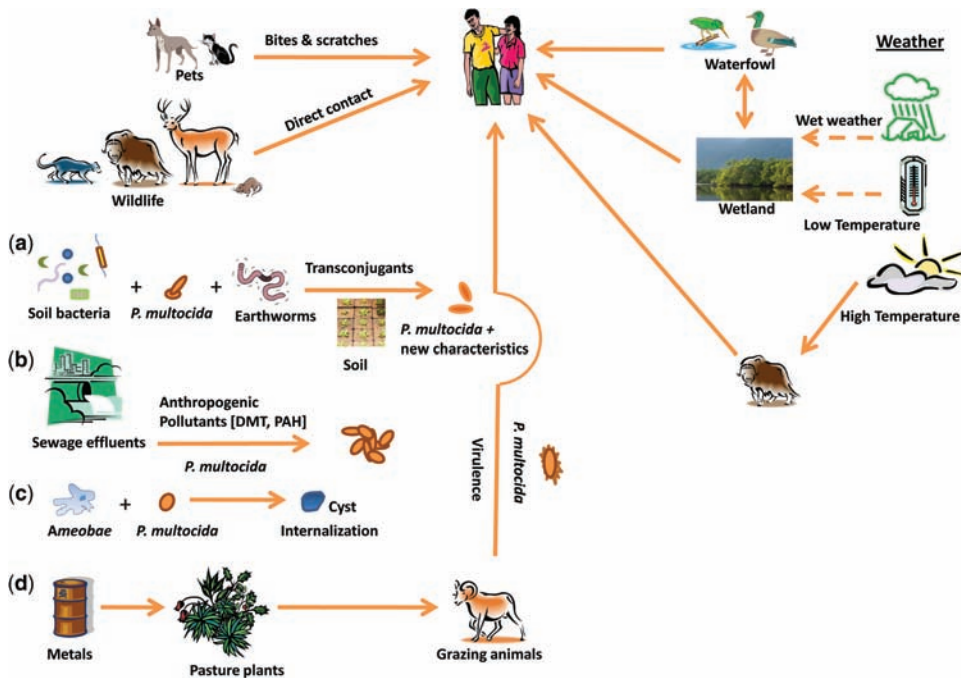
Figure 2.16.1. *Pasteurella multocida* infection cycle and possible environmental entangled factors

A *P. multocida* strain isolated from mangrove sediment was reported to biodegrade a certain chemical, dimethyl terephthalate (DMT), commonly used in polyester production (Li & Gu, 2006). Another *Pasteurella* spp. isolated from activated sludge was reported to biodegrade polyaromatic hydrocarbons (PAHs) such as phenanthrene, fluoranthene and pyrene (Sěpič *et al.*, 1997, 1998). Recently, a *Pasteurella* sp. was reported to be able to multiply in soils contaminated with cadmium. This particular species precipitated Cd^{+2} in the form of $CdCO_3$ in these cadmium contaminated soils (Li *et al.*, 2010). In connection with this biochemical characteristic, Russian scientists studied large areas including the semi-desert and desert areas of Kazakhstan and Uzbekistan, as well as the steppe and forest-steppe regions of Russia and Mongolia for a relationship between infections and environmental chemistry where plague epizootics occur. In relation to *Pasteurella* and other microbial pathogens, they found an increased pathogenicity well correlated to several metallic elements present in pasture plants, such as Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd and Pb. Excess of Mo in animals is known to intensify the removal of Cu from their organisms, and increased concentrations of Pb and Zn intensify the displacement of Cu (also recorded during the epizootic). Therefore it is logical that ungulates feeding on vegetation exceptionally rich in Mo, Pb, and Zn will experience a considerable shortage of Cu (observed in all the plague outbreaks studied) (Rotshild, 1996; Rotshild and Zhulidov, 2000). Based on these assumption, these authors suggested the following explanation related to plant chemistry and epizootics: pathogens that survive in environment through soil saprophytic cycle, water and their host bodies (asymptomatic) having enough nutrients including trace elements for their survival and multiplication however, when deprived of some elements (e.g., Fe) they compete with their host through activation of some hidden pathogenic attributes expressing their virulence and initiating disease. This theory sounds plausible; however, there are additional questions to be verified before its acceptance, such as: a) Does excess metals impair host immunology therefore favoring already opportunistic pathogens to prevail?; b) Do certain metals have an effect on pathogens virulence genes' expression? c) Do excess metals in plants impact their biochemistry in such a way that plants as food in turn impact animals immune system? Botzler (1991) in a review on epizootiology of *P. multocida* in wildfowl (infecting more than 100 species of free-living birds), described the disproportionate mortality and year-round disease periodicity. The survival of *P. multocida* seems to be significantly linked to the presence of animal organic matter in soil and water. Another important factor involved in the persistence of this pathogen in enzootic areas, as pointed out by this author, is the availability of carrier animals. Daane *et al.* (1996) showed that selected terrestrial earthworm species enhance gene transfer between different bacteria present in soil. Using *Pseudomonas fluorescens* harboring the plasmid pJP4 [*Pseudomonas fluorescens* C5t (pJP4)], the authors showed that among a large variety of successful transconjugant indigenous soil microorganisms were also *Pasteurella* spp.. This important study strongly supports an already published report on earthworms' role in soil pathogenic bacteria spread and possible enhancement of virulence transfer through their burrowing, casting, and feeding behaviors (Schuch and Fischetti, 2009). There is increasing evidences of a large variety of microbial pathogens interaction with free-living amoebae ubiquitous in water and soil environments. Hundt and Ruffolo (2005) showed experimental amoebae infection using a particular *P. multocida* sp. harboring green fluorescent protein (GFP) expression. *In vitro*, their results showed that this *P. multocida* sp. can invade and survive, replicate, and lyse in the amoebal host with a possible related occurrence *in vivo*.

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Chapter 2.17

Plague

[*YERSINIA PESTIS*]

Yersinia pestis (formerly *Pasteurella pestis*) is a gram-negative rod, non-motile, facultative anaerobe of the *Enterobacteriaceae* family. Microbiologists devide *Y. pestis* into three biovars: Antiqua, Medievalis, and Orientalis that can be distinguished depending on their abilities to ferment glycerol and reduce nitrate (Devignat, 1951). This pathogen is highly virulent with lethal effects, infecting humans and a large variety of animals (cat, dog, coyote, rabbit, camel, bobcat, raccoon, and bear but mainly free-living rodents that are also reinfection reservoirs) (von Reyn *et al.*, 1976, 1976a; Gage *et al.*, 2000; Gould *et al.*, 2008; Clover *et al.*, 1989; Wong *et al.*, 2009). Transmission to humans occurs mainly by rat flea as well by other ectoparasite vector bites (Wimsatt and Biggins, 2009) however, direct transmission through cat bite was also reported (Thornton *et al.*, 1975). *Y. pestis* infection has three clinical forms: pneumonic, septicemic, and the infamous bubonic plagues (connected to human demographic history). The disease expresses itself according to entry portal as follows: a) bubonic plague - fever, chills, headache, myalgias, malaise, lymphoadenopathy, septicemia, tachycardia, renal failure, gangrene, chest pain and death; b) primary pneumonic – fever, headache, myalgias, pulmonary signs (chest pain, cough, dyspnea, hypotension) and cardiac failure with ~20% death rate in spite of early antibiotic treatment; c) septicemic – gastrointestinal symptoms (abdominal pain, diarrhea, vomiting, nausea), hypotension, acute respiratory distress syndrome (ARDS), intravascular coagulation, organ failure with 50% death rate (without treatment) and <5% (with early antibiotic treatment).

2.17.1 *YERSINIA PESTIS* AND ENVIRONMENT

It can be stated with a high percentage of confidence that plague is the most "historic" zoonotic disease with the greatest devastating impact on human population. The first records originate from the 6th century, when it was called the "Plague of Justinian" after the emperor Justinian I himself who was also infected but survived the epidemic (McCormick, 2007). The second large, devastating epidemic occurred in the 14th century in Europe and China (along the Yangtse river valley) under the name of "Great Pestilence or Plague", while the name "Black Death" was set later in the 16th century in Nordic chronicles. The origin

seems to be the Gobi desert through fleas and rats that spread along trade routes (Crimea as one of the main ports) to Constantinople, Alexandria and further toward Western Europe (Haensch *et al.*, 2010) (Figure 2.17.1). The 14th century death toll have been estimated to be between 75 and 200 million people (Gage, 1998). In the next centuries, outbreaks occurred more locally in larger cities such as: Seville (Spain), London (UK), Vienna, (Austria) (17th century) and Marseille (18th century). More recent cases occurred in 1994 in northern Peru with 1,104 cases and 35 deaths and sporadic cases have occurred in the 21st century (Anonymous, 2004). In North America, *Y. pestis* was introduced around 1900, by rat-infested ships from Asia; evidence is supported by molecular studies revealing the strains similarity (Guiyoule *et al.*, 1994; Pollitzer, 1954).

Figure 2.17.1. European sites where human skeletons from mass graves were analyzed for aDNA (ancient DNA) and F1 protein capsule antigen and found positive for *Y. pestis* and possible routes of plague spread (from: Haensch, S., Bianucci, R., Signoli, M., Rajerison, M., Schultz, M., *et al.* (2010) Distinct Clones of *Yersinia pestis* Caused the Black Death. *PLoS Pathog* **6**(10):e1001134. doi:10.1371/journal.ppat.1001134.)

In relation to *Y. pestis* transmission path, when looking at environmental impact one should concentrate first on the two main parameters: rodents (as reservoir) and fleas (as vectors) and how these two main modules are affected by different climatic, geographic or anthropogenic factors (Mann *et al.*, 1979; Gage and Kosoy, 2005). Primarily, both rodents and flies are much more effective in disease transmission due to their large litter size and reproduction capability, secondly, they have been in close proximity to human habitation from the early stages of human history.

Pham *et al.* (2009) correlated (using Poisson regression model) data on domestic fleas and rodents, temperature, sunshine duration, rainfall, humidity and plague incidents in the central part of Vietnam already recognized as an endemic area. These authors found a strong correlation between the dry season periods and plague cases, expressed in a high flea index and rodent density during hot and dry months.

They hypothesized, that rainy weather reduces flea population through fungal, bacterial and viral increased susceptibility and decreased mobility. The same applies to the rodent population that increases according to food cascade during the tropical summer season. However, it should be mentioned that during dry seasons when rodent's food is in shorter supply, they will consequently scatter in search of food toward intensely populated areas, hence increasing plague spread (Figure 2.17.2). Parmenter *et al.*, (1999) in a different geographic area, an arid zone (New Mexico) found an increased occurrence of human plague cases (>60%) significantly correlated to winter-spring periods of above-average precipitation. Their analysis was performed at three spatial scales: 1) global (El Niño–Southern Oscillation Indices [SOI]), 2) regional (pooled state-wide precipitation averages) and 3) local (precipitation data from weather stations near plague case sites). The local scale was the only one that revealed a significant correlation, being explained by topical characteristics of mammal and flea populations' ecology that will respond to increased moisture only if it actually occurs in their immediate vicinity.
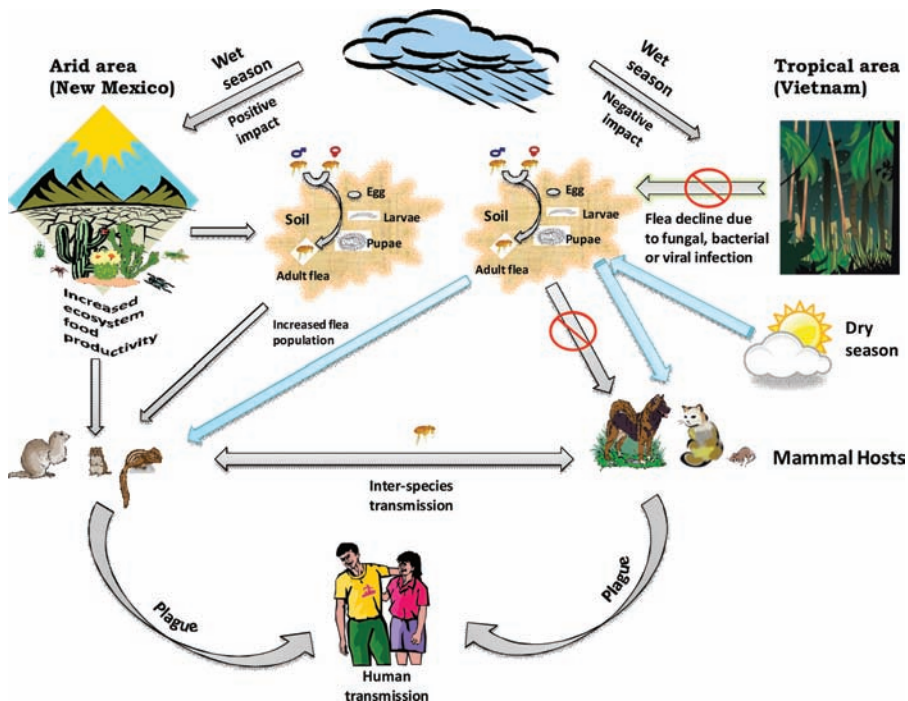
Figure 2.17.2. Ecological aspects of weather, soil, fleas and animals in human infection with *Y. pestis*

Davis (1953) reviewed plague history in Africa for the period of 1935–1949 and observed that seasonal incidence of human plague is strongly related to atmospheric conditions (temperature and rainfall) being more frequent in warm moist weather (15–27°C) than in hot dry, or cold, weather >27°C or <15°C. Highlands of equatorial Africa and of Madagascar appear to provide the optimum environment for the persistence of plague on the domestic plane (murine) and the high-veld and Kalahari of southern Africa on the sylvatic plane. Within these areas, *Yersinia pestis* finds an environment suitable for its continued survival where conditions seem to be comparable to those in endemic centers such as India.

Ten strains of *Yersinia pestis* were tested for static growth at 4°C when incubated in heart-infusion broth (Torosian *et al.*, 2009). Nine out of the ten strains were able to grow slowly and the shortest doubling times ranged from 13.5 to 22.16 hours. These results are important showing that *Y. pestis* can grow in rich media or in this case inside putrefying meat even at a low temperature.

On the other hand, Kartman (1969) showed experimentally that temperature has a profound effect upon vector potential and the capacity of fleas infected with *Y. pestis* being optimal at 23.5°C and much decreased at 4 or 29°C. These conclusions were drawn based on the successful pathogen proventricular blocking mechanism of the flea and flea longevity, and its resultant vectorial capacity at these temperatures (Bacot and Martin, 1914). The flea blocking mechanism is an exciting phenomenon described almost 100 years ago, which explains how fleas transmit *Y. pestis* (Figure 2.17.3). Briefly, the initial flea (a) with some blood residual in its stomach, having sucked from rodent uninfected with *Y. pestis* attaches this time to a new infected host and starts to suck in order to feed itself. Bacteria from infected blood colonize the proventriculus (PV) and the stomach (c) till both organs are distended with *Y. pestis* culture and partially digested blood (b). Under these conditions, a plug is formed in the proventriculus due to the gelatinous texture of blood-bacteria semi-digested complex (d) that blocks new sucked blood from entering the flea's digestive system, causing the flea to suffer from thirst and thus persist in its efforts to satisfy itself, without much success. The infected blood in the distended oesophagus may flow out again and infect a new host, on cessation of the sucking act when the insect withdraw its proboscis (sucking organ).
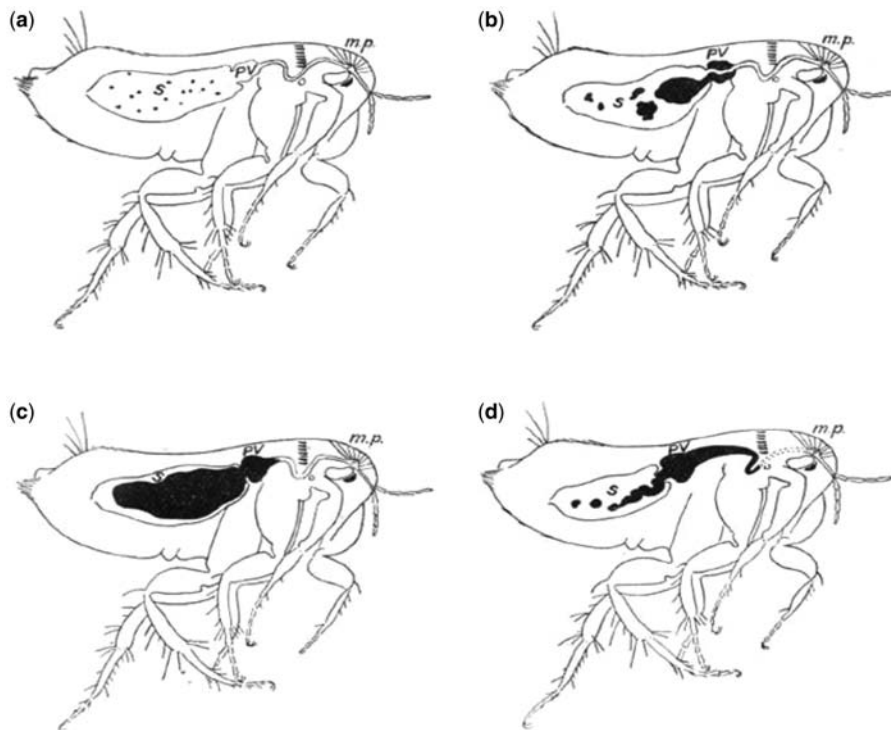
Figure 2.17.3. Flea proventricular blocking mechanism that explains *Y. pestis* animal transmission by this vector [adapted with permission from Bacot and Martin, 1914, *J Hyg (Lond)*]

Another temperature related aspect was published by Zharinova *et al.* (2008) concerning fleas (from Caucasian mountain plague foci) co-infected with *Y. pestis* and *Listeria*. The dual infection revealed antagonism between the two pathogens: at 4°C *Y. pestis* took over *Listeria* while 18–20°C favored *Listeria* over *Y. pestis*. According to these authors, it cannot be excluded that such a dual-infection might occur in this environment (in mountain sousliks under natural conditions) it may also explain the suppressed vector activity of fleas spreading plague at the above temperatures.

In another report by Wild *et al.* (2006), in which Canadian lynxes (129 individuals) were reintroduced in a program of species recovery in southwestern Colorado for a period of 4 years, *Y. pestis* was involved in high mortality. Among 52 dead individuals, 6 were diagnosed with pneumonic *Y. pestis* and one with bubonic. The authors suggested that these carnivores were possibly exposed to infected prey.

Snäll *et al.* (2008) using a Bayesian hierarchical model on the black-tailed prairie dog, showed that the rate of plague transmission between this rodent's colonies increased with increasing precipitation (that increases primary productivity as a result of more rodents and fleas, as shown before) and decreased with increasing topographic relief (ravines between colonies) (in spite of the fact that ravines facilitate prairie dog colonies dispersal, implying another animal carrier between colonies) and that the rate of infection from unknown sources decreased with hot weather (negative effects of heat on fleas). The authors suggested other likely plague carriers such as large carnivores that can carry diseases tens of kilometers in a year while feeding on infected prey (Smith *et al.* 2005).

Some reports suggested the possible survival of *Y. pestis* in soil environment (Mollaret, 1963) even for 10 months at 4 to 8°C (Breneva *et al.*, 2006), including internalization in soil protozoa (Pushkareva, 2003), form of latency (viable but not culturable, VBNC) (Suchkov *et al.*, 1997) and plant colonization as already shown with other bacteria, such as *Listeria* and *Salmonella enterica* (Rivkus *et al.*, 1993; Litvin, 1997; Girardin *et al.*, 2005; Kroupitski *et al.*, 2009).

In environment, *Y. pestis* is known to have two kinds of episodes: epizootic (disease with large scale spread) and enzootic (disease with local scale spread) that thus far are not yet well understood, especially in terms of how they move to and from, ecologically. Wimsatt and Biggins (2009) reviewed four published hypotheses on epizootic and enzootic episodes' rotation based on sylvatic plague. The first one suggests that "*Y. pestis* have a continuous propagation event of varying velocity (mainly enzootic periods punctuated by irregular epizootic periods) based on continuous supply of naive hosts and vectors (fleas)"; the second one is called "the carrier host hypothesis" and reminds one of the case of "Typhoid Mary", - where an infected individual is carrying the pathogen sequestered within, without clinical syndromes - (Anonymous, 1939), but in the present hypothesis the carrier host becomes septicemic in response to stressors, initiating an epizootic; the third hypothesis is so called "the telluric" based on the potential of *Y. pestis* to survive in soil and enhance its pathogenicity by environmental factors: soil elements, temperature, etc.; and the fourth hypothesis named "the flea reservoir hypothesis" is based on fleas capability to act as reservoirs besides their role as vectors. In their role as reservoirs, fleas were shown to be capable of unblocking themselves for longer periods of time (hence less pathogen transmission) and other environmental factors. Amongst these various hypotheses, the one that seems more adequate is that *Y. pestis* persists at foci by maintaining a more benign relationship within adapted rodents during the long time spans between outbreaks, a relationship influenced by environmental factors such as temperature, rain, soil and plants (Kartman, 1969; Mollaret, 1963; Rivkus *et al.*, 1993; Stapp *et al.*, 2008) (Figure 2.17.4). However, these authors emphasized the potentially pivotal role of fleas, which represent a low level plague reservoir.

Delwiche *et al.* (1959) studied the growth characteristics of virulent and avirulent strains of *Yersinia pestis* at 37°C under air, nitrogen and CO_2 atmospheres. Their results revealed that: (a) under aerobic conditions the avirulent cells outgrew the virulent ones; (b) under nitrogen atmosphere, both virulent and

avirulent cells grew equally well and the combined culture retained virulence and (c) under CO_2 atmosphere, the initial growth rate of both avirulent and virulent cells had been suppressed, although the virulent cells continued to proliferate, maintaining the initial ratio of virulent to avirulent roughly constant, where the culture as a whole retained its virulence for animals.
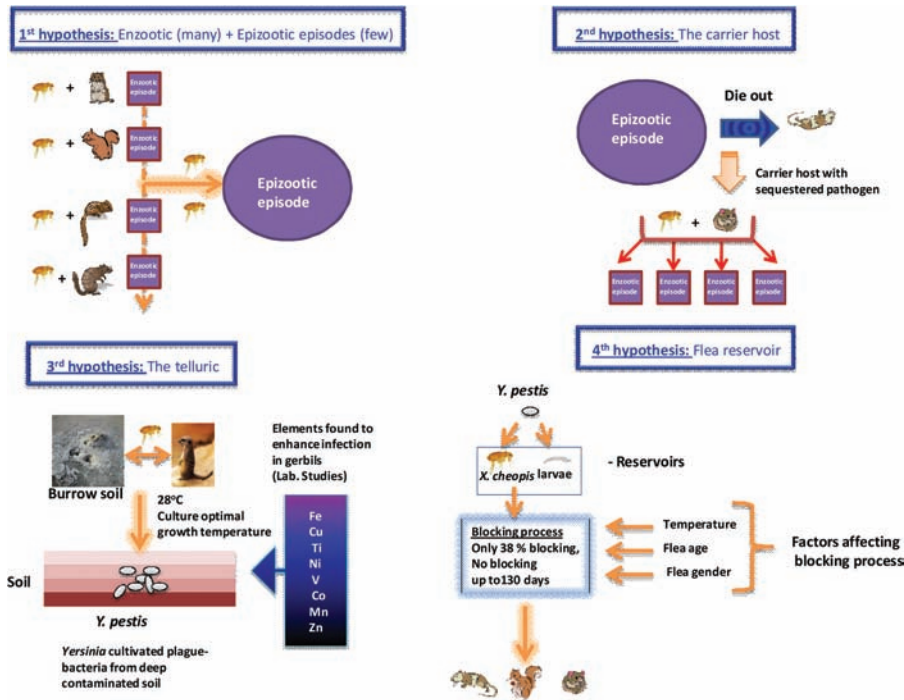
Figure 2.17.4. The four hypotheses related to *Y. pestis* epizootic and enzootic episodes in nature

Inevitably, *Y. pestis* is still considered a biological warfare weapon with immense disastrous consequences. Pala (2005) described the case of Vozrozhdeniye (Renaissance) Island located on the Aral Sea (now belonging to the Republics of Uzbekistan and Kazakhstan), a remote site where once the Soviet military did perform field tests with plague bacteria, whose lethality had been genetically increased (including other highly pathogenic bacteria). Due to wrong political decisions (diversion of water to cotton irrigation from two rivers that had naturally supplied water to the Aral Sea for more than 30 years), the Aral Sea was drying out with 75% shrinkage and splitting into two parts: the Small Aral in the north and the Big Aral in the south. To prevent the north part from becoming completely dry, the authorities decided to build a dam in order to fill this part, which in turn might affect the southern part by exposing land in the Big Aral, in the past partially submerged, consequently increasing traffic across a land link to Vozrozhdeniye Island. The main concern is that the newly open traffic path will cause people to come in contact with existent carrier animals (rats and camels) possibly by now infected or carrying military-grade (highly virulent), antibiotic-resistant plague bacteria, extremely different from the endemic strains present in Central Asia.

Duplantier *et al.* (2005) analyzing the well-documented human plague cases in Madagascar, suggested using the terms of expansion or regression phases for sylvatic rodent plague foci and reserving the term

re-emergence for human cases. According to these authors, the main factors in the re-emergence of human cases are: 1) surveillance and control reduction or discontinuance; 2) poverty and poor sanitary conditions that provide increased contacts with infected rodents and fleas; and 3) environmental changes (i.e., climatic changes, deforestation, urbanization) that could modify flea and rodent populations by (i) rodent habitats expansion (forests replacement with steppes or farmlands); (ii) modifications in population dynamics (possible outbreaks due to an increase of available food resources); but also, (iii) emergence of new vectors, reservoirs and new *Y. pestis* genotypes. An excellent example of these assumptions is the plague history in Madagascar where it was introduced in 1898 by a ship sailing from India. In an extensive survey of *Y. pestis* strains' analysis from 1939 till 1996, all strains up to 1982 were found to belong to biovar Orientalis (ribotype B). However, from 1982 on, new ribotypes (designated R, Q and T) were identified in one of the most active plague foci of this island. *Y. pestis* is known for its high frequency of spontaneous genomic reorganization (including conjugative transfer) that enhances its survival ability, transmission (to new hosts) and colonization of new environments (Guiyoule *et al.*, 1994; Guiyoule *et al.*, 1997; Hinnebusch *et al.*, 2002). For that reason, any environmental change should be taken as a warning signal, and active surveillance programs should be initiated to prevent emergence of new potent strains.

Breneva and Maramovich (2008) infected *Tetrahymena pyriformis* with avirulent and virulent variants of *Y. pestis* in an artificial soil ecosystem (ASE) containing burrows soil from one of the main carrier from the Gorno-Altayski natural plague reservoir. Avirulent strains were rapidly eliminated while highly virulent strains were the most resistant to the *T. pyriformis* phagocytosis process.

From the ecological aspect, Dinsmore and Smith (2010) studied the indirect impact of prairie dog plague epizootics on the mountain plove birds that preferentially nest in the area of black-tailed prairie dog colonies. Prairie dogs do modify vegetation and landscape structure around their colonies in ways that affect other grassland species. Akin to a chain reaction, morbidity of prairie dog as a result of *Y. pestis* epizootics impacted negatively plover colonization and nesting habits.

Another recent ecological facet, using field data on prairie community ecology, flea behavior, and plague-transmission biology, revealed that plague can persist in prairie-dog colonies for prolonged periods, because host movement is highly spatially constrained. An abundance of an alternate host for disease vectors (in this case, the grasshopper mouse-*Onychomys leucogaster*) drives plague outbreaks through connectivity increase of the prairie dog hosts and thereby allows percolation of the disease all the way through the primary host population. Indeed, these results provide an alternative point of view of plague's ecology, suggesting that more than one single main host is involved in *Y. pestis* dissemination in Africa and Asia. Furthermore, this phenomenon of abundance thresholds of alternate hosts that may be a key phenomenon determining outbreaks in many multi-host-disease systems has been supported by additional studies (Salkeld *et al.*, 2010; Davis *et al.*, 2008; Christie, 1982; Smith *et al.*, 2010).

Filipkowska (2003) examined the removal of various pathogens and indicator bacteria in a wastewater treatment plant in Lezany, Poland. The results of microbiological analyses confirmed a satisfactory removal rate of 94 to 97%; however, using biochemical tests the author found *Y. pestis* among many pathogenic bacteria still present in the final phase of purification (stabilization ponds). Still, the prospect of *Y. pestis* surviving in water is questionable and needs further research.

Boone *et al.* (2009) showed experimentally that scavenger carnivores (coyotes, swift foxes and badgers) may spread plague as flea carriers and by consumption of carcasses at long range, thereby possibly disseminating the pathogen and its vector to distant and new host colonies. Salkeld and Stapp (2006) surveyed exposure to plague by seroconversion of different carnivorous families. They found the following seroconversion rates in this order (from highest to lowest): mustelids (e.g., budger) > ursids

(e.g., bear) > felids (e.g., lynx) > canids (e.g., coyote) > procyonids (e.g., raccoon), probably reflecting exposure rates variation as a function of dietary habits.

Last but not least, a rough speculation linked to environmental pollution that is worth a second consideration is the phenomenon of cannibalism among rodents such as rats. Exposure of laboratory rats to environmental stresses, including teratogen (6-aminonicotinamide) and aflatoxin was found to increase cannibalism in these animals (Schardein *et al.*, 1978; Shankarmurti *et al.*, 1972). A number of explanations were suggested for this behavior, based mainly on experimental animal psychology including malnutrition, necessity of removing dead carcasses or defective offsprings and cross-species fostering. Regardless of what the correct answer might be, on the proviso that this phenomenon exists in wild rodents under exposure to pollutants with similar effects, it possibly explains the continuous maintenance of this pathogen enzootically through cannibalism of infected individuals.

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Chapter 2.18

Rat bite Fever (RBF) or Sodoku

[*SPIRILLUM MINUS OR STREPTOBACILLUS MONILIFORMIS*]

Rat bite fever is a disease transmitted by two bacteria species: *Spirillum minus* and *Streptobacillus moniliformis. Spirillum minus* is a gram-negative, aerobic spirochaete found in the blood and possibly in the conjunctiva of rats, but can be found in other animals such as squirrel, mice, pigs, cats, dogs, ferrets and weasels. *Streptobacillus moniliformis* is a rod in chains (streptobacilli) (in older culture it may form filaments) gram-negative facultative anaerobic bacterium found in normal oropharyngeal flora of rats or other rodents (Cunningham *et al.*, 1998). Importantly, these germs can also be transmitted via food or water that was probably contaminated with rat feces or urine (Andre *et al.*, 2005). Haverhill fever (HF) is a form of *S. moniliformis* infection considered to occur subsequent to ingestion of contaminated food or water (Gaastra, *et al.*, 2009). The disease is characterized by acute fever, headaches, malaise, lymphoadenopathy, vomiting and other external developments (maculopapular and urticarial rashes). More serious complications may occur such as myocarditis, hepatitis, meningitis, nephritis and pneumonia and death (very low with *S. minus* under treatment and 10% with untreated *S. moniliformis* cases) (Anonymous, 2005). *S. minus* is asymptomatic in animals while *S. moniliformis* can cause arthritis in turkeys.

2.18.1 RAT BITE FEVER AND ENVIRONMENT

Close contact and increasing exposure to exotic or wild animals and in this context to rodents coupled with gradual urbanization has increased the frequency of zoonotic diseases such as RBF (Graves and Janda, 2001; van Nood and Peters, 2005; Dendle *et al.*, 2006). Valverde *et al.* (2002) reported an unusual infection in two non-human primates by *S. moniliformis*.

Along with *Giardia*, *Cryptosporidium*, *E. coli*, *S. typhi*, *S. paratyphi* and *Campylobacter*, *Streptobacillus moniliformis* was implicated in several public and private drinking water related outbreaks in England and Wales from 1910 to 1999 as reported by Nichols *et al.* (2009). These outbreaks were significantly correlated to two contrasting climatic situations: low rainfall and excess cumulative rainfall prior to outbreaks. Relative drought (low rainfall) can result in the following events: a) elevated output of sewage effluents in rivers; b)

opening up of water flow channels as the water table drops and direct contamination of groundwater; and c) dry soil surface encouraging run-off and formation of cracks that affect the soil's filtration capability. Excessive rainfall besides causing wash out and spread of rats' urine and fecal materials over extensive areas may also impact the drainage rat population through flooding. This aspect has not yet been studied, but it should be taken seriously as one of the rats' main dwelling-places is sewer system. Countries subjected to continuous floods are at high risk, especially in densely populated areas at low economic levels. With regard to climatic conditions, Lima *et al.* (2008), using a logistic model reported on the impact of summer precipitation on two closely related kangaroo-rat populations (*Dipodomys merriami* and *Dipodomys ordii*). It was hypothesized that increased summer precipitations intensify green vegetation needed for the reproduction of these rodents (specifically, females that require the water content of green vegetation for lactation).

Rat bite fever association with excessive rain fall was reported in a large outbreak caused by *S. moniliformis* (304 people out of 700, 34%) in a boarding school in the UK (McEvoy *et al.*, 1987). Water (from taps) and milk were first suspected to be the transmission vectors; however following an extensive epidemiological survey, the authors deduced that water from a nearby spring was the outbreak's main pathogen reservoir. The assumption that this spring was infested with rats or even carrying a rat carcass could not be ruled out, as the water main (not connected to the spring) in this location did not cause additional cases outside the school. In this special case, digging around the building and exceptionally stormy weather had been reported, both events suspected of causing disorder of the local rat population resulting in accidental contact with spring water.

Finally, petrochemical land waste treatment was shown to induce cotton rats' dental lesions through fluorosis (Rafferty *et al.*, 2000). This observation is important as such lesions can increase the potential rat bite fever pathogens being transmitted between these rodents as well to other animals including humans with which they may come in contact.

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Chapter 2.19

Rickettsioses

[*RICKETTSIA* SPP.]

The genus *Rickettsia* are gram-negative, non-spore forming, non-motile, highly pleomorphic bacteria (cocci, rods or thread-like). All species are obligate intracellular parasites that replicate within cytoplasm of eukaryotic cells as host (mainly endothelial cells). The main species are: *Rickettsia rickettsii, Rickettsia felis*, *Rickettsia prowazekii*, *Rickettsia typhi*, *Rickettsia conorii*, *Rickettsia africae* and *Rickettsia akari*. Different ticks, fleas, and lice are the vectors of this pathogen and mammals (including humans) are the host. There are several diseases caused by Rickettsia such as typhus, scrub typhus (tsutsugamushi fever) rickettsialpox, Rocky Mountain spotted fever, Mediterranean spotted fever (Boutonneuse fever), African tick bite fever, Queensland tick typhus, Australian Tick Typhus and Flinders Island Spotted Fever. Rickettsioses can occur sporadically, endemically but also epidemically (e.g., typhus, Q fever) (Salgo *et al.*, 1988) (Table 2.19.1). *Rickettsia* has also been associated with a range of plant diseases and as an arthropods' endosymbiont. Resembling viruses, they grow exclusivelly inside living cells. The name rickettsia is often used for any member of the Rickettsiales. They are thought to be the closest living relatives to bacteria that evolutionarily are thought to be the origin of the mitochondria organelle present inside most eukaryotic cells. Diseases caused by the genus *Rickettsia* will manifest themselves according to the infecting agent's tropism such as: skin-texanthemas and necroses; lung-pneumonia; heart-myocarditis; brain-meningoencephalitis; gastrointestinal tract-diarrehea; pancreas-pancreatitis; liver-hepatitis; blood- thrombocytopenia and hemorrhages and kidney-hypoperfusion and acute renal failure.

2.19.1 *RICKETTSIA* AND ENVIRONMENT (FIGURE 2.19.1)

The ecology of rickettsiae is clearly linked to environmental factors as vector-borne pathogens. Mumcuoglu *et al.* (1993) investigated an outbreak of spotted fever group rickettsiae (SFGR) in a small settlement located in southern part of Israel. The settlement Kibbutz Zeelim (Z), the main source of SFGR with high morbidity was compared to another settlement of equal magnitude (Kibbutz Reim, R) located only 20 km away, as control. The dominant tick species isolated from site Z was *Rhipicephalus sanguineus* and from site

Table 2.19.1 Rickettsial zoonotic diseases and their global distribution.

Disease	Agent	Geographical Distribution	Hosts and reservoirs	Vectors
Rocky mountain spotted fever (RMSF)	*Rickettsia rickettsii*	USA, Canada, Costa Rica, Mexico, Panama, Columbia, Brazil	Rabbit, field mice, flying squirrel, other rodents and ticks	*Dermacentor variabilis, D. andersonii, Rhipicephalus sanguine, Amblyomma cayennense*
Mediterranean Spotted Fever (MSF), Boutonneuse fever	*Rickettsia conorii*	Southern Europe, Morocco, Israel, Georgia, Ethiopia, Kenya, South Africa, India, Pakistan	Dogs, rodents and ticks	*Rhipicephalus sanguine*
	Rickettsia sibirica	Russia, China, Pakistan Eastern Australia		
	Rickettsia australis	Flinders Island (Australia)		
	Rickettsia honei	Guadeloupe, eastern sub-Saharan Africa		
	Rickettsia africae	France,Slovakia		
	Rickettsia slovaca	Japan		
	Rickettsia japonica			
Epidemic Typhus	*Rickettsia prowazekii*	Ethiopia, Sudan, Somalia, Uganda, Kenya, Burundi, Rwanda, Mexico, Colombia, Peru, Bolivia, countries of former USSR and some US states	Humans, flying squirrels	Human body louse (*Pediculus humanus corporis*), Flea (*Orchopeas howardii*)

Disease	Agent	Distribution	Reservoir	Transmission/Vector
Murine Typhus, Endemic Typhus, Toulon Typhus, Tabardillo	*Rickettsia typhiRickettsia felis*	Yugoslavia, Greece, Malta, Sicily, USA (California, Texas)	Rats, urban Opossum	Rat fleas (*Xenopsylla chopis, Leptopsylla segnis*), Cat flea (*Ctenocephalides felis*)
Q Fever	*Coxiella burnetii* (now a separated genus of the family *Rickettsiaceae*)	World wide (except New Zealand)	Rodents, birds, game, domestic animals (e.g. cattle), ticks and humans	Airborne infected dust, aerosols, direct/indirect contact with infected animals or their excreta, possibly dairy infected products
Potential human zoonoses agents not yet isolated from humans[a,b]	*Rickettsia montanaRickettsia parkeriRickettsia massiliaeRickettsia rhipicephalisRickettsia helveticaRickettsia aeschlimannii*	Montana (USA), Swiss, Spain	?	?
Rickettsialpox	*Rickettsia akari*	New York (USA), former USSR, Croatia, Southern Europe, Korea, Southern Africa	Mice, mites	Bloodsucking mite (*Liponyssoides sanguineus*)
Scrub Typhus (tsutsugamushi fever)	*Orientia tsutsugamushi*	East Asia, Pacific Islands, Northern Australia	Rats, mice, rabbits, marsupials	Mites (*Trombicola* spp.)

[a]Tijsse-Klasen, E., Fonville, M., van Overbeek, L., Reimerink, J.Hj. & Sprong, H. (2010) Exotic Rickettsiae in *Ixodes ricinus*: fact or artifact ?. Parasit Vectors **3**, 54.

[b]Oteo, J.A., Portillo, A., Blanco, J.R., Ibarra, V., Pérez-Martinez, L. *et al.* (2005) Low risk of developing human *Rickettsia aeschlimannii* infection in the north of Spain. *Ann. N.Y. Acad. Sci.* **1063**, 349–351.

R, *R. turanicus Pomerantzev*. Physical factors such as soil composition and ambient surface temperatures that may clarify these differences were studied. Indeed, site Z had higher maximum soil and ambient temperatures well correlated with a higher tick load for a longer time span. Dogs, sheep, goats and various rodents were more heavily infested with ticks on site Z than on site R, while interestingly both sites were equal in dogs and mice' seropositivity to SFGR. Both sites had dogs as pet animals; however, site Z revealed that 7.1% of dog owners acquired Mediterranean spotted fever compared with only 1.4% of people without dogs. Population education and natural growth decline can prevent infections, in the particular case of rickettsiosis that has a familial character with close contact (louse-borne). Onishchenko *et al.* (1997) emphasized the decrease of louse-borne typhus in Russia due to natural demographic processes.
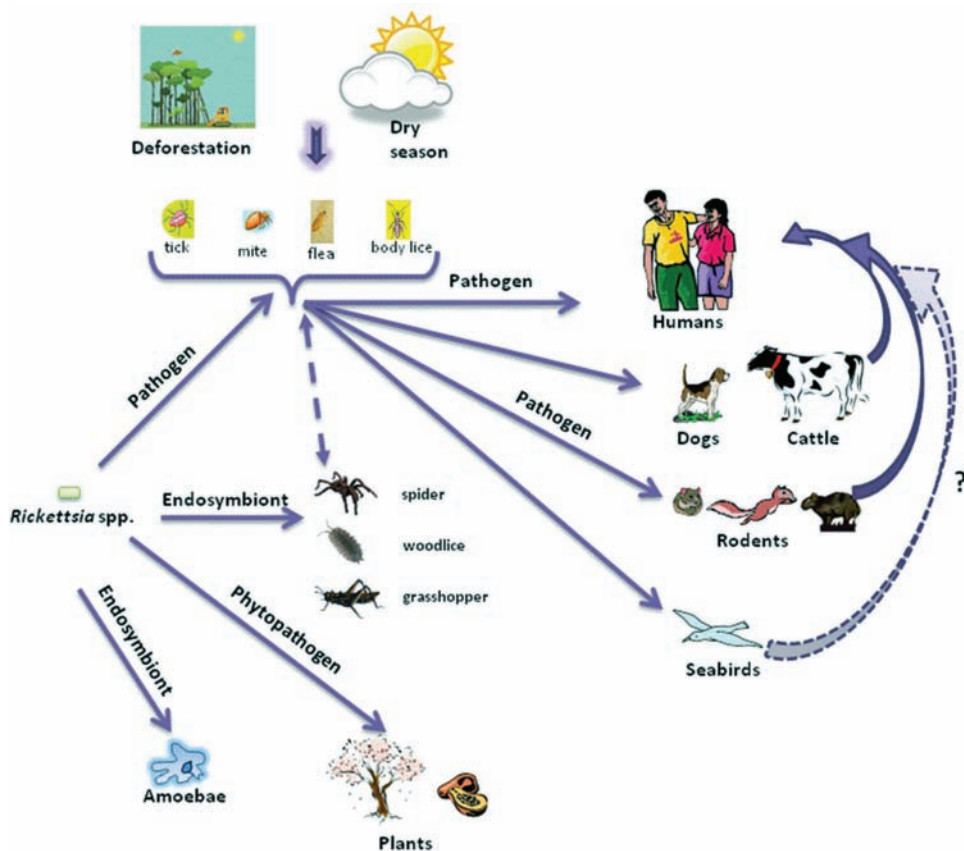
Figure 2.19.1. Zoonotic *Rickettsia* spp. proved and potential environmental life

As rodents are one of the main reservoirs of Rickettsia, Norway rats were surveyed for zoonotic pathogens in the Baltimore area, USA. Antibodies against *Rickettsia typhi* were detected in 7.0% of the live-trapped rats (Easterbrook *et al.*, 2007). Beside rodents a Japanese researcher reported detection of Rickettsia in association with seabirds suggesting a broader environmental circulation by microorganisms, ticks and long distance migratory seabirds around the Pacific Ocean (Kawabata *et al.*, 2006).

In a study performed in Russia (Leningrad province), cattle were shown to be the main source of *Coxiella burnetii* infection in humans (Tokarevich *et al.*, 2006). These authors showed that livestock farming modifications may impact infection with Q-fever. Over a period of 10 years, a reduction in large specialized livestock farms (subject to more advanced veterinary monitoring) occurred concomitantly with an increase in small private farms, exposing cattle to uncontrolled migration and contact with non-professional population, consequently increasing the health risk from this pathogen's transmission. It should be mentioned at this point that the *C. burnetii* is highly infectious requiring as few as one organism to cause disease via inhalation in susceptible humans (Sanders *et al.,* 2008). In addition, these authors observed a dramatic rise in domestic dogs carrying *C. burnetii* (based on seroprevalence) especially in urban areas (cities). In relation to dog population, Mannelli *et al.* (2003) in Piemonte (Italy) reiterated that link through *R. conorii* seropositivity observed in surveyed dogs, concluding that a relatively high dog population density within a rural or semi-rural environment encourages emergent foci of Mediterranean spotted fever (MSF) in this area. A related trend was also reported in Okinawa (Japan) supporting the above result (Satoh *et al.*, 2001).

Vector-borne diseases such as Boutonneuse fever were found to be also associated to climate change. Portuguese researchers looked at the endemic pathogen *R. conorii* (the strain involved in Malish and Israeli spotted fever) transmitted by the brown dog tick (*Rhipicephalus sanguineus*) and seroconversion during the off-season months (October-February) for a period of 6 years (2000–2005) (de Sousa *et al.*, 2006). They found a significant increase in positive cases, evidently related epidemiologically to climate change expressed in low precipitation values. The main factorial argument for this increase was the milder climate, permitting vectors such as ticks and other arthropods to survive and multiply preferentially. Vescio *et al.* (2008) looked at climatic factors that may correlate with mediterranian spotted fever (MSF) cases in the northern part of Sardinia and found that exposure to high temperature during summer months in a given year may increase MSF incidence in the following year. These authors explained their results based on the biology of tick vector life cycle.

Previous reports of *Rickettsia typhi* showed that lower temperatures than that of the human body are favorable for this pathogen and possibly for its other relatives. Moragues and Pinkerton (1944) subjected two groups of laboratory dba mice strain to murine typhus rickettsiae at two different temperature ranges: 18.3–22.7°C and 29.4–36.6°C and monitored illness and fatality. At lower temperatures the fatality was 100% and only <25% at higher temperatures.

In the USA, reported infections with epidemic typhus rickettsiae (*Rickettsia prowazekii*) were not associated with the classic man-louse-man cycle of epidemic typhus, but related to contact with flying squirrels (McDade *et al.*, 1980). A ten year study in the French Alps showed that human activity such as deforestation, can enhance the spread of certain ticks species from forested to urban areas, creating new or expanding known foci of disease transmission in the vicinity of large cities (Gilot and Pautou, 1982).

Wang *et al.*, (2004) described a new symbiont bacterium (provisional name "Candidatus *Hepatincola porcellionum*") that densely colonizes the midgut glands (hepatopancreas) of a terrestrial isopod (*P. scaber*). Isopods are an order of peracarid crustaceans, including commonly known animals such as woodlice and pill bugs. Based on comparative sequence analysis of 16S rRNA they classified this new bacterium as a distant relative of the order Rickettsiales (group known as intracellular symbionts and pathogens in many animals).

Another group of researchers analysed the genome of *Rickettsiella grylli*, an intracellular parasite of grasshoppers and the closest known phylogenetic relative of the *Coxiella* group (Seshadri and Samuel, 2005). Genomic proximity of crustacean and arthropods symbionts and pathogens suggests a broad evolutionary pattern of transition from symbiotic to pathogenicity and obligatory virulence in intracellular life.

An indication of a possible evolutionary pathway of an endosymbiont phylogenetically related to the order Rickettsiales, came from the protozoa *Acanthamoeba* spp. Considering the long-standing relationship of most Rickettsiales with arthropods, the related lineage of endosymbionts in protozoan hosts has had implications in the preadaptation and/or recruitment of rickettsia-like bacteria into metazoan hosts and further to invertabrates (Fritsche *et al.*, 1999).

Another fascinating interaction was described in a linyphiid spiders *E. atra*, that persists in heterogeneous environments because of its ability to recolonise areas through active long-distance airborne dispersal using silk as a sail, in a process termed "ballooning". However, a *Rickettsia* species endosymbiont that colonizes the sub-oesaophageal ganglion of this spider impairs the ballooning process. As mentioned earlier, in the case of *Y. pestis* and its "blocking mechanism" exercised on fleas, it is clear that this "biochemical behavior" outcome helps the pathogen to spread and infect new hosts, but in the present case of this *Rickettsia* sp. the ecological gain is not clear from the endosymbiont point of view and it might be that it is simply an "induced disease" through its activity on the sub-oesaophageal ganglion of this spider, unrelated directly to its ecology (Goodacre *et al.*, 2009).

Among the insects involved in transmission of Rickettsial disease, Hucko (1984) showed that house fly fed with *Coxiella burnetti* suspension keep their infectivity throughout their life (∼32 days) and possibly contaminate the environment during this time interval. The author also found that *C. burnetii* can survive in the fly's feces for 80 days and in dead flies as long as 90 days, therefore constituting a real health risk as *C. burnetti* is known to be airborne transmitted.

Finally, there are an increasing number of reports on Rickettsia like organisms in plants connected with phytopathogenesis (Hopkins and Mollenhauer, 1973). Whether they belong to the *Rickettsia* order is not clear (Evert *et al.*, 1981); however, Davis *et al.* (1998) using comparative sequence analyses, showed that papaya bunchy top disease is caused by a bacterium that is a member of the genus *Rickettsia*. Applying PCR method, they detected this bacterium only in diseased plants but not in healthy ones as the first evidence of genus Rickettsia as a potential plant pathogen.

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Chapter 2.20

Salmonelloses

[*SALMONELLA* SPP.]

Salmonella is a genus closely related to *Escherichia* genus, both members of the extended family of Enterobacteriaceae. Salmonella is a rod-shaped, gram-negative, facultative anaerobic, non-spore forming, predominantly motile (with peritrichous flagella) bacterium. *Salmonella* was isolated worldwide from both cold- and warm-blooded animals (including humans) and environments contaminated with hosts' excreta. *Salmonella* carried by animals (as residents of the GI tract) is transmitted to humans by direct contact but the vast majority of human cases are acquired through ingestion of contaminated food and water. *Salmonella* nomenclature is still evolving, but the main species are listed in Table 2.20.1 (Brenner *et al.*, 2000; Su and Chiu, 2007). In terms of time, the disease manifests itself relatively fast (from 5 to 72 hours) and depends on virulence and number of bacteria ingested (typically high numbers as a result of overgrow in food environment). The symptoms are: sudden nausea, vomiting and watery diarrhea that may contain blood, and fever (~39°C). Complications can occur mainly in immunocompromised patients for example: meningitis, septicemia, osteomyelitis, arthritis, peritonitis, urinary tract infection (UTI) and endocarditis. In animals it expresses as acute gastroenteritis or is silent (carrier state in reptiles).

2.20.1 *SALMONELLA* AND ENVIRONMENT

Presently, there is an extensive literature on food- and waterborne Salmonellosis that will not be reviewed in this chapter but occasionally mentioned; however, it is important to emphasize that its transmission is in general through the oral route by consumption of contaminated food (e.g. meat, dairy products, eggs and egg products), water and direct contact (Mataragas & Drosinos, 2009; Gomez *et al.*, 1997; Schuster *et al.*, 2005; Woodall, 2009). There are important aspects of global infections embroiling multi-drug resistance bacteria through meat import based on international trade (Espié *et al.*, 2005). Other important *Salmonella* food borne factors are: livestock feed, animal health conditions and slaughterhouse procedures (evisceration, etc.) (Maciorowski *et al.*, 2007). It should be mentioned that *Salmonella* is a highly resistant Enterobacteriaceae when outside human/animal intestine (its optimal milieu), a resistance accredited to diverse survival mechanisms (Foster and Spector, 1995). Orozco *et al.* (2008)

showed that hydroponic tomato farm greenhouses were infected with *Salmonella* through runoff flood and gained entry of wild animals (opossums, mice, and sparrows), both factors introducing the pathogen in spite of a reasonable protection level.

Table 2.20.1 *Salmonella* genus classification (serovars, hosts and transmission).

Salmonella species and subspecies	Other subspecies terminology	Serotypes or serovars	No. of serovars in subspecies	Usual Host	Human host	Transmission
S. enterica subsp. *enterica*	(I)	Choleraesuis, Enteridis, Paratyphi, Typhi, Typhimurium	1,504	Warm-blooded animals	+	Food, water, direct contact
S. enterica subsp. *salamae*	(II)	9,46:z:z:39	502	Cold-blooded animals and environment	+	Food, water, direct contact, reptiles
S. enterica subsp. *arizonae*	(IIIa)	43:z29:-	95	Cold-blooded animals and environment	+	Reptile
S. enterica subsp. *diarizonae*	(IIIb)	6,7:1,v:1,5,7	333	Cold-blooded animals and environment	+	Reptiles
S. enterica subsp. *houtenae*	(IV)	21:m,t:-	72	Cold-blooded animals and environment	+	Food, reptiles
S. enterica subsp. *indica*	(VI)	59:z36:-	13	Cold-blooded animals and environment	+	Food (+ vegetables), reptiles
S. bongori	(V)	13,22:z39:-	22	Cold-blooded animals and environment	+	Reptiles
S. subterranea			1	Environment (U^{VI}-contaminated subsurface sediment)	?	?
Total			3,829			

Groundwater was found to be the main significant independent risk factor linked to *S. choleraesuis* transmission, following adjustments for other potential confounders in a case-control study intended to identify the possible demographic and environmental risk factors associated with *S. choleraesuis* infection in Taiwan (Li *et al.*, 2009). It should be mentioned that pigs are considered as the only animal reservoir of *S. choleraesuis*, and therefore it may very well be that the dwellings of clinical cases could be near pig farms or use groundwater somehow connected to these farms. The groundwater's proximity to contaminated sources (landfill) proved to be a high risk of contamination through the leaching process (Adeyemi *et al.*, 2007). As previously mentioned in connection with other bacteria, *Salmonellae* too interact with protozoa such as *T. pyriformis* and *A. rhysodes*, using their ability to survive and replicate

within their protozoan protective environment, increasing their environmental transmission potential (Snelling *et al.*, 2006).

Recent publications showed that protozoa bestow protection (against low concentrations of calcium hypochlorite) and enhanced survival of the food-borne pathogen *S. enterica* through food vacuoles (vesicles) released by a *Tetrahymena* sp. isolated from moist soil (Brandl *et al.*, 2005). Moreover, Gourabathini *et al.* (2008) reported the isolation of similar protozoa from fresh lettuce and spinach plants, which can internalize pathogenic bacteria such as *Salmonella enterica* and then release them through food vacuoles as an environmental defense mechanism against bacterial pathogens. These authors also called attention to the possible proximity of the vacuolated released pathogens to stomata cells that may possibly support further plant internalization!

Nevertheless, when various amoebae, such as *Acanthamoeba*, *Tetramitus*, *Naegleria* and *Hartmannella*, were isolated from different vertebrates it was observed that their feeding preferences on bacteria appear to reflect their environment, not their taxonomic relationships (Wildschutte and Lawrence, 2007). These authors suggested an attractive hypothesis about the survival mechanism of *Salmonella* spp. as human and animal pathogens against being predated by amoebae (Figure 2.20.1). They proposed that O-antigen determinant (located on bacterial outer membrane) is the main key for predation recognition by the amoebae while the prey choice is governed by their environment (host animal) and not their taxonomic relationships (genome). In a previous study, Tezcan-Merdol *et al.*, (2004) showed that *Acanthamoeba* spp. can differentiate between different serovars of *Salmonellae* and that internalization is associated with cytotoxic effects mediated by defined *Salmonella* virulence loci.

Concerning the relation between plants and bacterial pathogens, Barak *et al.* (2007) demonstrated that *S. enterica* uses other surface polymers composing the extracellular matrix, such as cellulose and O-antigen capsule, in order to colonize plants. Schikora *et al.* (2008) used *Salmonella enterica* serovar *typhimurium* to prove that it is a real endopathogen of *Arabidopsis thaliana* and could successfully infect this plant. The definitive answer came from an Israeli group that clearly showed that *Salmonella enterica* induced by light can chemotactically penetrate open stomata, possibly explaining the reported high risk of Salmonellosis in connection with contaminated vegetables (Kroupitski *et al.*, 2009; Manas *et al.*, 2009). Another epidimeliogical study, performed in Oregon and British Columbia, showed that *Salmonella* transmission through plants can occur by consumption of alfalfa (*Medicago sativa*) sprouts originating from contaminated seeds (133 reported cases of *Salmonella enterica* serotype Newport-SN) (Van Beneden *et al.*, 1999). Alfalfa sprouts are excellent means for transmission of *Salmonella* and other enteric bacteria, as seeds are stored for extended time periods (even years) in dry environment. The sprouting process which takes 3 to 5 days, favors *Salmonella* growth to 3 to 4 orders of magnitude and further refrigeration does not reduce their number significantly and final consumption occurs without washing or cooking, therefore leaving the consumer completely unprotected.

There is an extensive literature on environmental factors impacting *Salmonella* presence in the coastal area, mainly due to the recreational and marine food role of these sites. Setti *et al.* (2009) surveyed 122 km of the coastline of Agadir (southern Morocco) for *Salmonella* spp., related to environmental parameters. They sampled seawater, marine sediment and mussels and found an overall *Salmonella* spp. prevalence of 7.1% (at the following ratios: mussels-10%, sediment-6.8% and seawater-4.1%). Amongst observed environmental variables, rainfall was the only parameter directly related to *Salmonella* occurrence, showing a linear positive effect well supported by other studies (Collin *et al.*, 2008). *Salmonella* highest numbers in shellfish is obviously related to these bivalve mollusks high filtration potential ability to concentrate various contaminants with high efficiency. A similar trend was found in mussels, clams and oysters from the coastal seawater of Galicia (Spain) and coastal areas of Bahia de Todos Santos (Mexico) (Martinez-Urtaza *et al.*, 2004; Simental and Martinez-Urtaza, 2008). In addition,

on the food chain scale, California's sea otters feeding on mollusks were found to harbor different *Salmonella* serovars beside other enteric pathogens (Miller *et al.*, 2010). Otters from more urbanized coastlines and areas with high freshwater runoff mainly in the rainy season (near outflows of rivers or streams) were more likely to test positive for bacterial pathogens. Otters exposure to fecally-associated protozoan pathogens such as *Toxoplasma gondii* and *Sarcocystis neurona* and enteric pathogenic bacteria, is suspected of impairing sick otters recovery in these geographical areas.
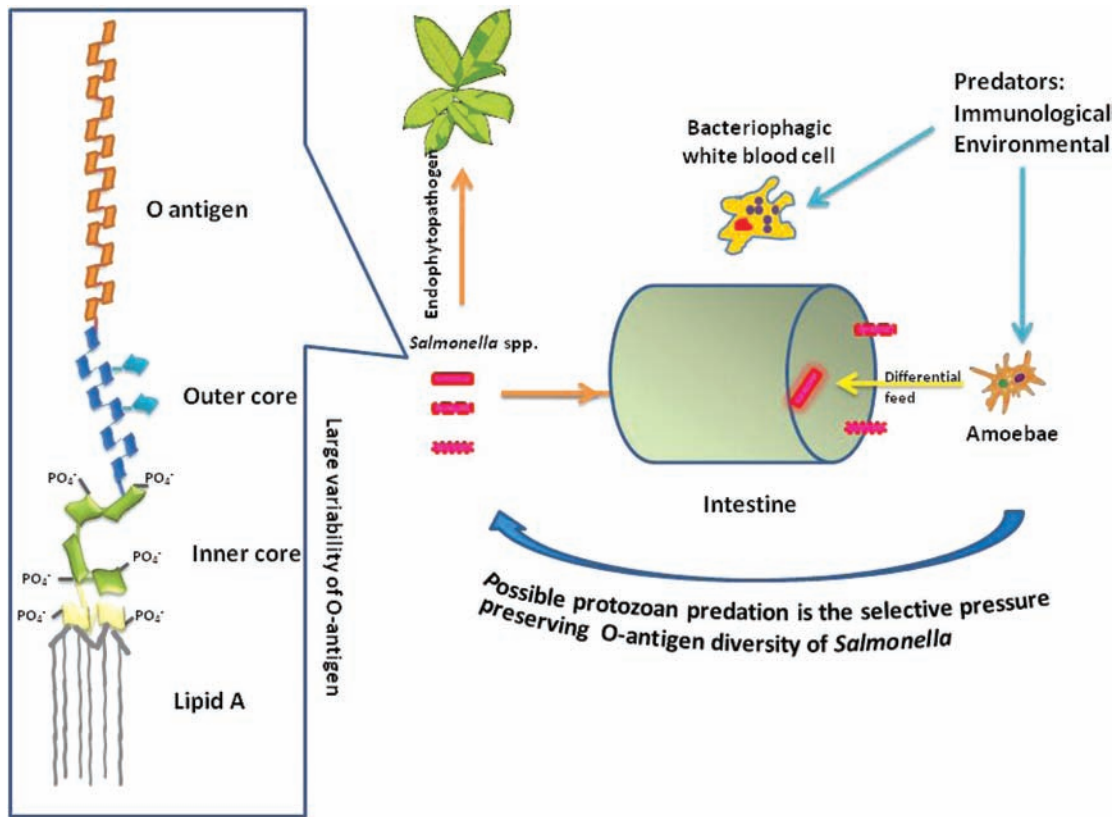
Figure 2.20.1. Predators prey choice and phytopathogenesis based on surface antigens of *Salmonella* spp.

In all these cases, presence of *Salmonella* spp. in coastal areas was merely confined to rainy periods, stream discharges and runoff, all factors involved in the transport of this pathogen via streams from source points to sea (Martinez-Urtaza *et al.*, 2004).

In another geographical edge (Australia), comprising both tropical and sub-tropical climates, researchers found that temperature and rainfall were the only significant parameters, suggesting that a potential 1°C rise in maximum or minimum temperature may cause a very similar increase in the number of Salmonelloses cases in both climatic regions (Zhang *et al.*, 2010).

Canadian researchers studied the survival of *Salmonella salamae* in estuarine waters of the St. Lawrence River (Monfort *et al.*, 1994). *Salmonalla salamae* cells exposed to starvation, increasing salinity and solar irradiation showed a rapid decline in viability; however, when bacteria were kept in darkness while

exposed to increasing salinity, their die-off rate was even higher than of those exposed to sunlight. Hence *S. salamae* cells exposed to sunlight seemed to be more resistant to gradual salinity stress than cells that were not subjected to sunlight. Bacteria exposed to sunlight irradiation are damaged by the UV short wave spectra causing DNA damage, however bacteria are able to cope with this germicidal activity. Bacterial sunlight damage repair (reactivation) has two well known enzymatic mechanisms: photoreactivation and dark repair. The photoreactivation mechanism, which requires light is much more effective than the dark mechanism. Zaafrane *et al.* (2004), studing *Salmonella thyphimurium* survival in sea water, found results similar to those of the Canadian researchers and furthermore emphasized that "bacteria grown in media with high salinity or osmolarity and transferred to sea water in stationary phase were more resistant to irradiation than those grown in media with low salinity".

Jones (1971) already reported that the death of sewage bacteria in seawater is mainly due to starvation and to natural heavy metal toxicity. In his study, other factors were found to be effective in controlling bacterial survival such as sunlight, temperature, salinity, antibiotics, bacteriophages, adsorption and sedimentation, parasitism, lysis, and protozoan predation. Enterobacteriaceae including *Salmonella* possess the σ^S subunit, encoded by rpoS gene of RNA polymerase, which functions as a master regulator of general stress responses (cessation of growth, acid resistance, thermotolerance and adaptation to growth in a medium of high osmolarity). The σ^S level increases in response to environmental stresses in cell; hence sunlight irradiation damage of the gene rpoS, lacking an effective repair, will decrease cell σ^S level leading to cell death under extreme environmental conditions.

When dealing with bodies of water it should be remembered that fish are also involved in transmission of *Salmonellae*. Miruka *et al.* (2009) studied Nile tilapia fish for contamination with members of Enterobacterioceae. Among the fish batch examined, 52.5% were positive for Enterobacteriaceae among which 11.1% with *S. typhimurium* and 25.4% with *S. enteridis* compared to an open-air fish market where *S. typhimurium* was present in 20% of fish. The authors concluded that the high level of *Salmonella* pathogens in fish indicates that beaches in this area are contaminated with untreated municipal sewage, runoff and storm-water.

Soil is also highly related to *Salmonella* ecology, mainly through utilization of organic fertilizers such as manure for different soils. Castro-Del Campo *et al.* (2010) studied the regrowth potential of *Salmonella enterica* subsp. *enterica* in three soil types: vermicompost, Class A biosolids and amended. At 30% moisture, the longest survival persistence of this pathogen (up to 20 days) was obtained for amended soil. Additional studies showed even more extended survival periods of time of *Salmonella* in soil and swine manure environments (for ~45 days, temperature dependent) (Guan and Holley, 2003). On bacterial soil endurance aspect, it should be pointed out that a *Salmonella typhimurium* strain has been shown to survive in soil for up to 54 days with decline in colony numbers (measured by direct plate culture) but constant viable cells when measured with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), as respiration-sensitive dye. A close look at these results revealed that direct CTC soil counts of *S. typhimurium* confirmed that intact cells were still present in an intact metabolic state after 54 days in the non-sterile soil, indicating a significant proportion of unculturable but active cells (Marsh *et al.*, 1998).

In an extra framework, soil was also reported as a possible *Salmonella* carrier state in amphibians and reptiles kept in terraria (private or zoological parks) (Hassl *et al.*, 2003). In Austria, where this study was carried out, there are ~90,000 households that keep a large variety of these "kind of pets". The authors detected a strong association between *Salmonella* subspecies (I and III) among captive reptiles and free living lizards accordingly. Kourany and Telford (1981) surveyed lizard species in Panama for *Salmonella* presence. Among these species, 29.4% were positive for *Salmonella* (with a range from 5.6 to 92.3%) with highest rates during dry season. The highest infection rate was detected in secretive (42.0%) and terrestrial (42.6%) lizards, whereas the lowest was among the scansorial lizards (17.5%).

Lizard soil contact reveals the possible contamination through excreta-contaminated ground. The infected lizards' distribution varied from remote rural and forested regions to urban developments, revealing their potential reservoir role. In the same geographical area (Panama) other animals such as opossums and rodents were also positive for *Salmonella* (∼10 to 20%) especially throughout the dry season (Kourany *et al.*, 1976).

Interaction between algae and *Salmonella* has been shown in Lake Michigan (USA) related to algal bloom. *Chladophora glomerata* is a filamentous, green alga that grows in nearshore water, on rocks and other hard surfaces. After their peak bloom, these algae detach and accumulate on the shore, creating an environmental problem through aesthetic, malodorous conditions and growth enhancement of pathogens such *Salmonella* (Byappanahalli *et al.*, 2009; Verhougstraete *et al.*, 2010).

As a final point, a group of ungulate veterinarians reported on pathogens and climate influence on the fecundity of ungulates in natural conditions. Beside animal density, they identified two other major confounding effects on the reproductive success of an alpine chamois ungulate (*Rupicapra rupicapra*): 1) prevalence of antibodies against pathogenic bacteria (among them, *Salmonella enterica* serovar *abortusovis* (36%), and 2) weather conditions (31%). Bad weather conditions that may act either as a direct stress or through food availability, were linked to increased infection with pathogens including *Salmonella* spp. (Pioz *et al.*, 2008).

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Chapter 2.21

Staphylococcal Infections

[*STAPHYLOCOCCI* SPP.]

The genus *Staphylococcus* is a group of gram-positive bacteria, grape-like cluster of cocci, non-spore forming, facultative anaerobes and desiccation resistant. The group has an additional differentiation based on coagulase activity (negative or positive reaction). Coagulase is an enzyme that converts blood fibrinogen to fibrin causing plasma clot (e.g., *S. aureus* is a coagulase-positive microorganism). The majority of *Staphylococci* are harmless, present in soil, water, skin of humans and animals and even human saliva (Lamka *et al.*, 1980; Kelesidis and Tsiodras, 2010). In many animals it was isolated from skin infections and mastitis, including from more exotic ones such as the white-eared opossum (Siqueira *et al.*, 2010). Two species are clearly recognized as zoonotic pathogens: *Staphylococcus aureus* a resilient foe and infrequently *Staphylococcus intermedius*. There are other *Staphylococci* family members involved in food and water-borne diseases, but they will not be described here. Staphylococcal infectious diseases exhibit themselves as: skin impetigo, folliculitis, abscesses, furuncles and in more serious cases as pneumonia, endocarditis, sinusitis, otitis, osteomyelitis and septic arthritis. Beside pathogenicity, one of the main negative aspects of *S. aureus* is its evolutionary resistance to methicilline (a narrow-spectrum β-lactam antibiotic of the penicillin class) (Livermore, 2000), though a recent publication reported skin infections among college football team members attributed to a community-acquired pathogenic *S. aureus* strain that lost its methicillin resistance, but retained important virulence factors (Fontanilla *et al.*, 2010). The important Staphylococcal strains are presented in Table 2.21.1, including antibiotic resistance to some common antibiotics and their coagulase activity.

2.21.1 *STAPHYLOCOCCUS* AND ENVIRONMENT (FIGURE 2.21.1)

As previously mentioned, staphylococci are also inhabitants of soil environment. Their soil permanent association with diverse types of bacteria may convey a variety of genetic characteristics, including antibiotic resistance. Polak and Novick (1982) showed that a common plasmid found in bacilli and *S. aureus* is highly homologous, except for the region occupied by their resistance determinants, suggesting possible natural transfer between the two bacterial types. Soil is also the source of dust and

therefore a carrier of airborne bacteria. Banana *et al.* (2001) found *S. aureus* as one of the most frequently isolated dust microorganisms originating from soil, which probably may explain its prevalence in human nostrils. These authors also reported its high prevalence in cinema halls (25.35%) and residential areas (18.75 %) alluding to potential cross-contamination by air-borne particles.

Table 2.21.1 Some important characteristics of *Staphylococcus* species.

Species	Host	Resistance			Coagulase
		Novobiocin	**Polymyxin B**	**Methicillin**	
S. aureus	Animals, Humans	−	+	+	+
S.epidermidis	Humans	−	+	+	−
S. haemolyticus	Humans, Apes, Lemurs	−	−	d	−
S. lugdunensis	Humans, Goats	−	d	?	+
S. schleiferi subsp. schleiferi	Humans	−	−	+	+
S. saprophyticus	Humans	+	−	?	−
S. intermedius	Dogs, Humans	−	−	+	+
S. hyicus	Turkey, Swine, Cattle	−	+	?	+

dVariable
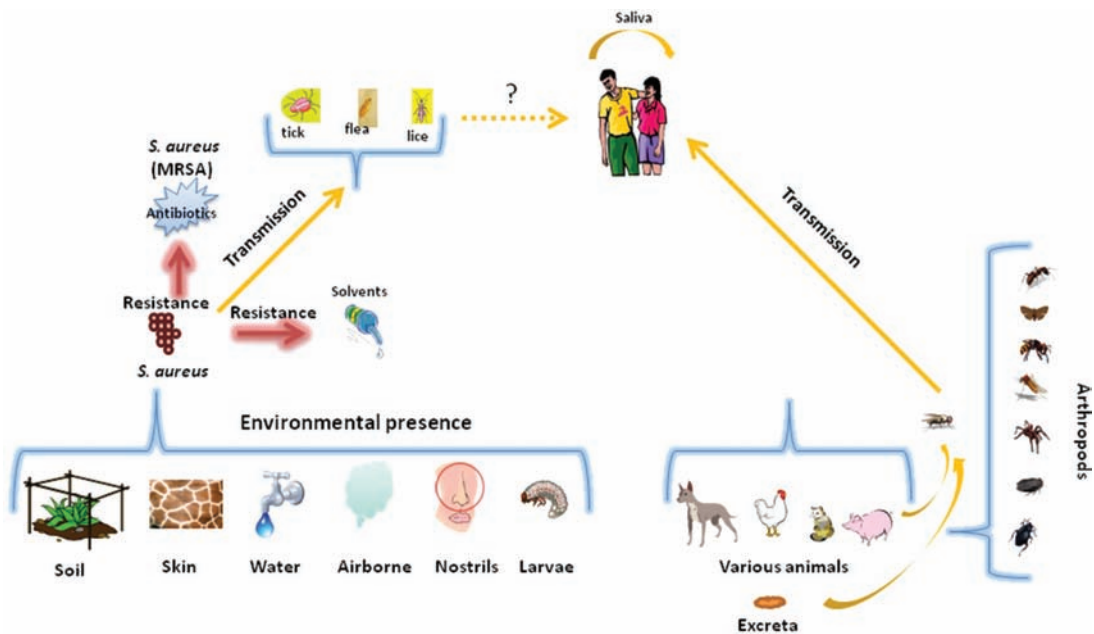
Figure 2.21.1. *Staphylococci* spp. environmental behavior and transmission

Most species of staphylococci are harmless and inherent in the normal flora of human and animal skin and mucous membranes. Among other species of this genus, *S. aureus* is also a persistent commensal

organism of the anterior nares in 20%–40% of humans without any clinical symptoms (Wertheim *et al.*, 2005). Nevertheless, nasal carriage seems to be a major risk factor for successive infections. The asymptomatic human group includes non- and intermittent carriers, both at low risk of reinfection. Ruimy *et al.* (2010) investigate different risk factors such as age, gender, underlying comorbidities, household sharing, smoking, hospital admission, contact with animals, antibiotics use and genetic polymorphism, that may explain the colonization variability of *S. aureus* in an isolated group of humans (Wayampi Amerindians living in an isolated village in the Amazonian forest). Based on bivariate analyses adjusted by Holm's test for multiple testing, the most significant and predominant factor involved was found to be the genetic variability among the group individuals.

Staphylococci ubiquity in the environment had been presented by Murrell *et al.* (2003) who surveyed ticks, lice and fleas for their microbial flora, in Australia. Staphylococci of the subspecies (*Staphylococcus succinus, S. saprophyticus, S. xylosus, Staphylococcus sciuri, S. lugdunensis, S. warneri, S. saccharolyticus, S. capitus, S. caprae, S. epidermidis* and others) had been isolated from most of the tested ticks and lice beside a large variety of other bacteria. *S. aureus* was absent, but *S. intermedius* was isolated from ticks. Graham *et al.* (2009) collected flies near broiler chicken houses and screened for antibiotic-resistant bacteria carried by these insects. The authors reported on enterococci (to be discussed further) and staphylococci (*S. aureus, S. xylosus, S. saprophyticus, and S. epidermidis*) that harbor antibiotic resistance [the most common reported resistance gene was: erm (A), erythromycin-resistance]. Their study strongly suggests that flies can exercise as vectors and contribute to human exposures through antibiotic-resistant bacteria (present in their digestive tracts and their external surface) dispersal when in close contact with intensive poultry production areas (Nmorsi *et al.*, 2007). The staphylococcal carrier role of the house fly was also supported by a study in peninsular Malaysia that showed a large bacterial diversity harbored by flies, revealing among other things that carrier state is horizontal and not vertical with these "hiking" bacteria (Nazni *et al.* 2005). Similar results with carrier flies were also obtained in Burkina Faso, in a nearby vending area close to waste and sewage, which provided food and harborage for these insects (Barro *et al.*, 2006). Cutaneous staphylococci (60% penicillin-resistant) possible direct transmission by flies has been also reported, based on the observation that Hippelates flies feed on purulent skin lesions (Allen and Taplin, 1974).

Additional arthropods such as mosquitoes, Tenebrionid beetles, flies, German cockroaches, wasps, ants, spiders, non-biting midges, moths, beetles isolated from a health care facility, were also shown to harbor gram-positive bacteria (13% prevalence) while among staphylococci *S. hominis* was the dominant one, displaying multiple-antibiotic resistance (Sramova *et al.*, 1992).

An interesting study was published by Robacker and Flath (1995) in which they showed that *S. aureus* grown in tryptic soy broth cultures produced volatile compounds that attract fruit flies. Odor production is one of the mechanisms by which, in a close ecosystem, a sessile organism can communicate with another motile organism and associate with it, therefore suggesting a possible bacterial transmission by these flies.

With regard to arthropods, one should remember at this point the potential use of greenbottle fly larvae to heal wounds infected with *S. aureus* and MRSA strains in severe cases, where antibiotics are useless (so called "maggot therapy") (Bexfield *et al.*, 2004; Bruggmann *et al.*, 2006). Park *et al.* (2010) reported on antibacterial acitivity of ethanol and water extracts from fly maggots against methicilline-resistant *S. aureus* (MRSA).

There is still a debate on the benefit of this therapeutic method, but it became increasingly clear that these larvae are of a beneficial potential. From the evolutionary point of view, it is very logical that larvae should have an antimicrobial weapon given that they are exposed to a large variety of environmental microbial flora, throughout their development (Sherman, 2009).

On the subject of plants, *Staphylococcus aureus* was reported to be strongly inhibited by plants hormones such as IAA, indolebutyric acid (IBA), naphthaleneacetic acid (NA), 2,5-dichlorophenoxyacetic acid (2,5-D)(Bouillenne and Bouillenne-Walrand, 1951). This phenomenon is probably linked to a possible double role of these hormones. The authors recommended use of plant hormones in controlling human cutaneous infections. Historically, is well known in traditional medicine the use of plants to combat skin infections and various diseases (Navarro *et al.*, 1996).

Bischoff *et al.* (2004) showed that infection with rhinovirus enhances the spread of coagulase-negative staphylococci through increased colonization of the latter due to nasal tissue damage inflicted by this virus. However, this problem can be easily solved by simple means such as: surgical scrubs, gowns and masks that are excellent barriers against bacteria spread (Bischoff *et al.*, 2007).

In New Zealand, researchers reported on methicillin-resistant *S. aureus* isolates, the British epidemic MRSA 15 strain (EMRSA-15) from dogs, cats, cow's milk and anterior nares of a healthy hospital staff member involved in animal care. The authors concluded that "MRSA can cause clinically overt and difficult-to-treat infections in animals in New Zealand and the rapid emergence of EMRSA-15 as the dominant MRSA strain in humans, resulted in infection spillover to animals". This conclusion is also supported by an old study performed on wild boars and domestic pigs, where the authors reported on an unusual discovery that staphylococci were absent in all wild boars' fecal samples when living in their natural environment. As the specific habitat of staphylococci is in man and wild boars are the ancestors of domestic pigs, it is very plausible that humans are the origin of *S. aureus* in these animals as well other pets domesticated for thousands of years (van Dijck & van de Voorde, 1979; Morgan, 2008; Leonard and Markey, 2008; Guardabassi *et al.*, 2003).

In a recent review, Loeffler and Lloyd (2010) raised the same concept of community-acquired MRSA rising number of cases, pointing in the direction of animals as the reservoir of this pathogen.

According to the numerous publications on staphylococci isolation from water resources, it is evident that beside their presence in the bodies of water they will also be closely related to aquatic life. Indeed, fish surface seems to contain *S. aureus* naturally able to produce enterotoxins and infect humans coming in contact with it (Simon and Sanjeev, 2007). Yamazaki *et al.* (1992) showed slight staphylococcal growth on fish surface (fillets of walleye pollack) when packed in 100% CO_2 in gas-impermeable pouches and preserved at 0°C for 20 days.

The potential of staphylococci to survive in sea water was presented by Bochet *et al.* (2008) who described two patients with a skin infection caused by community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) identical strains, when practicing scuba dive in the Philippines. The authors pointed to the scuba diving equipment as a possible new mode of acquisition of CA-MRSA.

In a periurban area of Niger, a survey on the presence of *S. aurues* in milk and on udder was carried out during two seasons: dry and rainy. *Staphylococcus aureus*, coagulase-negative staphylococci (CNS) and environmental microorganisms were detected in significantly more samples during the rainy season (55.2%, $p < 0.05$) than during the dry season (27.1%). The authors did not explain this variation however, speculatively it might be that the rainy season higher prevalence might be attributed to wash out of animal skin bacteria (know to contain staphylococci) and further contamination of udder and milk.

Finally, in relation to more unconventional environments, a special *S. haemolyticus* strain was isolated from larval guts of the oil fly *Helaeomyia petrolei* known to grow exclusively submerged in oil, where they ingest large quantities of oil and asphalt. This *S. haemolyticus* strain was able to tolerate excessive concentrations of solvents such as 100% toluene, benzene, and *p*-xylene on plate overlays and saturating levels of these solvents in monophasic liquid cultures (Nielsen *et al.*, 2005). The elucidation of this extreme bacterial tolerance was based on increased membrane fluidity via fatty acid composition once the bacterium is exposed to various solvents, a system much different from the gram-negative bacteria

mechanism of solvent resistance. In this case, we can merely speculate on the environmental advantage of the above attribute, such as the role of bacterial efflux pumps that are also linked to cross-resistance to antibiotics (Fernandes *et al.*, 2003), commensalism with higher organisms (e.g., larvae), collective defense through biofilm formation or just an opportunistic saprophyte.

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Chapter 2.22

Streptococcal Infections

[*STREPTOCOCCUS* SPP.]

Streptococcus is a bacterial genus of cocci, gram-positive, non-spore forming, facultative anaerobes arranged in chains or pairs. Their classification is based on blood hemolytic activity and surface antigens presence (according to Lancefield group nomenclature). Streptococci are both human commensals and pathogens (e.g., *S. pyogenes* causes the widest range of disease in humans among all bacterial pathogens) (Stock, 2009) and a large variety of animals (most of them of domestic type such as cattle, horses, pigs, etc.). Zoonotic pathogenic strains and some associated features are listed in Table 2.22.1. The streptococcal disease spectrum is wide and includes skin and wound infections (impetigo), scarlet fever, erysipelas, pharyngitis, rheumatic fever, acute glomerulonephritis, meningitis, endocarditis, etc.

2.22.1 *STREPTOCOCCUS* AND ENVIRONMENT (FIGURE 2.22.1)

The ubiquity of streptococci constitutes a continuous threat of infection. Herva (1979) reported on an outbreak of skin infection caused by streptococci (group A, β-hemolytic) in hunters from a wild moose. Group L streptococci were isolated from meat handlers (processing chickens and pigs) together with *Staphylococcus aureus* (Barnham and Neilson, 1987). Feng *et al.* (2010), following two severe large scale outbreaks in China of *S. suis* originating from swine industry, described a new disease form of streptococcal toxic shock syndrome (STSS). The Chinese variant of *S. suis* exhibited strong invasiveness and high pathogenicity. Epidemiological data showed that *S. suis* cases occurred mainly in South China, from summer to autumn, a period characterized by high moisture and high temperature.

   Cockroaches isolated from public hospitals, were also reported to carry streptococci internally and externally, consequently constituting a continuous source of human infections (Salehzadeh *et al.*, 2007).

   Water is also a source of fecal *Streptococci* (Enterococci) as reported during Nile flood season (Saleh, 1980), Apalachicola River, Florida (Elder, 1987) and in New Zealand where the main source of enterococci in river waters was most likely runoff from dairy farms located on the flood plains (Deely *et al.*, 1997). Invasive infections from fish infected with *S. iniae* have been reported in humans (Weinstein *et al.*, 1997). A recent publication described massive fish extinction in Kuwait Bay,

Table 2.22.1 Zoonotic *Streptococcus* spp. classified according to their Lancefield group antigen.

Lancefield group antigen	Species	Hemolysis	Associated disease	Zoonotic	Animal source
A	*S. pyogenes* (GABS)[a]	β	Pharyngitis, tonsilitis, wound and skin infections, scarlet fever, septicemia, pneumonia, glomerulonephritis, necrotizing fascitis, rheumatic fever	yes	Cattle, duck, monkey
B	*S. agalactiae*	β	Sepsis, skin infections, postpartum or neonatal sepsis, endocarditis, septic arthritis	yes	Cattle, fish
C G	*S. equi* *S. canis*	β	Pharyngitis, pneumonia, cellulitis, impetigo, puerperal-,neonatal-sepsis, endocarditis, septic arthritis	yes	Horse, dog and many other animals
D	Enterococcal: *E. faecalis* *E. faecium* Nonenterococcal: *S. bovis* *S. equinus*	α or γ	Endocarditis, UTI, intra-abdominal infection, cellulitis, wound infection	yes	CattleHorse
A, C, G and L	*S. dysgalactiae*	β	Bacteremia	yes	
E, P, U, V, none or new	*S. porcinus*	β	Septicemia	yes	Pig
Viridans*-	*S. mutans* *S. sanguis* *S. salivarius* *S. mitior* *S. milleri* *S. viridans* *S. gordonii*	α or γ	Endocarditis, localized infection (or abscesses)		
	S. iniae	β	Cellulites, invasive infections from fish, pneumonia, acute sinusitis, otitis media, meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulites, and brain abscess	Yes	Fish
	S. pneumoniae	α			
R, S and T	*S. suis* (serovar 2)	Non-β hemolytic	Meningitis, myocarditis, arthritis, etc.	yes	Pig

[a]GABHS- group A β-hemolytic streptococci.
*No reaction with Lancefield grouping sera.

S. agalactiae being suspected as the cause of this fatal infection (Jafar *et al.*, 2009). The authors, using random amplification of polymorphic DNA (RAPD) analyses, found nearly identical RAPD banding patterns between *S. agalactiae* from fish and sewage sources and deduct that sewage may be the principal fish contamination source. Unfortunately, they did not report on human cases related to fish handling or consumption. An additional relationship between fish and human streptococcal disease was presented by Miller and Neely (2004) who successfully used zebra fish (*Danio rerio*) as a model to study these infections, presenting similar pathologies.
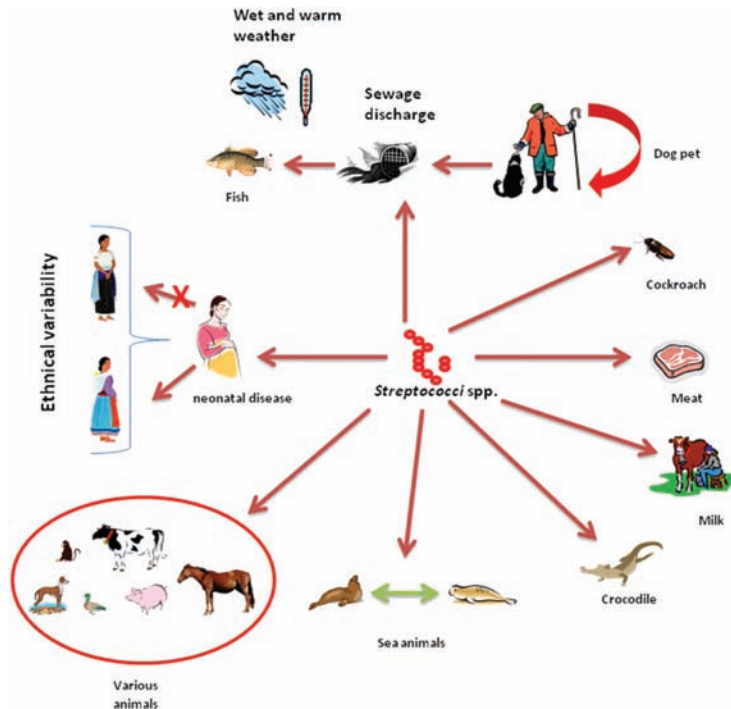
Figure 2.22.1. *Streptococci* spp. and various environmental aspects connected to their infection

In connection to sea environment, *Streptococcus equi* subsp. *zooepidemicus* strain was isolated from two remote seals (harbor and grey types) in the North Sea, Germany. According to authors, grey seals were probably the vectors of this strain (Akineden *et al.*, 2007).

Some environmental parameters (temperature, pH and osmolarity) were found to modulate some genes (multifunctional adhesions) in *S. gordonii*. Some *S. gordonii* strains are harmless and commensals of the human oral cavity, however some are strongly connected to severe disease (infective endocarditis) (El-Sabaeny *et al.*, 2000). The main question remaining is whether these environmental conditions are the only prerequisite for virulence direction change.

Harmeet *et al.* (2009) reported on the better capability of group B streptococci (GBS) strains isolated from asymptomatic carriers (pregnant women) to form biofilms compared to those from symptomatic patients. As mentioned before, biofilms are an environmental association designated to protect bacteria from environmental stresses. It can be postulated that more virulent strains able to penetrate human or

animal organs are less exposed to these stresses and, owing to their defense mechanism against immunological cascade and affable environment, do not require biofilm formation but dispersal in order to succeed.

Bishop *et al.* (2007) reported on a necrotizing fasciitis outbreak caused by *S. agalactiae* in a captive crocodile species. There were several speculative suggestions on this infection's origin such as: a) high stocking density that may increase the bite wounds and captivity stress; b) infected mice used as feed; and c) inhabitation of mucous membranes by these microorganisms. However, none of these suggestions was clear cut evidence.

The *in vitro* optimal atmosphere (aerobic vs. anaerobic) for the isolation and growth of group A β-hemolytic streptococci is still under considerable debate. Schwartz *et al.* (1985) found that this group isolated from throat, grew better under anaerobic atmosphere; however, other studies did not support this conclusion (Roddey *et al.*, 1995).

An interesting finding on the facultative anaerobe group B Streptococcus (GBS) (an opportunistic pathogen of pregnant women, newborns, and elderly persons) revealed that its invasiveness [using the dynamic *in vitro* attachment and invasion system (DIVAS) in neonatal mice] is significantly enhanced in the presence of $\geq 5\%$ oxygen. The invasiveness of this group was described as linked to enhanced polysaccharide production especially under aerobic atmosphere (von Hunolstein *et al.*, 1993).

A study of risk factors of increased carrier potential of group B streptococcus in southern Israel was performed on different human populations. Women, particularly immigrants from the former USSR, were significantly more prone to carry the pathogen than native Israeli women (comprised of ethnical groups such as Bedouins and Jews) with expected transmission rate of neonatal disease populations at low incidence.

Lam *et al.* (2007) described the identification of *S. canis* from dog owners associated with ulcer infections. It should be pointed out that the described cases were linked to preceding diseases such as diabetes mellitus that by itself causes permanent wounds and ulcers.

Finally, air flow was also suggested as a possible route of contamination. Thompson *et al.* (1978), in relation to cows infected with *S. agalactiae*, found that rapid airflow toward teats as a result of sudden vacuum loss characterized vacuum abnormalities associated with increased risk of mastitis infection in these animals.

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Chapter 2.23

Tularemia

[*FRANCISELLA TULARENSIS*]

Francisella is a genus of gram-negative, strict aerobic, small non-motile rod-shaped or coccobacillary bacteria that are also facultative intracellular parasites of macrophages. The genus *Francisella* contains at the moment five species: *F. tularensis, F. hispaniensis*, *F. noatunensis*, *F. philomiragia* and *F. piscicida*. *F. tularensis* is the most infective and virulent zoonotic species for humans. The species *F. tularensis* has four subspecies (or serovars): *1) F. tularensis subsp. tularensis* (Type A) (found in North America and the most virulent to humans and domestic rabbits; 2) *F. tularensis* subsp. *palaearctica* or *holarctica* (Type B) (found in North America in aquatic rodents such as beavers and muskrats, and in other small rodents in northern Eurasia); 3) *F.tularensis subsp. novicida* (formerly classified as a species) a relative non-virulent strain found in North America; and 4) *F.tularensis subsp. mediasiatica* (primarily found in central Asia, isolated from human, gerbil and tick) at the moment without information on its pathogenicity for humans (Petersen and Schrifer, 2005). A large variety of animals (>125 species) has been found to host *F. tularensis* (Avashia *et al.*, 2004; Park *et al.*, 2009), among which rodents are the main reservoir with ticks, fleas, lice and flies as vectors (Hubalek *et al.*, 1998; Meka-Mechenko *et al.*, 2003; Eliasson and Back, 2003; Aldea-Mansilla *et al.*, 2010). Direct contact with rodents' excreta is also known as a transmission route in addition to inhalation of contaminated dust (the ID is 10 to 50 organisms), consequently making it a possible bioterrorism weapon (Dennis *et al.*, 2001). Water was also implicated as a reservoir of *F. tularensis* spp. foci, associated to protozoa and water related arthropods (Tikhenko *et al.*, 2001; Ul'ianova *et al.*, 1982). The disease is also called Francis disease, marketmen's disease, rabbit fever, deer fly fever, Pahvant valley plague (in USA), yato-byo, Ohara's disease (in Japan) and lemming fever (in Norway). The clinical manifestation is related to the entry portal: from skin purulent ulcerate papule to pharyngitis, cervical lympadenitis, stomatitis, pneumonia, gastrointestinal bleeding, meningitis, diarrhea, renal failure and hepatosplenomegaly and death (∼30% without treatment).

2.23.1 TULAREMIA AND ENVIRONMENT (FIGURE 2.23.1)

Water as a transmission vector of tularemia to humans was reported in Turkey in a small village outbreak (Meric *et al.*, 2008). The water supply of this village was a spring with a water tank. The most frequent

clinical symptom among the farmers was cervical lymphadenopathy. Australian scientists reported on a *novicida*-like subspecies of *F. tularensis* from a foot wound sustained in brackish water (Whipp *et al.*, 2003). In a study performed in an active tularemia natural focus on Martha's Vineyard, Massachusetts, during a multiyear outbreak of pneumonic tularemia, brackish pond water was found to harbor *Francisella philomiragia* and other *Francisella* species closely related to *F. tularensis* and *F. novicida*, but not the main causative agent, *F. tularensis* subsp. *tularensis* (Berrada *et al.*, 2010).
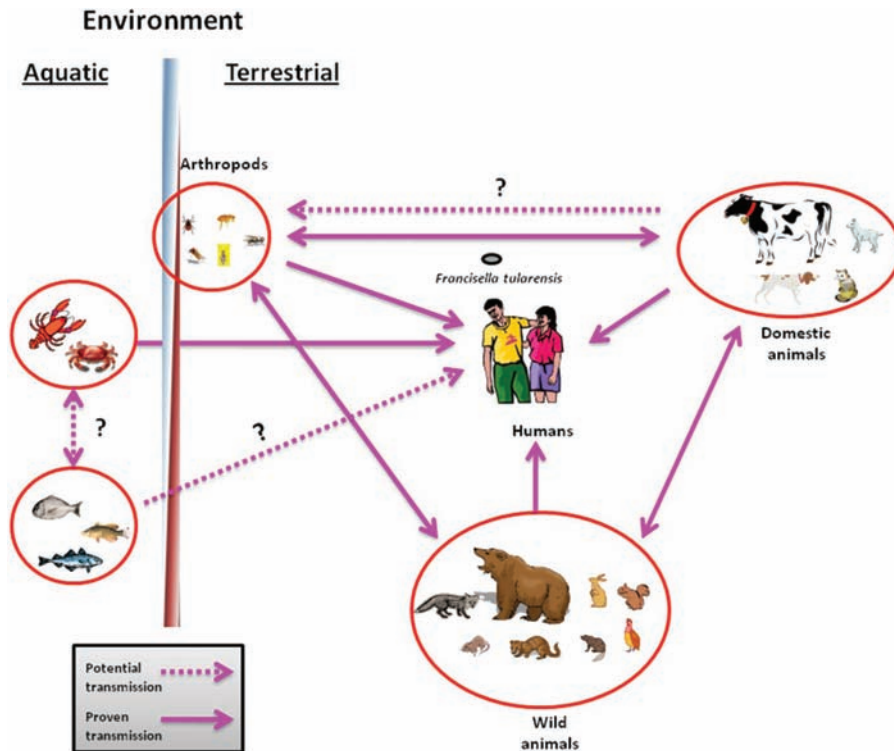
Figure 2.23.1. *Francisella tularensis* relation to various animals in aquatic and terrestrial environments

Other water-related sources are aquatic animals such as fish and crustaceans (e.g. crayfish) (Ottem *et al.*, 2007; Soto *et al.*, 2009; Anda *et al.*, 2001; Diaz de Tuesta *et al.*, 2001). Fish pathogenic *Francisella* spp. isolates were shown to be a new specific species for fish and not related to human pathogenesis, although this assumption cannot be ruled out, without additional research. On the other hand, both reports on *Francisella* spp. isolated from crustacean (crab and crayfish) caused human tularemia in people handling these animals (resulting in skin lesions), with strong evidences of water source contamination by sewage.

In relation to water, *F. tularensis* (strain LVS, live vaccine strain, type B) was stored in a highly oligotrophic (no carbon source) cold water environment (8°C). Cell counts were stable for >70 days, while viable plate counts dropped to undectability for the same time interval. Metabolic activity (measured through Rhodamine 123 accumulation) declined to ∼35% after 140 days of storage. After this time interval, when incubated again in a rich medium, ∼30% of the cell population increased again.

Mice intraperitoneal injection with these residual viable but non-culturable cells of *F. tularensis* did not cause tularemia and no lymphocyte stimulation was observed. This experimental study is important because it gives a new insight into the ecology of this pathogen (Forsman *et al.*, 2000). The major question based on the above study should be: Is this phenomenon related to a fairly mild strain (vaccine) or a broader phenomenon?

In another village in northern Finland, located in an endemic area, the transmission was through common farming activities, such as preparing fresh hay with a hay-cutter, handling dry hay, threshing, etc. suggesting an airborne path (Syrjala *et al.*, 1985). Another airborne case was reported when 15 people were infected by tularemia from a contaminated dog's fur disseminated when the dog shook itself among all the guests during a dinner (Siret *et al.*, 2006).

Now, it is clear that *F. tularensis* is an arthropod-borne pathogen throughout the northern hemisphere with a wide vector array. Petersen *et al.* (2009) in an excellent review raised several questions about this pathogen such as: What is the mechanism of mechanical or biological transmission? What happened to the pathogen inside or outside the carrier arthropod? How do different *F. tularensis* subspecies relate to various arthropods? Are there other arthropods highly significant in transmission? etc. All these questions need a definitive answer in order to understand and control the epidemiology of this important pathogen and its relation to environment better.

Franke *et al.* (2010) surveyed bird feeding ticks for presence of five tick-borne pathogens, *F. tularensis* among them. The authors reported co-existence of one or more of these pathogens inside the blood feeding ticks, and therefore possible simultaneous transmission of the pathogens (various combinations of co-infections occurred in 10.9% of all positive ticks). These results suggest that birds are not only important reservoirs for several pathogens but also act as vehicles for infected ticks and might therefore play a key role in the dispersal of tick-borne diseases, as previously shown with other tick-borne pathogenic bacteria (Padeshki *et al.*, 2010).

Combined county-based data for tularemia incidence (for a period of 14 years), by means of Geographic Information System (GIS)-based environmental data, were used to determine associations between coverage by different habitats (especially dry forest representing suitable tick habitat) and tularemia incidence. The results showed only a partial positive association between tularemia incidence and coverage by dry forested habitat. The authors tried to explain the partial correlation by differences in species' composition, abundance of vector ticks or vertebrate reservoirs, different transmission routes such as lagomorphs handling, and finally tick survival in those different habitats.

Hubálek *et al.* (1996) also reported on *F. tularensis* infected ticks (*Dermacentor reticulates*) in the flood plains forest ecosystem with warm and dry climate of South Moravia, Czech Republic. Temperature and relative humidity conditions were previously tested on *F. tularensis* survival on metal surfaces (Wilkinson, 1966). A dry atmosphere was found to support longer survival, while increased temperature decreased *F. tularensis* survival. These findings support partially the pathogen's geographical distribution: Northern hemisphere.

From the environmental point of view, one should ask a coherent question: Why are there not increased reports of *F. tularensis* transmission by mosquitoes, when these arthropods are more widespread in human surroundings than are ticks? Indeed, a new study presented the prevalence of *F. tularensis* in the *Culicidae* family. The results showed that among >2,500 tested mosquitoes (captured in Alaska) 30% of pooled samples contained *Francisella* DNA (Triebenbach *et al.*, 2010). However, further examination of transmission potential revealed that ingestion of this pathogen is not efficiently transferred to pupal and adults stages and in secondary blood meals. The authors concluded that inefficient transmission on the one hand and species dependency on the other is responsible for the low vectorial role of mosquitoes in tularemia.
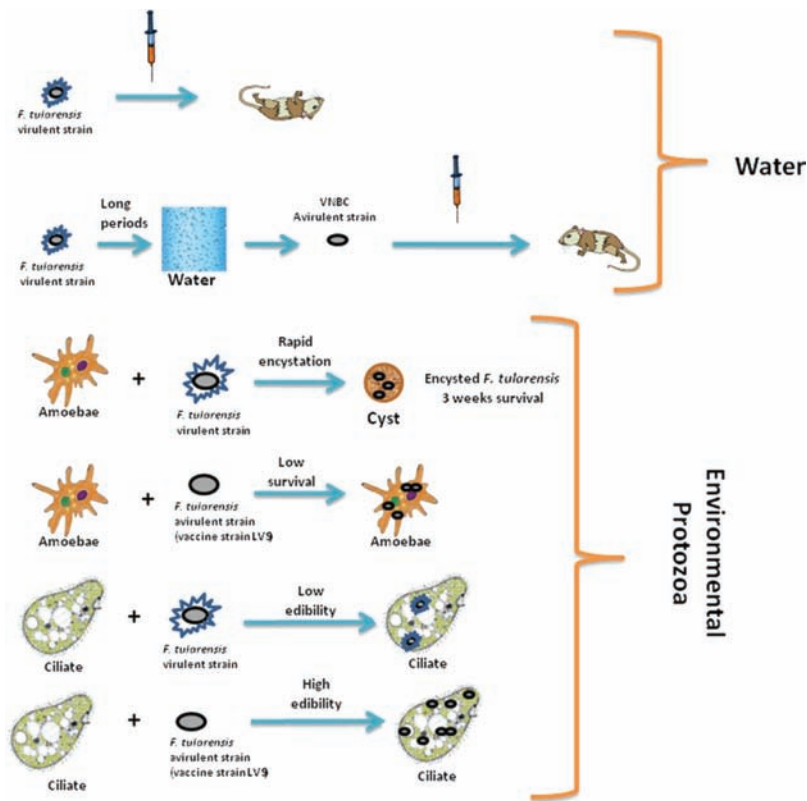
Figure 2.23.2. *Francisella tularensis* interactions (survival and virulence) with protozoa (amoebae and ciliate) and water

Berdal *et al.* (1996) investigated serologically different populations (hunters and healthy school children) for previous exposure to *F. tularensis*. The authors found up to 4.7% positivity in school children indicating spread of low-grade infections. Additionally, they studied and showed *Achantamoeba castellanii* pathogen internalization, growth and inactivation. In a more recent study on *Acanthamoeba castellanii* and its interaction with *F. tularensis*, several strains were tested for efficient internalization (survival and replication) into amoebae: a live vaccine (LVS) and clinical virulent strains. Rapid encystment was observed when amoebae were exposed to virulent strains, with 3 weeks post-infection survival of the pathogen (El-Etr *et al.*, 2009). The authors suggested that this interaction is essential for pathogen survival and may play a role in its environmental persistence. Adiba *et al.* (2010) using non-pathogenic and pathogenic *E. coli* bacteria and grazing amoebae (a social haploid amoeba *Dictyostelium discoideum*) demonstrated that virulence traits in bacteria might result from selective pressure exerted by protozoan predator. Thus, grazing resistance may be an evolutionary exaptation in bacterial pathogenicity. Similar experiments were also performed with *F. tularensis* and *Acanthamoeba castellanii* in which the pathogen had been shown to grow and survive (Abd *et al.*, 2003). *F. tularensis* internal growth affected the protozoa, reducing its numbers. This interaction demonstrated that ubiquitous protozoa might be an important environmental reservoir for *F. tularensis*.

Using another protozoan, Thelaus *et al.* (2009) showed that virulent strains of *F. tularensis* can internalize, although at a lower rate. Importantly, the authors also found that *F. tularensis* viable but non-culturable forms (VBNC) emerged after long term incubation in lake water microcosm, while a high nutrient level as well the presence of the grazer did not prevent this shift. *Francisella tularensis* interactions with protozoa are summarized in Figure 2.23.2.

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Chapter 2.24

Vibrioses

[*VIBRIO CHOLERAE*]

Vibrio cholerae species (genus *Vibrio*) is a gram negative, facultative anaerobe, non-spore forming, curved (comma-shaped) bacterium, highly motile with a polar flagellum. The genus Vibrio cantains 34 recognized species, among which *V. cholerae*, *V. mimicus, V. anguillarum*, *V. harveyi*, *V. parahaemolyticus, V. vulnificus, V. fluvialis, V. hollisae, V. alginolyticus, V. carchariae, V. metschnikovii, V. furnissii, Photobacterium damsela, V. cincinnatiensis* are human pathogens (Table 2.24.1). *V. cholerae* is divided into 155 serogroups (based on somatic antigens). From the epidemiological point of view, *V. cholerae* serogroup O1 (further subdivided into Inaba, Ogawa, Hikojima strains) is the main agent of epidemic outbreaks. *V. cholerae* serogroup O1 is further differantiated into El Tor and classical biotypes. The *V. cholerae* serogroup O1 classical biotype is found only in Bangladesh, whereas *V. cholerae* serogroup O1 (El Tor) is found throughout the rest of the world. Strains that do not belong to serogroup O1 are referred to as non-O1 *V. cholerae* such as *V. cholerae* O139 that have been isolated from an outbreak in Bengal, India. The disease presents itself as gastroenteritis, primarily watery diarrhea, vomiting, dehydration, hypotension, septicemia, renal failure and death.

2.24.1 *VIBRIO* AND ENVIRONMENT

Vibrios are perhaps the most aquatically-related microorganisms involved in human pathogenesis (Thompson *et al.*, 2004). Beside *V. cholerae* that mostly lives in fresh water (Perez-Rosas & Hazen, 1989) (however, isolated also from both temperate and tropical marine coastal waters), almost all other human pathogenic Vibrios are halophilic, and consequently isolated from coastal water, marine sediments, seaweeds and sea fauna (Hayat *et al.*, 2006). The main transmission of *Vibrio* infections is through the consumption of raw or undercooked shellfish or exposure of wounds to warm seawater (Prapaiwong *et al.*, 2009; Córdova *et al.*, 2002). The high infective dose of *V. cholerae* (range of 10^8 to 10^9 cells) (Sack *et al.*, 2004; Codeco, 2001) requires an amplification process that can occur within the gastro-intestinal system of ill people and further excreted in feces or environmentally in symbiosis with aquatic organisms. Among these organisms, amoebae are the best candidates resembling phagocytes,

Table 2.24.1 Animal sources of zoonotic *Vibrio* spp. related to human diseases.

Species	Zoonotic (Risk)	Animal	Gastroenteritis	Wound Infection	Primary Septicemia	Ref.
Vibrio alginolyticus	yes	Fin- and shellfish	±	++		1
Vibrio cholerae non-O1	yes	Shellfish, crab, shrimp	++	±	±	2
Vibrio cholerae O1	yes	Fish	++			3
Vibrio cincinnatiensis	No	–	±	±	++	4
Photobacterium (Vibrio) damsela	yes	Fish		++		5
Vibrio furnissii	yes	Eel, shrimps	++			6
Grimontia (Vibrio) hollisae	yes	Fish	++	±	±	7
Vibrio fluvialis	yes	Lobster	++	±	±	8
Vibrio metschnikovii	yes	Clam	±			9
Vibrio mimicus	yes	Crayfish	++	±	±	10
Vibrio parahaemolyticus	yes	Fish	++	+	±	11
Vibrio vulnificus	yes	Eel, fish	+	++	++	12
Vibrio harveyi (= V.carchariae)	yes	Shark		++		13

++ Common; + Occasional; ± Rare

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being present in aquatic environments and feeding on different bacteria. Indeed, Abd *et al.* (2007, 2010) recently showed experimentally an interesting interaction between *V. cholerae* and *V. mimicus* and the ubiquitous amoebae species, *Acanthamoeba castellanii*. Both *Vibrio* species revealed good growth and multiplication inside the monocellular organisms and also gained protection from antibiotics when encysted. The authors suggested that amoebae may play an important environmental role as host of these pathogens.

In the present chapter, water or foodborne infections will not be described as they were already reviewed in many excellent publications (Tantillo *et al.*, 2004; Mouchtouri *et al.*, 2010; Oliver and Kaper, 1997; McLaughlin, 1995; Newell *et al.*, 2010); nevertheless, their environmental water aspects associated to zoonoses will be evaluated (Austin, 2010).

Estuarine waters used for aquaculture, fishing and recreational purposes are influenced by a large variety of factors such as climate, tidal flushing, temperature, vertical mixing, precipitation (affecting salinity) and nutrients loading (mainly through sewage discharges). These factors were shown to correlate definitely with *Vibrio* spp. presence and abundance, increasing human and animal infection potential (Hsieh *et al.*, 2008; Inoue *et al.*, 2008; Sedas, 2007). On the other hand "refractory periods" were reported, when in spite of favorable climatic conditions for "cholera" spread, clinical reported cases dropped significantly, mainly due to lack of susceptible hosts (increased immunity in previously exposed populations to infectious *V. cholerae* strains) (Koelle, 2009).

Wind as an additional climatic parameter has been suggested as a possible disseminator of *Vibrio cholera* (Paz and Broza, 2007). Continental winds were shown to carry aero-plankton (tiny insects such as *chironomids* or non-biting midges and other small flies) contaminated with cholera bacteria from one body of water to an adjacent one. Earlier, Broza *et al.* (2001) already showed that chironomids egg masses were infected with *V. cholerae* and can be an aquatic reservoir of this pathogen. Later, adult non-biting midges (chironomids) were suspected of possibly carrying *V. cholerae* and becoming windborne (Broza *et al.*, 2005). Indeed, this hypothesis is sound enough based on the wide spread of this pathogen globally, especially intra-continentally where bodies of water are secluded.

Many articles emphasized the difficulty inherent in cultivating *V. cholera* by conventional techniques, mainly in growing them as colonies on differential culture media. In a recent important report, it was shown that *V. cholerae* bacteria present in surface water may exist as conditionally viable environmental cells (CVEC), and partially as dormant cells in an aggregate form (biofilms) (Faruque *et al.*, 2006). When these aggregates were directly inoculated into rabbit intestines they revealed complete virulence recovery. These authors also indicated two kinds of *V. cholerae* populations excreted in the stools of infected humans, planktonic and aggregates (clumps), with increased virulence of the latter.

Long *et al.* (2005) found that marine bacteria could inhibit the colonization of marine particles by *V. cholera*, through biosynthesis of an antibacterial agent controlled by temperature. At elevated temperature the production of this inhibitory agent diminished, giving preferentiality to *V.cholera* competitiveness and survival by means of particle colonization and biofilm formation. This biological pattern is compliant with El Niño-Southern Oscilliation (ENSO) and monsoonal events that cause water temperature elevation thereupon enhanced cholera outbreaks.

El Niño/La Niña-Southern Oscillation, or ENSO in brief, is a climate pattern (quasi-periodic) observed across the tropical Pacific Ocean (western part), approximately at an average of 5 years intervals (between 2 to 7 years). The main characteristics are variations in ocean water surface temperature (warming or cooling known as *El Niño* and *La Niña*, respectively) and air surface pressure in the tropical western Pacific - the *Southern Oscillation*. In the western Pacific, the two variations are coupled: the warm oceanic phase, El Niño, accompanies high air surface pressure, while the cold phase, La Niña, accompanies low air surface pressure (Figure 2.24.1). It is now clear that this climatic phenomenon has a global impact

causing in other continents a variety of disastrous events (Figure 2.24.2). The oscillation mechanism remains still unclear, but its results are seen in biosphere behavior including cholera outbreaks.
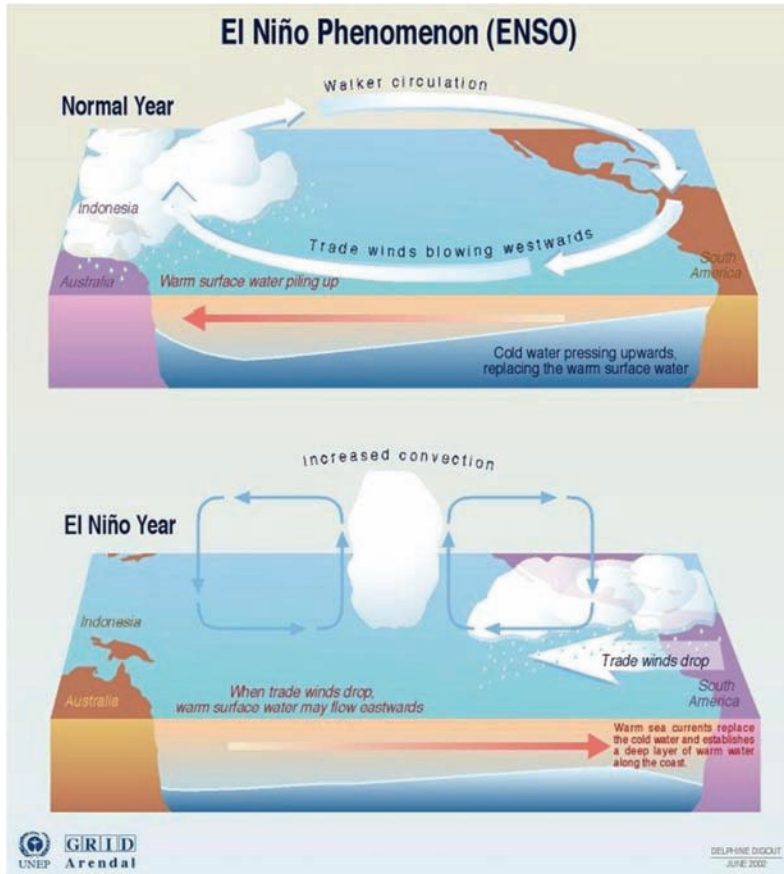
Figure 2.24.1. El Niño-Southern Oscillation (ENSO) Phenomenon. (2002) In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 14:53, January 2, 2011 from http://maps.grida.no/go/graphic/el-ni-o-southern-oscillation-enso-phenomenon, credit to Delphine Digout, UNEP/GRID-Arendal (Climate Prediction Center (CPC), National Centers for Environmental Prediction (NCEP); National Oceanic and Atmospheric Administration (NOAA)

Similar results for *V. cholerae* O1prevalence were reported in Mexico at different locations (after El Niño and La Nina) (Lizárraga-Partida *et al.*, 2009). These authors concluded that *V. cholera* O1 derives from marine and estuarine origin and not from sewage contamination, linking copepod abundance with climatic conditions. In other locations such as southeastern Africa (a 35 years survey) (Paz, 2009) and South Korea (Heon and Yeon, 2010), the same trend was observed after an increase in sea surface temperature.

A similar correlation between temperature rise and another *Vibrio* sp. (*Vibrio vulnificus*) proliferation and successful human infection was reported in Israel (Paz *et al.*, 2007). The authors clearly showed a rise in

V. vulnificus clinical cases in fish industry workers linked to high summer temperatures. In colder temperate geographical areas such as Denmark, *V. vulnificus* was also reported to infect people during a particularly warm summer (Bruun *et al.*, 1996).
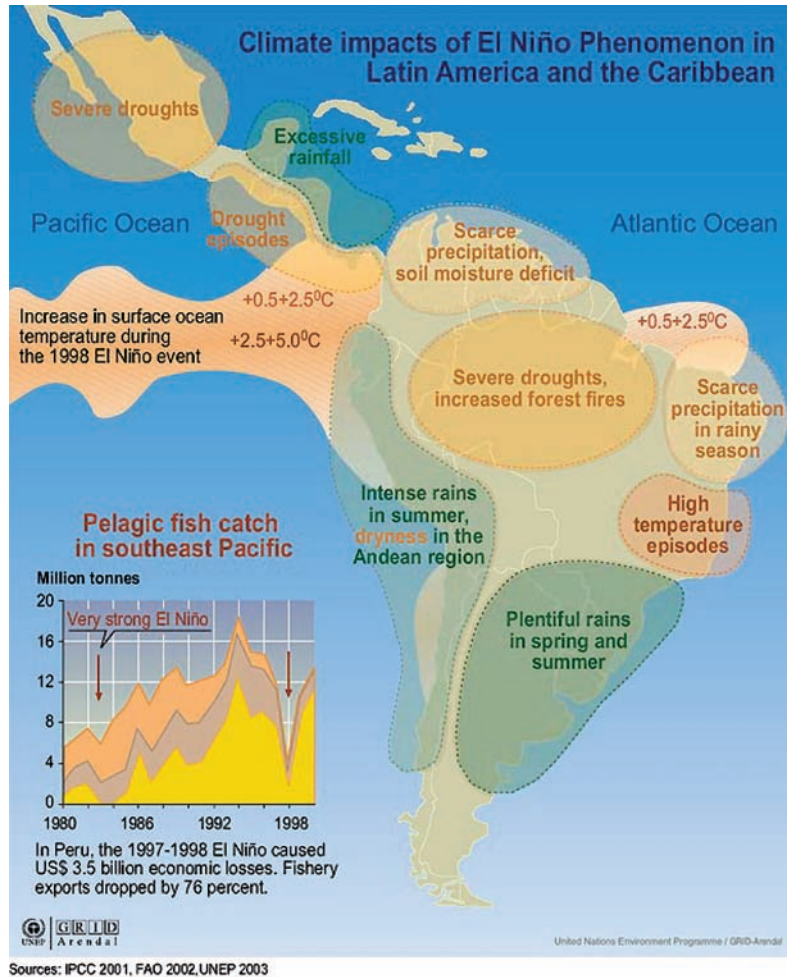
Figure 2.24.2. El Niño phenomenon impact on climate of South and Central America (2005). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 14:55, January 2, 2011 from http://maps.grida.no/go/graphic/climate_impacts_of_el_ni_o_phenomenon_in_latin_america_and_the_caribbean, credit to UNEP/GRID-Arendal (IPCC 2001, FAO 2002, UNEP 2003)

Figure 2.24.3 summarizes the global spread of cholera from 1950 to 2004. For 20 years it spread from the most endemic area (India) towards China in the north, Iran in the west and Indonesia in the east. After an additional 20 years the disease agent spread globally to North and South America, Europe, Russia and even Australia.

Mezrioui and Oufdou (1996) reported on non-O1 *Vibrio cholera* survival in a simple domestic wastewater treatment (stabilization ponds) versus the decline of indicator bacteria (fecal coliforms).

Seasonal distribution was reversed with increased survival of non-O1 *Vibrio cholera* with high densities in hot periods and low densities in cold periods.
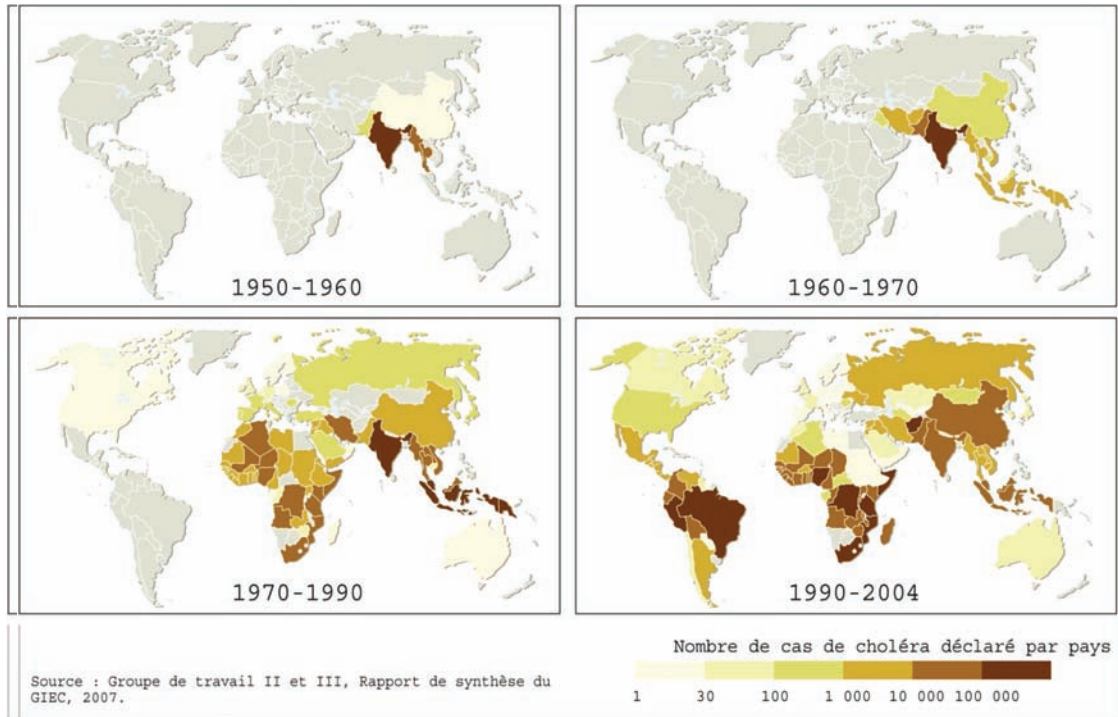
Figure 2.24.3. Global cholera spread from 1950 to 2004. (With permission from: The spread of cholera 1950-2004. (2009). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 20:56, January 19, 2011 from http://maps.grida.no/go/graphic/the-spread-of-cholera-1950-2004, credit to UNEP/GRID-Arendal (Working group II and III, Synthesis Report, IPCC, 2007)

Aquatic plants, seaweeds, and free floating phyto- and zooplankton can harbor vibrios in viable but nonculturable (VBNC), sporelike forms, as demonstrated by fluorescent antibody and polymerase chain reaction techniques (Islam *et al.*, 1989; Huq *et al.*, 1990). There is no reason to suspect that affinity for algae and weeds differs among various marine vibrios (Islam *et al.*, 1990*; Islam *et al.*, 1990). The growth of marine and freshwater photosynthesizers is prompted by nitrogen-rich wastewater, fertilizers, acid rain, and runoff soil (eutrophication) that may enhance also the growth of pathogenic Vibrios.

In contrast to tropical or subtropical seas, along the Swedish coastline (Skagerrak Sea) where the average temperature is ∼5°C, Vibrios population was studied and surprisingly found to be widespread (Eiler *et al.*, 2006). Total Vibrios spp. abundance was found to range from 10^3 to 10^4 cells/L with increasing numbers in more saline waters. *V. anguillarum* and *V. aestuarianus* genotypes were present in brackish waters. In addition, 50% of the samples were positive for *V. cholera* and *V. mimicus* ($>10^3$ cells/L) in very cold water and low salinity. These authors also indicated other factors such as nutrient levels and dinoflagelate abundances that also may impact the prevalence and abundance of these Vibrios along the

coastal line. These findings may explain the reports of wounds infected by *V. cholera* in Swedish swimmers (Andersson and Ekdahl, 2006; Rehnstam-Holm & Collin, 2009).

Using an experimental approach, Wiklund *et al.* (2009) looked for structural changes in the pelagic food web under temperature alteration by 5°C in a Baltic Sea mesocosm (a brackish sea water with a salinity of ~7‰ near the coastal line and 10–15‰ in the open, compared to 35‰ found in oceans) using the amphipod *Monoporeia affinis* as a key benthic species. They found a structural shift in the pelagic food web from one based on algae to one based on bacteria, which decreased amphipod productivity and the efficiency of the pelagic-benthic food web (FWE). As growth of amphipods and FWE were not directly affected by the temperature raise, the authors concluded that the main ecological impact occurred through indirect structural changes in the pelagic food web and consequently on the benthic productivity. As *Vibrio* species also belong to the benthic food web it is very much possible that temperature rise will increase human health risk in these environments.

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Chapter 2.25

Yersinioses

[*YERSINIA ENTEROCOLITICA* AND *YERSINIA PSEUDOTUBERCULOSIS*]

Both *Y. enterocolitica* and *Y. pseudotuberculosis* belong to the family of Enterobacteriaceae and genus *Yersinia* like their previously described relative: *Y. pestis*. These species are gram-negative coccobacilli, facultative anaerobes that primarily cause zoonotic diseases. Among the ∼50 serovars (serogroups) of *Y. enterocolitica*, only serogroups O:3, O:9 (Europe), O:8 (USA), O:5 and O:27 (Japan and Canada) were identified as human pathogens. *Y. pseudotuberculosis* has 14 serovars of which O:1 constitutes 60–70% while O:2 and O:3 constitutes 10–20% (in Europe), O:4 and O:6 (mainly in Japan). The main reservoirs of these bacteria are rodents and birds with cross-transmission to humans, farm animals, pets, laboratory and wild animals, animal food and water (Mollaret, 1983). The diseases caused by these two species are enteritis/enterocolitis with symptoms such as: febrile diarrhea, pseudo-appendicitis, septicemia, erythema nodosum, arthritis, mesenterial lymphadenitis, etc.

2.25.1 *Y. ENTEROCOLITICA, Y. PSEUDOTUBERCULOSIS* AND ENVIRONMENT (FIGURE 2.25.1)

Both pathogens, *Y. enterocolitica* and *Y. pseudotuberculosis*, are well documented zoonotic foodborne pathogens (otherwise called "saprozoonoses") (Krauss *et al.*, 2003), transmitted through direct contact or via water and food obtained from contaminated animals (farm animals: pigs, cattle, etc.) (Fredriksson-Ahomaa *et al.*, 2007). In this section, the foodborne aspect will not be discussed since it was reviewed elsewhere in details (Matargas and Drosinos, 2009; Dube, 2009).

Litvin *et al.* (1991) showed that plants such as cabbage, lettuce, pea and oat, challenged with *Y. enterocolitica* and *Y. pseudotuberculosis* originating from soil and water revealed internal penetration of these plants (roots, seeds and leaves) and survival for up to 30 days.

A similar plant connection was reported in Finland, where an outbreak of *Y. pseudotuberculosis* occurred among schoolchildren who fed on infected carrots (Jalava *et al.*, 2006). It is not clear if the carrots were infected previously or during preparation, though 42% of soil samples containing carrot residue (some also from the production farm), identified by pulsed-fiel gel electrophoresis (PFGE), were found positive

for this bacterium. In Finland also, Nuorti *et al.* (2004) reported on a nation-wide infection with *Y. pseudotuberculosis* O:3 originating from iceberg lettuce. Interestingly, the authors mentioned an ecologically distinct feature of a large population (>10,000 animals) of nonnative roe deer *(Capreolus capreolus)* introduced in the 1960s as well as large quantities of roe deer feces found all over the lettuce fields and around every one of the irrigation water sources.
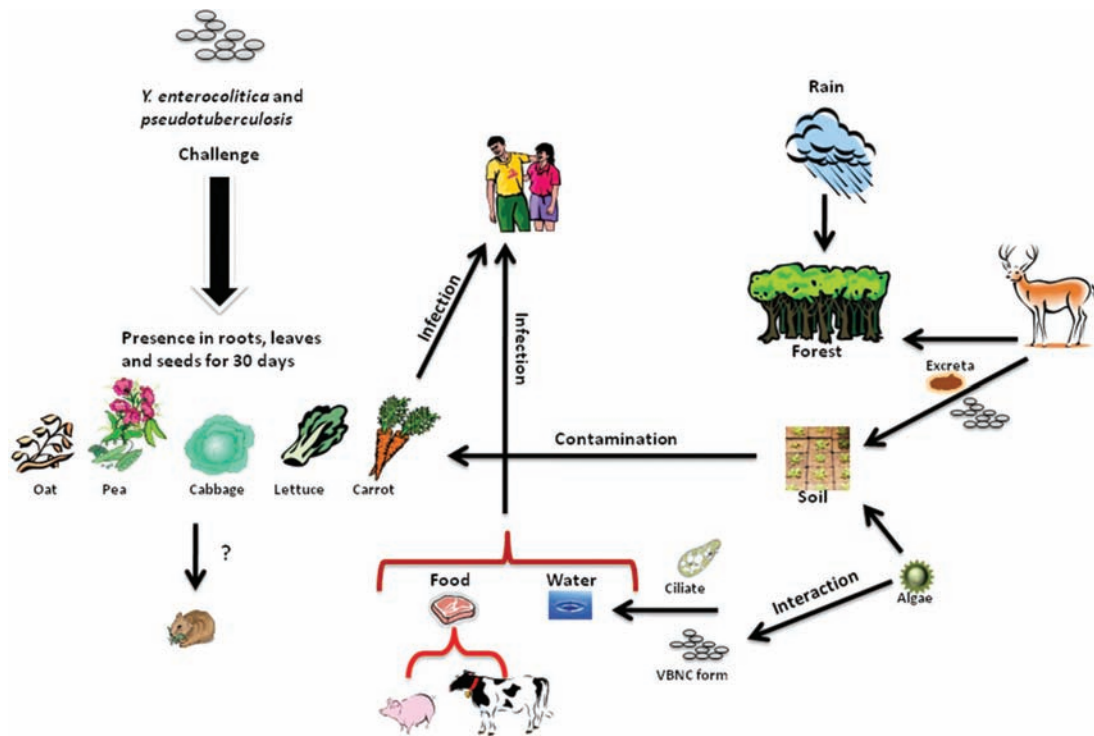
Figure 2.25.1. *Y. enterocolitica* and *pseudotuberculosis* spp. presence and interaction with environmental parameters

It is very feasible that soil is the main link between pathogens and cultivable plants. Barre *et al.* (1977) isolated different *Y. pseudotuberculosis* serotypes among which serotype I (an already demonstrated human pathogen) from soil samples around Paris (France) while Nastasi *et al.* (1986) isolated *Y. enterocolitica* from soil and dog feces. These reports suggest a clear potential health risk from soils as a reservoir for these pathogens. Also in relation to soil, *Y. pseudotuberculosis* was studied for its persistence in sterile soils (as an abiotic environment) and in natural soil (as biotic/abiotic multifaceted environment) (Litvin *et al.*, 1990). In sterile soil, *Y. pseudotuberculosis* was resistant to a wide range of temperatures (0 to 30°C), humidities (15 to 50%) and pHs (5.9 to 9.0). Under these conditions, the growth rate was only slightly affected but not the general nature of the population dynamic of this bacterium. A more marked impact was observed in natural soil containing other bacterial species such as *Acinetobacter* and *Pseudomonas* that can alter *Y. pseudotuberculosis* biotic persistence. According to the authors' results, another

important biotic factor is the endosymbiotic relationship between *Y. pseudotuberculosis* and free-living infusorian *Tetrahymena pyriformis*, shown to sustain this pathogen population in soil and water.

The saprophitic characteristics of *Y. pseudotuberculosis* were shown in soil samples from the surroundings of Nero lake (Rostov district, Russia), which is a natural source of this pathogen (Maksimenkova & Karpachevskii 1985). Soil in this area has a high content of organics, cations exchange capacity (CEC) and moisture, all favoring the growth and survival of thess organisms for prolonged periods. Soil survival of *Y. enterocolitica* was studied in natural soil, river water and well water (Tashiro *et al.*, 1991). Among *Y. enterocolitica* serotypes O:3, O:4, O:5A, O:5B, O:6, O:6, O:30, O:9 and O:13 seroptypes O3, O:5B and O:9 strains survived longer at 4°C than at 20°C in these soil and river water environments.

Aksenov *et al.* (1995) and Troitskaia *et al.* (1996) found higher numbers of *Y. pseudotuberculosis* in environmental soil samples from natural foci using the specific molecular method (PCR) than using the classical culture method. This detection disparity was attributed to a viable but non-culturable (VNBC) form of *Y. pseudotuberculosis*. VNBC forms of *Y. pseudotuberculosis* were also observed in soil in association with blue-green algae, *Anabaena variabilis*, closely related to seasonal changes such as temperature (Solokhina *et al.*, 2001). Exometabolites of these algae accelerated the formation of "resting cells" of *Y. pseudotuberculosis* at 22°C. When VNBC forms were passed through infusoria ciliated protozoa, partial reversion to vegetative form (cells capable of growing on solid culture medium) was observed. The transfer from vegetative to VNBC form in relation to algal soil presence is not clear, but it can be speculated that some "partial antibiotic activity" may be attributed to algal exometabolites that do not eradicate bacterial cells completely but act as a biostatic factor altering their growth capability. Indeed, lower enzymatic activity, agglutinability, cytopathogenicity and plasmid p45 loss were observed in revertant bacterial cells.

A potential connection to soil types and algal content was also described by Sidorenko and Buzoleva (2007) who compared two seashore soil types (maritime meadow and tidal marsh) for their growth support of two pathogens: *L. monocytogenes* and *Y. pseudotuberculosis*. Both soil types were supportive for growth and survival, with maritime meadow being superior.

In Germany, soil originating from deciduous forest was found to harbor *Y. enterocolitica* in contrast to grassland soil that was found to be negative (Botzler, 1987). In northwest California, *Y. enterocolitica* was isolated from soils inhabited by infected wapiti (an elk species, *Cervus elaphus roosevelti*, L.). Prevalence of *Y. enterocolitica* in soil from forest habitat was significantly higher than that from prairie habitat especially following a previous excessive rainfall of 17 mm (Botzler, 1979). From these reports it is clear that forest animals' excreta contribute to the prevalence of *Y. enterocolitica* in forest soil, affected strongly by moisture as a result of rainfall (French *et al.*, 2010).

Water is one of the main carriers of *Yersinia* and clearly proven to infect humans (Fukushima *et al.*, 1988). A study on stream water from urban, agricultural and forested watersheds, applying the 16S rRNA gene sequencing method, revealed *Yersinia* spp. presence mainly in forested watershed (Belt *et al.*, 2007). In water environment, diverse planktonic and benthos invertebrates, such as water flies, cyclops, mollusks, oligochaeta, insects' larvae were found to support prolonged survival of *Y. pseudotuberculosis*. Beside these organisms, fish and higher plants were also found to be supportive, therefore presenting a health risk (Pushkareva *et al.*, 1994).

Bresolin *et al.* (2006) reported that *Y. enterocolitica* expresses virulence factors pathogenic to insects (such as Tc proteins) at low temperature (10°C). The virulent *Y. enterocolitica* lives in a symbiotic complex with entomophagous nematodes. Insects are invaded by these nematodes that in turn release its gut symbiotic bacteria into the haemocoel (insect circulatory system) killing the insect host to provide more nutrients (Forst *et al.*, 1997). These findings are probably pointing towards insects as a life stage of

several *Y. enterocolitica* biotypes, similar to its other close relative, *Y. pestis*, that alternates between fleas and mammals. Temperature in this case is the environmental signal for *Y. enterocolitica* to switch replication from mammalian host to soil or poikilothermic insects (Palonen *et al.*, 2010).

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Chapter 2.26

Rare zoonotic bacteria

In this chapter, other miscellaneous bacteria "incriminated" as potential zoonotic agents are described. Some of their characteristics are described in Table 2.26.1. Their prevalence as human zoonoses is low, although they cannot be ruled out as hazards especially due to their domestic animals' origin.

2.26.2 MISCELLANEOUS ZOONOTIC BACTERIA AND ENVIRONMENT (FIGURE 2.26.1)

Actinobacillus lignieresii, *equuli* and *suis* species are a common component of oropharingeal microbiota of animals such as cattle, horses, sheep and pigs. These *Actinobacillus* spp. are also the main human zoonotic pathogens of this genus, even if rare (Ashhuurst *et al.*, 1988). *A. lignieresii* and *A. suis* were detected following horse and pig bites (Dibb *et al.*, 1981; Escande *et al.*, 1996), in a farmer (*A. lignieresii*) (Orda and Wiznitzer, 1980) and also directly recovered from horses, the majority exhibiting some kind of illness, in New Zealand (Carman and Hodges, 1982). In Japan they have been isolated more frequently from respiratory infections and endocarditis cases (Sakazaki *et al.*, 1984).

Andrade *et al.* (1997) studied the effects of roots and of arbuscular-mycorrhizal (AM) fungi on the composition of soil bacterial colonies and their combined effects. Roots were experimentally colonized by three arbuscular-mycorrhizal (AM) fungi: *G. etunicatum*, *G. intraradices* or *G. mosseae*. Among the large variety of bacteria isolated from the rhizosphere and hyposphere (the soil area not directly influenced by roots), *Actinobacillus lignieresii* was only isolated from the hyposhere of the AM-fungi *G. mosseae*.

Actinobacillus suis has been shown to cause diseases in pigs, horses, geese, cats, rodents, sheep (Maddux *et al.*, 1987; Daignault *et al.*, 1999; Lentsch and Wagner, 1980; Peel *et al.*, 1991) with elevated serological heterogeneity in horses (Bada *et al.*, 1996).

Carvalho *et al.* (1999) tested different soil bacteria strains for their emulsification activity on water-diesel oil combinations. *Actinobacillus lignieresii* was among the high biosurfactant producers with high emulsification potential. This feature seems to be related to its susceptibility to hydrophobic compounds such as the antibiotic novobiocin (Hart and Champlain, 1988).

Table 2.26.1 Potential zoonotic bacteria that have been rarely isolated from infected and ill humans.

Genus	Species	Gram Staining	Growth conditions	Transmission	Disease	Occurrence
Actinobacillus	*lignieresii*	–	Facultative aerobic	Animal bite	Abscess, septicemia	Domestic animals
	equuli	–				
	suis	–				
Arcanobacterium	*pyogenes*	+	Anaerobic	Animals, flies	Septicemia, endocarditis, meningitis, arthritis, pneumonia, empyema, pharyngitis, abscess	Domestic animals
Bordatella	*bronchiseptica*	–	Obligate aerobic	Aerosols, close contact with animals	Paroxysmal cough, respiratory whoop	Horses, Pigs, dogs, cats, rabbits
Corynebacterium	*pseudotuberculosis*	+	Aerobic or facultatively anaerobic	Close contact with animals, raw milk	Inguinal nodes, granulomatous necrotizing lymphadenitis	Domestic animals
Dermatophilus	*congolensis*	+	Facultative anaerobic	Animals, flies	Diphteria-like pharyngitis, pneumonitis	Soil, domestic and wild animals
Helicobacter	*pylori*	–	Microaerophilic	Oral	Chronic gastritis, ulcer	Humans and animals
	heilmannii	–				
	canis	–				
	felis	–				
	fennelliae	–				
	suis	–				
Rhodococcus	*equi*	+	Facultative aerobic, intracellular	Sapronosis ?	Necrotizing pulmonary infections	Foals and domestic animals
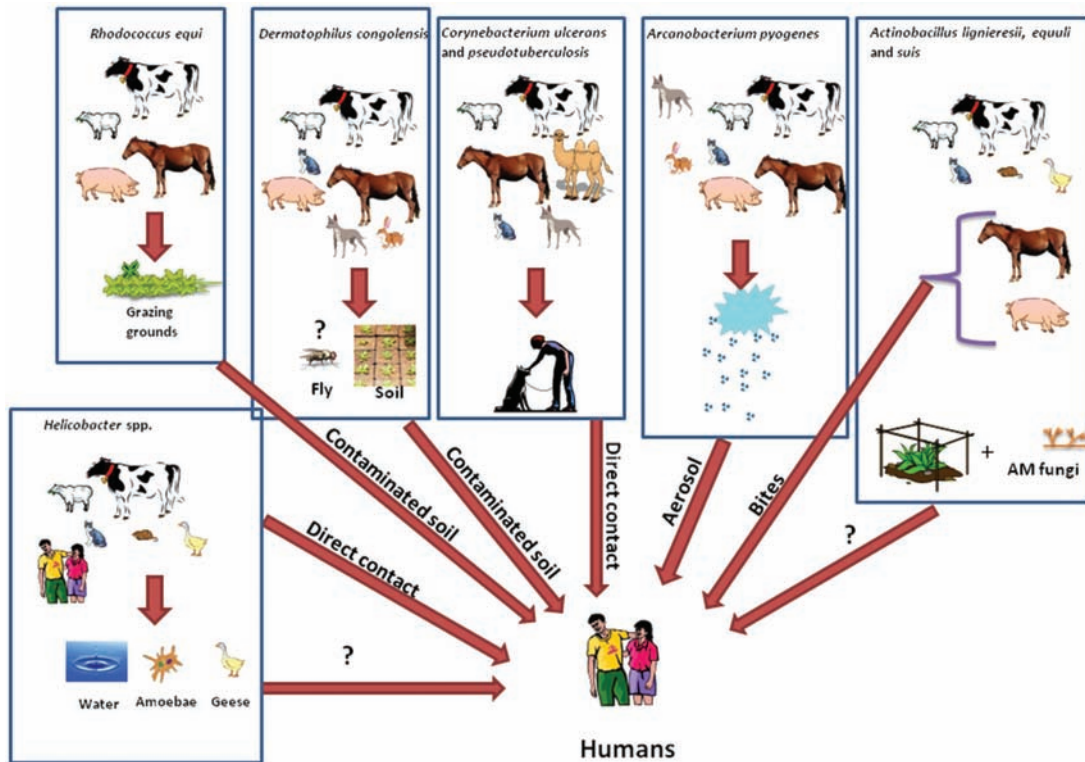
Figure 2.26.1. *Actinobacillus*, *Arcanobacterium*, *Bordatella*, *Corynebacterium*, *Dermatophilus*, *Helicobacter* and *Rhodococcus* spp. as miscellaneous zoonotic diseases and their suspected transmission

Arcanobacterium pyogenes is another commensal of mucous membranes of domestic animals (cattle, sheep and pigs) that may cause disease in other animals and humans (Lin *et al.*, 2010; Levy *et al.*, 2009; Kavitha *et al.*, 2010). In humans, infection with *Arcanobacterium pyogenes* can result in severe diseases with infrequent fatality (Gomez-Mateos *et al.*, 2009; Hermida *et al.* 2004; Plamondon *et al.* 2007). The transmission path is not clear, but direct contact with carrier or sick animals and their mucous membranes, exudates or aerosols can infect humans (Jost *et al.*, 2002). Beside the regular domestic animals mentioned above as carrying this bacterium, cats and dogs were found also to be infected with *A. pyogenes* suggesting them also as a possible human infection source (Billington *et al.*, 2002). In animals, especially ungulates, the bacterium becomes pathogenic when the animal is stressed by captivity or something else (Palmer and Whipple, 1999; Nettles *et al.*, 2002). Lavin *et al.* (2004) described a foot infection in three adult male free-living fallow deer in Asturia, Spain. The authors described the area, showing some weather and geographical conditions that were possibly related to animals infection, such as mild temperature, high humidity, mixed pasture and rock land, domestic and wild herds living side by side and hunting activity (that may stress wild animals).

Bordetella bronchiseptica is another commensal of respiratory tract of horses, pigs, dogs, cats, rabbits and guinea pigs (Baetz *et al.*, 1974). The transmission seems to be through aerosols or close contact with infected animals (Tamion *et al.*, 1996; Stehmann *et al.*, 1991; Hermann *et al.*, 2008). Aerosol transmission has been shown to be supported by its enduring nature at lower and humid temperatures, especially in intensive

husbandry (Stehmann *et al.*, 1992). *B. bronchiseptica* has the potential to survive and grow in the natural environment through expression of two virulence determinants (controlled by its genomic *bvg* and *ris* loci) modulated by different environmental signals (e.g., low temperature). Experimental evidence indicated that, for *B. bronchiseptica*, *bvg* locus controlled determinants expressed under modulating conditions (e.g., motility) facilitate adaptation and survival in environments outside the host (Coote, 2001).

Corynebacterium ulcerans and *pseudotuberculosis* were also described as zoonoses, though much less frequent than their classical relatives (Kraeva *et al.*, 2007). *C. pseudotuberculosis* found its natural hosts in many domestic animals such as: cattle, sheep, goats, horses and even camel (Cetinkaya *et al.*, 2002; Tejedor-Junco *et al.*, 2009; Doherr *et al.*, 1998; Stefanska *et al.*, 2007), while *C. ulcerans* is mainly found in cattle although cats were also implicated (De Zoysa *et al.*, 2005; DeWinter *et al.*, 2005). Wagner *et al.* (2010) reviewed *C. ulcerans* cases in United Kingdom for the period from 1986 to 2008 and found that the major risk factor of infection is contact with companion animals. In relation to *C. pseudotuberculosis*, people in direct contact with domestic animals are also at high risk (shepherds, children and animal workers) (Bartolome *et al.*, 1995; Join-Lambert *et al.*, 2006; Dorella *et al.*, 2006).

Another bacterial pathogen suspected of infecting humans is *Dermatophilus congolensis* found mostly in tropical and subtropical and much less in temperate areas (Matheron *et al.*, 1989; Towersey *et al.*, 1993; Zaria, 1993; Kaminski and Sutter, 1976; Oduye, 1975; Pal, 1995; Pospisil *et al.*, 1992). Wild and domestic animals are affected by this bacterium present in contaminated soil (Martinez and Prior, 1991). Soil type and water content were the main components impacting survival of *D. congolensis* in environment. Martinez and Prior (1991) also reported that organic matter content protects *D. congolensis* in oxisol soil as a temporary reservoir, but not in vertisol, though it retains its pathogenicity. Their conclusion was that ponds and dipping tanks may constitute sources of cattle infection as a result of soil and water environmental mix. Direct close contact with infected animals (domestic and wild) was also reported to cause infection in humans (Amor *et al.*, 2011). Interestingly, flies were also suggested as vectors, possibly through their direct contact with soil or domestic animals urine, feces and gut mucus. Morris *et al.*, (1997) tested the odors originating from these excretion including those produced by growth of different bacteria, among them *D. congolensis*, as attractants for the Australian sheep blowfly (*Lucila cuprina)*, revealing significant movement of this insect towards *D. congolensis* cultures.

Rhodococcus equi (a soil actinomycete) was also suggested as a human zoonotic pathogen (Martens *et al.*, 2000). In humans the disease represents itself as pulmonary infections (Silva *et al.*, 2010). Domestic animals such as cattle, horses, sheep and pigs are also infected (Martens *et al.*, 2000; von Bargen and Haas, 2009; Muscatello *et al.*, 2007). The main reservoir seems to be contaminated soil where domestic animals graze (Martens *et al.*, 2000; Barton and Hughes, 1982, 1984); however a study performed in Japan on the presence of *R. equi* in soil and sand from parks and yards, revealed a significant presence (73.9%) but only of non-virulent strains (Takai *et al.*, 1996). When horse farms' soil was surveyed for the presence of *R. equi*, only 18.8% of soils contained virulent strains and no correlation was found between bacterial presence and diseased horses (Takai *et al.*, 2001). Additionally, Barton and Hughes (1984) showed that *R. equi* was isolated from gut contents, rectal feces and dung of all grazing herbivorous but not from penned animals, indicating soil as the main source of this bacterium. It is very much possible that soil containing virulent strains can be dispersed as dust and directly infects humans through respiratory system, primarily in immunocompromised individuals.

Finally, *Helicobacter* a genus of gram-negative bacteria, microaerophilic, helical shape, living in stomach was also incriminated as being of zoonotic origin. *Helicobacter pylori* is well established as a human pathogen in association with gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa associated lymphoid tissue (MALT) lymphoma (Marshall and Warren, 1984; Ernst and Gold, 2000), although some of its closely related relatives, presented in Table 2.26.2, were isolated from animal hosts

and already reported to be infectious in humans (Marshall and Warren, 1984; De Groote *et al.*, 2000; Haesebrouck *et al.*, 2009). Potential transmission patterns are: person–to-person (oral-oral, fecal-oral, iatrogenic and familial), waterborne and zoonotic (vector-borne) (Brown, 2000; Svec *et al.*, 2000; Sasaki *et al.*, 1999; Haesebrouck *et al.*, 2009). De Groote *et al.* (2000), based on experimental data, advocated *Helicobacter* human infection via zoonotic path as evidential proof of transmission. *Helicobacter canadensis* (from avian reservoirs such as geese) was also linked to human disease (Waldenström *et al.*, 2003; Fox *et al.*, 2000). Some authors also pointed out that, besides direct contact with infected or carrier animals, transmission through carrier arthropods able to excrete *Helicobacter* in their feces is also feasible in connection with water and food (Imamura *et al.*, 2003; Shineng and Stutzenberger, 2000).

Table 2.26.2 *Helicobacter* spp. natural colonizers of animals' stomach and human association.

Helicobacter spp.	Natural host	Human association	Isolation site	Experimental water survival (hours)
H. pylori	Humans, non-human primates, cat	Yes	Dyspeptic humans, soil, flies	>96
H. suis	Pig, macaque, mandrill monkey	Yes	Pig stomach	
H. felis	Dog, cat, rabbit, cheetah	Yes	Dog owner, human gastric biopsy, South African population	10
H. bizzozeronii	Dog, cat	Yes	Finnish patient with severe dyspeptic symptoms	
H. salomonis	Dog, cat, rabbit	Yes	Dams and puppies	
"*Candidatus Helicobacter heilmannii*"	Dog, cat, wild felidae, nonhuman primates	Yes	Human gastric mucosa, small villages, rural area, patient with pets	
H. baculoformis	Cat	No	Feline stomach mucosa	
H. cynogastricus	Dog	No	Canine gastric mucosa	
"*Candidatus Helicobacter bovis*"	Cattle	Yes	Human	
H. mustelae	Ferret	No	Stoats and ferrets from New Zealand	~47–48
H. aurati	Syrian Hamster	No	Hamster	
H. nemestrinae	Macaque	No	Pigtailed macaque	
H. acinonychis	Cheetah, tiger	No	Stray dogs ?	
H. cetorum	Whales, dolphins	No	Human upper digestive tract, gastric mucosa of harp seals (*Phoca groenlandica*)	
H.muridarum	Mice	No	Intestinal mucosa of rodents	~47
H. canadensis	Geese	Yes	Geese, shorebirds,	~48

Water seems to be one of the major environmental potential carriers of *Helicobacter* based on this pathogen's survival potential for many hours (Table 2.26.2), grown in mixed bacteria biofilms and on different abiotic materials (Azevedo *et al.*, 2008; Percival and Thomas, 2009; Azevedo *et al.*, 2006; Dube *et al.*, 2009). Li *et al.* (2001) reported a significantly higher prevalence of *H. pylori* infection in immigrant population from a flooded area and inversely related to flood stages, indicating floods and living conditions as a source of high prevalence. Fujimura *et al.* (2004) reported the correlation of *H. pylori* prevalence in children and *H. pylori* DNA presence in water of an adjacent river in Japan. *H. pylori* prevalence in children living near the middle reaches was 9.8% and 23.8% for children nearby downstream reaches, both values being greater than in an area distant from this river (0%) (p < 0.01) used as control.

Soil polluted by human and animals excreta was shown also to harbor the pathogen (9% prevalence in soil tested) providing a possible source of infection for children in contact with playgrounds (Perez *et al.*, 2010). As a final point associated to water and soil survival, *H. pylori* was reported to be competent to infect and internalize free-living amoebae, as a result enhancing this pathogen's environmental endurance (Winiecka-Krusnell *et al.*, 2002).

It should be pointed out that, due to actual confusion on *Helicobacter* spp. classification and newly emerging species isolated from a variety of animals, an unambiguous transmission route is still not available and more research is required to clarify this important issue.

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Chapter 3

Viral Zoonoses

Chapter 3.1

Alphavirus

[FAMILY *TOGAVIRIDAE*, GENUS *ALPHAVIRUS*]

Alphaviruses are important viruses, widely distributed throughout the world, causing localized diseases in humans and animals as well as epizootic and epidemic outbreaks. The genus *Alphavirus* belongs to the family *Togaviridae*. The Togaviridae family includes a second genus, namely *Rubivirus* (Powers *et al.*, 2001). Alphaviruses have a linear, + (ss) RNA genome of approximately 11.7 kb size, packed in an icosahedral nucleocapsid. The viral capsid is enclosed in a detergent sensitive lipid bilayer envelope derived from the host cell membrane and modified by insertion of viral glycoproteins (Anthony and Brown, 1991). Alphavirus envelope is covered by characteristic spikes made of glycoproteins (E1 and E2), arranged in trimers $(3 \times E1/E2)$. The glycoproteins are responsible for viral binding to host-cell receptors and the internalization process (Kielian, 2010). The virions have a diameter between 60 and 70 nm and are found in the cytoplasm of the host cell (Strauss and Strauss, 1994).

Alphaviruses are maintained in nature by alternate infection of vertebrate and arthropod (mosquitoes) hosts. According to this life cycle, blood sucking mosquitoes get infected during a blood meal on an infected vertebrate and further responsible for virus transmission to the next vertebrate host.

The genus Alphavirus includes ≥ 29 different species, each species including different subspecies. Based on serologic criteria, the species can be classified into seven complexes or clusters, which among others appear to share medically important characteristics (Calisher and Karabatsos, 1988). The five complexes Eastern equine encephalitis, Western equine encephalitis, Venezuelan equine encephalitis, Semliki Forest virus and Barmah Forest viruses include important disease causing viruses. Human infections with Alphavirus can to be asymptomatic. When clinically expressed, the disease appearance depends on the virus and occurs in either of two forms: expressed by febrile illness with fever, malaise, headache, or symptoms of encephalitis, fever, rash and arthralgia (Table 3.1.1) (Zacks and Paesler, 2010).

Alphaviruses are arthropod borne viruses with mosquitoes as primary vectors in transmission of this pathogen. In their natural life cycle these viruses are maintained by transmission between mosquitoes and vertebrate hosts (birds or mammal), acting also as amplifying hosts. These potential amplifying hosts develop sufficient viremia in order to infect mosquitos vectors and still survive the infection. Human are usually dead end host due to the fact that they do not develop sufficient viremia to infect mosquitoes

(Pfeffer and Dobler, 2010). Alphaviruses' airborne transmission is possible and has been documented for *Venezuelan equine encephalitis virus* infections (Zacks and Paessler, 2010). Emerging epizootic outbreaks are usually due to climatic conditions.

Table 3.1.1 Alphaviruses complexes, viruses and clinical features[a].

Complex	Virus	Clinical Manifestation	Host	Vector	Distribution
Eastern Equine Encephalitis	Eastern Equine Encephalitis (EEE)	Encephalitis	Birds	*Culiseta melanura*	USA, Canada, Central and South America
Western Equine Encephalitis	Western Equine Encephalitis (WEE)	Encephalitis	Birds, Mammals	*Culex tarsalis Ochlerotatus spp., Aedes spp.*	USA, Canada, Central and South America
	Sindbis (SIN)	Febrile illness, rash, arthralgia	Birds	*Culex pipiens*, *C. univittatus, C. torrentium, Culiseta morsitans, Ochlerotatus spp., Aedes spp.*	Africa, Asia, Australia, Middle East, Eastern Europe, Scandinavia
Venezuelan Equine Encephalitis	Venezuelan Equine Encephalitis (VEE)	Febrile illness, Encephalitis	Rodents, Horses	*Ochlerotatus taeniorhynchus, Culex spp*	South and Central America
Semliki Forest	Ross River (RR)	Febrile illness, rash, arthritis	Mammals, Humans	*Culex annulirostris, Aedes vigilax, Ae. camhorhynchus*	Africa
	Chikungunya (CHIK)	Febrile illness, rash, arthralgia	Primates, Humans	*Aedes spp., Ae. aegypti, Ae. albopictus, Ae. frucifer, Ae. luetocephalus, Culex, Mansonia, and Anopheles*	Africa, India and South Asia
	O'nyong'nyong (ONN)	Febrile illness, rash, arthralgia	Primates, Humans	*Aedes aegypti, Ae. furcifer, Ae. Africanus, Anopheles funestus, Anopheles gambia*	Africa
	Mayaro (MAY)	Febrile illness, rash, arthralgia	Primates, Humans	Haemagogus janthinomys	South and Central America
	Semliki Forest (SF)	Febrile illness, rash, arthritis	Birds	*Aedes africanus and Aedes aegypti*	Africa and Asia, possibly present in central and southern Europe
Barmah Forest	Barmah Forest (BF)	Febrille illnes, rash, arthritis	Humans	*Culex spp, Aedes spp.*	Australia

[a](Adapted from ICTVdb, 2006).

Several factors affected the spread of these viruses: vector, host and environmental changes. Periodically, the virus causes vast outbreaks, to disappear later on for a certain time interval and then reappear or/and spread to another location. Such an example is the reoccurrence of O'nyong'nyong fever outbreak after 35 years in Uganda and the 7 year intervals of Sindbis virus disease in Finland (Lutwama *et al.*, 1999; Kurkela *et al.*, 2005)

3.1.1 ALPHAVIRUS AND ENVIRONMENT

The restricted geographical distribution of Alphaviruses is based on their natural transmission cycle, including susceptible reservoir host and the species particular mosquito vector (Weaver *et al.*, 2000; Johnston and Peters, 1996). Vector activity is seasonal and is affected for the most part by temperature and rainfall.

Due to climatic and environmental changes (deforestation, agriculture, water storage and irrigation), human population growth and urbanization, trade and travel behavior, an increasing risk of alphaviral spread to new ecological areas and countries throughout the world continues to subsist (Sutherst, 2004).

There are several reports regarding alphaviral disease emergence attributable to infected travelers. *Ross River Virus* (RRV), endemic and enzootic in Australia and Papua New Guinea, caused a large epidemic in 1979/1980 in Samoa, Cook Island and New Caledonia probably due to a viremic Australian traveler. It also has been supposed that RRV is frequently introduced to North America as a consequence of human travelers from Australia (Harley *et al.*, 2001). In Germany an investigation of patients with unresolved complaints of fever with headache, skin redness and severe multiple joint arthralgia demonstrated an infection with *Ross River Virus* and Barmah Forest virus (BFV). These diagnosed patients had all returned from Australia (Peffer *et al.*, 2001). BFV that is similar to RRV is endemic in Australia and the Pacific Islands, causing fever, rash polyarthritis and arthralgia similarly to German cases (Anonymous, 1991).

Similar reports were published for a Mayaro virus (MAYV) infection, enzootic in tropical humid forests of South America and maintained in a sylvatic cycle involving wild vertebrates, that occurred recently in a French citizen who suffered from severe rheumatologic disorders after visiting the Brazilian Amazon (Receveur *et al.*, 2010). In the same year, MAYV infection was also diagnosed in a Dutch couple, complaining of a persisting arthralgia, temporary fever and rash after residing in Surinam (Hassinge *et al.*, 2010). A similar scenario was suggested for Chikungunya (CHIK) fever, a tropical disease, occurring in Africa, India and parts of South Asia (Pialoux *et al.,* 2007). This virus circulates in natural sylvatic pools using primates and rodents as both hosts and reservoirs. Major human epidemics occurred in cases of vector abundance subsequent to heavy rainfall. CHIK infection symptoms are flu-like; headache, fever, joint pain and arthralgia (Taubitz *et al.*, 2007).

Since the first reported outbreak of CHIK fever in Tanzania and its isolation from humans and mosquito in 1952 (Ross, 1956), many outbreaks of Chikungunya fever have been reported from Africa and Asia from 1960 to 1982 (WHO, 2006). Between 2004 and 2005, islands located in the Southwest Indian Ocean were affected by an epidemic and approximately 1/3 of the population was infected. In 2006 around 1.4 million people developed symptomatic disease of CHIK, probably due to a virus mutant, lack of vector control and trade and travel globalization (Kalantri *et al.*, 2006). Since 2006, there have been new reports of CHIK cases in European countries (i.e. Germany, Belgium, Czech Republic, France, Italy, Spain) and the USA, directly associated with homecoming tourists who had visited India and affected islands of the Indian Ocean (Powers and Logue, 2007). Special consideration should be given to two cases of Chikungunya (in Italy, 2007 and France, 2010) where this virus was probably transmitted locally. In July 2007, an outbreak of CHIK virus infection took place in the Romagna area, in northern Italy. The initial source of this local transmission was

an Indian immigrant, residing in the Romagna area, who returned from an epidemic area one month before the outbreak. At the time of his arrival in Italy, he was at the viremic, asymptomatic stage of the infection (Sambri *et al.*, 2008). As a result of a viral mutation circulating in India it was possible to associate the two cases. In France, in the town of Fréjus (Provence), a child who had traveled also to India was diagnosed with Chikungunya virus infection. Two weeks after the initial event, two additional cases of Chikungunya fever occurred in the same town in persons who did not travel abroad. The chronologic time besides the local proximity of these cases, implies that the infection was transmitted and acquired locally through the mosquito vector *Aedes albopictus* (Grandadam, 2011). This Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae) originating from Asia, is one of the fastest invading mosquitoes which now inhabit North and South America as well Africa and Europe (Benedict *et al.*, 2007). Female mosquitoes, with breeding sites closely related to human habitats (containers with standing water, tires or containers, plant pots, etc.) lay desiccation-resistant eggs that may remain viable for several months (Hawley, 1988, Koehler and Castner 1997) (Figure 3.1.1). Therefore global trade in used tires and bamboo seems to be the main factor in the worldwide distribution of mosquito eggs and for that reason a critical factor affecting the spread of new viruses (Hawley *et al.*, 1987; Hawley, 1988; Dalla Pozza *et al.*, 1994; Schaffner and Krach, 2000).
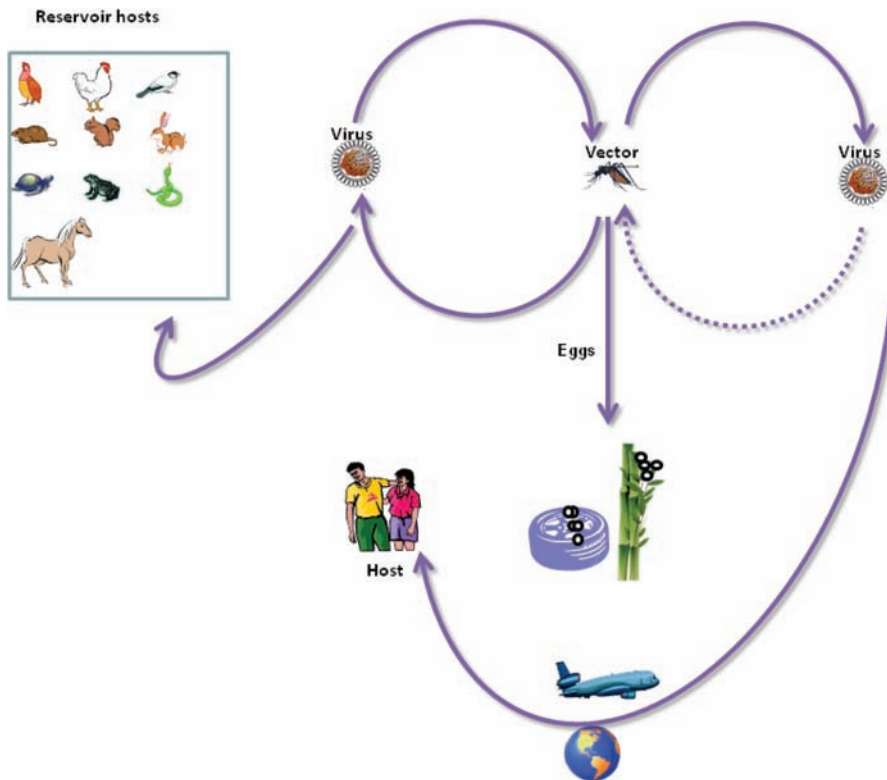

Figure 3.1.1. Infection routes of Alphaviruses

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Chapter 3.2

Flaviviruses infections

[FAMILY *FLAVIVIRIDAE*, GENUS *FLAVIVIRUS*]

Flaviviruses share a common symmetry (enveloped, icosahedral nucleocapsid), with a size range of 40 to 65 nm and an encapsulated nucleic acid + (ss) RNA, approximately 10,000–11,000 bases). *Flaviviruses* are transmitted by bite from an infected arthropod (mosquito or tick) and not by direct contact (person to person) (Table 3.2.1). A typical prototype of this virus family is yellow fever virus (YFV) (that beside other symptoms, causes jaundice in infected persons). *Flavivirus* human infections are characteristically incidental, since humans are not capable to produce enough high titers in order for it to be spread further by infected arthropods. However, yellow fever and dengue viruses are the exception, because they still require mosquitoes/ticks as vectors and they are adequately adapted to humans as to not necessarily depend upon animal hosts (even though both continue to have animal transmission routes as well) (Busquets *et al.*, 2008). In ticks and mosquitoes, vertical transmission is possible through transovarial and transstadial routes (Figure 3.2.1). Vertebrates are used as amplification hosts from which ticks and mosquitoes get infected and in spite of high viremia they do not necessarily develop clinical diseases. Other virus transmission routes include handling infected animal carcasses, blood transfusion (Dengue fever virus, DEN), child birth and breastfeeding (West Nile virus, WNV) (Anonymous, 2002) and through consumption of unpasteurized milk products. The transmission from animals to humans without an intermediate vector arthropod is thought to be unlikely. For example, early tests with yellow fever showed that the disease is not a contagious disease. The disease course is marked by biphasic fever and prodromal phase, manifesting itself as CNS diseases, hepatitis, hemorrhagic fever, encephalitis and myalgia.

3.2.1 FLAVIVIRUSES AND ENVIRONMENT

It can be stated with a high degree of confidence that flaviviruses (in the past found primarily in the tropics) are now a global problem due to the intensive spread of the carrier vectors. Flaviviruses are transmitted to humans and animals by two main vectors: ticks and mosquitoes. Importantly, both arthropods can as well be used as reservoirs of flaviviruses through the "transovarial" and "transstadial" processes, in particular when

Table 3.2.1 Zoonoses caused by *Flaviviruses* spp.

Species	Disease and main symptoms	Host	Geographical area	Transmission Vector	Origin
TBE (Tick-borne Encephalitis virus), european subtype, CEE (Central European Encephalitis)	Meningo-encephalitis	Humans, dogs	Europe, Asia	Ticks (*Ixodes ricinus*), subclinically infected cattle, goats, sheep	Hedgehog, shrew, mole, cattle, goat, sheep, dog
LI (Louping ill) and Negishi virus encephalitis	Influenza-like syndrome followed by CNS manifestation	Humans, sheep, dogs	Europe, Asia (Japan and China)	Ticks (*Ixodes ricinus*)	Cattle, sheep, grouse, red deer, hare, rabbit, bat, hedgehog
PE (Powassan virus encephalitis and Modoc virus encephalitis	Influenza-like syndrome followed by meningitis and encephalitis	Humans	North America, Russia	Ticks (*Ixodes marxi, I. cookie, I. spinnipalpus, I. andersoni, I. scapularis, Haemophysalis neumanii*), mosquitoes	Squirrel, groundhog, rabbit, dog, skunk, fox, goat
KFD (Kyasanur Forest Disease)	Fever with CNS disturbances (encephalitis) and hemorrhages of mucous membranes	Human	India	Ticks (*Haemaphysalis* spp.)	Cattle, monkeys (rhesus, langur), rodents, birds
ALKV (Alkhurma virus hemorrhagic fever	Hemorrhagic fever and encephalitis	Human	Saudi Arabia	Ticks	Camels and sheep
OHF (Omsk Hemorrhagic Fever)	Fever with head and limb aches with hemorrhagic diathesis	Humans, Muskrats	Russia (Western Siberia)	Ticks (*Dermacentor reticulates, Dermacentor apronophorus*)	Rats (*Arvicula terrestris*), Muskrats (*Ondrata zibethica*)
JE (Japanese Encephalitis)	Encephalitis, fever, hemorrhages	Humans, sows, piglets, frogs, horses	Asia (Siberia maritime regions, China, Japan, Korea, Taiwan, Phillipines, Vietnam, Thailand, Indonesia, Burma, Malaysia, Bangladesh, India southern and eastern parts, Bali, Australia)	Mosquitoes (*Culex tritaeniorhynchus, Culex annulus, Culex annulirostris*)	Mosquites, pigs, cattle, goats, cats, dogs, birds (heron), bats, snakes, toads

Virus (Disease)	Clinical manifestations	Susceptible hosts	Geographic distribution	Vector	Reservoir
MVE (Murray Valley Encephalitis) and Kunjin Virus Disease	Encephalitis, meningitis	Humans	Australia, Mew Guinea	Mosquitoes (*Culex annulirostris*)	Mosquitoes, waterfowl (herons and pelicans)
SLE (St. Louis encephalitis virus)	Encephalitis, meningitis	Humans, wild and domestic birds	North America (USA and Canada), Mexico, Caribbean, Brazil	Mosquitoes (*Culex tarsalis, Culex pipiens, quinquefasciatus*)	Sparrows and pigeons
RE (Rocio Encephalitis virus)	Encephalitis, anorexia	Humans	Brazil (southeastern)	Mosquitoes (*Aedes scapularis, Psorophora ferox*)	Wild birds, mosquitoes
WNF (West Nile Fever virus)	Fever, encephalitis, lymphadenopathy	Humans	Africa (Uganda, Egypt, Zaire	*Culex pipiens* (Eastern, US), *Culex tarsalis* (Midwest and West, US), and *Culex quinquefasciatus* (Southeast, US)	Wild birds, horses, mosquitoes
Usutu virus	Neurological disease in immunosuppressed patients	Humans	Africa and Europe	Mosquitoes (*Culex pipiens*)	Wild birds, rodents
Wesselsbron fever	Encephalitis, lymphadenopathy, photophobia	Humans	Africa	Mosquitoes (*Aedes* spp.), laboratory infections	Sheep, cattle
YF (Yellow fever)	Jaundice, bradycardia, epistaxis, melena and urogenital bleeding, kidney failure, etc.	Humans, monkeys	Central Africa and South America	Mosquitoes (*A. aegypti, A. simpsoni, Haemagogus* spp.)	Jungle monkeys
DEN (Dengue fever, types 1-4) DHF (Dengue hemmorhagic fever)	Biphasic fever, lymphadenopathy, myalgias, leucopenia, Haematuria, myocarditis	Humans, monkeys	Asia, Africa, Central and South America	Mosquitoes (*A. aegypti, A. albopictus, A. scutellaris, A. africanus, A. niveus* ?)	Jungle monkeys: chimpanzees, gibbons, macaques, mosquitoes

a direct vertebrate host is not directly available (Figure 3.2.2) (Joshi *et al.*, 2002). Viral transition from invertebrates to vertebrates is an interesting phenomenon as in this particular case the arthropod vectors (invertebrates) can multiply very fast (days) and reach high numbers versus a much longer time span of replication in vertebrates and in much lower numbers (e.g., humans). In an intensive replicative system, any parasitic life form will proliferate successfully gratifying its life objective: to spread its genes. For example, *A. aegypti* that multiply in clean water sources (artificial containers, wasted tires, puddles, etc.) lays eggs that are highly resistant to dessication (for months) and able to hatch when submerged in water (e.g., after rainfall periods), on the site walls. Anthropophilic females of this mosquito species have the characteristic of interrupted and multiple feeding prior to gonotrophic cycle completion and once infected with one of the flaviviruses (Dengue, Chikungunya and Yellow fever viruses) or simultaneously with two (West Nile and Usutu viruses) it is for life (Calzolari *et al.*, 2010; Tamba *et al.*, 2010; Klowden and Briegel, 1994).
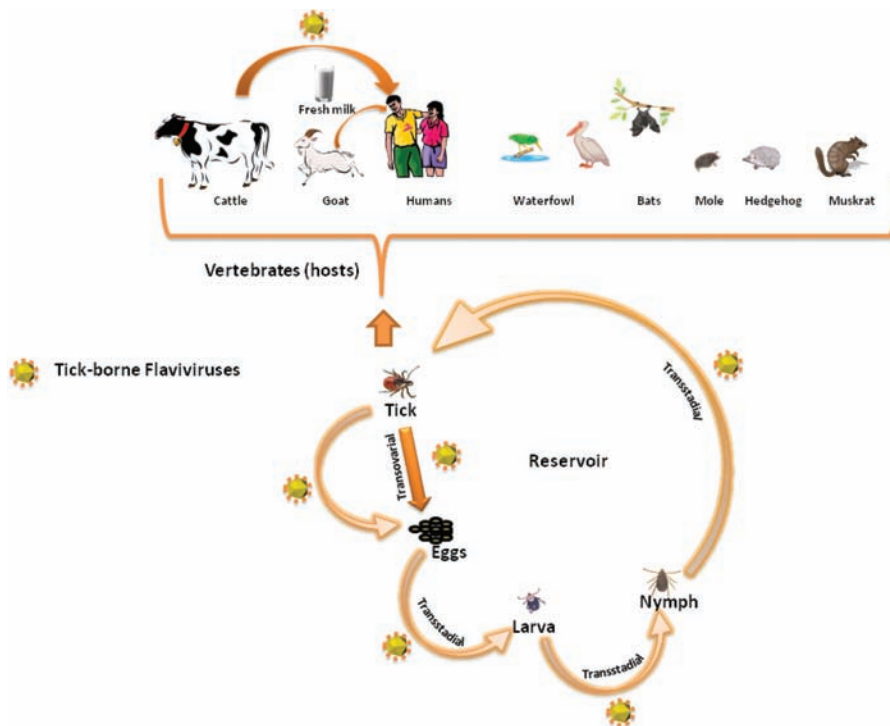
Figure 3.2.1. Transmission of tick-borne flaviviruses to vertebrates and related to tick life cycle

In tropical countries, temperature and rainfall impact the distribution of Dengue Fever virus (Halstead, 2008). The transmission of the DEN virus is almost closely related to warm and rainy seasons. Rainwater collection expands the breeding sites. Temperature was found to cut down the extrinsic incubation period the interval between the acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts and extend mosquitoes' life span, consequently increasing biting frequency. According to recent data, global warming and interannual climate variations do not seem to impact Dengue and other mosquitoes-borne flaviviruses' transmission

significantly; nevertheless, socio-economic aspects such as people's increased mobility, international trade, urban poverty, lack of piped water, open reservoirs and air conditioning, do enhance disease spread and persistence (Figure 3.2.3) (Reiter, 2001; Arunachalam *et al.*, 2010; Becker, 2008). Here should be mentioned a special case in which the used tire global trade has been shown to contribute to spread of mosquitoes between USA, Japan and South Korea (Reiter and Sprenger, 1987). In the case of West Nile virus (WNV), an additional factor was found to be involved in the dramatic expansion to new geographic areas beside global commerce, climate change and ecological factors. This change was related to new genotypes that evolved from the febrile illness of children in areas endemic to neurological disease as a result of a more virulent genotype emergence. From the genetic point of view, in spite of WNV stable evolutionary structure, the 1999s' North American introduction of a new genotype was linked to better and more efficient propagation at elevated temperatures (indicating temperature as a selective criteria) as well a prior mutation (under adaptive evolutionary pressure) associated with increased replication in avian hosts, demonstrating the rapid adaptation of these viruses to both hosts: vertebrates and invertabrates (Brault, 2009).
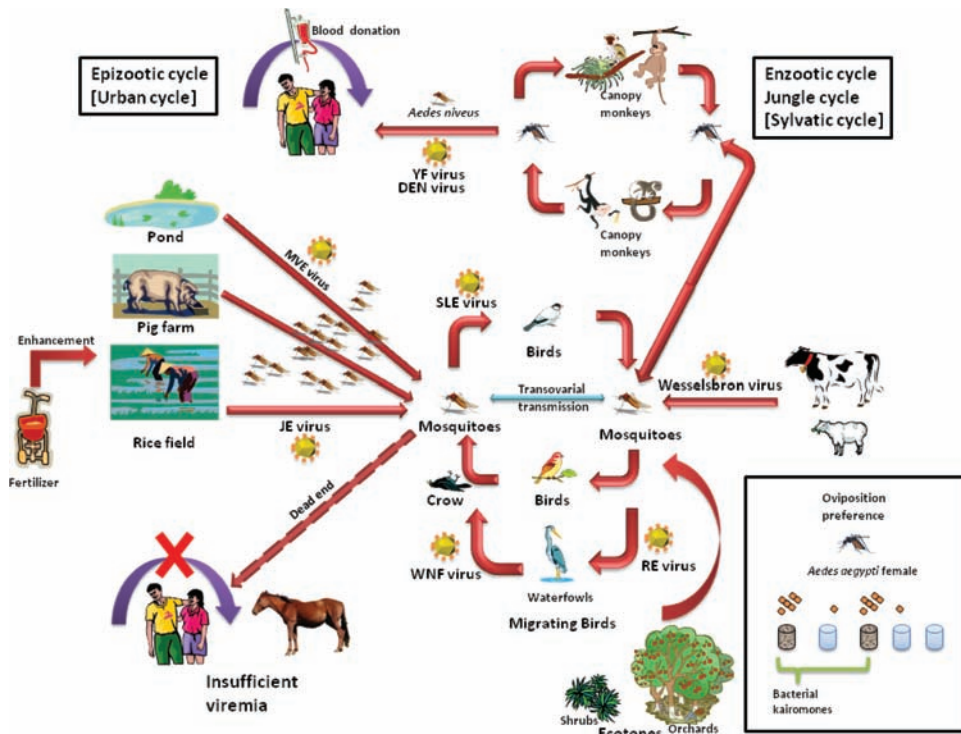
Figure 3.2.2. Transmission of mosquitoes-borne Flavoviruses

The disease spread and persistence factors are also linked to human determinants, such as blood virus titer (viremia) although till now the viremia threshold is undefined, herd immunity and household multiple infections (clusters of dengue infections) (Neff *et al.*, 1967; Zhao, 1986).

In Thailand, known as an endemic area, Bangkok, a metropolitan area of over 9 million people, was found to be the country's endemic center. Based on the periodicity of pediatric cases hospitalized in a

Bangkok hospital from 1983 to 1997, waves of severe cases progressed from the capital in all directions, moving at a speed of 148 km per month (Cummings *et al.*, 2004). An interesting feature of the oviposition site preferences of *Aedes aegypti* females was recently published (Ponnusamy *et al.*, 2008). A previous study showed that *Aedes aegypti* females lay and disperse their eggs in multiple water-filled containers, a phenomenon called "skip oviposition" (Colton *et al.* 2003). This environmental bounce behavior was related to some chemical response of mosquito females (Reiter *et al.*, 1991; Trexler *et al.*, 1998; Benzon and Apperson, 1988). Based on these details, Ponnusamy *et al.*, (2008) clearly proved that kairomones, such as bamboo and white-oak leaf infusions (kairomone is a semiochemical mediator involved in inter-specific interactions), attract *Aedes aegypti* females to lay their eggs when present. These kairomones are specific bacteria-associated carboxylic acids and methyl esters that can serve as potent oviposition stimulants, and therefore a potential tool in biological warfare against these mosquitoes. To strengthen this phenomenon, Barrera *et al.*, (2006) showed that *Aedes aegypti* larval and pupal abundance were larger in containers with leaf litter or algae. Pupal productivity and the biomass of emerging females varied in containers with litter of different tree species. The organics produced by litter are the basic nutrients for different bacteria present in a water container to produce the above kairomones.
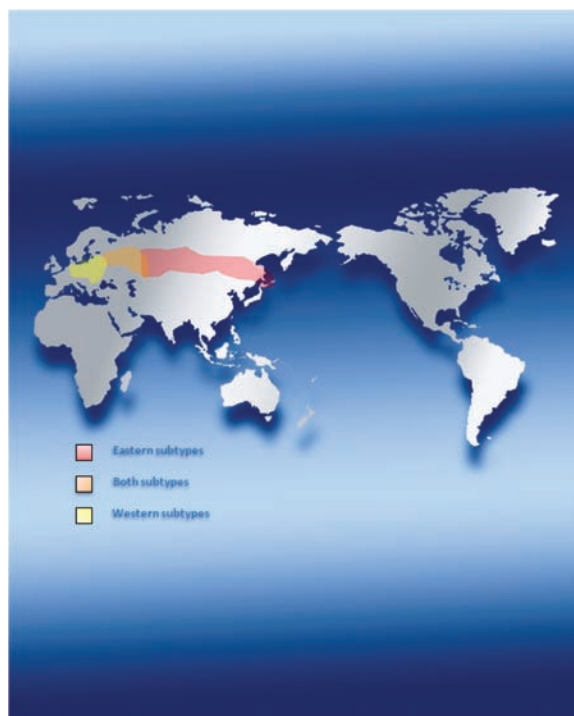
Figure 3.2.3. Tick-borne encephalitis (TBE) (Eastern and Western subtypes) endemic areas in Eurasia

In an interesting review, Reisen (2010) described the term "nidus" (in Latin- nest) (described as a place where pathogen transmission occurs) as a spatial defined focus in landscape epidemiology where host, vector, and pathogen populations interact spatially within a permissive environment. The nidus is characterized by vegetation, climate, latitude, elevation, and geology (Christofferson *et al.*, 2010; Ciota

et al., 2007). This author showed that in certain ecotones in which mosquito vectors and avian abundance prevail there is a significant potential increase in West Nile virus transmission in USA, which is certainly true around the globe (Weissenböck *et al.*, 2002). Such ecotones (transects intersecting desert scrub vegetation and citrus orchard habitat) are utilized by both birds and mosquitoes: for nesting and host seeking, respectively.

Beside humans, other mammals (pigs) are also involved in the persistence of flaviviruses. Japanese Encephalitis virus (JE) is distributed throughout Southeast Asia, the Indian subcontinent, Indonesian archipelago, and into the Australasian zoogeographical region with a high level of fatal cases. While infected with JEV, humans and horses do develop fatal encephalitis, yet they are dead-end hosts due to low viremia, and thus the virus is maintained in an enzootic cycle between ardeid wading birds, *Culex* mosquitoes and pigs that are important for epizootic transmission (van de Hurk *et al.*, 2009; Smartt *et al.*, 2010). Again, birds are involved as amplifying hosts and through their long distance flight they can spread the virus to new ecosystems.

Platonov *et al.* (2008) studied West Nile fever (WNF) outbreaks in southern Russia from 1999 to 2007. For 4 years (200–2004) the incidence rates dropped to zero, while in 1999 and 2007 they were at their highest. 1999 and 2007 had the warmest winters and summers. Two mosquitoes types were shown to transmit WNF in this area: *Culex pipiens* and *Culex modestus*, both attracted to humans and birds. These authors emphasized the climatic conditions as predisposing factors for WNF spread in this temperate climate zone (Ranjit and Kissoon, 2011). Flood periods in temperate areas revealed an enormously high population density of mosquitoes (like the one that occurred in 1997 Morava River in South Moravia, Czech Republic) that significantly increased also the infection rate of these mosquitoes with flaviviruses (WNV) (Hubalek *et al.*, 2000).

The spread of flaviviruses to temperate climate is coupled with the survival resistance of their vectors to much lower temperatures, especially in winter times (Huhtamo *et al.*, 2009). Mosquitoes from the *Aedes* family are among those well adapted to temperate winters through hibernation, egg tolerance to sub-zero temperatures and adults' survival throughout the winter in appropriate microhabitats. (Romi *et al.*, 2006; Hanson and Craig, 1995; Hawley *et al.*, 1989).

TBE viruses are transmitted by ticks and maintained, amplified, and disseminated in wild vertebrate hosts (e.g., small rodents and insectivores) as endemic cycles due to inefficient vertical transmission. Individuals who work or play in tick-infested areas are the frequently infected targets, but with low potential of further transmission due to low viremia (similar to other flaviviruses transmitted by mosquitoes also to other domestic animals). However, milk and raw dairy products may pass the virus from sheep, goats, and cattle to humans as a result of virus secretion into their milk by these domestic animals (Ludwig and Iacono-Connors, 1993).

Arthropods are known for their toughness and resistance in a large variety of extreme environments. Fielden *et al.* (2011) studied dog tick (*Dermacentor variabilis*) survival when submerged in water. These authors showed that these ticks can survive underwater for extended periods using plastron respiration. Plastron is a thin permanent layer of air around the body of some aquatic insects, held in place by hydrophobic hairs or other cuticular projections acting as a physical gill. Indeed, ticks have a complex spiracular plate that is used for plastron respiration. Due to its hydrophobic property, the complex spiracular plate can hold oxygen bubbles to be used for respiration, supporting the survival period for up to 15 days. Interestingly, wetting the complex spiracular plate with alcohol (resulting in reduced hydrophobicity) lowered water submerged ticks' survival to ∼7 days.

Zvereva and Kozlov (2010) performed a meta-analysis of the responses of terrestrial arthropods (decomposers and predators) to air pollution and concluded that some adverse impacts may have negative consequences on the ecosystem structure and services. However, their meta-analysis did not

provide support that an overall decrease in diversity or promotion of herbivore outbreaks is indeed impacted by air pollution. According to their meta-analysis, a decrease in abundance of predators was found likely to be a widespread phenomenon where as an indirect factor, pollution may favor herbivores by generating an enemy-free space. In relation to climatic effects, these authors indicated that temperature and precipitation may enhance pollution therefore thereby increasing ecosystem adverse effects on arthropods. These results were also supported by a more particular study of observed changes in the geographical range of ticks in parts of Europe, showing that a complex combination of climate variables as well host availability and vegetation controls ticks' life cycle and their population dynamic (Estrada-Peña, 2008).

Natural evolution of the tick-borne encephalitis (TBE) virus was studied in eastern and western Siberia, middle Urals and European part of Russia (Pogodina *et al.*, 2007) (Figure 3.2.4). Environmental and epidemilogical conditions that impacted this evolution were: 1) changes in TBEV subtypes over a time span of 50-60 years (from eastern to western Siberian type); 2) steady-state circulation of western Siberian type and accumulation of mutant types; 3) co-existence of both types in the common vector (*Ixodes persulcatus*); and 4) TBEV strains genetic mixture including genes expressing proteins E and NSI of both viruses types.
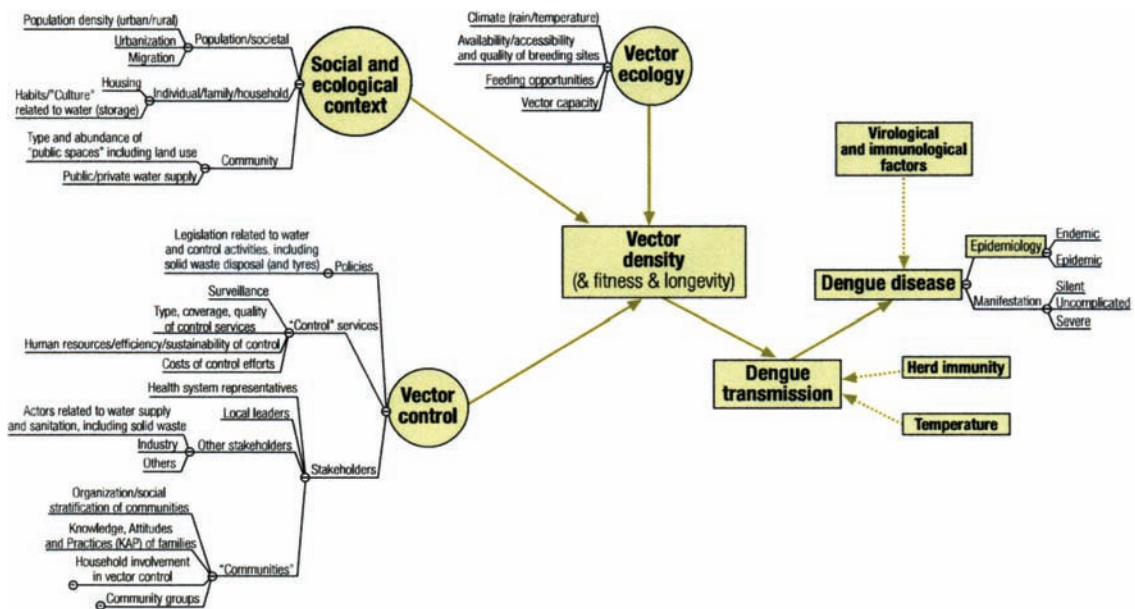
Figure 3.2.4. Frame-work of eco-bio-social determinants of dengue vector breeding: a multicountry study in urban and periurban Asia (with permission from Arunachalam *et al.*, (2010) Bull. World Health Organ. 88, 173-184)

Haemig *et al.* (2008) pointed to the roadside with its different ecology compared to the surrounding landscape, as a significant environmental factor where ticks, humans, pets and livestock encounter each other, therefore constituting an important factor in tick-borne viral diseases.

Ticks are basically divided in two major groups: hard ticks (*Ixodidae*) and soft ticks (*Argasidae*) (Table 3.2.2). From the ecological point of view, hard ticks can be found in open environments (except in the nidicolous *Ixodes* sp., particularly immature), with seasonal activity on usually three hosts

Table 3.2.2 Tick-borne flaviviruses and some environmental attributes.

Tick species	Virus transmission	Distribution	Ecology	Feeding Hosts	Humans affinity	Climate
Ixodes persulcatus	TBE, CEE, RSSE	Estern Europe, Russia, Asia, northern Japan	Coniferous and deciduous forests and woods	Small mammals (I), birds (I), medium to large mammals (I,A)	Yes	Humid continental, subarctic, humid subtropical
Dermacentor marginatus	TBE, CEE, RSSE	Morocco, Spain, France, central Europe, central Asia	Scrub steppe, temperate forests, grassland, pastures	Small mammals and birds (I), large mammals (A)	Yes	Mediterranean, Moderate marine west coast, arid
Dermacentor silvarium	TBE, CEE, RSSE	Northern China, Mongolia	Scrub steppe,	Small mammals and birds (I), large mammals (A)[a]	Yes	Semiarid, arid, humid continental
Haemaphysalis spp.	TBE, CEE, RSSE	South-east Asia, USA, Europe (Spain, Hungary, Iran, Africa (Ethiopia) Indonesia	Forest, pasture	Rodents, birds (I), ungulates, carnivores (A), large mammals (cattle, sheep, dogs, horses) (A)	Yes	Humid subtropical, humid continental, Mediterranean

(*Continued*)

Table 3.2.2 (*Continued*)

Tick species	Virus transmission	Distribution	Ecology	Feeding Hosts	Humans affinity	Climate
Ixodes ricinus	Louping ill	Western Europe to central Asia, northern Japan	Grassy woodlands, heathlands, forest, pastures, humid microhabitat	Small mammals, birds, reptiles (I), medium to large mammals (I,A)	High	Marine west coast, humid continental, subarctic, humid subtropical
Ixodes pacificus, marxi, cookie, spinnipalpus, andersoni and *scapularis* (experimentally)	Powassan encephalitis virus	Canadian Pacific coast south to California to Mexico, Russia, Northeast and eastern USA (Vermont, Main and South Carolina), North America, Eastern and southeastern USA, Canada	Shrub, desert, coniferous forest area	Small mammals (squirrel, groundhogs) reptiles, birds (I), large mammals (A)	Yes	Subarctic, marine west coast, semiarid, humid continental
Haemaphysalis spp.	Kyasanur Forest Disease (KFD) and Alkhurma Virus Hemorrhagic Fever	India	Forest, pasture	Rodents, birds (I), ungulates, carnivores (A)	Yes	Humid subtropical, tropical wet, semiarid
Dermacentor reticulatus, apronophorous	Omsk Hemorrhagic Fever (OHF)	Western Siberia	Forest and steppe areas	Small mammals (I), domestic and wild mammals (A)	Yes	Humid continental, subarctic

(different species, one per stage) a life span from several months to three years (less resistant to starvation and desiccation) and mostly seeking non-nidicolous free-ranging hosts, while soft ticks can be found in sheltered environments (nests, burrows, caves and man-made shelters) have no seasonal activity with multi-host feeding pattern, long life (up to 10 years), are highly resistant to starvation and mostly nidicolous in the host habitat as host-seeking behavior (Parola and Raoult, 2001).

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Chapter 3.3

Bunyaviridae

[FAMILY *BUNYAVIRIDAE*, GENUS *HANTAVIRUS*, *NAIROVIRUS*, *ORTHOBUNYAVIRUS*, *PHLEBOVIRUS*, *TOSPOVIRUS*]

The family of *Bunyaviridae* viruses contains several genera and more than 300 members of enveloped, spherical particle, somewhat similar to *Paramyxoviridae* family with a size range of 90 to 100 nm, without matrix proteins and a tripartite nucleic acid – (ss) RNA at a size range of ∼10,500 to 22,700 bases). *Bunyaviridae* are mainly vector-borne viruses (ticks, mosquitoes and sandflies) with the exception of Hantaviruses genus that is transmitted through direct contact with rodents' feces. This family of viruses can infect a diverse range of hosts, from arthropods (mosquitoes) to marsupials, and thrips to plants (tomatoes) (Table 3.3.1). Some of the Bunyaviridae viruses (e.g. Crimean-Congo hemorrhagic fever virus) can cause serious disease and are associated with high levels of morbidity and mortality, therefore laboratory handled with caution at biosafety level 4 (Ergönül, 2006). Clinical aspects of Bunyaviridae may present as: fever, photophobia, myalgia, conjunctivitis, arthralgia, vomiting, diarrhea, renal insufficiency, meningitis, myocarditis, hemorrhagic diathesis with a lethality of 30 to 55%.

3.3.1 BUNYAVIRIDAE AND ENVIRONMENT

Most of the genera belonging to Bunyaviridae family are linked to animal hosts and mainly horizontal transmition (though vertical one is also possible when vector-borne) by two major pathways: vector-borne (ticks, fleas, mosquitoes, and other biting insects) or through the inhalation of infectious aerosolized saliva or excreta of a rodent/insectivor reservoir/host (Miller *et al.*, 1977; Song *et al.*, 2007) (Figure 3.3.1). Among those confirmed as human Bunyaviridae viruses, three genera belong to those transmitted by arthropod vectors (known as arboviruses-arthropod borne e.g., Orthobunyaviruses are transmitted by mosquitoes or midges, Nairoviruses by ticks, and Phleboviruses by sandflies or ticks) and one that do not have arthropod vectors but are maintained in nature as persistent infections of rodents (hence the term robovirus, from rodent-borne) and are transmitted to humans via aerosolized infectious rodent secretions (e.g. Hantaviruses) (Nowotny 1994; Ullmann *et al.*, 2008). The fourth genus Tospoviruses (that are plants' pathogens or phytopathogens) is carried and spread by thrips.

Table 3.3.1 General Bunyaviruses classification including those pathogenic to humans.

Genus	Virus	Host	Vector	Geographical occurrence	Humans	Disease	Fatality (%)
Orthobunyavirus	La Crosse virus (LAC)	Squirrels, rabbits, chipmunks	Mosquitoes (*Aedes triseriatus*)	North America	Yes	California (La Crosse) encephalitis	
	Snowshoe hare virus (SSH)	Snowshoe hare, rodents, carnivores (Bears), ungulates and birds (Ruffed Grouse) and domestic species- chickens, dogs, horses and cattle.	Mosquitoes (*Aedes cinereus, Aedes communis, Aedes euedes*) Ticks (*Haemaphysalis leporis-palustris*)	North America	Yes	California encephalitis	
	Tahyna virus (TAHV)	Mammals and avian spp.	*Aedes caspius, Culex* spp.	Old World and Northern China	Yes	California encephalitis	
	Oropouche virus	Sloths, primates	Culicoid flies (*Culicoides paraensis*), Mosquitoes (*Aedes* spp.)	Brazil, North Peru, Ecuador, Panama, Trinidad and Tobago	Yes	Oropouche fever	
Nairovirus	Crimean-Congo Hemorrhagic Fever (CCHF) virus	Herbivors (cattle, goats, sheep and hares)	*Hyalomma* ticks	Southern Europe (Mediterranean area), Asia (Northwestern China, central Asia), Africa, Indian subcontinent	Yes	Crimean-Congo Hemorrhagic Fever	20-80
Phlebovirus	Rift Valley Fever virus (RVF)	Rodents (?), Herbivors (carcasses or fetuses)	Mosquitoes (*Culex* spp.), aerosols, hematophagous flies (?)	Africa, Asia Minor	Yes	Rift Valley Fever	1-10

Genus	Virus	Reservoir/Host	Transmission/Vector	Geographic distribution	Human disease	Disease	Mortality (%)
	Sandfly fever virus (SFSV)	Gerbils, bats (?)	Sand flies (*Phlebotomus* spp., e.g. *Phlebotomus papatasi*)	Southern Europe, Northern Africa, South America, West and Central Asia	Yes	Sandfly fever	
Hantavirus							
Subgroup A			No vectors				
	Puumala virus (PUUV)	Rodents (Bank vole), contaminated water with rodents excrements, aerosols	Direct transmission, No vector	North and Central Europe	Yes	Nephropathia epidemica (NE)	0.1
	Hantaan virus (HTV)	Rodents (*Apodemus agrarius*)	Direct transmission, No vector	Europe, South and North America, Asia	Yes	Korean hemorrhagic fever (KHF)	5-15
	Dobrava virus (DOB)	Small mammals, rodents (*Apodemus flavicollis, A. agrarius*)	Direct transmission, No vector	Europe (Balkan area)	Yes	Balkan hemorrhagic fever	
	Seoul virus	Rodents (*Rattus norvegicus*)	Direct transmission, No vector	Worldwide	Yes	Hemorrhagic Fever with renal syndrome (HFRS)	1
Subgroup B							
	Sin Nombre virus (SNV)	Rodents *Peromyscus maniculatus* (deer mouse)	Direct transmission, No vector	North America	Yes	Hantavirus pulmonary syndrome (HPS)	50
	Laguna Negra virus (LNV)	Laucha chica (*Calomys laucha*)	Direct transmission, No vector	South America	Yes	HPS	
	Andes virus (ANDV)	Long-tailed rice rat (*Oligoryzomys longicaudatus*)	Direct transmission, No vector	South America	Yes	HPS	

(Continued)

Table 3.3.1 (*Continued*)

| Genus	Virus	Host	Vector	Geographical occurrence	Humans	Disease	Fatality (%)
	Bayou virus (BAYV)	Rice rat (*Oryzomys palustris*)	Direct transmission, No vector	North America	Yes	HPS	
	Black Creek Canal virus (BCCV)	Cotton rat (*Sigmodon hispidus*)	Direct transmission, No vector	North America	Yes	HPS	
	Subgroup C						
	Prospect Hill virus (PHV)	Rodents (*Microtus pennsylvanicus*)	Direct transmission, No vector	North America	Possible (Low prevalence)		
	Tula virus (TV)	Rodents (*Microtus arvalis*)	Direct transmission, No vector	Northern Europe	Possible (Low prevalence)		
	Lemming virus (LV)	Rodents (*Lemmus lemmus*)	Direct transmission, No vector	Northern Europe	N.A.		
Tospovirus	Tomato spotted wilt virus (TSWV)	Thrips species, *Frankliniella occidentalis* *F. fusca, Thrips tabaci*			No	Plant disease	
	Impatiens necrotic spot virus (INSV)	Thrips species, *Frankliniella occidentalis* *F. fusca, Thrips tabaci*			No	Plant disease	
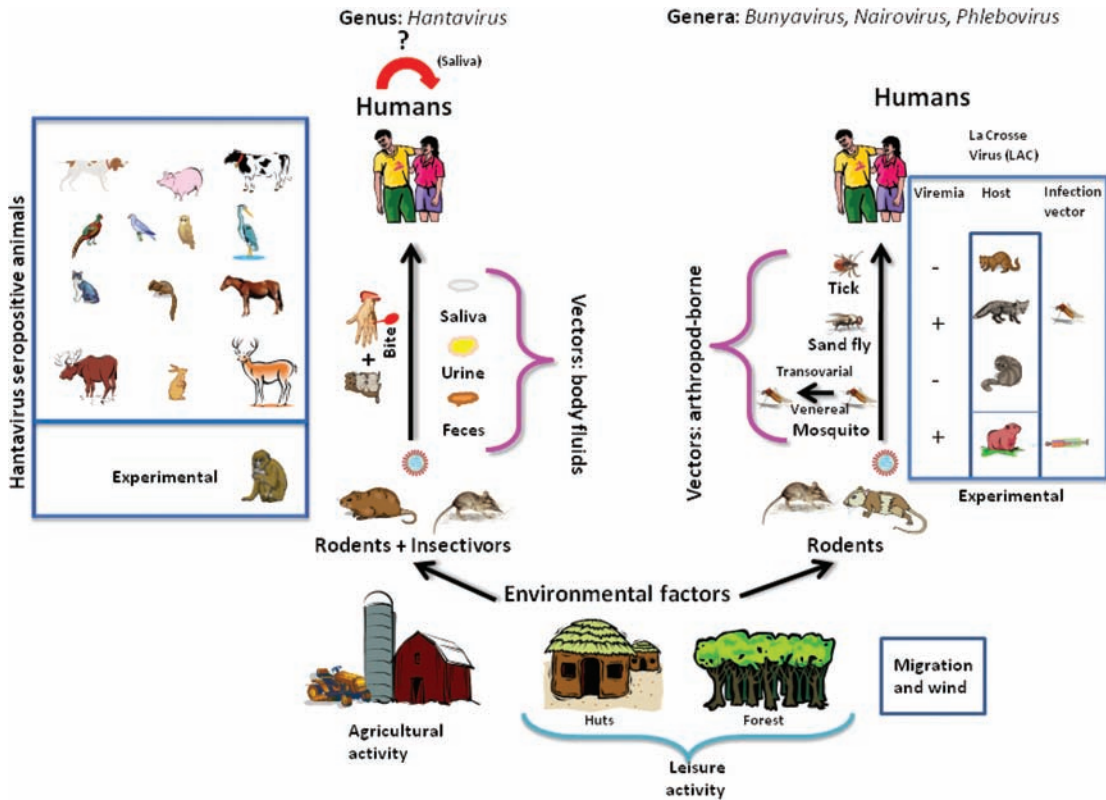
Figure 3.3.1. Bunyaviridae transmission paths and relation to different animals and environment

Here is the place to mention some pieces of evidence accepted among the members of the scientific community related to Bunyaviridae: a) the pathogens/diseases and their vectors (e.g. insects) will abound if warming indeed takes place; b) if warming occurs, dynamics of the present pathogens variability will change including interspecies cross; c) intermediate stages of vectors (mainly insects) will develop faster; d) geographic migration of hosts and vectors will take place northern to previously colder regions; e) long-distance movements of persistent organic pollutants (POP's) may affect the immunology of remote and cold region fauna (hosts), therefore making them more susceptible to new diseases and finally f) favorable physical environmental factors (e.g. temperature and humidity) affect the longevity and infectivity of roboviruses outside the rodent host.

Those bunyaviridae transmitted by insect vectors behave mostly like the Oropouche virus (Pan American Health Organization (PAHO, 2010). This virus exists in nature in two cycles, the wild and urban-epidemic cycles. In the wild cycle, wildlife such as primates, sloths, and certain arthropods serve as reservoirs, with the flies acting as vectors. In the urban epidemic cycle, the infection cycle remains vector-man-vector, with the human being acting as the principal host. Tesh (1994) described seroconversion for the Oropouche virus in Panamian population an area with unknown cases. Based on reported isolation of this virus from insects and mammals it seems that it has a wide geographic distribution in tropical South America. Endemic areas for Oropouche virus are still sparsely populated, but human new settlement and development can increase its prevalence. Other factors involved in this virus distribution are deforestation, urbanization and

agricultural development, all connected to breeding habits of *Culicoides paraensis*. The preferred habitats of this biting midge are rotting cacao husks and banana stump. Both habitats contain decomposing plant material able to preserve moisture during dry periods, consequently serving as an excellent matrix for these insects' larvae. In addition, in rural areas, farmers intentionally place rotting plant material among the cacao plants, in order to encourage these insects and other ceratopogonids as pollinators of cacao flowers. In urban areas, people plant banana trees in their gardens therefore increasing the breeding sites of *Culicoides paraensis* and possibly other vectors.

Mosquitoes as vectors, have shown the ability to transfer La Crosse virus by two important modes: venereal (between matting partners) (Thompson and Beaty 1977) and transovarial (vertical to eggs) (Miller *et al.*, 1977). The second mode is even more resistant environmentally as mosquito eggs are highly resistant and dormant for extended periods of time (weeks/months/years) during which virus maintain its viability.

Rift Valley fever virus (RVFV) transmitted by mosquitoes was first identified in 1930, during an outbreak of sudden deaths and abortions among sheep along the shores of Lake Naivasha in the greater Rift Valley of Kenya (Daubney and Hudson, 1932). Since then, RVFV spread all over African continent (from Egypt to South-Africa), Madagascar and between 2000–2001 towards Arabian Peninsula, causing livestock and human outbreaks (Pepin *et al.*, 2010; Balkhy and Memish, 2003). Since the early 1900s some major ecological changes occurred in eastern and southern Africa, through establishment of colonial agriculture systems including the import of large numbers of European breed livestock that are highly susceptible to this virus. In the case of the Egyptian outbreak, the construction of the Aswan High Dam along the Nile River was associated it, while the 2006–2007 African outbreak was related to changes in rainfall patterns (Meegan, 1981; Bird *et al.*, 2008).

When dealing with arthropods, air streams and climatic conditions play an important role in the transport of biting midges (hosts of several Bunyaviruses) (Elliott, 2009). Braverman and Chechik (1996) analyzed in Israel the temperature, relative humidity, wind speed and direction at different altitudes (0.5, 1 and 1.5 km) for four years and their relationship to outbreaks of bluetongue (Genus: Orbivirus) and Akabane (Genus: Orthobunyavirus) viruses, both non-zoonotic viruses. The main conclusion of these authors was that the outbreaks and seroconversion occurred only following the Persian through air-stream system, in spite of the main vector's, *Culicoides (Avaritia) imicola*, previous presence (March and April months) and without correlation to the amount of precipitation in the spring season. If these results are correct, other more sever zoonotic bunyaviridae may travel vectorially long distances and may spread geographically further.

The genus Hantaviruses (Bunyaviridae transmitted by infectious aerosolized saliva or excreta of rodents) is a group of at least 25 antigenically distinct viruses that do not cause evident illness but only some pathology in their reservoir hosts (Netski *et al.*, 1999)(Table 3.3.2).

Field data suggest that transmission in host populations occurs horizontally and that this occurs more frequently among male than female rodents (Glass *et al.*, 1998). All Hantaviruses known to cause hantavirus pulmonary syndrome (HPS) are carried by New World rats and mice of the family *Muridae*, subfamily *Sigmodontinae*, that contains at least 430 species widespread in North and South America (Schmaljohn and Hjelle, 1997). In Argentina there are increasing reports of the geographic expansion of these viruses resulting in more cases of Andes virus (Bellomo *et al.*, 2009). Rodent-borne viruses' prevalence and transmission factors were categorized into five major classes: (1) Environmental (weather and food supply) that impacts transmission rates (reproductive success and population densities); (2) Anthropogenic (disturbance by agricultural activity) that cause decreased diversity and increase of opportunistic species; (3) Genetic (impacts mice susceptibility to infection by chronic shedding); (4) Behavioral (male and female mice relations such as fighting and communal nesting) (Kariwa *et al.*, 1996) and (5) Physiologic (host response to infection and infectivity duration) (Mills, 2005).

Table 3.3.2 Genus hantaviruses and their rodent/insectivore hosts.

Hantaviruses species	Rodent or Insectivore Host	Hantaviruses species	Rodent or Insectivore Host
Amur	*Apodemus peninsulae* (Korean wood mouse)	Jemez Springs	*Sorex monticolus* (dusky shrew)
Andes	*Oligoryzomys longicaudatus* (long-tailed pygmy rice rat)	Juquitiba	*Oligoryzomys nigripes* (black-footed pygmy rice rat)
Laguna Negra (Andes virus variant)	*Calomys laucha* (no common name)	Khabarovsk	*Microtus fortis* (reed vole)
Lechiguanas (Andes virus variant)	*Oligoryzomys flavescens* (yellow pygmy rice rat)	Muleshoe	*Sigmodon hispidus* (cotton rat)
Oran (Andes virus variant)	*Oligoryzomys longicaudatus* (long-tailed colilargo)	Prospect Hill	*Microtus pennsylvanicus* (meadow vole)
Rio Mamore (Andes virus variant)	*Oligoryzomys microtis* (small-eared pygmy rice rat)	Puumala	*Myodes glareolus* (bank vole)
Araraquara virus	*Bolomys lasiurus* (no common name)	Rio Segundo	*Reithrodontomys mexicanus* (Mexican harvest mouse)
Ash River	*Sorex cinereus* (masked shrew)	Saaremaa (or DOBV-Aa)	Apodemus agrarius (striped field mouse)
Bayou	*Oryzomys palustris* (rice rat)	Seewis	Sorex araneus (Eurasian common shrew)
Black Creek Canal	*Sigmodon hispidus* (cotton rat)	Seoul	*Rattus norvegicus* (Norway rat); *Rattus rattus* (black rat)
Bloodland Lake	*Microtus ochrogaster* (prairie vole)	Sin Nombre	*Peromyscus maniculatus* (deer mouse)
Camp Ripley	*Blarina brevicauda* (northern short-tailed shrew)	Monongahela (Sin Nombre virus variant)	*Peromyscus maniculatus* (deer mouse)
Choclo	*Oligoryzomys fulvescens* (fulvous pygmy rice rat)	New York (Sin Nombre virus variant)	*Peromyscus maniculatus* (deer mouse); *P. leucopus* (white-footed mouse)
Dobrava	*Apodemus flavicollis* (yellow-necked field mouse)	Soochong	*Apodemus peninsulae* (no common name)

(*Continued*)

Table 3.3.2 (*Continued*)

Hantaviruses species	Rodent or Insectivore Host	Hantaviruses species	Rodent or Insectivore Host
El Moro Canyon	*Reithrodontomys megalotis* (Western harvest mouse)	Tanganya	*Crocidura theresae* (Therese shrew)
Hantaan	*Apodemus agrarius* (striped field mouse)	Thailand	*Bandicota indica* (bandicoot rat)
Hu39694	*Oligoryzomys flavescens* (?) (yellow pygmy rice rat)	Thottapalayam	*Suncus murinus* (musk shrew)
Isla Vista	*Microtus californicus* (California vole)	Topografov	*Lemmus sibiricus* (Siberian lemming)
		Tula	*Microtus arvalis* (European common vole)

For example, rodents' host sharing by Tula virus (TULV) (Hantavirus subgroup) was recently shown in central Europe to be much more extensive than thought before, demonstrating that TULV represents a promiscuous hantavirus with a large panel of susceptible hosts, suggesting an alternative evolution mode other than a strict co-evolution (Schmidt-Chanasit *et al.*, 2010; Plyusnin *et al.*, 1994).

The HPS rodent hosts are in general not associated with urban environments, though some species such as deer mouse (*Peromyscus maniculatus*) and white-footed mouse (*Peromyscus leucopus*) may be found in proximity to human habitation in rural and suburban areas. Direct contact of humans with rodents' excreta or by unpredicted bite can transmit the virus (Schultze *et al.*, 2002; Yanagihara *et al.*, 1984; Torres-Pérez *et al.*, 2010) and is possibly linked to certain seasons (spring-early summer) like the Sin Nombre virus in Montana rangeland (grassland and shrub-steppe habitats) close to peridomestic environments (Waltee *et al.*, 2009). Cantoni *et al.* (2001) studying hantaviruses seroprevalence of rodents in southern Argentina according to seasonal variation found that summer had the highest prevalence (59.3%) compared to spring and winter (13.7% and 12.4%, respectively) supporting the Montana (USA) results (Schönrich *et al.*, 2008).

Another South American study analyzed the spatial and temporal distribution of Hantavirus Pulmonary Syndrome (HPS) cases from 1998 to 2001 in the Buenos Aires Province, Argentina. Their results showed that the spatiotemporal interaction appears to be strongly related to seasonality and the presence of particular ecological conditions. The main explanatory variables for HPS distribution cases were human population, the rodent *Oxymcterus rufus*' allocation and evapotranspiration. The last two variables are possibly indicators of favorable ecological conditions for the reservoirs as well for virus survival in environment (Busch *et al.*, 2004).

Another disease caused by Hantaviruses is hemorrhagic fever with renal syndrome (HFRS) mainly by the Hantaan, Puumala, Dobrava and Seoul viruses (Papa *et al.*, 2001). Even though viruses are present in the blood and urine of HFRS patients, no common person-to-person transmission has been ruled in cases of HPS in North America or HFRS in Eurasia (Woods *et al.*, 2009; Kolobukhina *et al.*, 1998). The only case of person-to-person transmission has been reported during an outbreak of Andes virus in South America (Enria *et al.*, 1996; Padula *et al.*, 1998; Pinna *et al.* 2004). This transmission pathway was recently reinforced by a brief study in which the Andes virus was shown to be more resistant to

saliva antiviral effect than to other genus subgroups, such as Hantaan and Puumala viruses (Hardestam *et al.*, 2009).

Linard *et al.* (2007) studied Puumala virus (PUUV) prevalence in Belgium in relation to landscape attributes and host abundance. A linear regression was found related to landscape configuration (e.g., deciduous forests of varying size, shape, and proximity to/connectivity with other forest patches). Other important attributes mentioned by these authors were: surrounding land cover types linked to patches connectivity and thus rodents' abundance and prevalence (e.g., the land cover matrix around forest patches), climate conditions (previous year temperature) and soil characteristics (moisture, chemistry). Their results suggest that low winter temperatures and high soil moisture favor the survival of this virus in the environment and thus the indirect transmission to bank voles (as reservoir) and humans (as dead-end hosts). Kallio *et al.* (2006) showed experimentally that wild-type PUUV excreted by experimentally infected donor bank voles can be transmitted indirectly between rodents through contaminated beddings, and is able to maintain its infectivity to recipient voles at room temperature for 12–15 days, demonstrating that hantavirus transmission does not require direct contact between rodents, or between rodents and humans. The indirect transmission of PUUV through contaminated environment can take place among rodents for a prolonged period of time. Finally, as already pointed out by others, besides the above described factors, Puumala virus prevalence among local rodents (bank voles) depends on their abundance throughout the season (Pettersson *et al.*, 2008). In central Paraguayan chaco, the vesper mouse (*Calomys laucha*), the main reservoir of another hantavirus subgroup Laguna Negra virus was reported as common in agricultural habitats and uncommon in the native forest habitat, and its population densities were greater during the dry season months and declined during the wet season (Yahnke *et al.*, 2001; Raboni *et al.*, 2009). Similar results were obtained in a pilot study that analyzed mosquito distribution along a 10-km transect of a West African rainforest area, which included primary forest, secondary forest, plantations, and human settlements (Junglen *et al.* 2009). The results of this pilot study pointed to differences in mosquito and virus prevalence in disturbed and undisturbed habitats, where disturbed habitats constitute basically human interference in pristine sites. In another geographical area (western and eastern Paraguay), a researcher reported Hantavirus clades formation according to rodents' reservoirs and land cover. A certain new viral clade was found in areas experiencing rapid land cover fragmentation and change within the interior Atlantic forest eco-regions of Paraguay (Chu *et al.*, 2006). Here is the place to mention the Russian parasitologist and geographer E.N. Pavlovsky, who through his pioneering work, recognized the significance of landscape in the transmission of infectious diseases (Pavlovsky, 1966). Pavlovsky showed that the distribution of disease could be associated to key biotic and abiotic environmental elements, and that the landscape could be used to recognize and map the spatial and temporal patterns of disease risk. Pavlovsky also found that landscape change could be a key factor in the occurrence of disease. His research in the former USSR (of more than 30 years) coincided with a period of intensive land cover change in the country motivated by agricultural intensification and expansion. Pavlovsky also coined the term "landscape epidemiology", useful to understand the transmission dynamics of vector borne diseases, particularly those transmitted by fleas, ticks and mosquitoes. The process of Hantavirus transfer from the original natural host to an incidental host is coupled with changes in its ecology, environment and other factors that may impact the pathology of the virus. It should be remembered that seropositivity for Hantaviruses was detected in a large variety of mammals beside the main rodent hosts (Leighton *et al.*, 2001; Nowotny, 1994). It is very possible that a second round of infection may take place when rodents come in contact with those mammals' excreta or carcasses (Amundson *et al.*, 1985). Humans as the main incidental host reveal a modified evolutionary pressure resulting in a much more dangerous viral form (with lethal results). Therefore more studies on the influence of non-natural hosts on the ecology of hantaviruses are needed

in order to get a clear idea of the directions that hantavirus evolution could pursue. Other pending questions related to the ecology of these viruses are: How do environmental factors, population density, and interspecific competition influence the susceptibility to and outcome of hantavirus infection in reservoir hosts? What are the limiting factors for a spillover infection and are there indications of hantavirus-associated disease in spillover-infected animals? Why do New World hantaviruses and Old World hantaviruses cause HCPS and HFRS or NE, respectively? And for example, what makes PHV non-pathogenic for humans? (Zeier *et al.*, 2005).

In Finland, Puumala virus prevalence was also found to be clearly related to certain geographic regions. The southern part that has fewer forested areas and increased urban space (therefore lower rodent abundance) was found to have the lowest prevalence. The authors mentioned that residents from these areas possibly became infected as Finns vacation in forest and lakeside cottages located in the north (Finnish Lapland, mainly during summer and autumn) where vole' population exceeds that in the southern region (Rose *et al.*, 2003). A recent report from Germany described a steep rise in Hantavirus infections especially in the southern part known as an endemic region for Puumala virus amplified by the bank vole (*Myodes glareolus*) the main animal reservoir, through increased population density (Faber *et al.* 2010).

Transmission from rodent to rodent is believed to occur primarily after weaning and through physical contact, perhaps through aggressive behavior, such as fighting. Studies of the genomic sequences indicate that the virus has probably evolved concurrently with its rodent host over a long period of time. The possible horizontal transmission was described by several scenarios, all linked to rodents' behavior, their nest and environmental factors, as presented in Figure 3.3.2 (Calisher *et al.*, 2009).
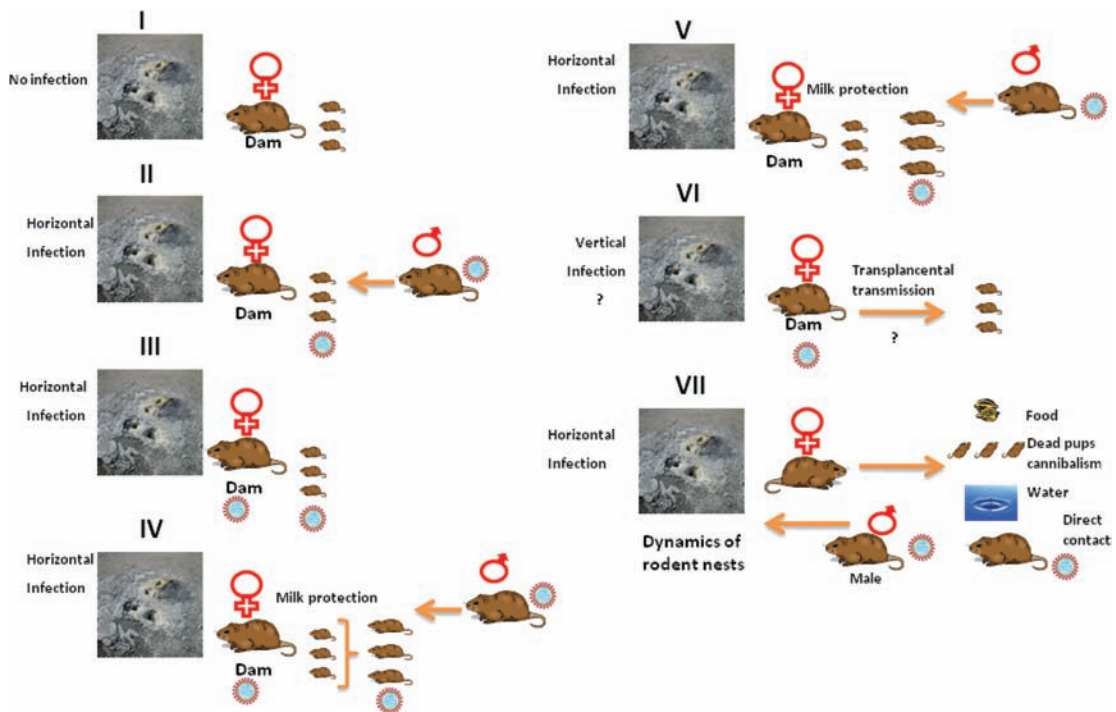

Figure 3.3.2. Different possible scenarios of rodents infection with Hantavirus

Childs *et al.* (1995) conducted a case-control study in southwestern United States, where an outbreak of hantavirus pulmonary syndrome (HPS) took place. The only significant environmental factor that distinguished between cases and controls was elevated densities of small rodents in the case area (mainly heavier males), while infected rodents' prevalence was similar in both cases and controls. Transmission of the virus between rodents may occur through biting or allogrooming (saliva contains high numbers of virions) or via excreta and urine release (aerosolized during fighting) (Hinson *et al.*, 2004). Laboratory studies with *Apodemus* infected with Hantaan virus (HTN) revealed shedding of this virus up to one year (Lee *et al.*, 1981). Similar results were strengthened by data presented in a longitudinal study of the Bayou virus (BAYV) (subgroup B of Genus Hantavirus) showing excretion of viruses in saliva and excretion for at least 3 months (McIntyre *et al.*, 2005). A third way of infection was suggested by these authors through environmental contamination with excreta by high rodent density in a certain geographical area, based on high seroprevalence detected in these high density areas. In the case of BAYV, it was shown that this virus was spatially patchy and temporally dynamic, linked to host abundance (*Oryzomys palustris*- rice rat) in its specific microhabitat (coastal prairie) and the lack of peridomesticity of this rodent. Concerning environmental factors, Abuzeineh *et al.* (2007) reported that floods did not change significantly the rice rat population density (host of BAYV) in the coastal prairie locality of southeastern Texas.

Another piece of evidence related to the impact of rodent density on Bunyaviruses human infections was described by Andes hantaviruses (ANDV) (subgroup B of Genus Hantavirus) distribution in Chile, studied in three defined eco-regions (at a latitude span of nearly 4000 km), Mediterranean, Valdivian and North Patagonian rain forests, through host (*O. longicaudatus*) seroprevalence (Torres-Pérez *et al.*, 2010). The authors reported that the highest relative seroprevalence in *O. longicaudatus* (the rodent host) was detected in the Mediterranean region, and therefore people leaving in this area were at higher risk of contracting Hantavirus cardiopulmonary syndrome (HCPS). However, from epidemiological data, the southern regions (temperate forest regions: Valdivian and Patagonia) have more cases of HCPS. One of the key explanations, according to these authors, was that these regions, in spite of their scarcer human population (by absolute numbers), has a population that is mainly rural with much closer and prevalent contact with rodent hosts, which increases the likelihood of exposure to Andes virus. Padula *et al.* (2004) using caged seronegative rodents that were exposed to wild seropositive individuals by freely sharing the same cage or being separated by a wire mesh. These authors reported on efficient Andes virus transmission, suggesting that this particular hantavirus may be principally transmitted via saliva or saliva aerosols rather than via feces and urine.

The ecology of rodents in terms of density was interestingly explained by Gilg *et al.* (2003). These authors explained the density dynamics of collared lemming in the high-Arctic tundra in Greenland where there are no food and space limitations for the lemming population and only four main predator species are available (arctic fox, snowy owl, long-tailed skua and staot). A predator-prey model clearly predicted a 4 year periodicity in lemming population dynamics. It should be remembered that the arctic environment is still a pristine one with much less human interference; however, high temperatures can change drastically this rodent cyclic balance in relation to its virus (Lemming virus). An interesting finding was reported by Holsomback *et al.* (2009) in relation to BAYV in United States. Seroprevalence for this virus was detected in other rodent species besides *O. longicaudatus* (the common host) a phenomenon called by the authors "spillover hosts". Spillover locations differed significantly from all other capture locations in terms of percent water, shrub, and grass cover. This observation was suggested for use as a predictive indicator of BAYV spillover, as *O. longicaudatus* long-lasting, virus shedding, adult males may dominate the favored habitats thereby transmitting the virus intra- and interspecific in these ecological niches. In another study, seropositive adult males were found to move farther

(increasing their home ranges) and were larger in terms of mass and testes size in comparison to uninfected (seronegative) ones (McIntyre *et al.*, 2009).

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Chapter 3.4

Reoviruses

[COLTIVIRIDAE AND ORBIVIRIDAE]

Reoviridae is a family of non-enveloped spherical viruses, with icosahedral capsid symmetry built of an outer and inner protein shell, 60–80 nm in diameter, containing a segmented (ds) RNA as genetic material. This family is divided into two subfamilies: *Sedoreovirinae* (smooth capsid) and *Spinoreovirinae* (spiked capsid). The genus Coltiviridae belong to *Sedoreovirinae* while genus Orbiviridae belong to *Spinoreovirinae* (Attoui *et al.*, 2002). Both Coltivirus and Orbivirus are important zoonotic diseases transmitted by ticks (*Ixodes* spp.). Coltiviruses contain two major zoonotic viruses: Colorado tick fever virus (CTFV) and Eyach virus. CTFV is present in the Rocky Mountains, Colorado and western provinces of Canada with seasonal occurrence (spring and summer). The important reservoirs are: ground squirrels, western chipmunks, wood rats and deer mice. The vector of this virus is the hard–shell tick named *Dermacentor andersoni* that can be found at high elevations (between 1,500 to 3,000 m). The clinical symptoms are: fever (50% biphasic fever), headache, myalgias, photophobia, malaise, nausea, retro-orbital pain, hemorrhagic manifestation and rarely in children affected CNS (meningitis and encephalitis).

3.4.1 REOVIRUSES AND ENVIRONMENT

Colorado tick fever and Eyach viruses (genus Coltivirus) and Kemerovo and Lipovnik viruses (genus Orbivirus) are recognized as zoonotic viruses transmitted by ticks (Emmons, 1988). Direct contact with infected animals or tissue or via blood transfusion has been also documented (Randall *et al.*, 1975). Colorado tick fever virus (CTFV) is spread in North America along the west coast, while Eyach virus is geographically distributed in France, Germany, Netherlands, Czech Republic (Rehse-Kupper *et al.*, 1976; Chastel, 1998; Chastel *et al.*, 1993; Chastel *et al.*, 1984) and Kemorovo and Lipovnik viruses in Russia, Czech Republic, Slovakia, Austria, Scotland, Macquarie Island (Australia), Peru, Morocco, (Nuttall *et al.*, 1981; Doherty *et al.*, 1975; Clifford *et al.*, 1980; Hubalek *et al.*, 1986; Chastel *et al.*, 1982, 1993).

Hubalek *et al.* (1986) studied seroprevalence against Lipovnik virus humans and domestic animals (goats, sheep and cattle) in the Roznava district (formerly Czechoslovakia). 30% of the inhabitants were

positive for antibodies against this virus, while 88% of goats, 55% of sheep and 45% of cattle. They also looked for the ectoparasites (*Ixodes ricinus*, *Dermacentor marginatus*, *Haemaphysalis punctata* and the louse-flies *Melophagus ovinus*) for infection with this virus. Only an *Ixodes ricinus* male and female from cattle were found to harbor two virus strains antigenically related to Lipovnik and Tribec viruses that belong to the Kemerovo viruses group. Chastel *et al.* (1982) also looked for seroprevalence in small mammals (rodents and insectivors) in Morocco for arboviruses. Using a complement fixation test, they found an *Apodemous sylvaticus* positive for a Kemerovo group virus. Eyach viruses or closely related viruses had been isolated in Germany and France from *Ixodes ricinus* and *Ixodes ventalloi*, respectively.

From the gender point of view, Colorado tick fever is more associated with human males (age 20 to 40) a fact that reflects leisure activities, such as fishing, hunting, lumbering and tracking. The tick vector (*D. andersoni*) has seasonal occurrence (from late February or early March to early November, but mostly May through July). The host-seeking activity in endemic areas depends on region, climate, cyclical vector and host abundance (human and animals activity). *D.andersoni* is the main virus reservoir through transstadial (without proved transovarial) transmission and survival for up to three years. From human case records, a sharp endemic area can be drawn for this tick vector from British Columbia and Alberta toward south along the Pacific coast states to New Mexico. The variable vegetation (e.g., sagebrush-juniper-pine containing moderate shrubs, herbs and grass cover) near streams and rocky hillsides (mainly on south-facing slopes) support both the vector and the host encounter and virus transmission and spread. Involvement of porcupines (*Erethizon dorsatum*) as another important host of Colorado tick fever in Rocky Mountain National Park was also studied (McLean *et al.*, 1993). Among 20 animals not one carried CTFV but 17 had neutralizing antibody against it, revealing exposure to this virus in this ecosystem. Another important question related to climate is: What is happening with the virus in hibernating infected hosts? Experimentally, viremia had been shown to persists up to 160 days in squirrels (*S. lateralis*) that hibernate for 4 months. Viremia persisted in these squirrels up to 47 days after their arousal (Emmons, 1966). Virus persistence in dormant host and/or nymphal and adult ticks is a highly possible way to survive through winter climate.

An interesting study has been carried out on the wood tick (*Dermacentor andersoni*) and its host-seeking microhabitats in two canyons: Poudre and Rocky Mountain (National Park) of Larimer county, CO, USA (Eisen *et al.*, 2008). These authors reported that a certain vegetation (big sagebrush- *Artemisia tridentate*) can serve as a general indicator of high risk exposure to *D. andersoni* ticks, both sharing the same climate. Open grass, bordering rocks and shrubs had the highest host-seeking *D. andersoni* adult' number. Sampling sites in Rocky Mountain National Park in comparison to Poudre Canyon were characterized by more intense usage by elks (*Cervus elaphus*) and reduced brush coverage, and size and abundance of adult host-seeking ticks. These authors, speculated that due to the tremendous elk population increase in Rocky Mountain National Park over several decades, an ecological cascade evolved, i.e. overgrazing, rodent population suppression, tick abundance drop and therefore reduced risk of human exposure. This phenomenon is supported by another study on *Theileria parva* (a parasitic protozoan that causes East Coast fever-theileriosis in cattle, an important economic disease of cattle in Africa) carried by *R. appendiculatus* (the tick vector) in Zimbabwe (Norval *et al.*, 1985). The authors reported that overgrazing resulted in a non-uniform distribution of ticks: high prevalence in commercial farming areas and very low in most overgrazed communal farming areas (Figure 3.4.1).

Clifford *et al.* (1980) redescribed the tick *Ornithodoros* (*Alectorobius*) *amblus* found on the Pacific coast and on offshore islands of Peru using as hosts several bird species, besides humans. This tick was reported to harbor Huacho virus (Reoviridae, genus Orbivirus, Kemerovo serogroup), possibly transmitting this virus to its hosts or even to spiders and lizards that prey on it. An observed side effect by the authors was that high

density population of ticks can cause breeding birds to desert their nests, resulting in damage to Peruvian guano industry.
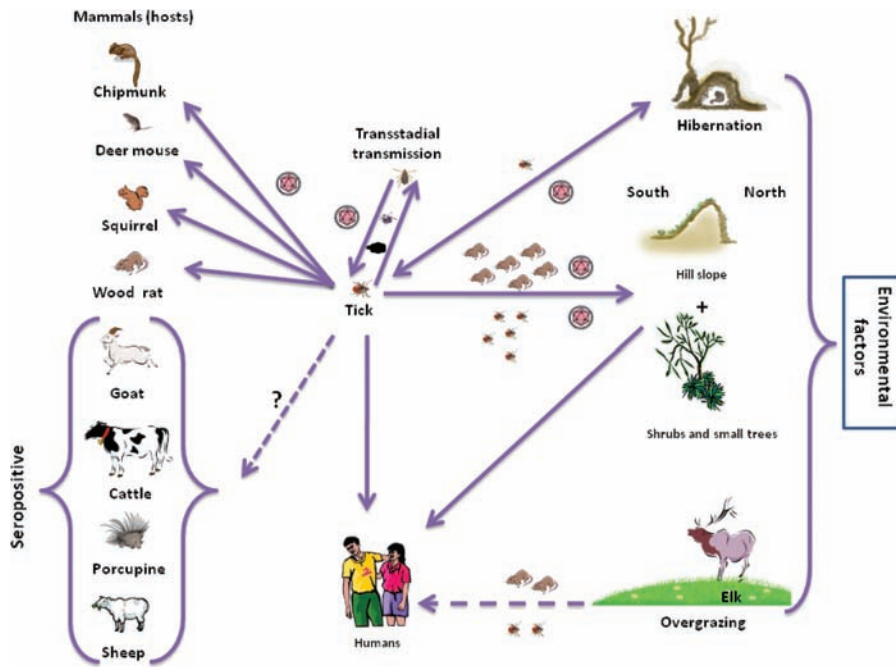

Figure 3.4.1. Host, transmission and environmental aspects of zoonotic reoviruses

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Chapter 3.5

Arenavirus

[FAMILY ARENAVIRIDAE, GENUS ARENAVIRUS]

The arenaviriuses belong to the family Arenaviridae that causes chronic infections in rodents in Africa, America, Europe and parts of Asia (Table 3.5.1). The family consists of a single genus (Arenavirus) and of fifteen species known to infect animals, five causing diseases in human (CDC[a], 2005). The virions are spherical to pleomorphic and have an approximate size of between 50 to 300 nm (average 110–130 nm). The genome (11 kb in size) consists of two linear(ss) RNA segments, each packed in a circular closed nuclear capsid and surrounded by a lipid envelope with embedded glycoprotein spikes.

Six arenaviruses are known to cause severe disease in humans (Table 3.5.1). LCM virus is an agent of acute central nervous system disease (Jahrling & Peters, 1992) and congenital malformations (Barton *et al.*, 1993) while Lassa, Junin, Machupo, Guanarito, and Sabiá viruses cause hemorrhagic fever in humans. In relation to Sabiá virus, up to now, only one known case of naturally acquired Sabiá virus infection has been documented (Gonzalez *et al.*, 1990), while additional cases were accidental exposure of laboratory workers to this virus (Ryder *et al.*, 1995; Barry *et al.*, 1995, Gandsman *et al.*, 1997), but in these cases the transmission, the reservoir host and/or vector are still unknown (Gonzalez *et al.*, 1996).

3.5.1 ARENAVIRUSES AND ENVIRONMENT

In general the natural life cycle of Arenaviruses includes rodents as reservoir hosts and vectors who get chronically infected without showing any sign of illness. The virus is found in the saliva, urine, and feces of infected rodents, which remain infectious for life. Pet rodents (hamster, guinea pig, etc.) can contract infection from wild rodents that are used for pet feeding (e.g., snakes) or those intruding in human dwellings in autumn or winter (Williams & Barker, 2001). In this case, virus transmission of the accidental individual host occurs through contaminated aerosols (rodent excreta, urine, saliva, blood) (de Manzione *et al.*, 1998; Carballal *et al.*, 1988), ingestion of contaminated food or via a skin lesion or a direct bite. An infected individual can pass viral infection to others (person to person transmission) via aerosol (coughing) or from direct contact with infected human body fluids (CDC[a], 2005). Infection may be also transmitted to patients who receive an organ transplant from an infected donor and in laboratory

workers during virus handling (CDC[b], 2005). The predominant groups of people who get infected are somehow involved in agricultural activities (Figure 3.5.1). Farmers get infected during the harvest season, when they are exposed to the aerosolized virus from harvesting machinery (CFSPH, 2010).

Table 3.5.1 Some features of Arenaviruses.

Virus	Clinical Manifestation	Host	Distribution
Lymphocytic choriomeningitis (LCM)	meningitis or meningoencephalitis	Rodent (mice; *Mus musculus*)	World wide
Lassa	hemorrhagic fever	Rodent (rats; (*Mastomys natalensis*)	Africa
Junin	Argentine hemorrhagic fever (AHF)	Rodent (mice; *Calomys musculinus*)	Argentina
Machupo	Bolivian hemorrhagic fever (BHF)	Rodent (mice; *Calomys callosus*)	Bolivia
Guanarito	Venezuelan hemorrhagic fever (VHF)	Rodent (mice; *Zygodontomys brevicauda)*	Venezuela
Sabiá	hemorrhagic fever	not known	Brazil
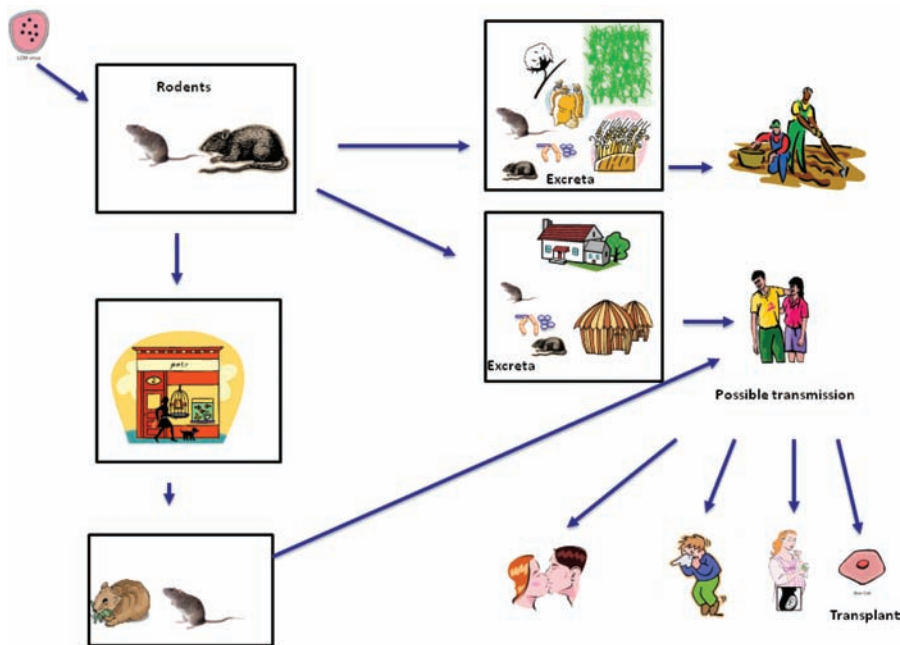
Figure 3.5.1. Arenaviruses transmission paths

In pregnant women, Lymphocytic choriomenningitis virus infection may result in pregnancy loss or cause severe birth defects in newborns (Barton *et al.*, 2002; Greenhow & Weintrub, 2003; CDC[c], 2005).

As a result of arenaviruses' high infectivity and lethality via the aerosol route, these viruses are potential biological agents for biological warfare and terrorism (Franz *et al.*, 2001).

The high mutation rates of the low-fidelity viral RNA-dependent RNA polymerase (RdRp) of Arenaviruses generate the existence of quasispecies, for example among lymphocytic choriomeningitis virus (LCMV) strains. The genomic heterogeneity is an environmental advantage allowing these viruses to adapt rapidly to changing environments to survive selective pressure (Sevilla & de la Torre, 2006). Such a characteristic is aerosol stability (particularly at low relative humidity-30% RH) and the ability to infect guinea pigs and monkeys via the respiratory route at considerable distances at a relatively low infectious dose (ID_{50}-15 PFU) (Stephenson *et al.*, 1984).

In addition, one of the primary causes of hemorrhagic fever outbreaks (e.g. Lassa virus) is ecological disruption as a result of human activities. Continuous world population expansion does perturb ecosystems, facilitating closer contact between infected carrier animals (i.e. rodents) and humans. Another source of infection is poor medical hygiene hospitals (mainly in poor areas) that possibly transmit viruses nosocomially (Le Guenno, 1997).

Fichet-Calvet and Rogers (2009) carried out a spatial analysis (three models) on Lassa fever virus data based on human cases, infected rodent hosts and environmental conditions (temperature, rainfall and vegetation) for a period of 42 years. According to one of the models, Lassa fever outbreaks occurred mainly in zones receiving annual rainfall of 1,500 to 3,000 mm. Rainfall but to a much lesser extent temperature were the most significant variables in other models, while vegetation and altitude were insignificant according to these models. Importantly, according to their models, Lassa fever virus covered ~80% of Sierra Leone and Liberia, 50% of Guinea, 40% of Nigeria, 30% of Ivory Coast, Togo, Benin and only 10% of Ghana.

In six geographical areas of Nakhon Pathom Province of Thailand, rodents' seroprevalence for Arenavirus has been examined. The overall prevalence was 13.3% with higher prevalences in *Bandicota savilei* (35.7%) and *Rattus norvegicus* (31.5%). No significant seroprevalence diversity was reported regarding ecology, behavior and rodents' gender but geographical variability did show a significant correlation (Nitatpattana *et al.*, 2000).

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Chapter 3.6

Filoviridae

[ORDER *MONONEGAVIRALES*, FAMILY *FILOVIRIDAE*, GENUS *MARBURGVIRUS*, *EBOLAVIRUS*]

Filoviridae is a family of viruses with two genera, the Ebola virus and Marburg virus. *Filoviridae* are enveloped -(ss) RNA viruses with a genome size of 19.1 kb, hooded in a nucleocapsid. The virons have a filamentous or pleomorphic structure measuring about 80 nm in diameter and up to 1400 nm in length. *Ebolavirus* genus contains five species while *Marburgviruses* genus has only one species (Table 3.6.1). *Filoviridae* members are highly contagious, causing severe hemorrhagic fever with characteristic bleeding and high mortality rate; although outbreaks are sporadic, there is the potential for filoviruses to spread to other continents accidentally via air travel of infected individuals.

3.6.1 FILOVIRUSES AND ENVIRONMENT

Viruses of the *Filoviridae* family cause sporadic outbreaks of Ebola and Marburg Hemorrhagic Fever (HF) in Africa. Ebola HF as well as Marburg HF is a zoonotic disease transmitted accidentally by direct contact with infected live or dead humans or animals; however, the natural reservoir remains unknown. Regarding Marburg HF, only in the first simultaneous outbreak of this disease in Germany and Yugoslavia (now Serbia) could virus transmission be traced back to green monkeys (*Cercopithecus aethiops*) imported from Uganda for research purposes (Martini *et al.*, 1968). All incidents or outbreaks could not be connected to a primary transmission of the virus from a natural reservoir host.

Secondary transmission (monkey to human or human to human) may occur in the surrounding community and hospital staff without appropriate precautionary measures to prevent direct contact of skin with infected organs, and droplets of fluids (blood, semen, sputum) (Martini *et al.*, 1968; Ligon, 2005). Following cumulative evidence arising from different outbreaks, Towner *et al.* (2009) isolated in a recent research a Marburg virus from an Egyptian fruit bat (*Rousettus aegyptiacus*) living in Kitaka Cave, Uganda, thereby demonstrating that bats could be the natural reservoir of this virus. Additional indication, pointing at bats as a potential natural host, was the occurrence of several human Marburg HF incidents in miners or visitors in the vicinity of caves inhabited by fruit bats (Smith *et al.*, 1982, Johnson

Table 3.6.1 Some characteristics of zoonotic *Filoviridae*.

Genus	Species	Distribution	Secondary Host	Fatality rate in human
Marburg (MARV)	*Lake Victoria marburgvirus*	Uganda	Vervet Monkey, Human	25% (CDC, 2010)
Ebola (EBOV)	*Ivory Coast ebolavirus* (CIEBOV)	*Ivory Coast, Liberia*	Chimpanzees, Human	not lethal for humans
	Reston ebolavirus (REBOV)	Philippines	Macaque, Human	not infectious for humans
	Sudan ebolavirus (SEBOV)	Sudan	Human	54% (Wamala *et al.*, 2010)
	Zaire virus (ZEBOV)	Zaire	Fruit bats, Gorilla, Chimpanzee, Duiker, Human	90% (Sanchez *et al.*, 1995)
	Bundibugyo ebolavirus (BEBOV)	Uganda	unknown, Human	34% (Wamala *et al.*, 2010)
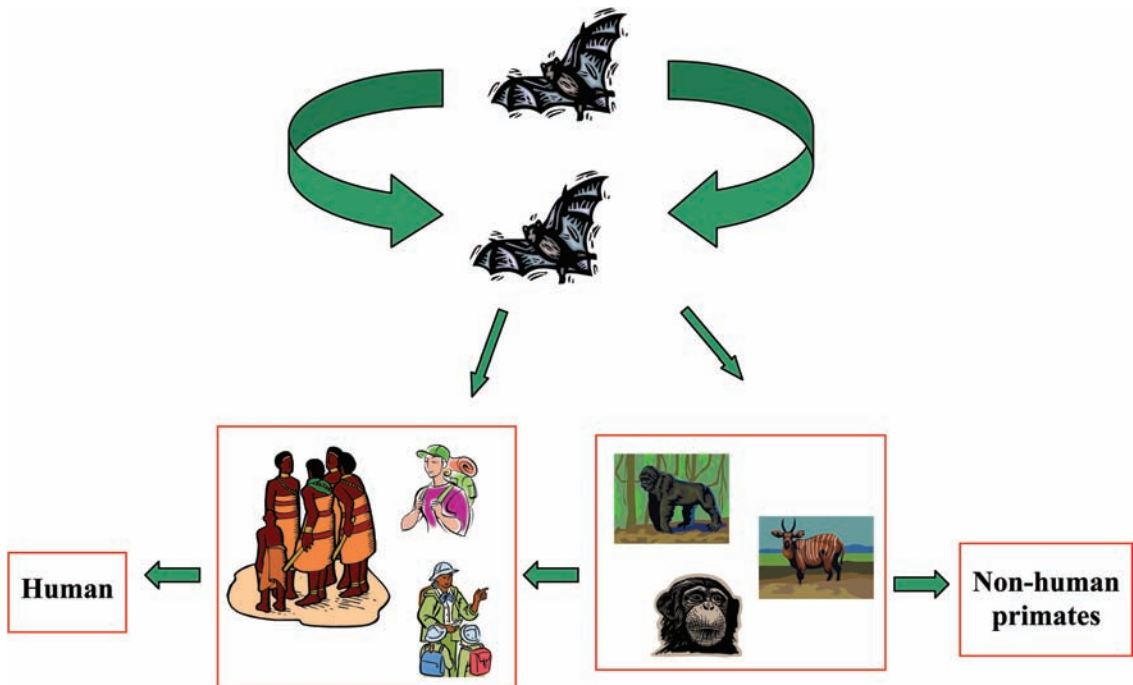
Figure 3.6.1. Filoviruses transmission pathways

et al., 1987, Bausch *et al.*, 2006). Over 70% of primary human infections are linked to mines or caves, implying that the natural reservoir host could be associated with these ecosystems (Towner *et al.*, 2006). Secondary infections were observed to be transmitted to people being in proximity to an initial infected person. Marburg and Ebola virus outbreaks are sporadic, with high fatality rates and unknown natural reservoir host. Recent investigations that proposed bats as a natural reservoir host used different evidence such as geographic distribution of viral variants and the connection between bats and other virus groups (Figure 3.6.1). Specific antibodies against Ebola virus have been identified in blood samples from different bat spp. in central Africa (Pourrut *et al.*, 2005, Swanepoel *et al.*, 1996, Peterson *et al.*, 2004*). The Ebola virus secondary transmission scenario is similar to that described for Marburg virus infections: index case usually connected to handling infected monkeys and further spread of the initial infection to caring family members or hospital staff (Pourrut *et al.*, 2005).

Thus far, Marburg and Ebola HF outbreaks originate from Africa. Ecological niche modeling of outbreaks and/or isolated cases has suggested that Ebola (HF) occurs in humid rain forests of Central and Western Africa while Marburg (HF) is related to drier and more open areas of these regions (Peterson *et al.*, 2004 & 2006).

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Chapter 3.7

Rhabdoviruses infections

[ORDER MONONEGAVIRALES, FAMILY RABDOVIRIDAE, GENUS LYSSAVIRUS, VESICULOVIRUS]

3.7.1 RABIES VIRUS

[Rabies virus-lyssavirus type 1]

Rabies virus is perhaps the most notorious zoonotic virus transmitted by animal' bite to humans and probably the most fatal one. Rabies distribution is worldwide with some exceptions where it has been annihilated or was never present due to geographical morphology: insular or peninsular countries (e.g., New Zealand, Australia, UK, Japan and Scandinavia) (Figure 3.7.1). Animal import and translocation may contribute to virus introduction into non-endemic areas (Anonymous, 2008). The virus belongs to the *Rhabdoviridae* family and *Lyssavirus* genus with a cylindrical morphology, enveloped and -(ss) RNA genome (~12 kb in size). The virus is neurotropic, i.e., via introduction of the virus into a muscle through an infected animal bite it will travel towards the brain through neuronal movement. Incubation of rabies can take between weeks and months while during this period the animal does not pose a risk as the virus is not excreted in saliva. When infectious particles are excreted in saliva direct contact (without bite) with skin lesions can cause rabies in humans. There are two paths for human infection: sylvatic and urban. In urban infections (mainly in developing countries), stray dogs and bats are the reservoirs and can transmit the virus. In sylvatic infections (mainly in developed countries) the main reservoirs are wild animals (e.g., bats, foxes, raccoons, jackals, wolves, stoats, etc.) as primary reservoirs and domestic animals (e.g., pet dogs and cats; sheep, horses, cattle and pigs) as secondary reservoirs for the final host, humans (Lei *et al.*, 2009). Clinical manifestations consist of nausea, vomiting, headaches, paresthesia, bite site pain and two phases: furious and paralytic with final fatal outcome.

3.7.1.1 Rabies and environment

At the interface between animals and humans, emerging zoonoses can be classified as follows: Type 1) from wild animals to humans (e.g., Hanta, rabies viruses); Type 2) from wild animals to humans with further

human-to-human transmission (AIDS virus); Type 3) from wild animals to domestic animals to humans (Avian flu, rabies viruses) and Type 4) from wild animals to domestic animals to humans, with further human-to-human transmission (severe acute respiratory syndrome, SARS-see chapter 3.13) (Kahn *et al.*, 2009). Rabies virus can be spread through two main paths: directly from sylvatic animals (Type 1) and from sylvatic through domestic animals (Type 3) (Brown, 2011). Based on the literature it can be stated that bats are the most ubiquitous animal carrying the rabies virus, including in countries defined as free of canid rabies (Australia, Japan, UK, Ireland, etc.), thereby posting a future potential of reemergence! Cunha *et al.* (2006) surveyed bats from Sao Paulo state, Brazil and reported a prevalence range from 14.3 to 33.7%, consequently raising the possibility of a high risk of infection of animals and humans related to their close environments. Sulkin *et al.* (1960) have shown an interesting impact of environmental temperature on bats (two species) experimentally infected with virulent rabies (one strain from a fatal human case). When these bats had been experimentally induced to hibernate, only little or no viral multiplication occurred. Transfer to a warmer room and hibernation stoppage caused the "cold storage" rabies to multiply and consequently appear at detectable levels in various tissues. These authors also showed that bat rabies virus in hibernating animals following a period of latency in those dormant animals, when activated by temperature, may reach the salivary gland more rapidly, with greater frequency, and attain higher concentrations than in animals which have not experienced a period of hibernation.
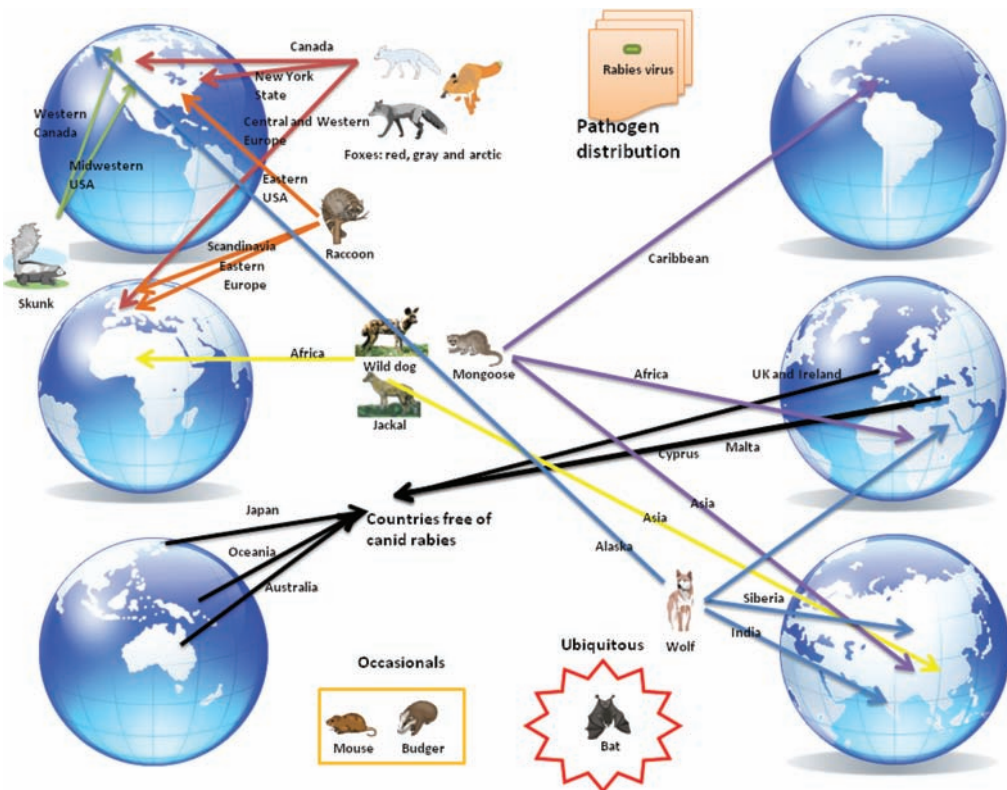

Figure 3.7.1. Global distribution of rabies virus and wildlife contributors

In relation to environment, an interesting study was carried out on rabies infected striped skunks from central Great Plains of North America and host ecology, landscape features and two sympatric rabies strains (Barton *et al.*, 2010). In this area, two sympatric rabies viruses shared a common host, striped skunk, circulating in this area. The two strains involved were South Central Skunk rabies strain (SCSK) that evolved from a North American bat virus and North Central Skunk rabies strain (NSCK) closely related to global dog rabies virus. The SCSK strain was found to be more infective with intense purifying selection of N gene (purifying selection refers to selection against non-synonymous substitutions at the DNA level), both characteristics suggesting a more transmissible strain than the NSCK strain. Being sympatric strains, the more transmissible SCSK strain seems to be more indicative of epizoosis while the NSCK of enzoosis. In relation to landscape (crossing natural barriers such as rivers) the short latency of the SCSK strain is partially restricted by landscape heterogeneity, but once it crosses the barrier the infection is more efficient among many hosts.

In relation to landscape, Hubbard (1985), following a raccoon's rabies outbreak in Loudoun County, Virginia, studied the epidemiology of the virus spread. The author suggested based on his results that reduction in favorable habitats (less rainfall) and influx of displaced raccoons caused physiological stress (intra-specific competition, hormonal and neurological changes) increasing these animals availability to rabies infection. Jones *et al.* (2003) assessed land use and demographic data to predict discriminatively between different close regions with epizootics of rabies among raccoons. They analyzed rabies-positive raccoon data from Maryland, Virginia and Pennsylvania versus public environmental and demographic database sources. These authors found the following parameters significantly correlated to large epizootics (expressed as odds ratio): agricultural land use (OR = 9.1), high water coverage combined with low density of human population (OR = 8.8) and low water coverage with high human population density (OR = 11.7). Explanations for these results are simply based on human-animal encounters and environmental factors involved, i.e., agricultural land use in comparison with forested area increases the contact between the animals themselves (in this case rabid raccoons) and other animals, as well with humans. Close proximity to water has been also shown to be a critical factor in determining the quality of raccoon habitat with marshes, riparian areas, and other semi-aquatic environments supporting exceptionally high densities and linked to lower human population density.

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3.7.3 VESICULAR STOMATITIS

3.7.3.1 [Vesicular Stomatitis Virus-VSV, *Vesiculovirus* genus]

Vesicular stomatitis virus (VSV), which infects mammals and insects, belongs to the Rabdoviridae family. Among the 20 serotypes identified, four are human pathogens: Indiana, New Jersey, Chandipura and Piry. VSV is highly contagious in horses, mules, cattle and pigs (lower frequency), but many other wild animals might be infected including sand flies (*Phlebotomus*). In sand flies, vertical transmission (transovarial) has been confirmed. VSV is also enveloped with a rod shape coat that contains -(ss) RNA with a size of ~11 Kb. VSV distribution has been reported in North and South America, Africa and Asia but absent is Central Europe. Transmission mode and reservoir agents are still unknown, but laboratory infections have been reported possibly linked to increased water persistence that had already been confirmed (Mahnel *et al.*, 1977). In cattle, transmission seems to occur through milking (automatic or manual), poor hygienic conditions, the use of coarse roughage and hard-pelleted concentrates, the presence of uneaten feed (possibly contaminated with virus-laden saliva), increased interpen movement of cows and poor ground surface conditions, all poor management factors associated with clinical signs (Hansen *et al.*, 1985). Sand fly seems to be one of the major transmission agents (therefore VSV is also called arbovirus) through persistence without cytolysis. The ability of VSV to infect arthropods as well mammals is an important feature, representing its quintessential ability to infect and perform well in multiple hosts (Novella *et al.*, 2007). On the positive side, VSV is used as an expression vector, a basis for recombinant vaccines and recently VSV has shown oncolytic activity preferentially killing tumor cells and reducing tumors size and spread (melanoma, lung cancer, colon cancer and certain brain tumors in laboratory models) (Özduman *et al.*, 2008; Stojdl *et al.*, 2000). Clinical manifestations are mostly subclinical but influenza-like symptoms may develop: high fever, malaise, headache, myalgias, arthralgia, retrostenal pain, nausea, eye aches with photophobia, rarely mucosal vesicles and in some rare cases hemorrhagic fever.

3.7.3.2 VSV and environment

In recent years, it became increasingly clear that VSV is a vector-borne disease transmitted mainly by sand flies but also by other hematophagous insects such as black fly (Mead *et al.*, 2009). Mead *et al.* (2004) using VSVJV (Vesicular stomatitis New Jersey virus) studied the transmission mode of these viruses by black fly. Their experimental results suggested that black flies might be infected with VSVJV via two paths connected to blood meal: 1) direct blood feed from vesicular lesions of an infected animal (in their case pigs) and 2) by cofeeding with other infected insects on an unexposed host (Mead *et al.*, 2000). This report was the first to show that insects as vectors could become infected while feeding on infected hosts that did not express viremia. Their findings demonstrated "that during epidemics, VSNJV can circulate between livestock and black flies, and possibly other hematophagous insects, in the absence of viremic livestock hosts" (Mead *et al.*, 2009; Lord and Tabachnick, 2002) (Figure 3.7.2.1). Recently, Smith *et al.* (2009) using domestic swine, have been able to prove that VSV arthropod transmission may occur although via mechanical transmission of and not only by black fly hematophagous behavior. An important factor reported almost 40 years ago, to which much less attention was paid was VSV's stability in drinking and

surface water (Mahnel *et al.*, 1977). At temperatures of 9 to 15°C, VSV survived for 20 to 50 days in these waters, with survival decreasing at higher temperatures.
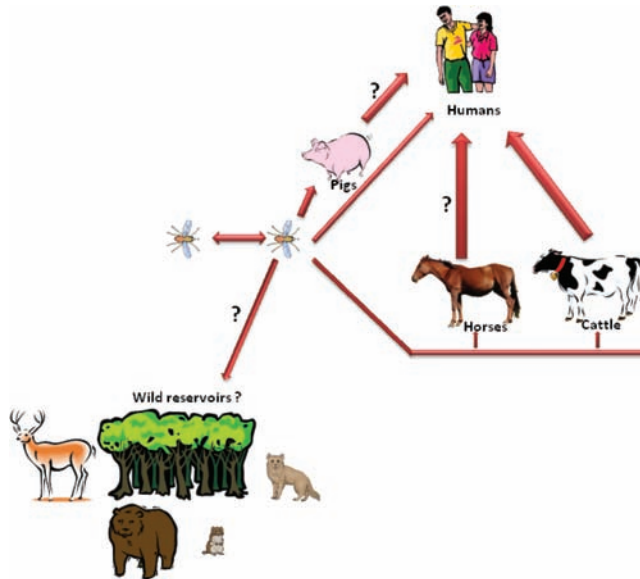

Figure 3.7.2.1. Vesicular stomatitis viruses (VSV) established and prospective infectious cycles. (Adapted from Rodriguez, 2002; question marks represent possible infective sources and reservoirs of different virus lineages also related to ecological niches)

McCluskey *et al.* (2003) linked climatic factors to VSV outbreaks in New Mexico, USA. Using discriminant analysis, these authors showed that increased precipitations from two months to one year previous to VSV cases are a good indicator. It seems that, like other arthropod-borne diseases, VSV spread by insects (black and sand flies) depends on temperature, precipitation and winds for its reproduction and growth (Sellers, 1980).

In another geographical area (Central America and Panama), not far away from New Mexico, Clein (1976) studied the ecological factors related to VSV in rural areas. The author reported that VSV-New Jersey seroconversion was highest "for persons living at elevations between 350 and 649 meters, with relatively open, dry vegetation and distinct seasonal alternation of dry and moist (not wet) ground conditions", while that of VSV-Indiana was highest in moist environments with dense tree cover (tropical rain forest habitats). These results suggest that VSV-New Jersey and VSV-Indiana have similar but not identical maintenance and transmission cycles (possibly due to vector developmental requirements). These results were further substantiated by a study in which VSV-New Jersey strains found in the two regions (premontane tropical moist forest and tropical dry forest) were shown to have distinct genotypes. The discovered evolutionary pattern suggested that VSV genetic divergence represents a selective adaptation to new ecological zones of this virus (Rodriguez *et al.*, 1996). In the USA, distinct viral lineages occur in the southwestern and southeastern parts of the country (Rodriguez, 2002). The southwestern lineage seems to be closely related to viruses maintained in endemic areas of Mexico having a sporadic appearance during the last 70 years according to surveys. In comparison to

VSV's specific ecological areas (e.g., Central America) the southwestern lineage does not have a stable endemic cycle as concluded from phylogenetic, geographical and temporal distribution of outbreaks data. It can be speculated that in such cases a wildlife reservoir exists (still undetected) that contributes to this pattern (Jimenez *et al.*, 2000).

Vanleeuwen *et al.* (1995) studied cattle VSV (mainly Vesicular stomatitis New Jersey virus) prevalence in Costa Rica. They reported that VSV affected cattle were in general older and in lactation, but without predisposing diseases. Poultry presence, longer calving interval, presence of sand flies and proximity to forest land were the most significant ones related to VSV prediction.

Among other peculiarities of VSV, Bussereau (1973) reported that VSV infection of *Drosophila melanogaster* (the fruit fly) induces CO_2 sensitivity (hypercapnia had paralyzed these flies), although some temperature recovery had been observed. What the environmental impact of this phenomenon is remains unclear as additional reports on different flies (sand or black) have not been yet reported.

Finally, Novella *et al.* (1995), experimenting with a wild-type strain of VSV grown continuously in sand fly tissue culture (for 10 months of continuous passages), showed that this virus increased its replicative fitness for arthropod cells but decreased its fitness for mammalian tissue cultures. However, a single passage of the arthropod-adapted virus in mammal cell culture (e.g., BHK-21 cells) at 37°C restored its fitness to near neutrality, as well its mouse neurovirulence. These results clearly demonstrated the enormous capacity of VSV to adapt to changing selective environments.

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Chapter 3.8

Paramyxovirus

[ORDER *MONONEGAVIRALES*, FAMILY *PARAMYXOVIRIDAE*, SUBFAMILY *PARAMYXOVIRINAE*, GENUS *AVULAVIRUS, HENIPAVIRUS, MORBILLIVIRUS, RESPIROVIRUS, RUBULAVIRUS*]

Paramyxoviridae are enveloped, -(ss) RNA viruses with a linear genome (15–19 kb in size) and a helical, non segmented nucleocapsid. The virons are spherical to pleomorphic or filamentous, 150 to 300 nm in diameter with embedded glycoproteins covering virus surface involved in attachment to host cells (Enders, 1996). The family Paramyxoviridae consists of five genera; *Avulavirus, Henipavirus, Morbillivirus, Respirovirus, Rubulavirus*. Members of the *Respirovirus* and *Morbillivirus* genus are not zoonotic viruses causing diseases either in humans (e.g., measles) or animals (e.g., canine distemper or ovine rinderpest) and will therefore not be further discussed. Relevant zoonotic species of Paramyxoviridae family are listed in Table 3.8.1.

The genus *Avulavirus* contains viruses that formerly were related to genus *Rubulavirus* (Mayo, 2002).

3.8.1 NEW CASTLE DISEASE AND ENVIRONMENT

Newcastle disease virus (NDV) is one of *Avulavirus* genus species that causes disease in humans, migratory birds, feral pigeons and chickens. In birds, Newcastle disease (ND) is an acute infectious disease characterized by sever respiratory and digestive signs or encephalitic symptoms with a high level of mortality causing significant economic loss in the poultry industry (Cattoli *et al.*, 2010).

In humans, NDV can cause mild conjunctivitis and influenza-like symptoms, usually among poultry farmers and veterinarians handling infected chicken or birds. Transmission of the virus occurs by direct contact with feces and secretions from the nose, mouth, and eyes or due to environmental contamination (water, food). Thus far, person to person transmission was not yet reported (Anonymous, 2004). Waterfowls are considered as important reservoirs and vectors of NDV. Various investigations showed viral infection in houseflies, probably due to the consumption of fluids and defecates of an infectious

source or direct contact with infected birds. Therefore it was suggested that arthropods could serve as a vector in NDV transmission (Chakrabarti *et al.*, 2007).

Table 3.8.1 Some characteristics of Paramyxoviruses.

Genus	Virus	Reservoir host	Host	Disease in human	Distribution
Avulavirus	*Newcastle disease virus* (VDV)	pheasant, waterfowl	Chicken, Human	Conjunctivitis	Worldwide
Henipavirus	*Hendra virus* (HeV)	Fruit bats	Horses, Human	Acute respiratory syndrome, encephalitis, meningitis	Australia
	Nipah virus (NiV)	Fruit bats	Pigs, Dogs, Cats, Horses Human	Encephalitis	Malaysia
Rubulavirus	*Menangle virus* (MenPV)	Fruit bats	Pigs, Human	Febrile illnesses, encephalitis	Australia

3.8.2 MENANGLE VIRUS AND ENVIRONMENT

In 1998, Philbey *et al.* described a new virus isolated from a high number of stillborn piglets with deformities in a piggery in Menangle, New South Wales, Australia. Subsequent serological investigation revealed infections in adult pigs, human workers at the affected piggery and fruit bats roosting nearby. The virus was found to be a new virus in the family Paramyxoviridae called *Menangle virus* after isolation site name. Worker at the above piggery who had been in contact with infected material had experienced a flu-like febrile illness with subsequent recovery (Philbey *et al.*, 1998; Chant *et al.*, 1998).

3.8.3 HENDRA AND NIPHA VIRUSES AND ENVIRONMENT

The genus *Henipavirus* contains two zoonotic viruses both having fruit bats as natural host; *Hendra virus* (HeV), causing respiratory and neurological infection in human and respiratory infection in horses (Playford *et al.*, 2008), and *Nipah virus* (NiV) causing fatal encephalitis in human and neurological and respiratory syndromes in pigs (Goh *et al.*, 2000; Mohd Nor *et al.*, 2000).

HeV that had been firstly identified in 1994 was responsible for several outbreaks of fatal diseases with elevated mortality in horses and humans in Australia between 1994 and 2008 (Selvey *et al.*, 1995; Field *et al.*, 2010). Humans became infected after close contact with body fluids of infected horses while fruit bats had been accepted to be HeV natural reservoir to transfer this virus to horses (Halpin *et al.*, 2000). Serological investigation showed that half of fruit bat colonies located in affected regions carry antibodies against these viruses (Wild, 2009). The transmission mode from bats to horses is still unknown and remains purely hypothetical. Field *et al.* (2010) suggested transmission of body fluids from infected bats via contaminated feed, water or yard rails. Horse to horse transmission has been reported

and related to contaminated surroundings with body fluids while person to person transmission was not yet reported.

First NiV outbreak was reported in Malaysia in 1998, when a viral encephalitis illness occurred among pig farmers, starting in one district and further spreading to different districts (including Singapore) due to commercial trade and transport of infected pigs (Paton *et al.*, 1999). High mortality rates have been related to human infection through close contact with urine and feces of infected pigs. Infected fruit bats (displaying NiV virions in saliva and urine) located in pig farms vicinity was suspected as the natural host of this virus (Chua *et al.*, 2002; Looi and Chua, 2007). Infection was shown to spread easily among cats, dogs and horses. Outbreaks of Henipavirus infections were also correlated with extensive deforestation behavior, slash-and-burn and El Niňo-related drought forcing the bats, the natural reservoir host, to abandon their native biotopes and approach the proximity of human habitats (Looi and Chua, 2007; Wild, 2009) (Figure 3.8.1).
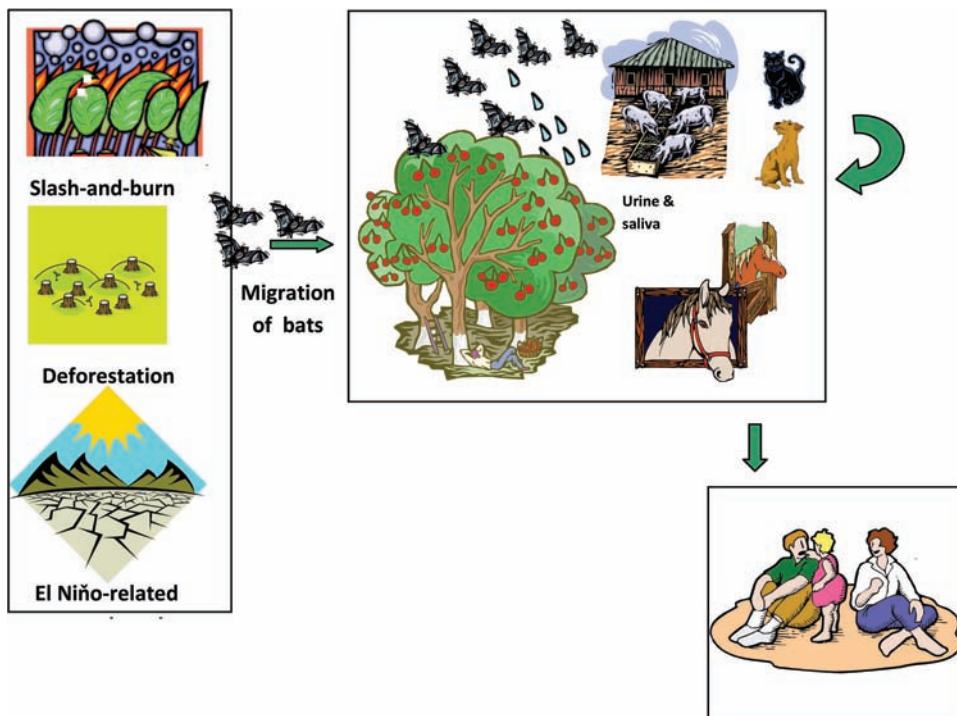
Figure 3.8.1. Henipavirus hypothetical spread

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Chapter 3.9

Orthomyxovirus

[SWINE INFLUENZA VIRUS H1SWN1, AVIAN INFLUENZA VIRUSES-H5N1, H7N7 AND H9N2, *GENUS INFLUENZA A VIRUS*]

Swine influenza virus (H1swN1) and Avian Influenza Viruses (H5N1, H7N7 and H9N2) belong to the genus Influenza A virus (IAV). They are enveloped viruses (some pleomorphic) of a size of 80 to 120 nm containing segmented (6–8 segments) of – (ss) RNA of a 13.5 kb size. Swine influenza virus was isolated from pigs in 1930 and it was responsible for the 1918–1919 pandemic, causing more than 20 million deaths. The distribution is worldwide, with latent infections in pigs. Avian influenza virus was originally observed in 1997, in a Hong Kong outbreak with fatalities. Those viruses infecting poultry can be divided in two groups: highly pathogenic avian influenza (HPAI) (flock mortality up to 100% with subtypes H5 and H7) and low pathogenic avian influenza (LPAI) (mainly mild respiratory diseases) (Capua and Alexander, 2007). Avian influenza has a worldwide distribution, with latent infections of waterfowls (ducks, geese, etc.). In humans, transmission of these viruses occurs through aerosols (swine influenza virus) or direct contact with live birds (avian influenza virus) and possibly through handshake (1918–19 pandemic was a classic example of person-to-person transmission). An interesting hypothesis has been raised by Lowen and Palese (2009) in connection with influenza A virus transmission. These authors, based on previous results showing blockage of aerosol transmission by high humidity (80% R.H.) and temperature (30°C) and the observed global seasonal behavior of this virus, hypothesized that there are two predominant modes of transmission in temperate and tropical climates. Distinctively, the aerosol mode is predominant in temperate climate while direct contact is predominant in tropical climate. Mammalian influenza viruses are more labile to environmental conditions; however, they can survive for several hours in body fluids. The avian influenza virus has a fast inactivation rate in manure though it can stay alive for up to 2 weeks in feces. Avian influenza virus can survive for 100 days in distilled water at room temperature, 200 days at 17°C and indefinitely when frozen. Clinical manifestations of infection with both virus types are high fever, bronchitis, pneumonia, malaise, acute myalgia and death.

3.9.1 SWINE INFLUENZA VIRUS AND ENVIRONMENT

Influenza A viruses (IAV) are basically of avian origin (waterfowl and shorebirds), birds being classified as primary hosts. Among these birds, the virus is spread by fecal-oral transmission in untreated water. Frequent transmission between aquatic birds and domestic poultry occurs with emergence of highly pathogenic subtypes (e.g. H5 and H7) capable of decimating commercial poultry flocks (Webster, 1997). Interspecies transmission is less frequent; however, in environments comprising mud, water and organic material IAV undergo a high degree of genetic changes to switch hosts further (pigs, horses, humans, domestic poultry, etc.), suggesting that they may be more evolutionarily flexible than had been previously realized (Morens and Taubenberger, 2010) (Figure 3.9.1). It would not be unexpected if additional secondary reservoir hosts were found to be involved in viral maintenance, evolution, and transmission. Once IAV is established in mammals, its transmission occurs through the respiratory airborne route, therefore favoring crowded, humid and closed places as foci (Belser *et al.*, 2010; Robinson *et al.*, 2007). Mubareka *et al.* (2009) studied aerosol and fomite transmission of influenza A/Panama/2007/1999 (H3N2) among guinea pigs as models. They were able to demonstrate that aerosol transmission is a more efficient mode than fomites contact.
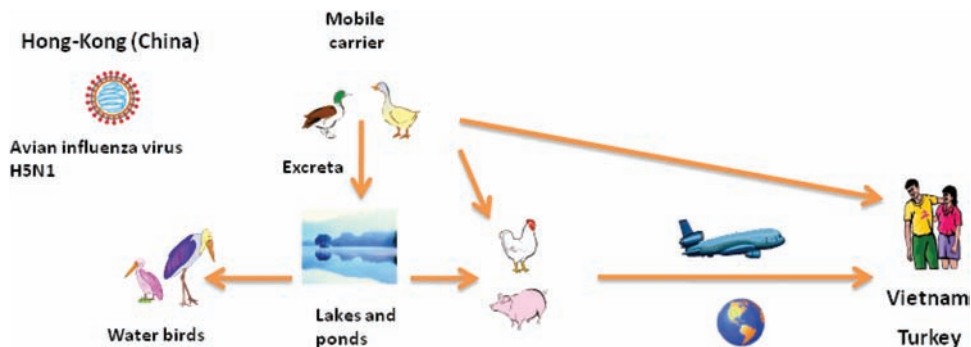
Figure 3.9.1. Transmission of Influenza A virus from waterfowls through domestic poultry and pigs to humans and rapid transmission by air-travel

Kida (1997) reported on a new pandemic strain of swine that circulated in migratory and domestic ducks in southern China. The author emphasized that pigs' co-infection with swine and avian viruses generates reassortant viruses (with genes from both viruses expressed in a new influenza virus variant). The process has been found to occur yearly in frozen lake water, a waterfowl reservoir, in the absence of ducks. Concerning a similar reassortment process in pigs, Castrucci *et al.* (1993) reported on influenza A virus in Italy. These authors analyzed phylogenetically the internal protein genes of classical H1N1, avian-like H1N1 and human-like H3N3 present in pigs in Italy. Indeed, the newer viruses (from a more recent pandemic) contained internal protein genes of avian-like H1N1 whereas older strains did not. These results obviously revealed that pigs could serve as "mixing vessels" for reassortment events (Ma *et al.*, 2009). In addition, areas densely populated by pigs showed an outbreak clustering in Canada related to increased cases and virus movement among pig population (Pasma, 2008).

Conceivably, one of the main conclusions derived from the epidemiology of IAV is the separation of wild aquatic and domestic poultry and of pig and poultry farming, in order to reduce the next potential pandemic

of influenza in humans. Potable water as a route does not seem to have a role in virus transmission, as the present water treatment strategies currently used for surface water treatment are entirely suitable for removing and/or inactivating influenza A viruses (Lenes *et al.*, 2010).

3.9.2 AVIAN INFLUENZA VIRUS AND ENVIRONMENT

It is now clear that highly pathogenic avian influenza viruses are globally spread by migratory birds (Wang *et al.*, 2008; Causey and Edwards, 2008). Different studies had also shown that the avian influenza virus can be transmitted to mammalian species (e.g., tigers, domestic cats and dogs) and also to crocodilians through ingestion of infected birds (Davis and Spackman, 2008). The significant risks of distribution and transmission of avian influenza viruses rely on competent avian hosts, their immune systems, annual cycles, feeding behaviors, migration patterns, decreased biodiversity (through active destruction and modification of wetland environments for human use) and legal and illegal trade in wild birds (Gibbs, 2010). Perez *et al.* (2003) showed that chickens, quails and other land-based birds that are extensively farmed are closely related to zoonotic outbreaks of avian influenza in Hong Kong through use as mixing vessels and disseminators of these viruses. These authors also demonstrated that Japanese quail could provide an environment in which H3N2 virus (A/swine/Texas/4199-2/98) could further reassort and generate influenza viruses with pandemic potential. In another study, Perez *et al.* (2003b) reported that quail provide an environment in which the adaptation of influenza viruses from ducks generates novel variants that can cross the species barrier.

These results are strongly supported by a study performed in Korea on live bird markets (LBMs) that provide an ideal environment for the evolution and interspecies transfer of avian influenza viruses (AIVs) (Lee *et al.*, 2010). This study indicated that the newly evolved AIV-s have been continuously generated by reassortment in ducks, and that these reassortments could result in expanding the host range of AIVs.

Davidson *et al.* (2010) reported on the survival of the H9N2 strain at different temperatures (37, 20 and 4°C) and pH-s (5.0 and 7.0). This avian influenza H9N2 strain had become endemic in Israel since its introduction into this country. The authors reported that H9N2 survival decrease rapidly with temperature increase corresponding to winter outbreak occurrence. At pH 5.0, the virus survived for one week while at pH 7.0 it remained viable for up to three weeks (both tested at 20°C).

Brown *et al.* (2009) studied the survival of twelve avian influenza strains isolated from wild fowl under natural ranges of pH, salinity and temperature in water. According to their results, avian influenza viruses increased survival was dependent on slightly basic pH-s (7.4 to 8.2), temperatures <17°C and fresh to brackish salinities (0 to 20,000 ppm). Conversely, Negovetich and Webster (2010) showed experimentally that the variation of thermostability of H2N3 influenza virus isolates begins at the level of the population and that the presence of highly thermostable subpopulations possibly suggests longer water persistence and better transmission susceptibility. In relation to water as an environmental factor in avian influenza virus transmission, Faust *et al.* (2009) showed experimentally an interesting ecosystem able to inactivate efficiently the virus present in water. These authors used the filter-feeding bivalves, *Corbicula fluminea*, to remove the experimentally introduced high pathogenic avian virus (A/whooper swan/Mongolia/244/05, H5N1). Wood ducks exposed to water containing HPAI but filtered through bivalves did not contract the disease while those exposed to unfiltered water exhibited morbidity or mortality. Their results indicate that filter-feeding bivalves could remove and reduce the infectivity of HPAI viruses in water. How these biotic environmental factors may influence avian influenza virus transmission requires further examination.

According to the high fatality in the poultry industry infected with avian influenza virus, there is an urgent need to inactivate these viruses present in disposed carcasses. Graiver *et al.* (2009) studied the survival of H6N2 in a methanogenic landfill leachate and water as afunction of temperature, conductivity and pH. High temperatures and nonneutral pH-s caused the fastest inactivation of this avian influenza virus, while conductivity had no impact on its survival. Leachate inactivation was similar or faster in comparison with water medium. After waste disposal, the viral inactivation rate ranged from 30 to 600 days, revealing high persistence.

Laboratory results on environmental persistence and application to field studies have been reviewed by Stallknecht *et al.* (2010). These authors critically reviewed the tenacity of avian influenza virus in environment compared to laboratory results, pointing at the knowledge gaps to understand the factors affecting environmental infectivity, specifically from aquatic habitats utilized by wild birds that are much more complex than the laboratory environment. Breban *et al.* (2009) tried to explain avian influenza persistence and periodicity in North America through an environmental transmission model. Their model consisted of host-pathogen relations based on the following factors: within-season transmission dynamics, between-season migration and reproduction and environmental variations. According to their results, the environmental transmission model supported persistence mechanism within small communities (where epidemics are not supported by direct contact), epidemics periodicity and population persistence.

Delogu *et al.* (2010) studied preening as an alternative route of avian influenza virus transmission. In spite of many reports on high viral loads in feces of infected birds and consequently surface water contamination, these authors questioned whether dilution and environmental conditions do not play a role in a reduced contact between virus load and aquatic birds, favoring other routes of contamination such as preening. They were able to demonstrate that feather samples and cotton swabs impregnated with preen oil attracted and concentrated avian influenza viruses, therefore pointing to the body of wild birds as a possible reservoir. This interesting study also indicates a possible direct contact between humans and birds in the poultry industry during plucking. Indeed, Busquets *et al.* (2010) also suggested feathers as a suitable sampling site for avian influenza virus. Yamamoto *et al.* (2010) strengthen this point by comparing the survival of different clades of avian influenza (H5N1) on feathers, in drinking water, and in feces collected after 3 days postinoculation and stored at 4°C or 20°C. They reported a viral infectivity persistence for 160 days at 4°C and for 15 days at 20°C, on feathers! This observation may also explain the report by Shivakoti *et al.* (2010) from Japan, of an emaciated mountain hawk-eagle infected with the highly pathogenic avian influenza (HPAI) virus subtype H5N1 and three additional outbreaks of H5N1 within three weeks that had occurred in this area, in chickens. It seems reasonable to conclude that the hawk-eagle (not an aquatic bird) contracted this highly pathogenic avian influenza virus through direct contact (feeding?) on infected chickens, as has also been shown with other experimentally infected raptors (Hall *et al.*, 2009).

The global fatality of influenza A virus is estimated at ~500,000 human deaths and many more sublethal cases. Avian viruses make a major contribution to the genetic material of human viruses (including H5N1 and H1N1). Anthropogenic changes, such as human population growth, land use, trade globalization, agricultural intensification, fast global travel and changes in vaccine technology, may impact the evolution and transmission of these viruses (Vandegrift *et al.*, 2010). From the environmental point of view, Li *et al.* (2011) compiled 16 environmental variables to assess their correlation to the highly pathogenic avian virus H5N1. According to their results, the strongest positive association was with the human footprint index, certain wetland types and mild temperatures (10 to 30°C). In addition, H5N1 outbreaks in poultry and wild birds were frequently connected to certain habitats (e.g., tropical and subtropical moist broadleaf forests, temperate broadleaf and mixed forests, and flooded grasslands and

savannas) while high mountains and plateaus did not affect the speed and direction of virus transmission. Li *et al.* (2007) developed a new climatic approach for HPAI outbreaks by investigating prior climatic conditions of outbreaks. Their results showed that a sharp drop in temperature and dust storms exacerbate HPAI H5N1 spread.

Finally, climate change may potentially affect avian influenza epidemiology through changes in distribution, composition and migration behavior of wild birds (Gilbert *et al.*, 2008). However, HPAI confined mainly to domestic poultry and distributed worldwide regardless of climatic conditions will be only slightly affected and mostly indirectly through the influence of climate change on agro-ecosystems associated to duck and crop production and distribution of domestic-wild waterfowl contact points.

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Chapter 3.10

Picornavirus

[FAMILY *PICORNAVIRIDAE*, GENUS *APHTHOVIRUS, CARDIOVIRUS, ENTEROVIRUS*]

Picornaviridae family includes the smallest mammalian viruses (virion size range of 22-30 nm) that possess a monopartite, non-enveloped, linear $+$ (ss) RNA genome (7.1–8.9 kb in size) with an icosahedral nucleocapsid (Lewis-Rogers and Crandall, 2010). This family currently consists of 12 genera as well as many human pathogenic species. In spite of the large number of picornaviruses, there are only three zoonotic picornaviruses: *Aphthovirus, Cardiovirus, Enterovirus* (including *Rhinovirus*). Other non-zoonotic picornaviruses are: *Erbovirus, Hepatovirus, Kobuvirus, Parechovirus, Sapelovirus, Senecavirus, Tremovirus, Teschovirus* (Anonymous[1], 2011). Viruses of the *Picornaviridae* family are infectious for a broad spectrum of animals such as: fish, reptiles, birds and mammals (Lewis-Rogers and Crandall, 2010).

Swine vesicular disease is a highly contagious disease of pigs caused by the *Swine vesicular disease virus* (SVDV), which is classified as a porcine variant of human *Coxsackievirus B5* and a member of the genera *Enterovirus*. The disease is similar to foot and mouth disease, characterized by leg and mouth lesions; pigs get feeble and lose weight. Infections in pigs are usually subclinical to mild but sometimes more severe. Transmission to humans occurs rarely and has been reported in laboratory personnel working with this virus (Anonymous[2], 2007). The clinical related virus causing food and mouth disease (FMD) belongs to the genera Aphtovirus. FMD is a highly contagious infection for cloven-hooved ruminants, especially cattle and swine. According to the United States Department of Agriculture (USDA) the disease is not recognized as a zoonotic one and is often confused with hand, foot and mouth disease (Anonymous[3], 2007). Reports assuming FMD as zoonosis refer to an incident of a veterinarian's self-infection who developed clinical signs after milk ingestion from infected cows or, relying on the description of clinical incidents, with lesions of foot and mouth disease following contact with infected animals; virus isolation from infected lesions was not reported (Armstrong *et al.*, 1967, Bauer, 1997).

Encephalomyocarditis (EMC) is a disease caused by a virus of the genus Cardiovirus. The virus has a worldwide distribution and causes myocarditis, encephalitis and reproductive disorder in many mammalian species and nonhuman primates. Rodents are considered to be the natural host of this virus

and were linked to outbreaks in piggeries and zoos (Grobler *et al.*, 1995; Acland and Littlejohns, 1975; Koenen *et al.*, 1999; Canelli *et al.*, 2010). There is one report of EMC virus isolation from a patient with febrile infection (with headache and fever symptoms) (Oberste *et al.*, 2009). The zoonotic characteristic of this virus has not been not clearly demonstrated thus far. Different authors suggested rodents as the natural host and responsible for transmission to other animals via ingestion of infected feces, urine and carcasses (Seaman *et al.*, 1986, Maurice *et al.*, 2005).

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Chapter 3.11

Herpesvirus

[FAMILY *HERPESVIRIDAE*, SUPFAMILY *ALPHAHERPESVIRINAE*, GENUS *SIMPLEXVIRUS, SPECIES MACACINE HERPESVIRUS 1*]

The Herpesviridae family comprises spherical to pleomorphic, enveloped viruses with a monopartite, linear + (ds) DNA genome. Herpesviridae size ranges from 120 to 200 nm in diameter with glycoproteins embedded in a lipid envelope. The family of the *Herpesviridae* comprises many species infectious for different kind of animals, including humans. Among all these species, only one species is defined as zoonotic with severe pathogenesis in humans: the *Macacine herpesvirus 1*, also known as herpesvirus B or monkey B virus. In humans, monkey B virus infections cause encephalitis and myelitis with a high fatality rate. The first reported case of monkey B virus infection in a human (1933) was attributed to a bite from an apparently healthy *Macacus rhesus* monkey. Among other severe symptoms, the bitten patient developed encephalomyelitis and died some days after the incident (Sabin and Wright, 1933). In general the disease in humans starts with a flu-like illness, fever, headache over to parenthesis, encephalomyelitis, neurological dysfunction, coma and may conclude in death of the infected person. Macaque monkey is the natural host of the virus with lifelong carriage, latent infection (virus shedding) with intermittent disease expressions usually as a result of stress. In macaques the infection has no or mild symptoms, with lesions on tongue, lips, oral or genital mucosa.

3.11.1 HERPES B VIRUS TRANSMISSION AND ENVIRONMENT

The route of transmission from monkey to human is by means of bites/scratches of imported, infected animals or a scratch/cut from contaminated objects (Anonymous, 1987, 1989). A case report described the transmission through a splash in the eye of contaminate objects; human to human transmission is very rare and occurs by direct contact wounds to herpes lesions (Anonymous, 1998). The fatality rate of infected cases was reported to reach 70%. Thus far there are only 31 reported cases describing monkey B virus infection with 21 fatal outcomes (Anonymous, 2010).

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Chapter 3.12

Poxvirus

[FAMILY *POXVIRIDAE*, SUBFAMILY *CHORDOPOXVIRINAE*, GENERA *ORTHOPOXVIRUS, PARAPOXVIRUS, AVIPOXVIRUS, CAPRIPOXVIRUS, LEPORIPOXVIRUS, SUIPOXVIRUS, MOLLUSCIPOXVIRUS, YATAPOXVIRUS*]

The brick-shaped viruses of the *Poxviridae* family contain a large genome of not segmented linear (ds) DNA (size range 130 to 289 kb) and presents themselves in two distinct infectious forms, the intracellular mature virus and the extracellular enveloped virus with an approximate size of 140-206 nm diameter and 220–450 nm length (SIB, 2010; Seet, 2003). The *Poxviridae* family consists of two sub families, *Chordopoxvirinae* with eight genera of which three are enzootic (*Orthopoxvirus*, *Parapoxvirus*, *Yatapoxvirus*) and *Entomopoxvirinae* with three non enzootic genera (Paran & Sutter, 2009).

Orthopoxvirus contains beside the non-zoonotic species some zoonotic agents such as Cowpox, Monkeypox, *Vaccinia*, Camelpox, Buffalopox and Elephantpox viruses (Table 3.12.1). *Variola* virus, the most famous *Orthopoxvirus* that causes smallpox exclusively in humans, due to cross protection between same genera, had been eradicated in a major vaccination program initiated in the 19th century. Still some concerns are left that genetically modified smallpox virus could be used as an agent of bioterrorist attacks (Moore *et al.*, 2006).

3.12.1 POXVIRUS AND TRANSMISSION AND ENVIRONMENT

Vaccinia virus is genetically related to the *Smallpox* virus, but it is milder and is mainly used for vaccination against smallpox. For the majority of healthy people the vaccine is safe but some side effects have been recorded such as: rash and fever. The virus may be spread from newly immunized to non immunized humans through contact with local lesions at the vaccination site (i.e. contact transmissions). There are several reports of viral transmission from newly immunized infants to other immune naïve family members (Anreev, 1969) and also transmitted after sexual contact with recently immunized persons (Figure 3.12.1) (Egan *et al.*, 2004; CDC, 2007). In immunosuppressed or enfeebled persons it can have a dangerous outcome. Redfield *et al.* (1987) reported on a fatal vaccinia virus in an HIV infected person.

Essbauer *et al.* (2007) tested a vaccinia virus strain (orthopoxvirus) for survival in food and other environmental samples (storm water, potting soil and gauze) at different temperatures. In food, gauze and water, vaccinia survived well at 4°C for two weeks but not in storm water supplemented with potting soil. It can be hypothesized that potting soil has antiviral activity, mainly through various microorganisms present in natural soil. Interestingly, storm water supplemented with fetal calf serum (FCS) and kept at 4.5°C revealed infectious virions for up to 166 days. High protenaceous content of FCS bestowed protection against inactivating factors to vaccinia virus as already reported in many studies (Benbough, 1971). However, McDevitt *et al.* (2008) recently found that vaccinia aerosols are extremely susceptible to ultra-violet light at 254 nm (UVC) at low relative humidity, a fact that can reduce infections in hospital emergency rooms where infected people may congregate and spread the virus.

Table 3.12.1 Zoonotic Poxviruses.

Genus	Species	Clinical manifestation in human	Host	Distribution
Orthopoxvirus	*Camelpox virus* (related *Variola virus*)	Skin ruptions, mild local infection	Tick, Mosquitoes	Africa, Middle-east, Central Asia, India
	Cowpox virus	Localized lesions	Rodents, Mammals	Europe and central and northern Asia
	Monkeypox *virus*	Rash causing illness, Skin eruption	Rodents	Republic of Congo (Arica), USA
	Vaccinia virus (Vaccine)	Rash and fever	Human	worldwide
	Buffalopox virus (strain of *Vaccinia virus*)	Pox-like lesions	Buffaloes, Cows,	India
	Elefantpox virus (strain of *Cowpox virus*)	pox-like lesions	Rodents, Elephants,	Zoos and Circus
Parapoxvirus	Contagious Ecthyma of sheep (Orf)	Infectious lesions	Goads, Sheep (Artiodactyla)	worldwide
	Pseudocowpox virus (syn. milkers' nodules)	Infectious lesions	Cattle	worldwide
	Bovine Papular Stomatitis	Infectious lesions	Cattle	worldwide
Yatapoxvirus	Tanapox	Febrile illness, pox-like lesions	Monkeys, Arthropods	Kenya

Buffalopox virus, closely related to *Vaccinia* virus, causes mild infection with pox lesions on the body of infected persons, usually animal handlers, milkers and laboratory workers (Bhanuprakash *et al.*, 2010).

Camelpox virus is closely related genetically to variola virus, the causative agent of smallpox, yet its pathogenicity to humans is not well known and seems to be very low (Gubser and Smith, 2002). An investigation of herdsmen, handling infected camels, revealed only a partially developed localized lesion and only mild local infections in contrast to camels that presented severe clinical signs, such as drop in milk production, weight loss and high mortality. These findings suggested that this virus is rarely or almost not infectious for humans (Jezek *et al.*, 1983). Additional investigations are necessary in order to clarify this point. *Cowpox* virus is also mildly infective for humans, typically causing localized lesions. Some reports described incidental cowpox transmission and infection by infected cats, fancy rats and mice, kept as home pets, to humans (Willemse and Egberink, 1985; Rjan *et al.*, 2005; Postma *et al.*, 1991; Wolfs *et al.*, 2002; Lewis-Jones *et al.*, 1993). Czerny *et al.* (1991) reported on an orthopox virus (OPV) transmitted from cat to man with a fatal outcome. Rodents have been already suggested as the main cowpox virus reservoir combined with chasing cats' exposure (Herder et al., 2011; Baxby, 1977; Bennett *et al.*, 1986). Some evidence showed the potential transmission of *Elephantpox* virus from elephants to humans following direct contact. Signs of pox infection (ulcerating papulae) localized on fingers, arms, eyes and face of animal keepers have been reported (Kuntze, 1998; CDC, 2008). Infected rodents such as rats are considered to be the virus transmission source to elephants (Figure 3.12.1). Monkeypox virus, a virulent *Orthopoxvirus*, got its name owing to outbreaks in nonhuman primates in laboratories and a zoo in Europe and USA (between 1950 and 1960). In those episodes, primates were imported from Asia and Africa for poliovirus vaccine production. Africa seems to be the exclusive site of naturally occurring monkeypox virus, yet after the import and arrival in Europe and USA of these infected monkeys, they did approach other wild animals, possibly transmitting and spreading the virus (Breman, 2000). The first human monkeypox virus infection case was reported in Africa (in 1970) in a nine month old child (Ladnyj *et al.*, 1972). Since then, the disease occurred in Africa sporadically in humans and only in 2003 was a first report on human monkeypox virus in United States described in association with an imported African rodent (prairie dogs) (Parker *et al.*, 2007). The virus causes severe smallpox-like illness in humans, expressed by an initial fever, headache, fatigue, subsequent cutaneous eruptions and high fatality (Breman, 2000). Transmission occurs by animal bite, direct contact with animal lesions or body fluids and can be spread from human to human (Reed *et al.*, 2004). In Africa, 59 cases of human monkeypox virus have been reported between 1970 and 1980, with severe infections and 17% mortality; the majority of these cases (47 incidents) were reported from the tropical rain forest of the Democratic Republic of Congo (Heymann *et al.*, 1998). In the following years (1981–1986) additional cases in Africa (350 incidents) were reported with a preponderance (342 incidents) also in the tropical rain forest of the Democratic Republic of Congo (Breman, 2000). The outbreaks are thought to be attributable to intermittent smallpox vaccination, which provided cross-immunity between the different genera (Fine *et al.*, 1988). The highest cases rate was recorded between June and August, probably caused by intensive agricultural work and hunting during this period. In the following years, human monkeypox virus continued to be reported from Africa as a consequence of human urbanization, civil unrest and economic plight. These facts may have forced people to set foot more frequently in the rain forest increasing their susceptibility to infected animals. The reality that vaccination with vaccinia virus is impractical, as a result of previous recorded high fatality rate in HIV patients, led to the conclusion of improved and enhanced health education (Heymann *et al.*, 1998; Parker *et al.*, 2007).

Parapoxviruses are commonly associated with domestic and wild ruminants (Artiodactyla) and cause skin infections. One of the most common poxviruses, with a worldwide distribution, is the ORF virus (genus *Parapoxvirus*) also known as contagious ecthyma of sheep (with lips and mouth lesions in small ruminants: sheep and goats). Clinically, *Pseudocowpox virus* (PCPV) and bovine *Papular stomatitis virus* (BPSV) are almost indistinguishable from ORF, therefore termed "farmyard pox" and only

molecular techniques (e.g., specific real-time PCR) can differentiate them. These viruses are transmissible to humans causing pruritic, painful vesicular lesions on hands and fingers. People get infected through contact with infected goats or sheep (for ORF) or cattle (for PCPV and BPSV) or through contact with infected fomites. Several cases described transmission from animals to humans through contagious animal bites, sheep shearing and incidental injury of hands while feeding recently vaccinated animals. The disease is usually self-limited (CDC, 2006).
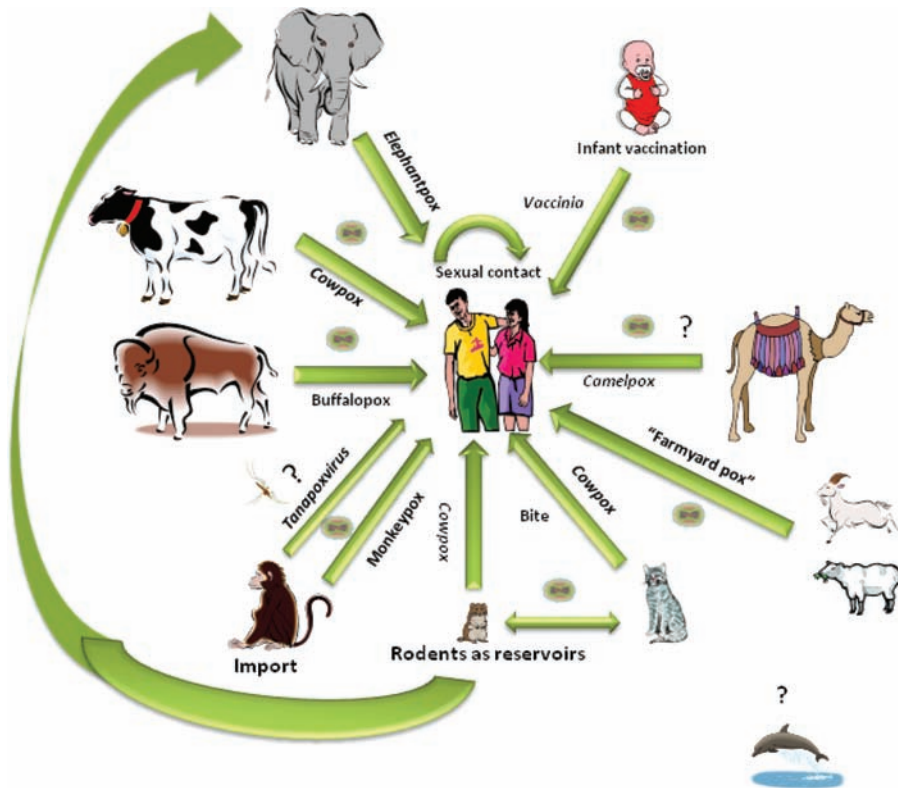
Figure 3.12.1. Poxviruses proved and potential transmission

The genus *Yatapoxvirus* consists of different species, with the *Tanapoxvirus* being the zoonotic one. Monkeys are considered to be the natural host and mosquitoes were suggested to be the vector for human infection. The disease initiates with a febrile illness, headache and pox-like lesion occurring after several days. Two outbreaks were reported in Kenya (1957 and 1962) in humans living close to the Tana River (Downie *et al.*, 1971). In 2004 a student, returning to US after observing a chimpanzee in the Republic of Congo, was also diagnosed with tanapox infection (Dhar *et al.*, 2004).

Finally, it should be mentioned that Maldini *et al.* (2010), studying the epidermal conditions of a small population of coastal bottlenose dolphins (*Tursiops truncates*) in Monterey Bay, found pox-like lesions affecting these animals (80%). In spite of no laboratory evidence, the authors suggested "a possible indication of a widespread presence of a poxvirus or a similar pathogen in this population". Additional studies will be required in order to verify the etiology of these lesions and, if definitely pox viruses, are they zoonotic?

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Chapter 3.13

Coronaviruses

Coronaviruses belong to *Coronaviridae* family which includes two subfamilies: *Coronavirinae* and *Toronvirinae*. The subfamily *Coronavirinae* contains three genera: *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus*. Coronaviruses are enveloped viruses containing $+$ (ss) RNA with helical symmetry and a relatively large genome size, between 27 to 32 kb. In the last years the *Severe Acute Respiratory Syndrome Coronavirus* (*SARS-CoV*) (genus *Betacoronavirus*) drew special attention. The 2002–2003 SARS outbreaks with initial cases in China led to over 8.000 reported cases and a fatality rate of up to 10%. The outbreak got a pandemic character and spread rapidly to 37 countries around the globe. Different hosts, such as bats, civets, horses, beluga whales, birds, humans and other still undetected animals, are assumed as potential host (Rihtaric *et al.*, 2010; Nagata *et al.*, 2010). Clinical manifestations are linked to respiratory syndrome: fever, malaise, cough, myalgia, dyspenea, breath shortage, hypoxia, nausea, vomiting, diarrhea, headache and death. Fatality ranges from 3 to >10% in patients older than 40 years old.

3.13.1 SARS AND ENVIRONMENT

Summarizing data accumulated from the 2002–2003 pandemic, it is further evidently that virus spread occurs through aerosols originating from infected individuals (as hosts and also vectors) where different animals play a significant role as reservoirs (Yu *et al.*, 2004). Yip *et al.'s* (2007) study suggested that meteorological conditions had played a major role in SARS transmission in Hong-Kong epidemic of 2003. The virus was spread via aerosols by ambient wind in an apartment building at low temperatures that contributed to its survival. These results were further supported by Kuk and Tan (2009) in their statistical analysis estimates of Singapore and Hong-Kong outbreaks related to occurrence time of infections. When they correlated infection rate as a function of time it was evident that infection rate had more than one peak, revealing that the hospital environment has ~10 times greater infection rate in comparison with the nonhospital. Based on these outcomes, they raised the hypothesis that the Singapore

outbreak was primarily nosocomial with "super spreaders" strongly affected by environmental rather than biological factors (genetic or biochemical).

In addition, it seems that SARS can survive at ambient temperature for 4 days in diarrheic stools (at alkaline pH) and for >7 days in sputum specimens (Lai *et al.*, 2005). Both environments hold moisture thereby supporting SARS survival while desiccation (on paper or on cotton gown) had been shown to inactivate the virus rapidly. Common disinfectants have also a high and rapid inactivation potential of these viruses, and therefore widespread disinfection practices could eliminate and prevent environmental contamination.

Food-borne infection could not be ruled out, as initial reports on the Chinese outbreaks pointed in the direction of civets as a potential reservoir of this virus, as civets are consumed in China as delicacies (Normile and Enserink, 2003) (Figure 3.13.1). In crowded markets with low hygienic standards where many animal species are kept close together interspecies transmission is very possible. Basically, we should look at such places as a factual incubator of new viral species emergence, a reality that would take a much longer time span to occur if in remote natural environments.
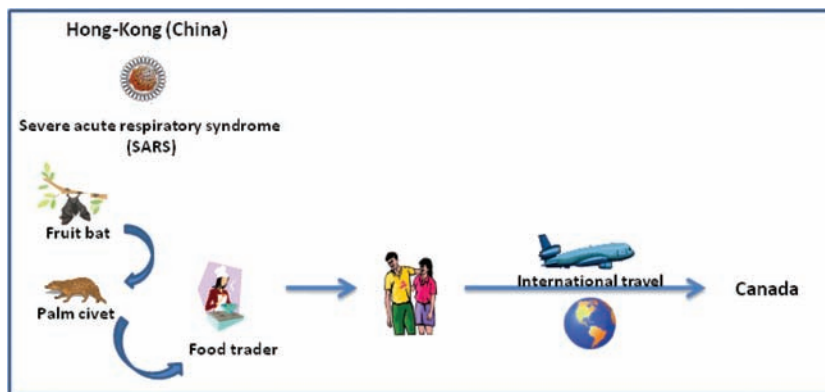
Figure 3.13.1. SARAS virus pandemic spread and potential sources of infections

The zoonotic aspect of this virus is interesting in the light of close contact between humans and domestic animals such as birds (e.g., poultry) in highly populated areas, low hygiene and crowded animals markets. Rockx *et al.* (2011) showed that human and zoonotic SARS-CoV could infect cynomolgus macaques with symptoms resembling those in mild human cases. Late phase human isolate revealed higher viral replication in those animals model. Woo *et al.*, (2009) in a recent review pointed out the increasing numbers of new coronaviruses identified following the 2003 outbreak: 2 new human strains (NL63 and HKU1) and additional strains from bats, horses, beluga whale and various birds. This high diversity seems to be related to the infidelity of RNA-dependent RNA polymerase, homologous RNA recombination high frequency and as previously mentioned the relatively large coronaviruses genome. The high diversity seems to be linked to bats and birds based on their species diversity as well as their ability to fly (high dispersal) and roosting and flocking habits, along with environmental pressures. According to their analysis, it seems that closely related coronaviruses from highly diverse animals have the "jumping capability" with a high risk of future catastrophic outbreaks.

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Chapter 3.14

Prions and zoonoses

[BOVINE SPONGIFORM ENCEPHALOPATHY-BSE, NEW VARIANT OF CREUTZFELDT-JAKOB DISEASE-NVCJD, GERSTMANN-STRÄUSSLER-SCHEINKER SYNDROME-GSS AND KURU]

Prions (proteinaceous infectious particles) is a specific group of proteins that affect central nervous system in humans and animals (so called progressive neurodegenerative diseases) resulting in spongiform encephalopathy and fatality. In humans, Creutzfeldt-Jakob seems to occur sporadically (possibly through ingestion), and is iatrogenic and familial. The natural hosts are humans and additional hosts are unknown; experimental transmission was shown in human primates: gorillas and chimpanzees. BSE is found naturally in infected cattle but additional hosts are possible: cats, zoo animals and humans, based on newly evidences that experimental transfer of a prion strain to different hosts leads to emergence of strain variants (Mahal *et al.*, 2010). Transmission of BSE seems to be through ingestion of contaminated beef. Prions are basically proteins encoded by host genes that in their normal form (PrPc) are transmembrane proteins with still unclear functions; however, recent studies revealed that normal PrPc are involved in cell proliferation and cellular homeostasis regulation (Nicolas *et al.*, 2009). Following infection (e.g. ingestion of infected beef) a pathological process starts when abnormal PrP^{Sc} proteins (different fold) induce transformation/production of normal PrP^{c} to more PrP^{Sc} proteins (in other words simply by templating that conformation "PrP^{Sc}" to other molecules of the protein) causing neuronal degeneration (amyloid plaques) (Figure 3.14.1). BSE in humans is now called a new variant of Creutzfeldt-Jakob Disease (nvCJD), however it is still a BSE related disease showing its zoonotic origin. All prion related diseases are rare (between 130 and 200 clinically identified) and most of them have been reported in European countries (probably due to much higher awareness and improved detection technology). Clinical manifestations are "slow infection", i.e., after years expressed as neurological and psychomotoric disturbances (manic depression, walking disturbances, memory loss, dementia and myoclonus).

3.14.1 BSE AND ENVIRONMENT

Juling *et al.* (2006) reported on a substantial genetic PRNP (prion protein gene) indel component for BSE susceptibility in cattle. Their conclusion was that "the main risk factor for BSE in cattle is environmental, i.e.

exposure to feedstuffs contaminated with the infectious agent". As a result, different studies have been carried out to gain information on prions environmental stability in soil, water and on various surfaces (Smith *et al.*, 2011). Ma *et al.* (2007) studied the mobility of prions in porous media of quartz sand (simulating soil and landfill waste environments) without infectivity follow-up. Their results showed maximal prion adsorption onto sand at their isoelectric point (pH 4.6); consequently, soil pH increase (e.g., lime or fly ash burial) may contribute to prions' mobility in soil. In a recent study, infectivity of prions following soil contact had been presented (Saunders *et al.*, 2011). Under various ionic compositions, prions desorption ability and replication (evaluated by protein misfolding cyclic amplification -PMCA) are affected after$>$30 days but not$<$7 days. Unbound or prions bound to different soils revealed high stability expressed as replication capability even after one years suggesting a potential transmission risk in environment. These results have been further supported by Cooke and Show's (2007) study on recombinant ovine PrP (rec PrP) in soil columns (loamy sand and clay loam) under various regimes: water content, redox potential and microbial activity (normal vs. reduced). recPrP survived in soil over a long period of 9 months, but its migration was restricted to a maximum distance of 1 cm due to adsorption. Their conclusion was that under natural soil conditions, infected animal burial does not present a high risk of groundwater contamination as soil can act as a significant barrier.
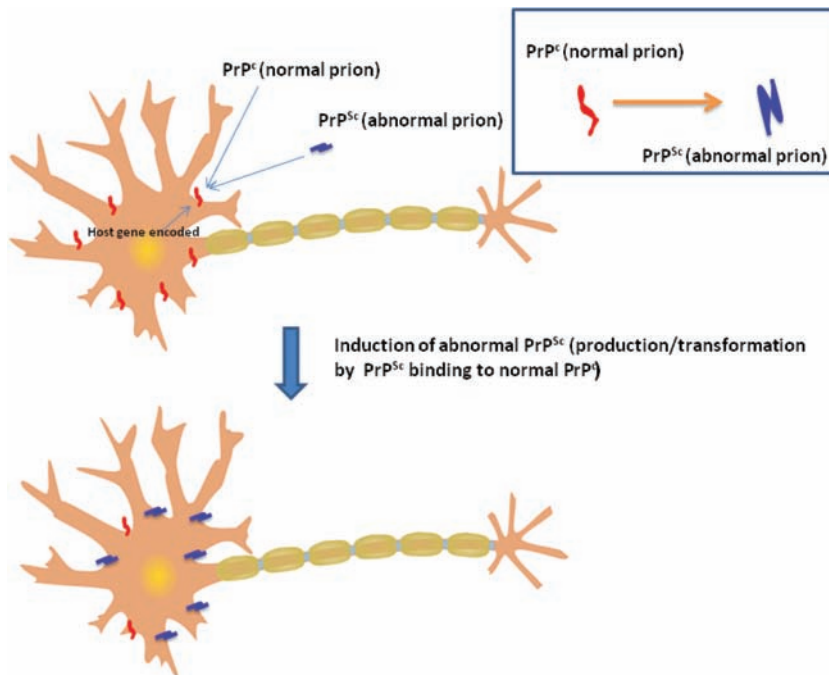
Figure 3.14.1. Prions infection of neurons as a causative of BSE

The demonstrated persistence of prions infective state in soil raised the question: What are the conditions that confer this environmental resistance? In this context, Polano *et al.* (2008) examined in depth the influence of organic polyanionics such as humic acids. Using a recombinant murine prion protein (MoPrP), these authors showed that prions adsorption is driven by interaction with humic acids and clay.

A definitive answer as to whether clay and humic acids might contribute to prions' conversion from non-pathogenic to pathogenic (through fibril growth) still awaits experimental evidence. Indeed, recent evidence was published in relation to the role of clay in infectivity preservation. Walter *et al.* (2011) recently reported an indirect proof of clay's role in prions' infectivity perpetuation by analyzing prion-infected deer herd data in the USA in relation to clay soil content. A 1% increase in clay content in close proximity to deer range increased the infection odds by up to 8.9%!

In environmental water ecosystem (urban sewage and seawater) prions have been shown to have limited survival compared to in buffered solutions such as phosphate buffered saline (Maluquer de Motes *et al.*, 2008). Important evidence of high survival ability of prions through potential horizontal transmission was provided by Maddison *et al.* (2010). These authors used ovine scrapie and corvine wasting disease agents (prions) to follow their surface endurance on metal, plastic and wooden planes (using protein-misfolding cyclic amplification-sPMCA as a viability method). In these experiments, prions persisted for up to 20 days revealing that even inanimate objects can present a high risk of infection. Mueller *et al.* (2007) studied the thermal inactivation potential of prions as a potential safety measure in the food industry. The effect of thermal inactivation of prions in the presence of components such as fat, fatty acids, and glycerol have been investigated by infectivity inactivation as well as by the degradation of prion polypeptide backbone. Results revealed that fat stabilized prions against degradation but destabilized infectivity against inactivation. In contrast, prion backbone was protected against heat-induced degradation by these components. In another study related to surgical instrument disinfection, Beekes *et al.* (2010) reported on the successful inactivation of prions (~5.5 logs) among other pathogens by exposure to a solution containing SDS, NaOH and n-propanol. These components have been shown to change proteins' hydrophilic/hydrophobic balance and modulate the oligomerization of membrane proteins in lipid bilayers, which may explain why under basic conditions prions are inactivated (Sahini and Ionita, 2011; Otzen *et al.*, 2007).

Prions' endurance and fascinating biochemistry have been linked to epigenetics under extreme conditions or as further defined: "a quasi-Lamarckian mechanism that connects environmental conditions to the acquisition and transgenerational inheritance of new traits" (Halfmann and Lindquist, 2010). In their publication, these authors did not treat prions as a disease agent but as complex evolutionary adaptations that helped organisms to survive and adjust to new extreme environments (at least in early biological systems).

In summary, it can be stated that prions stretch traditional views of proteins in biological systems, raising questions not only about simple soil resistance, though environmentally important, but also about their extraordinary epigenetic potential and evolutionary role (Wiggins, 2009).

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Chapter 4

Parasitic Zoonoses

Chapter 4.1

Protozoa

Chapter 4.1.1

Amebiasis

[*ENTAMOEBA HISTOLYTICA*]

Entamoeba histolytica is an eukaryotic, microaerophilic parasitic protozoan, a species of the genus *Entamoeba*. *Entamoeba spp.* have basically three forms: 1) trophozoite, a vegetative motile form, 20 to 30 µm in size and pathogenic (able to invade intestinal wall, phagocytize red and white blood cells, causing acute amebic dysentery); 2) minuta form, a vegetative motile lumen form, 10 to 20 µm in size, that lives as a commensal in intestinal lumen, found mainly in chronic infections; and 3) the immotile cyst, a highly robust form, 10 to 15 µm in size, that is excreted during infection and protect the protozoa in harsh environments outside the host (Goncalves *et al.*, 2004). *E. histolytica* infects mainly humans (the reported infectious dose is 10-100 cysts) and other primates, through water and contaminated food ingestion, but arthropods (e.g. flies and cockroaches) may play the role of mechanical vectors of cysts. It is estimated that *E. histolytica* infects about 50 million people worldwide (Nyarango *et al.*, 2008; Gascón, 2006). Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, *E. dispar* (a noninvasive species of mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission) (Anonymous, 1997). The invasiveness of *E. hystolitica* was reported to be linked to peroxiredoxins (an important class of antioxidant enzymes) present largely on the outer surface of this species and as much as 50 times more common than *E. dispar* (the non-invasive species). The peroxiredoxins are important for *E. histolytica* to reduce peroxides and peroxynitrites' stress exhibited by infected cells (Choi *et al.*, 2005). Clinical manifestations are: diarrhea with alternating constipation, colics, spasms, mucoid and bloody diarrhea, many stools per day (10 to 12), fever and in more complicated forms as "tropical liver abscess" with hepatical lesions, pleuropulmonary amebiasis due to liver abscess rupture, as well peritonitis and pericarditis (both resulting in high mortality). In immunocompromised patients, *Acanthamoba* spp. can cause acute or chronic granulomatous amebic encephalitis (GAE).

4.1.1.1 *ENTAMOEBA HISTOLYTICA* AND ENVIRONMENT (FIGURE 4.1.1.1)

It is somehow difficult to call *E. histolytica* a zoonotic disease, as most infections are water and food-borne disease and only a small part are transmitted by dogs, monkeys and rodents that exercise as reservoirs

(Schuster *et al.*, 2004). In animals, a rare infection in a captive Tammar Wallaby has been reported (Stedman *et al.*, 2003). In humans, rural populations associated to low socioeconomic factors and poor environmental sanitation conditions are at high risk of infection with this parasite (Maco *et al.*, 2002; Mandal *et al.*, 1990; Waikagul *et al.*, 2002). Immigration from *E. histolytica* endemic areas to developed countries is also a risk factor in the reintroduction of this protozoan parasite into the general population (Gualdieri *et al.*, 2011). Another social behavior linked to parasitic infections was described by Salit *et al.* (2009) in which *E. histolytica* (a highly virulent strain that caused liver abscesses) was transmitted via heterosexual and homosexual activity.
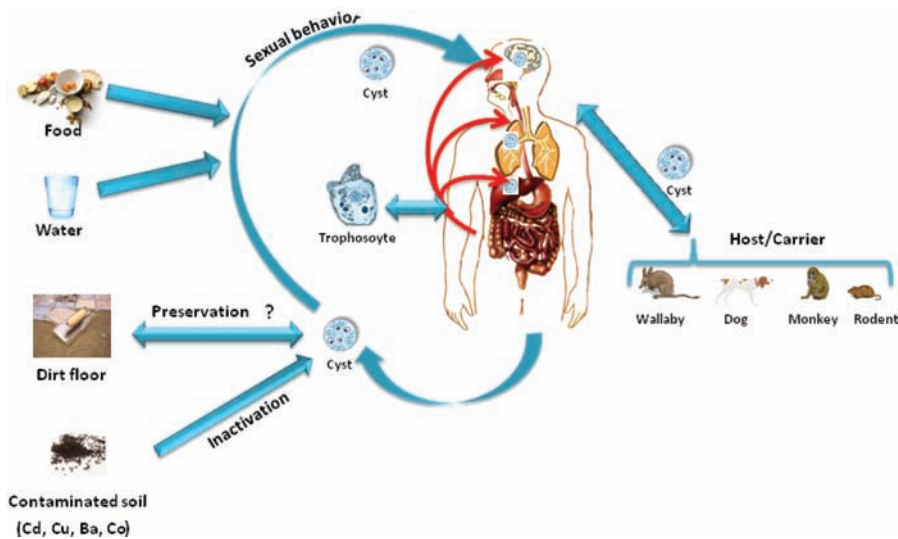
Figure 4.1.1.1. *Entamoeba histolytica* infection routes in humans and environmental factors

Aksoy *et al.* (2004) reported on the fate of *E. histolytica* cysts in soil contaminated with metal ions (Cd, Cu, Ba and Co) at concentrations of 0.05, 0.1 and 0.2 mM. At these concentrations, metal ions led by Cu had a lethal effect on cysts' viability. Perhaps these results are somehow encouraging, as Saxena and Bhattacharyya (2005) raised the issue of increased environmental risk of hazardous wastes combined with biological (parasitic) contamination in India. In India, another study examined the pollution of beach water and sediment at seven sites receiving land drainage, along a 45-km stretch of the east coast near Visakhapatnam (Clark *et al.*, 2003). Sediments revealed significant contamination with bacteria and protozoa, suggesting that they play a significant role in the retention of these pathogens. An interesting issue that seems to be partially answered by this study is the resistance and viability of cysts to high salt concentrations. In relation to halotolerance, Pandey *et al.* (2002), showed that transgenic tobacco plants that express an *E. histolytica* calcium binding protein exhibited greater than before resistance to high salt concentrations. In plants it has been demonstrated that Ca^{+2} is involved in their survival at elevated sodium concentrations. According to this report, it is evident that Ca^{+2} homeostasis manipulation by changing the expression level of Ca^{+2} binding proteins might be an important strategy to alter plants' survival rate under abiotic stress (Bressan and Hasegawa, 1998). Whether this calcium binding protein

exhibits the same phenotypic characteristic when expressed in *E. histolytica* is not known, but if affirmative it may explain the survival of cysts in sea water!

Rinnea *et al.* (2005) using a multivariate logistic model in a population with high parasitic infections, found that the main risk factors for *E. histolytica*/*E. dispar* infections include >1 month since antiparasitic treatment, unchlorinated drinking water, poor hygiene (children not washing their hands before meals) and having a fewer number of electrical appliances at home. Though not clearly stated, the authors used electrical appliances as a poverty index for their studied population. Another important factor related to amebiasis prevalence was reported by Jacobsen *et al.* (2007) in the same area of Ecuador. The authors found that houses with dirt floors are significantly related to higher infections with *E. histolytica* (63.4% vs 29.7%, p<0.001). It can be very possible that dirt floors support cysts' preservation, are difficult to clean and wash and possibly carry cysts before installation.

As mentioned earlier, *E. histolytica* have been isolated from primates (chacma baboons, mantled guereza, northern plains gray langurs, macaques, vervet monkeys, olive baboons, hamadryas baboons, cynomolgus monkeys, etc.) and some from fatal cases in zoo animals (Muriuki *et al.*, 1998; Jackson *et al.*, 1990; Howells *et al.*, 2011; Reiner *et al.*, 2010; Rivera *et al.*, 2010; Ghandour *et al.*, 1995; Takano *et al.*, 2005). The Philippines is a major exporter of monkeys for biomedical research purposes, so screening animals before transportation to other locations lessens the risk of spreading zoonoses to a wider remote area (Rivera *et al.*, 2010; Takano *et al.*, 2005).

Finally, an interesting report linked to *E. histolytica* and its prevention has shown that ultrasonic vibrations can rapidly destroy cysts a measure that can be implemented in modern disinfection practices (Horton *et al.*, 1952).

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Chapter 4.1.2

Babesiosis (aka Piroplasmosis)

[*BABESIA SPP.*]

Babesia is a protozoan parasite that infects humans through erythrocyte invasion. There are >100 *Babesia* species, but species infecting humans are: *B. microti*, *B. divergens*, *B. bovis*, *B. equi*, *B. gibsoni* and *B. canis* (order *Piroplasmida*, family *Babesiidae*). The parasite is transmitted by arthropod vectors' bite (hard ticks such as: *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Hyalomma*) from reservoir animals, such as rodents, cattle, horses and dogs (Jonsson *et al.*, 2008; Soares *et al.*, 2006; Robert *et al.*, 2005). Another pathway of transmission is iatrogenic (via blood transfusions using an infected carrier as a donor or by needle sharing), with infected donors residing in endemic areas and through their geographical migration (Cable and Leiby, 2003). *Babesia* spp. are distributed worldwide according to their tick vector species (Table 4.1.2.1). The parasite has mainly two reproduction cycles: sexual (mainly in tick) and asexual (mainly in human erythrocyte) (Figure 4.1.2.1). Beside salivary glands, parasite transmission by ticks may occur transovarially (from the maternal body to eggs within the ovaries) and also transstadially (from one developmental stage to its subsequent stage or stages) by eggs, larvae and nymph infestation (Iori *et al.*, 2010). In healthy persons, infections are mostly subclinical, but in immunosuppressed patients they can manifest by means of fever, malaise, dyspnea, haemolytic anemia, hemoglobinuria, thrombocytopenia, shock, ARDS and even death.

4.1.2.1 *BABESIA* SPP. AND ENVIRONMENT (FIGURE 4.1.2.1)

There is an increasing number of reports of new *Babesia* species isolated from a large variety of animals, including wild life. The transmission of *Babesia* spp. from domestic to wild animals was successful only in closely related species or in humans following splenectomy. Infected animals do not present clinical manifestations; however, under stressful conditions (confinement following capture) infection can become active, including fatal outcome. This aspect raises the question of what may happen to confined infected wild animals released into natural environments as part of conservation programs? (Penzhorn, 2006). A partial response was presented by mice experimentally infected with *Babesia microti*. A group of mice, housed in cages furnished with shelves and nestboxes, displayed increased aggression and

reduced resistance to parasitic infection. Serum concentrations of testosterone and corticosterone (immunodepressive hormones) were downregulated while serum total IgG (as a measure of immunocompetence) and resistance to *B. microti* decreased as attack numbers increased (aggression). According to this study and other studies, hormone-related reduction in resistance occurs when hormone levels and immunocompetence are decoupled (Barnard *et al.*, 1996). A more recent study revealed that pregnant mice indirectly exposed (separated by a perforated Perspex partition) to *B. microti* infected neighbors had higher levels of serum corticosterone and produced later on less aggressive offsprings with accelerated immune response against this parasite. Without direct contact, the authors suggested that pregnant mice females assessed neighbors' infection via olfactory cues. This study is important to our understanding of animal and human infection and resistance to pathogens under environmental stresses (Curno *et al.*, 2009).

Table 4.1.2.1 *Babesia* spp. and geographic distribution.

Babesia spp.	Animal hosts	Country	Vectors (main)
B. microti	Rodents	USA, Mexico, Europe, China, Taiwan, Egypt, Nigeria, South Africa, Poland	*Ixodes scapularis*, *I.dammini*
B. equi	Horses	USA, Greece, Mongolia, China, Israel	
B. divergens	Cattle	France, UK, Germany, Russia, Spain, Sweden Switzerland, former Yugoslavia, Austria	*Ixodes ricinus*
B. gibsoni	Dogs	Spain, USA, Japan, South Korea	*Haemophysalis bispinosa* and *H. longicornis*, *Rhipicephalus sanguineus*
B. canis	Dogs	France, Hungary, Taiwan, Russia, Brazil	*Rhipicephalus sanguineus*
B. bovis	Cattle	Spain, former Yugoslavia, Mexico, Mozambique, Tanzania, Costa Rica, Malaysia, Sri Lanka	*Rhipicephalus microplus*

Babesia like other vector-borne pathogens uses antigenic variations to evade its host's immunity. During a single infection, many variants (serotypes) are expressed and this is why many studies reported different subpopulations (Dalrymple *et al.*, 1992; Arai *et al.*, 1998; Barbour and Restrepo, 2000).

Sinski (1999) pointed to the importance of susceptible rodents in the role of competent zoonotic reservoirs for *B. microti*, especially in areas with changing farming systems that left large land areas under grassland and pasture in Poland (Mazury Lakes district). The same trend was also reported in Uganda, where babesiosis infections in rural area were assessed based on the prevalence of ticks under different grazing systems and agro-ecological zones (Rubaire-Akiiki *et al.*, 2004). The highest tick prevalence was observed in the lowland zone under the free range and tethering grazing systems in comparison with the midland and upland zones under a zero-grazing system. The authors also mentioned that during the dry seasons farmers got additional fodder from outside their farms that possibly resulted in the import of new ticks.
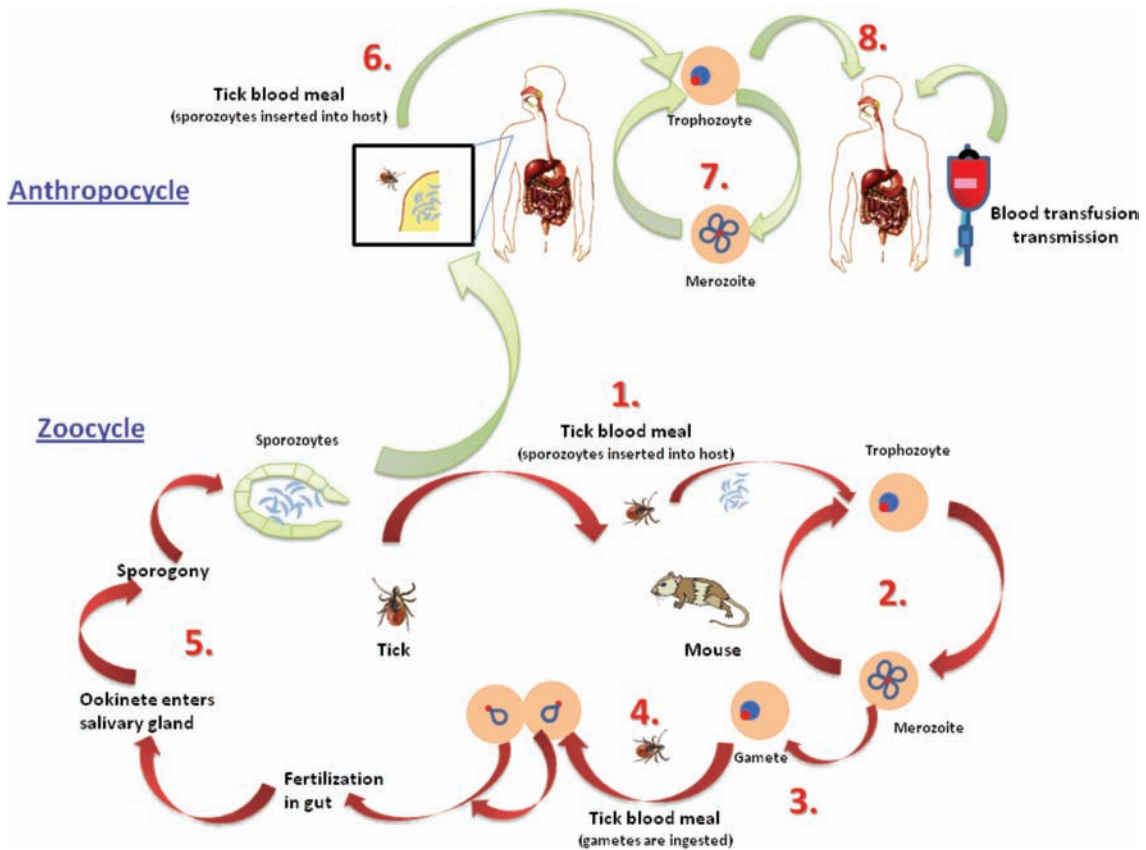
Figure 4.1.2.1. Infection and transmission cycles of *Babesia* spp. in different hosts (animals and humans)

Koum *et al.* (2010) used two models (maximum entropy ecological niche modeling-Maxent and spatial scan statistic-SaTScan) to predict geographic range and infection clusters of *Babesia caballi* (or equine piroplasms). The most important environmental factors contributing to these models were land cover (forested areas) and temperature (regulated temperatures and higher humidities) that are highly significant for tick survival and reproduction (Kouam *et al.*, 2010).

There are numerous studies obviously showing the biological impact of chemical pollutants, present in different environments, on a large variety of animals (terrestrial and aquatic). Peltzer *et al.* (2008) looked at the health effects of agricultural runoff-polluted ponds containing organochlorine pesticides on anuran tadpoles (*Scinax nasicus*) as asentinel organism. Following seven days of exposure to organochlorine pesticides and high organic load, the experimental tadpoles' survival, growth and development rates, erythrocytes nuclei aberrations, parasite infection, and brain cholinesterase activity were monitored. The results showed deleterious effects on all parameters tested that can possibly lead to an increase in tadpole vulnerability to opportunistic parasite infection. In relation to chemical contaminants, similar effects on erythrocytes were observed with other environmental chemicals that, according to the author, do not follow a simple diffusion mechanism but to a great degree depend on the physiological condition of the erythrocytes (Ryasnyanskii, 1982).

Telfer *et al.* (2010) pointed out an important aspect linked to simultaneous or sequential infection with various microparasites (bacteria, viruses and protozoa) and their host infection risk outcome. Four microparasites coinfections in wild animals (field voles) were analyzed: cowpox virus, *Anaplasma phagocytophilum*, *Babesia microti* and *Bartonella* spp. These authors analyzed time-series data of individual infected hosts and found that there is a significant interconnection between these microprasites affecting the infection risk at a much greater magnitude than environmental factors, previously thought to be of major importance. The effect can be positive, increasing the infection risk factor, but also negative concurrently reducing this factor. For example, the largest effect was observed with *Bartonella* reduced susceptibility in individuals infected with *B. microti* (especially in chronic infections). In this particular case, *B. microti* infection also decreased *Bartonella* spp. infection duration. The explanation for this phenomenon is based on host resource depletion as both pathogens are haemoparasites targeting erythrocytes. Another example of such interaction was shown in cattle's simultaneous infection with *Babesia divergens* (another *Babesia* sp.) and *A. phagocytophilum*, resulting in suppression of the first by the subsequent microparasite (Purnell *et al.*, 1977).

Healy (1989) raised an important aspect related to the present spread of babesiosis (*B. microti*) in north and eastern America due to cultural and environmental changes. The first eye-catching common evidence responsible for these infections was the patients' behavioral (cultural) activities such as recreational pursuits in woodlands, forests, meadows, where the tick *Ixodes dammini* prevails (Spielman *et al.*, 1985). *Ixodes dammini* is a three-host species rodents, humans and other mammals (raccoon, possum, dog, fox and white-tailed deer), while white-tailed deer is the most abundantly infested without clinical symptoms. Due to human intervention in the last 20–30 years, white-tailed deer population increased immensely (>13 millions in USA), requiring even mandatory hunting in several states. Consequently rodents, tick, migratory host (white-tailed deer) and human behavior are the main factors that affect babesiosis epidemiology, at least in North America.

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Chapter 4.1.3

Balantidiasis

[*BALANTIDIUM COLI*]

Balantidium coli is a protozoan ciliate parasite the only one known to be a human pathogen among the ciliate pylum. This ciliate has two stages: trophozoite (the vegetative stage found in the cecum and colon of pigs, rats, humans and other mammals) and cysts (the resistant infective form excreted in feces) (Figure 4.1.3.1). The trophozyte has an oval shape with an average size of 40×70 by 50×100 μm, while the round cysts size is ∼55 μm. The main vertebrate host is pig but humans and other mammals can be infected through cysts transmitted by contaminated water and food (Uysal *et al.*, 2009; Weng *et al.*, 2005). *Balantidium coli* has a worldwide distribution (common in the Philippines) but the infection rate is low (<1% in human population) and mainly among people that come in contact with pigs or their feces (Schuster and Visvesvara, 2004; Schuster and Ramirez-Avila, 2008). Human infection is sporadic and most infections are asymptomatic. Clinical manifestations are: abdominal pain, acute diarrhea, severe colitis and in extreme cases colon perforation that can become life threatening.

4.1.3.1. *BALANTIDIUM COLI* AND ENVIRONMENT

In spite of its lower zoonotic importance, *B. coli* can be found in children in endemic rural communities in Venezuela with low hygienic standards (deficient sanitation), absence of basic services in dwelling, low socioeconomic level and close contact with infected pigs (Devera *et al.*, 1999). In another distant geographical area, Nigeria, a cross-sectional study was conducted on the presence of *Cryptosporidium* oocysts and *Balantidium coli* cysts in pigs' fecal samples from a semi-intensively management. *B. coli* was also isolated from water and soil samples from an area where pigs are allowed to scavenge and defecate. In addition, the authors described usage of pigs' untreated manure in vegetable farms, thereby spreading further infections (Yatswako *et al.*, 2007). A similar elevated *B. coli* infection rate has been detected in Korean rural areas (Ismail *et al.*, 2010). Use of untreated pig manure has a great contamination potential especially in so-called "organic farming", the new trend in developed countries. Indeed, there are two recent reports of a rare form of balantidiasis affecting lungs through inhalation of

manure aerosols: one in Canada and a second one in South Africa (Sharma and Harding, 2003; Koopowitz *et al.*, 2010). An interesting aspect of pigs as host and carriers of *B. coli* was raised by Solaymani-Mohammadi *et al.* (2005) who pointed at Iran, an Islamic country where according to Muslim religious rules, pig breeding and growth are prohibited, and which would therefore be expected to be free of human infection with pigs' parasites. Nevertheless, a very low prevalence of infection exists (0.009% in Tehran suburbs) explained by these authors as originating from wild boars as carriers, especially in the forested areas of the country, where these animals roam freely and can contaminate water and soil environments contaminators. In a reply letter to the Editor, Cox (2005) suggested that camels that they were already proven to harbor *B. coli* (11.9 % fecal samples prevalence in Ethiopia) seem to be the main reservoir (especially in this geographic area) and also the intensive agricultural use of camel dung as fertilizer. The scientific debate on this issue would be solved by further research, but it seems that definitely wild boars are a more predominant wild life population than camels in Iran, albeit close contact is prohibited (*Solaymani-Mohammadi *et al.*, 2005).
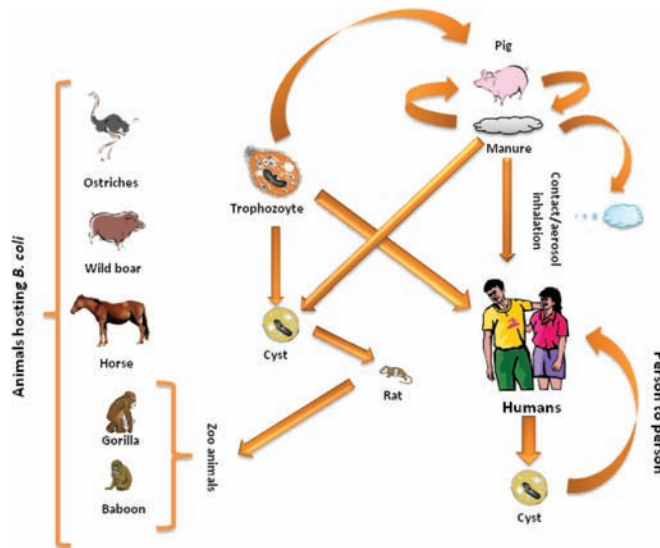
Figure 4.1.3.1. *Balantidium coli* infectious cycle in humans and animals involved as carriers

Finally, there are increasing reports of *B. coli* isolated from various animals, such as ostriches, ares, Old World monkeys (e.g. macaques and baboons), great apes (chimpanzees, bonobos and gorillas) and wild boars. Pomajbková *et al.* (2010) surveyed wild and captive great apes for the presence of *B. coli*. The captive great apes were found to harbor this parasite (prevalence 52.6%) versus zero isolation in wild ones. The authors brought up several ideas to explain this dissimilarity: 1) in urban areas, infection of captive animals is by synantropic rats and 2) persistence due to feeding captive apes with high starch diets that support parasite growth. However, in humans, pigs remain the main host and infection source with this parasite, especially due to their close contact (Ponce-Gordo *et al.*, 2008; Headley *et al.*, 2008; Levecke *et al.*, 2007).

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Chapter 4.1.4

Chagas' disease (American Trypanosomiasis)

[*TRYPANOSOMA CRUZI*]

Trypanosoma cruzi is a vector-borne parasitic species (a hemoflagellate) of euglenoid trypanosomes genus, infecting both humans and animals (related to >150 domestic and wild spp. as hosts and reservoirs except birds which are refractory), that causes a serious disease called Chagas' disease or American Trypanosomiasis. Chagas' disease is spread in Central and South American countries. The parasite is mainly transmitted by bloodsucking insects (inhabiting both sylvatic and domestic areas) (Magallon-Gastelum *et al.*, 2004) from the subfamily Triatominae (family *Reduviidae*) but other possible transmissions pathways are also known such as: congenital, food-borne, blood transfusion or organ transplant and laboratory accidents. Tritominae subfamily has >35 species, some of them important vectors of *T. cruzi* (Table 4.1.4.1). The insect deposits its feces (containing the metacyclic trypomastigote form of the parasite) on the host skin and subsequently bites in order to have a blood meal. Scratching the bitten area facilitates the entrance of fecal trypomastigotes into the blood stream and initiates infection. *Trypanosoma cruzi* has two major forms: trypomastigote ($\sim$ 20 µm in length, found in human blood) and amastigote (3–5 µm in length, found in tissue) (Figure 4.1.4.1). In humans, following infection, trypomastigotes enter the peripherial blood without reproduction and shortly after infect intracellularly myocardial and endothelial cells of internal organs (e.g., spleen, liver, reticuloendothelial system, etc.). On these sites they reproduce, forming amastigote forms grouped in cysts that, after the cells burst, produce again trypomastogotes that invade again the peripheral blood for a short time period, causing further infection in host cells. Trypomastigotes are transferred to bloodsucking insects from humans or infected animals for the duration of the blood feeding process. In the anterior midgut of the insect vector, the parasite forms a different stage called epimastogote (>30 µm in length) that goes through intensive binary fission in the posterior end of the vector (Triatomae) resulting in the final infective form: metacyclic trypomastigote found in insect excreta. The disease manifestation has two forms: acute and chronic. The acute form (cutaneous-Chagoma or ocular- Romaña's sign) follows after insect vector bites and presents as a local swelling (on skin or conjugtiva) that possibly can go unnoticed

by the host (Herrera *et al.*, 2007). In terms of time, the chronic form may develop 10 to 20 years after the initial bite into a life threatening disease in which the parasite colonizes the heart, esophagus, colon and peripheral nervous system. The main symptoms are fever, malaise, anorexia, hepatosplenomegaly, lymphoadenopathy, myocarditis, meningoencephalitis and fatal outcome as a result of heart failure. The disease progresses slowly (see above the time span from initial bite) through progressive cell destruction due to parasite colonization of different organs, multiplication and autoimmune processes occurring in the host.

Table 4.1.4.1 Triatomine vectors geographic and ecosystem distribution.

Species	**Geographic distribution**	**Ecosystem distribution**
Triatoma infestans	South America, Western and Southern USA	Exclusively domestic, with the exception in Bolivia where (sylvatic forms)
Triatoma sordita	South America	Both sylvatic and domestic habitats
Triatoma barberi	Mexico	Domestic and peridomestic habitats
Triatoma dimidiata	Central America, Columbia, Ecuador	Both, sylvatic and domestic habitats
Rhodnius prolixus	Northern South America, Venezuela, Western and Southern USA	Both sylvatic (associated with palm trees) and domestic habitats
Rhodnius pallescens	Panama	Both sylvatic and domestic habitats
Panstrongylus megistus	Brazil, Peru	Tropical Atlantic, cerrado (similar to the savanna) and Atlantic forest remnants habitats
Triatoma juazeirensis	Northeast Brazil	Exclusively rupicolous (thriving among rocks) but able to invade peridomestic environmental habitats
Triatoma pseudomaculata	Northeast Brazil	Both sylvatic and domestic habitats
Triatoma brasiliensis	Northeast Brazil	Both sylvatic and domestic habitats Semiarid areas
Triatoma pallidipennis	Mexico	Mostly domestic (rural areas)

4.1.4.1 *TRYPANOZOMA CRUZI* AND ENVIRONMENT

Chagas' disease is perhaps one of the most prominent paradigm of this book on the environmental aspects of zoonotic diseases. Briceño-León (2007) analyzed and discussed different aspects involved in the spread of this disease towards Amazonian and new environments. The Amazon region is a river basin encompassing nine countries, Brazil, Colombia, Peru, Ecuador, Bolivia, Venezuela, Surinam, Guyana, and French Guyana, an area of approximately seven million km^2, up to the last century having mainly scattered indigenous populations, and consequently considered a non-endemic area. However, in the last four decades, the disease reappeared with higher intensity due to socio-economic factors imposed by governmental decisions under local and global pressure, one of which was to build the Belém-Brasília highway to launch the country's "inward development" plan, which was subsequently followed by

others (North-South Cuiabá-Santarém Highway and the Trans-Amazonian Highway). As a result of these developments and massive population migration several factors appear to impact the disease spread: 1) new human dwellings built (mostly closed with bricks) in contrast to indigenous open dwellings that are unfavorable to vectors colonization; 2) lower mobility of the new population and the establishment of fixed colonies in contrast to the dynamic mobility of the native population; 3) introduction of mass domestic animals that are used as host for bloodsucking vectors, in contrast with the absence of domesticated animals grown by original natives; 4) deforestation in order to increase cattle and crop production that compel vectors' migration into new niches with abundant food supply; 5) lumber industry that may disturb and disperse sylvatic vectors, originally inhabiting trees and nests, and 6) development of highway transportation that accelerates and facilitates the disease's transmission to remote places, including immigration of infected population from endemic areas, posing a new threat of spreading this disease to remote sites (Hanford *et al.*, 2007) (Figure 4.1.4.2).
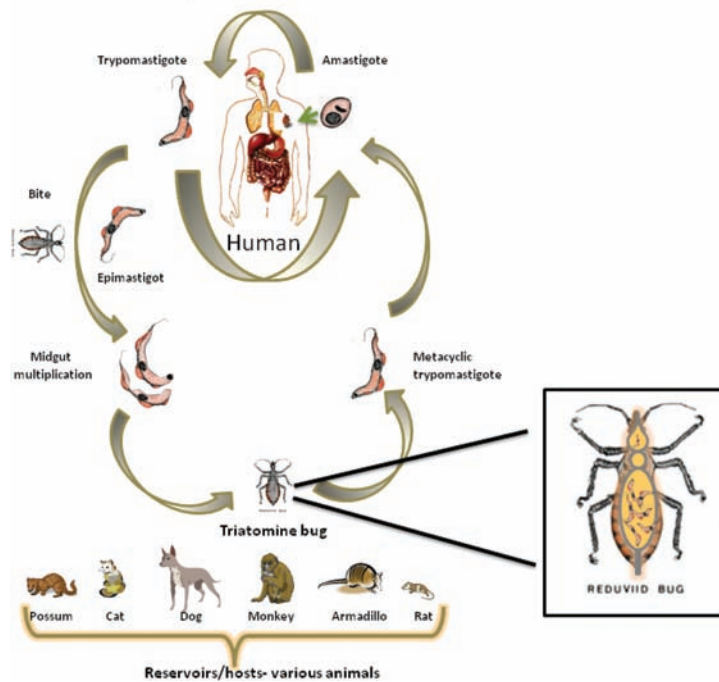
Figure 4.1.4.1. *Trypanosoma cruzi* life cycle and some of its hosts

In relation to domestic animal growth, Herrera *et al.* (2007) evaluated how landscape and cattle ranching affect transmission cycles and the patterns of tripanosomatid infection (*Trypanosoma cruzi* and *Trypanosoma evansi*) of small wild mammals in the Pantanal (world's largest tropical wetland, comprising Brazil, Bolivia and Paraguay). In relation to small mammals, the conclusions of their study, in contrast to the above described factors, was that cattle ranching in the study area did not enhance overall prevalence of *T. cruzi* infection among small wild mammals as well as biodiversity loss or risk of Chagas' disease. Chagas' disease vectors from the subfamily Triatomae are insects widespread in South and Central America. They are expected to be the main factor influenced by environmental, including

manmade, changes when dealing with disease infection and prevalence. Lima *et al.* (2008) presented the health risk of *Copernicia prunifera*, a species of palm trees, found in both periurban and rural localities of an endemic northeast Brazilian Chagas' disease region. These palms are colonized with infested Triatomae (in this case with *Rhodnius nasutus*) that carry *T.cruzi* at high prevalence. As a result of the continuous destruction of palm trees, there is a high risk of vectors invading of periurbanic human dwellings, in search for new habitats. Researchers also reported on the demographic areas of Cochabamba (Bolivia) where 58% of the population lives in peripheral urban districts ("popular zones") which differ in social, environmental, and agricultural conditions. These areas also exhibited extremely high infection prevalence (in children age 5 to 13) with *T. cruzi*, suggesting that *T. cruzi* infection should be considered an urban health problem and not only restricted to the rural areas and small villages of Bolivia, by continuous infection and reinfection (Medrano-Mercado*et al.*, 2008).
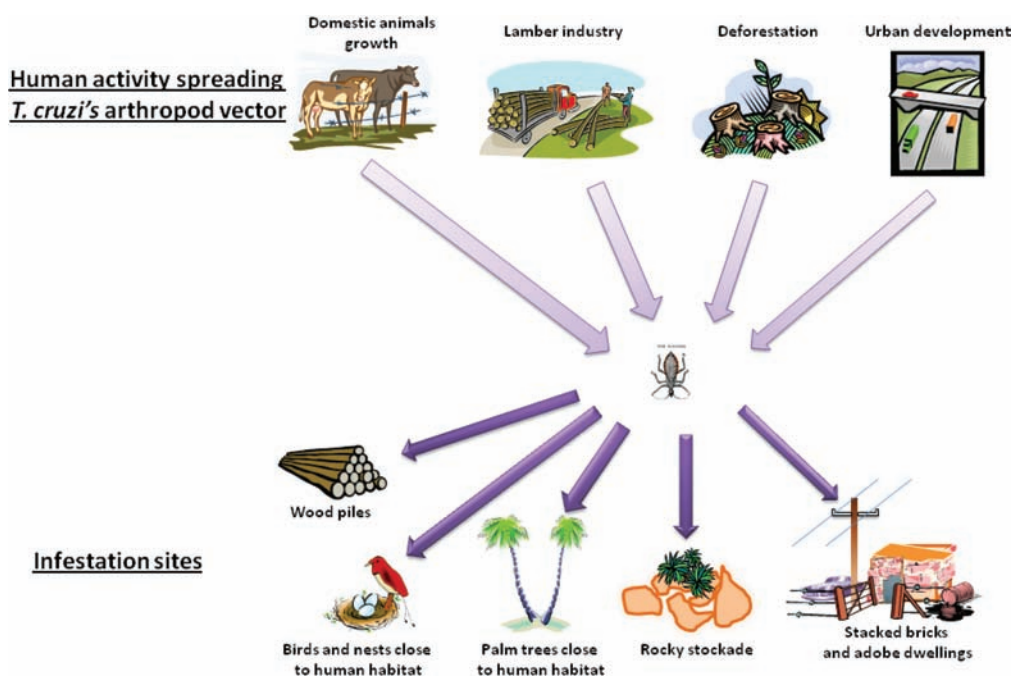
Figure 4.1.4.2. Environmental factors involved in spreading and infestation of Triatominae arthropod vectors (*T. cruzi* carriers)

de la Fuente *et al.* (2008) studied the behavior of two species: *T. psudomaculata* and *T. juazeirensis*. According to these authors, the above arthropods do not share the same habitat in sylvatic environment. *T. pseudomaculata* can be found in trees and bird nests without apparent tree preference, while *T. juazeirensis* lives exclusively in rupicolous (thriving among rocks) environments. Both were found to have behavioral plasticity to invade peridomestic structures (including wooden structures) in >80% of cases. The synanthropic behavior evolution of these insects was studied by Abad-Franch and Monteiro (2007) in the various regions of the Amazon. The authors pointed out that evolutionarily, most of the Amazonian Triatomines live in highly humid forest environments; nevertheless, only a few species adjusted to new drier environments (mainly through anthropogenic ecological disturbance, driven by deforestation),

forming domestic infestation foci. Thus, only a small fraction of *Rhodnius* spp. adapted evolutionarily to arid or semi-arid climates long before human existence in the Americas, ostensibly in drier regions of the forest, making them adapt better to human dwellings. This study confirms two obvious facts: 1) arthropod evolution is a continuous process on an immense time scale, although 2) anthropogenic activity may accelerate this process.

Click *et al.* (2008) applied GIS (geographical information system) to analyze the domesticity potential of three triatomine species within the United States known to harbor *Trypanosoma cruzi*. According to their results and based on actual minimum lower temperature threshold, the current area at increased risk of Chagas' disease includes much of the southern United States. Nevertheless, based upon the 1°C (1.8°F) increase in temperature predicted by the Intergovernmental Panel on Climate Change (IPCC) by the year 2030, they concluded that the higher risk range is expected to expand into the central United States.

Zeledo *et al.* (2008) reported on a new project that used garden chicken coops as a decoy for *T. dimidiata* to prevent home colonization, with good results. However, there are some issues related to infected chickens and their possible parasite food-borne transmission to farmers. The idea of using small animals in wire meshed cages as baits is based on an entomological survey performed in Peru (Levy *et al.*, 2006). These authors emphasized again the importance of human dwellings (fully stuccoed versus rooms of stacked bricks and adobe enclosure) for the prevention of infestation with *Triatoma infestans* vector.

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Chapter 4.1.5

Cryptosporidiosis

[*CRYPTOSPORIDIUM PARVUM*]*

Cryptosporidium parvum belongs to phylum Apicomplexa and order Eucoccidiorida. It is a parasite infecting the intestinal tract of a large variety of mammals (~152 species) including humans (Tzipori *et al.* 1980; Atwill *et al.* 2004; Torres *et al.* 2000). There are at least two genotypes (based on oocysts' wall protein phenotypes), genotype 1 (exclusively in humans) and genotype 2 (found in livestock animals, such as sheep, cattle, goats and rodents) that can infect humans too. Basically, the parasite has two forms: the one excreted in feces, called oocyst (5 to 6 μm with an ovoidal, pea-like shape, two types: thick and thin walled) and the vegetative form found in the intestinal epithelium called sporozoite. Sporozoite development is intracellular (between the epithelial cells' cytoplasm and their membrane) following two replication paths: asexual and sexual (Figure 4.1.5.1). Oocysts are the final form excreted in feces during acute infection, in high numbers (10^6 to 10^{10} oocysts/gram feces). Oocysts (thick wall) have several attributes that favor their environmental distribution: environmental survival for months, stickiness that helps infection and a relatively low infection dose (~132 oocysts in healthy humans). Feces in diarrheic form, from infected calves (the main infected hosts), are washed out into water and soil sources, thereby contaminating human environment (Atwill *et al.* 2006). Infection with *Cryptosporidium* oocysts can take place by direct contact with infected stools, food and water, person to person (sexual contact) and aerosols. Clinical manifestations are watery, acute diarrhea, anorexia, nausea/vomiting and abdominal pain. In immunocompetent persons the disease is self-limiting while in immunocompromised patients it can be fatal (rapid dehydration due to 10–15 liters of diarrhea) with infections of other organs, such as pancreas and lungs.

4.1.5.1 *CRYPTOSPORIDIUM PARVUM* AND ENVIRONMENT

Cryptosporidium is now recognized as one of the major causes of zoonotic human diarrheal disease worldwide. Based on a large published data base, cryptosporidiosis is mainly a water-borne zoonosis but

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*(This chapter is dedicated to late Huw V. Smith, a worldwide expert in *Cryptosporidium*, a friendly and thoughtful person that passed away prematurely)

also food-borne, as infected animals' excreta reach water sources or food, and human' infection occurs through consumption or direct contact with (Figure 4.1.5.2). The best known outbreak occurred in 1993 in Milwaukee, Wisconsin, USA, where Lake Michigan contaminated drinking water with *Cryptosporidium* oocysts passed the filtration system of one of the city's water treatment plants affecting >400,000 people with a massive outbreak of watery diarrhea (Mac Kenzie *et al.* 1993).
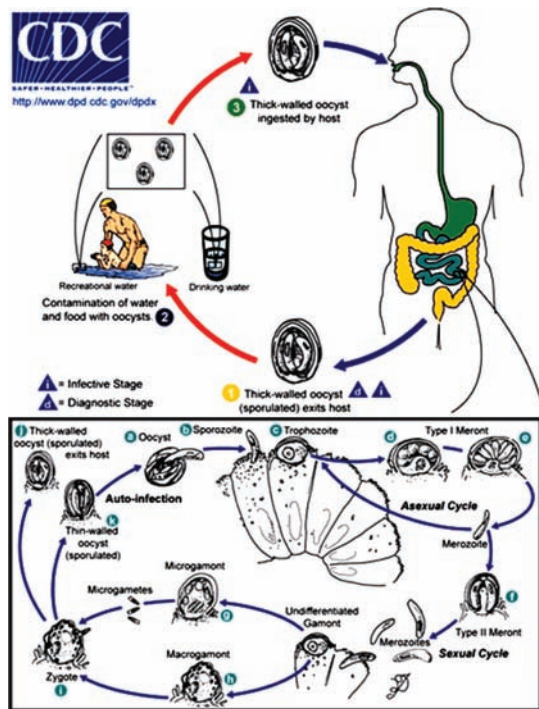
Figure 4.1.5.1. *Cryptosporidium parvum* life cycle. (with permission from CDC, Atlanta, USA, http://www. dpd.cdc.gov/dpdx/html/Cryptosporidiosis.htm)

Due to its environmental robustness and distribution, adequate mechanical and chemical treatments are required for oocyst removal from water prior to human supply. The main process for oocyst removal is filtration through a large variety of matrices that strains these particles and prevents their further movement into clean water outlets. In this context, Parker and Smith (1993) reported that shaking a mixture of *C. parvum* oocysts and sand particles can induce oocysts destruction, especially after chlorine addition. Agitation of oocysts with sand for 5 minutes and subsequent chlorination for additional 5 minutes resulted in 68.02% inactivation. The direct outcome of this phenomenon can be applied in large water filtration facilities, saving expensive processes used today to inactivate this protozoon parasite.

Naumova *et al.* (2007) proposed an analytical and conceptual framework for assessment of disease seasonality. Among six enterically transmitted reportable diseases (EDs) in Massachusetts, the authors quantified the timing and intensity of seasonal peaks of cryptosporidiosis incidence and examined the synchronization in timing of these peaks with respect to ambient temperature for a 10 years period.

Relative to peak temperature, *Cryptosporidium* exhibited a significant delay of ∼40 days. The long delay was explained by the following attributes: different transmission routes, person-to-person infection amplification, pathogen environmental survival, incubation time period, different disease manifestation and combination thereof.
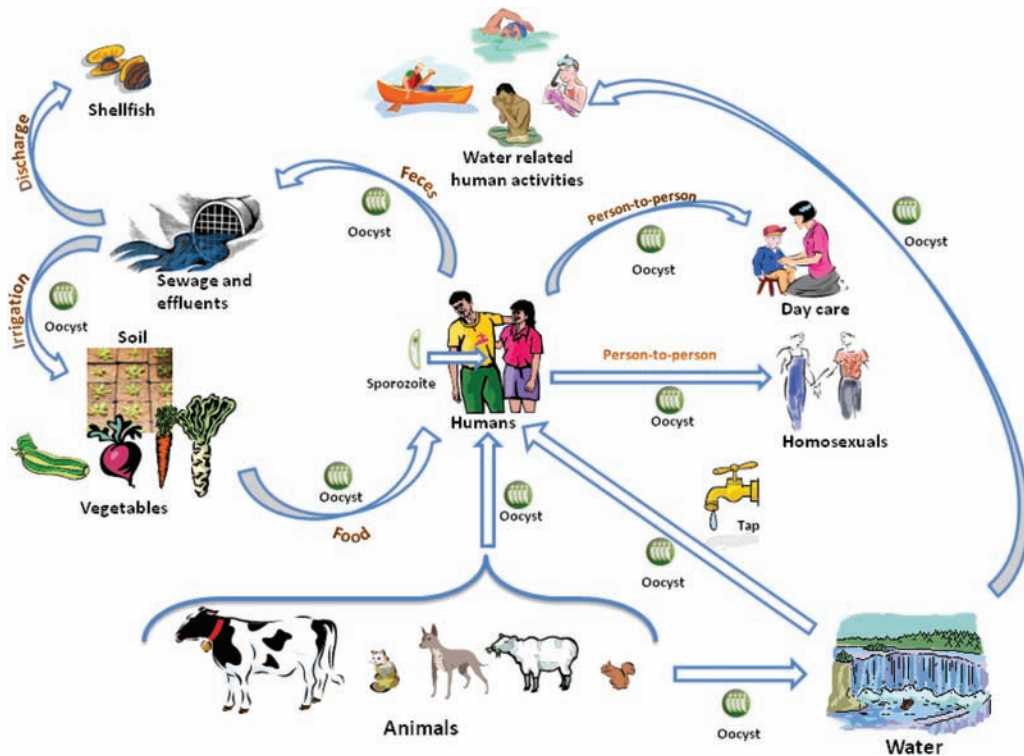
Figure 4.1.5.2. *Cryptosporidium parvum* environmental cycle

Hu *et al.* (2007) also pointed on temperature as a significant factor involved in *Cryptosporidium* transmission in Australia, by data analysis using two models: time series Poisson regression and seasonal auto-regression integrated moving average (SARIMA). It should be remembered that during warm summer months people drink more and practice more outdoor activities, increasing the risk of infection. In a more predictive study, Casman *et al.* (2001) analyzed qualitatively the impact of climate changes on cryptosporidiosis. Heuristically they showed that elevated temperature, changes in river flow, and increased water pollution could intensify cryptosporidiosis in the U.S., however in a developed country like U.S. with advanced public health management and high standards of living, the negative effects of climate can be overwhelmed, even in cases of climatic catastrophes.

In contrary, in a developing country like Indonesia, Katsuma *et al.* (1998) applied a multiple logistic regression model to find out the significant risk factors for *Cryptosporidium* infection. Their results revealed that direct contact with animals (in their particular case, cats), increased precipitations, floods and socio-economical aspects (e.g. crowded living conditions) are indeed the significant risk factors that

public health management has to deal with, but much less efficiently then in USA. Here it should be pointed out another social aspect not covered by Casman *et al.* (2001) in their report and this is the age factor in cryptosporidiosis in other words children high susceptibility to infections, especially in developing countries (Reinthaler, 1989).

It is clear now that rainy seasons are a major factor in the spread of *C. parvum* and its high prevalence, but rain has another impact on pathogen pollution namely dilution factor (Katsumata *et al.*, 1994; Curriero *et al.* 2001; Charron *et al.* 2004). If the main source of *C. parvum* is manure from calves (with elevated oocysts output), rain besides spreading and carrying manure can also dilute its content. A direct outcome of such an event is additional contaminated water sources with this specific pathogen but at lesser number, therefore in order to contract an infection, a person has to be exposed to larger volumes of water. A proof to this perception was reported by Noordeen *et al.* (2000) in Sri Lanka. The authors screened three agro-climatic zones in rural communities of Sri Lanka to determine *Cryptosporidium* species among goats. *Cryptosporidium* oocysts were detected in all agro-climatic zones, with highest prevalence of infection in the dry zone (33.6%), followed by intermediate zone (24.7%) and wet zone (21.7%) ($p <$ 0.001). It is surprising that drier zone had higher prevalence, since water is an important parameter in infection and its extensiveness. Their logical explanation was that in dry regions, goats encounter nutritional stress under extensive management system without supplementary feed, in consequence lower immunity to parasitic infection. They also pointed out on additional environmental conditions (e.g. sheds with low standards of hygiene) that may favor a continuous fecal-oral transmission in those animals. In this case, it can be suggested a third possibility in which dry zone preserve higher concentrations of the parasite in excreta without rain dilution factor, increasing the chance of infection (based on infection dose).

In a more recent publication, Nichols *et al.* (2009) reported on a case-crossover study comparing rainfall and outbreaks of different pathogens, among them *Cryptosporidium*, for an extended time span (from 1910 to 1999). Based on weather data they found a significant correlation between excessive cumulative rainfall in the previous 7 days and pathogens outbreaks ($p = 0.001$), but low rainfall (<20 mm) for three weeks before outbreaks was also highly correlated ($p = 0.002$). Based on these results, these authors suggested that any climate change should examine both situations.

Jenkins *et al.* (2002) studied the survival of *C. parvum* oocysts in three soil types (silty clay loam, silt loam, and loamy sand) at different temperatures (4, 20, and 30°C) and water potentials (-0.033, -0.5 and -1.5 MPa). According to their experimental results, soil survival was not affected by the experimental water potentials, but affected at a certain extent by soil texture and mostly by temperature. Their conclusion was that under temperate climates' temperatures range, oocysts may survive for months in agricultural soil consequently posing a threat to surface waters.

A more extreme environmental survival study on *Cryptosporidium parvum* in soil was performed in Norway, where winter soil temperatures are very low with many freeze–thaw cycles (minimum ranges -9°C to -25.2°C and maximum $+9$°C) (Robertson and Gjerde, 2004). The results of this study revealed that *C. parvum* oocysts do not endure the Norwegian terrestrial environment over winter, and if despite all that oocysts are isolated from soil, their origin is from previous winter/spring excretions.

In England and Wales, a study was carried out, using ordinary least-squares regression method, to assess the role of environmental factors on cryptosporidiosis from records over an eight years period. Weather and river flow (as indicators of precipitation amount) were used as environmental factors. In general, between April and November cryptosporidiosis rate was positively related to maximum river flow, however between December to March (winter season) no such association was found (Lake *et al.* 2005). The authors hinted to animal-human infection route as the main factor related to disease prevalence, as during winter time animals (the main parasite carriers) are penned, hence reduced water and soil contamination. However, as already pointed out by Robertson and Gjerde (2004) in Norway,

low temperature including freeze–thaw cycles that may occur in England and Wales should be also considered.

Graczyk *et al.* (2000) determined the geographical factors that contribute to watershed contamination with *C. parvum* in cattle farms in the floodplain area in Lancaster County, Pennsylvania, U.S.A. These factors are: large areas of ranches, herd streams crossing and unlimited access to, infected calves and manure that can provide continuous supply of oocysts into water sources.

As already mentioned, waterborne characteristic of *C. parvum* should be taken in consideration when applying effluents for soil irrigation. Khashiboun *et al.* (2007) studied the fate of *C. parvum* oocysts in history and non-history soils irrigated with effluents. Interestingly they found that history soil (formerly irrigated with effluents) enhances oocysts migration and infiltration into soils, concluding that effluent irrigation requires intensive treatment in order to remove soluble organic matter in addition to oocysts, in order to prevent soil and groundwater contamination.

Another aspect of water contamination with *C. parvum* oocysts is sewage discharge into sea or oceans. Beside their survival into the sediment fraction of these environments, oocysts of *C. parvum* can be up taken by a large variety of bi-valves living on the sea bottom, therefore jeopardizing the shellfish industry. There are two aspects linked to this problem: first, oocysts survival in sea water and second shellfish contamination. Sewage-borne oocysts reaching sea water can survive up to 4 weeks at a salinity of 30 ppt at 20°C (Fayer *et al.* 1998; Robertson *et al.* 1992; Johnson *et al.* 1997) therefore shellfish can harbor infectious *C. parvum* oocysts and can serve as mechanical vectors of this pathogen (Izumi *et al.* 2006; Freire-Santos *et al.* 2000; Gomez-Bautista *et al.* 2000; Gomez-Couso *et al.* 2003). From the public health point of view, this fact is very important as shellfishes are mostly consumed uncooked.

Finally, Gomez-Couso *et al.* (2009) showed an interesting phenomenon upon which oocysts subjected to increased temperatures (as a result of solar water disinfection-SODIS method) excyte only up to 53.8% from the initial titer, when exposed to a temperature of 46°C for 12 hours. Environmental excitation of oocysts and release of defenseless sporozoytes increase the inactivation rate of this pathogen, making this process an effective disinfection method.

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Chapter 4.1.6

Giardiasis (Lambliasis)

[*GIARDIA INTESTINALIS*-SYN. *G. LAMBLIA* OR *G. DUODENALIS*]

The flagellated parasitic protozoan *Giardia intestinalis* infects humans and animals through reproduction into the lumen of small intestine causing a disease called Giardiasis. It has a worldwide distribution with higher prevalence in tropical areas, with different genotypes independent of geographical areas (Minvielle *et al.*, 2008; Caccio *et al.*, 2005; Feng and Xiao, 2011). *G. intestinalis* is a species of the genus *Giardia* and phylum Metamonada. *G. intestinalis* has two main forms: cyst with an oval form of 8 to 14 μm in length and 7 to 10 μm wide (a highly resistant form excreted in feces of infected animals) and a motile, vegetative form called trophozoite. Trophozoite form has a pear-shaped (resembling human face) form with a ventral adhesive disc and eight flagella. The ventral adhesive disc helps the trophozoite to attach and colonize lumen epithelium. The reproduction is by binary fission and growth is supported by nutrients originating from lumen under anaerobic conditions. Besides humans, *G. intestinalis* infects a large variety of domestic and wild mammals (e.g. livestock, cats, beavers, dogs, rodents, pigs, etc.) (Bitto and Aldras, 2009; Robertson, 2009; Atwill *et al.*, 1997) but marine animals were also reported (Deng *et al.*, 2000). A classic example is infectious upstream beavers that contaminate mountain flows with their feces that unaware thirsty trekkers drink (Monzingo and Hibler, 1987). In humans, the infection is mainly through ingestion of contaminated water and food (by feces, sewage, effluents, etc.) but also through direct contact with animals or infected humans (person-to-person). WHO estimates ∼200 million infections in Asia, Africa and Latin America, with children and immunocompromised persons at higher risk (Gardner and Hill, 2001). In children aged <5 years experiencing recurrent diarrhoea, including *Giardia*sis, a significant increase in the risk of growth delays or stunting was observed (Ricci *et al.*, 2007). Clinical manifestations of *Giardiasis* are acute diarrhea, with unpleasant odor, mucous, abdominal cramps, anorexia, vomiting, belching, headache, chills, constipation and weight loss.

4.1.6.1 *GIARDIA* AND ENVIRONMENT (FIGURE 4.1.6.1)

Giardia as a zoonotic waterborne parasite was previously described by many reports in relation to its transmission, resistance and persistence in various environments and outbreaks (Briancesco and

Bonadonna, 2005; Lipp *et al.*, 2001; Rimhanen-Finne *et al.*, 2010; Maco *et al.*, 2002; Eisenstein *et al.*, 2008). Cysts can be transmitted through sewage and sludge, potable and recreational water, agricultural crop irrigation and livestock densities, contaminated food, direct contact with animals and their products (e.g., milk) and person-to person (daycare, homosexuals, etc.) (Winkworth, 2010; Chauret *et al.*, 1999; Kabore *et al.*, 2010; Amoros *et al.*, 2010; Amahmid *et al.*, 1999; Monzingo and Hibler, 1987; Bowman and Lucio-Forster, 2010; Carlander *et al.*, 2009; Cody *et al.*, 1994). Perhaps the main source of environmental contamination with *Giardia* cysts are dairy lots exposed to stormy seasons and rainfall events (Curriero *et al.*, 2001). Miller *et al.* (2007) looked at climatic factors and farm management effects on *Giardia* cysts' environmental load. Cattle age (younger over older), stocking number and precipitation were the main factors associated with environmental loading of *G. duodenalis* cysts. An interesting finding of this study was that vegetated buffer strips can significantly reduce waterborne cysts in storm runoff. In Canada, in the North Saskatchewan River Basin, Alberta, Heitman *et al.* (2002) looked for the prevalence of *Giardia* cysts and *Cryptosporidium* oocysts in three sources: wildlife, human and agricultural (sewage and manure respectively). The lowest prevalence of *Giardia* cysts was obtained in wildlife (3.28%), followed by human sewage influent (48.8%), and the highest was in cow-calf manure (~60%). According to these authors, animal manure does not have access to water sources; therefore it serves only as an infectious source for feedlot animals and their human operators, while sewage effluents seem to be the main contamination source in this area with the lowest contribution from wildlife (depending on runoff severity). Here it should be mentioned that hydrological features are also important when dealing with runoffs. In New Hampshire, environmental risk of infection with *Giardia* cysts has been related to shallow ground water (Dennis *et al.*, 1993). In another study, *Giardia* cysts have been shown to travel freely and not attached to natural soil particles, in such cases where soil particles are carried by runoff (Dai and Boll, 2003).

Another feature of *Giardia* cysts is their association with suspended particles versus free-state and their settling velocities, especially under storm conditions. Cizek *et al.* (2008) reported on a Giardia cysts association with suspended particles of ~30 % during dry weather and 60% during wet weather. The increase in this association value is potentially linked to increased particles wash out during wetter seasons, increasing the direct contact incidences.

If these high-cattle-use areas are close to the coast their impact on marine environments is obvious. One of these reports studied cyst accumulation in shellfish, an important human health issue as these animals are eaten mainly raw (Graczyk *et al.*, 2003). There are different retention potentials according to the bivalve type involved and in proportion to the ambient concentrations of the parasite. Shellfish contamination with *Giardia* cysts seems to be a reversible process depending on water quality, therefore good depuration processes before processing can remove these pathogens completely. A range of recent studies have detected *G. duodenalis* in feces of marine vertebrates such as whales, dolphins, seals and shore birds (Lasek-Nesselquist *et al.*, 2010; Plutzer and Tomor, 2009). A marine animal shown to harbor *Giardia duodenalis* is sea lion, which is therefore suggested as a marine reservoir of this parasite in marine environments (Deng *et al.*, 2000).

Giardia removal from its main source, sewage, was studied at different sewage treatment plants (STP) according to their diverse applied processes: the bigger ones with tertiary treatment, smaller plants with enhanced secondary treatment and small ones as compact facilities. The tertiary, big STP-s were found to have a >3 logs reduction capability of *Giardia* cysts compared with <1.5 logs in the smaller STP-s (Kistemann *et al.*, 2008). In Spain, in spite of relatively good practice in removal of cysts from raw sewage, the effluents discharged into a hydrological basin were still shown to harbor enough cysts to contribute to recreational area and fluvial beach contamination (Castro-Hermida *et al.*, 2008; Castro-Hermida *et al.*, 2010). In Switzerland, a survey study showed high prevalence of *Giardia lamblia*

in recreational streams (Wicki *et al.*, 2009). According to these authors, effluents were found to contain high numbers of *G. lamblia* cysts and consequently human wastes appear to be an important source of contamination.
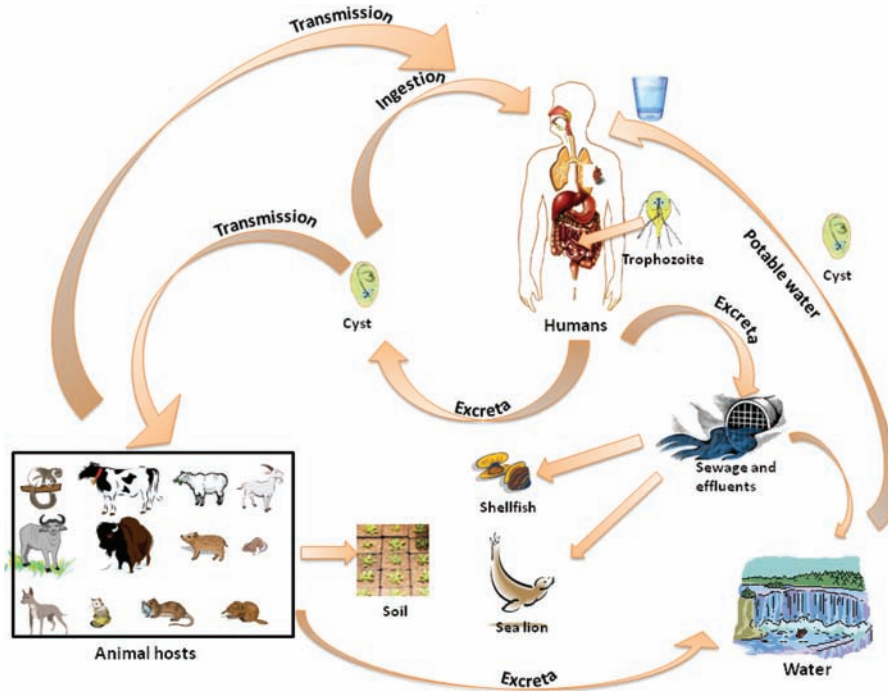
Figure 4.1.6.1. *Giardia intestinalis*' environmental infection patterns

Among that of other parasites, anthropogenic transmission of *Giardia* cysts to wild animals has been documented (Patz *et al.*, 2000; Volotao *et al.*, 2008). Black and gold howler monkeys were studied in relation to parasitic infections (*Giardia* and *Cryptosporidium*), closeness to human residence and residence type (wildness, rural and village) in Northern Argentina. The prevalence of *Giardia* sp. was significantly higher at the rural site (67%) compared with the remote forest (57%) and village sites (40%). The high prevalence on all sites suggests that these monkeys can be used as a continuous reservoir for re-infection. Rural prevalence, which proved to be the highest, implies repeated zoonotic transmission among non-human primates, livestock, and/or people (Kowalewski *et al.*, 2011). An additional example of *Giardiasis* introduction into a mountain gorilla population was described in Uganda. As a result of growing ecotourism and conservation activities, the area occupied by these wild animals was probably infected by human visitors (Nizeyi *et al.*, 1999).

An interesting aspect related to environmental changes' impact on infectious diseases was shown by Eisenberg *et al.* (2006). These authors showed a significantly higher odds ratio of infections (including *Giardia*) in non-remote vs. remote villages, where non-remote ones are linked by new road construction in a previously roadless area of northern coastal Ecuador. In this case, due to easier access from one geographic location to another, the disease agent could move faster!

The ultimate, cheapest and simplest mean used to remove *Giardia* cysts from contaminated water is by different filtration processes combined with various pretreatments followed by various disinfectants (e.g.,

conventional sand filtration, direct filtration, slow sand filtration, contact filtration, cartridge filtration, diatomaceous earth filtration, dissolved air flotation, sedimentation, lime softening, coagulation, bank filtration, second stage filtration, continuous backwash upflow dual sand filtration, UV, chlorination, chloramination, ozonation, chlorine dioxide, bromination, etc.) (LeChevallier, 1992; Jacangelo *et al.*, 1995; Weiss *et al.*, 2005). Indeed, based on cyst size, filtration can retain these particles, preventing their distribution in drinking water systems. The efficiency can vary from 99 (two logs reduction) to >99.999% (five logs reduction) depending on water turbidity, temperature, etc. in accordance with the USEPA Surface Water Treatment Rule that requires a >3 logs reduction effectiveness. In this context, Bichai *et al.* (2010) showed that *Giardia* cysts trapped into a granular activated carbon filter are subjected to predation by zooplankton (in this case Rotifers) through cyst internalization in these natural zooplankton. It is very conceivable that such processes take place in nature, reducing the parasite load in environments such as: river banks and dunes, granular filters and sediments.

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Chapter 4.1.7

Leishmaniasis

[*LEISHMANIA* SPP.]

Leishmaniasis is a vector-transmitted disease caused by intracellular protozoan parasites that belong to order Kinetoplastida, family Trypanosomatidae and genus *Leishmania*. The arthropod vectors that transmit the parasite by bite are certain sand fly species (subfamily Phlebotominae), mainly from the genus *Lutzomyia* but also from genus *Phlebotomus*. In humans, infection is transmitted zoonotically from infected animals but it can be also transmitted anthroponotically from other infected humans (even congenitally) (Lysenko, 1971; Meinecke *et al.*, 1999). Human infection is caused by about 21species out of 30 zoonotic species found in mammals (grouped in two subgenera: *Leishmania* and *Viannia*) (Table 4.1.7.1) *Leishmania*: [*L. donovani* complex (*L. archibaldi, L. donovani, L. infantum,* and *L. chagasi*); *L. mexicana* complex (*L. mexicana,L. garnhami, L.pifanoi, L. amazonensis*, *L. forattinii* and *L. venezuelensis*); *L. tropica* complex (*L.killicki, L. tropica*); *L. major* complex (*L. major*); *L. aethiopica* complex (*L. aethiopica*) and 2) *Viannia*: *L. braziliensis* complex (*L.(V.) braziliensis, L.(V.) peruviana*); *L.(V.) guyanensis* complex (*L.(V.) panamensis, L.(V.) guyanensis, L.(V.) shawi*); *L.(V.) naiffi* complex (*L.(V.) naiffi*); *L.(V.) lainsoni* complex (*L.(V.) lainsoni*); unclassified (*L.(V.) colombiensis, L.(V.) equatoriensis*). Subgenus *Leishmania* contains another group of similar organisms that is non-pathogenic for humans and includes *L.arabica, L. gerbilli, L. turanica* (Old World) and *L. aristidesi, L. enrietti, L. deanei, L. hertigi* (New World) (Table 4.1.7.1). At species level, differentiation is done by isoenzyme and DNA sequence analysis or monoclonal antibodies. The parasite is introduced into humans by the sand fly vector as a flagellate form (promastigote) through blood sucking. The promastigote penetrates monocytes where intracelullar binary reproduction takes place forming a tailless form called amastigote. The amastigotes continue to multiply asexually (binary fission) into monocytes located in liver and spleen (causing the further described symptoms). A subsequent bite from a sand fly will transfer amastigote infected monocytes within blood meal into the gastro-intestinal system of the arthropod vector, where they will transform into flagellated promastigotes that can now migrate to the proboscis part of the arthropod vector (Figure 4.1.7.1). The disease can manifest in three forms, depending on leishmanial species, virulence and tropism, vector and host immune response: visceral, cutaneous and mucosal. The visceral form is the most severe and if untreated it is fatal. After an initial

Table 4.1.7.1 *Leishmania* spp. geography, reservoirs and vectors.

Complex	Species	Clinical syndrome	Geography	Vector	Reservoir	Fatality
L. donovani	*L.donovani*	Kala-Azar (visceral leishmaniasis)	India, Southwest Asia, China, Nepal, East Africa	*Phlebotomus argentipes*	Humans, dogs, rodents	75 % in adults ~100% in children
	L. infantum	idem	Mediterranian littoral, Balkans, Middle East, North and East Africa, Central and Southwest Asia, China	*Phlebotomus* spp. (*P.major*, *P.papatasii*, etc.)	Dogs, foxes foxes, rodents, jackals	75 % in adults ~100% in children
	L. chagasi	idem	Central and South America	*Lutzomyia* spp. (*L. intermedus*, *L. longipalpis*) *P. sergenti*	Dogs, foxes, foxes, rodents, opossums	75 % in adults ~100% in children
L. tropica	*L. tropica minor*	Old World cutaneous leishmaniasis (oriental sore and leishmaniasis recidivans)	Eastern Mediterranean littoral, East Africa, Southwest Asia to India	*P. sergenti*	Humans, dogs, rodents	No fatality
	L. tropica major	Old World cutaneous leishmaniasis (oriental sore)	Central and Southwest Asia, Middle East, North, East and South Africa	*P. papatasii*, *P. perfilieri*, *P. caucasicus*		No fatality
L. aethiopica	*L. aethiopica*	Diffuse cutaneous leishmaniasis (Old World)	Ethiopia, Kenya	*P. longipes*	Rock hyraxes, rodents	No fatality

L. braziliensis	*L. braziliensis*	Espundia	Brazil, East of the Andes, Bolivia, Peru, Chile, Ecuador, Colombia, Venezuela, Paraguay	*Lutzomyia* spp.	Rodents, dogs, armadillos	No fatality
	L. peruviana	Uta	Peru, Bolivia, Ecuador	*Lutzomyia* spp.	Dogs	No fatality
L. guyanensis	*L. guyanensis*	Pian bois (forest yaws)	French Guiana, Suriname, Venezuela, northern Brazil	*Lutzomyia* spp.	Wild rats	No fatality
	L. panamensis	Panama leishmaniasis	Panama, Colombia	*Lutzomyia* spp.	Wood rats, kinkajous, olingos, sloths, monkeys	No fatality
L. mexicana	*L. mexicana*	Chiclero ulcers	Mexico, Honduras, Guatemala	*Lutzomyia olmeca*	Arboreal wild rodents	Rare (immunocompromised patients)
	L. venezuelensis	Diffuse cutaneous leishmaniasis (New World)	Dominican Republic, Venezuela, Amazonian basin	*Lutzomyia* spp.	Small wild rodents, forest rodents, cats	No fatality
	L. amazonensis	idem	idem	idem	idem	No fatality
	L. amazonensis	Amazonian leishmaniasis	Panama, Venezuela, Amazonian basin	*Lutzomyia flavis-cutellata*	Small wild rodents, opossums	No fatality

bite from an infected sand fly, a skin lesion may develop (leishmanioma), the incubation period can range from months to years with the following symptoms: hepatosplenomegaly, fever (remittent and intermittent), chills, vomiting, anemia, leukopenia, hypergammaglobulinemia and more, depending on the infected organ.
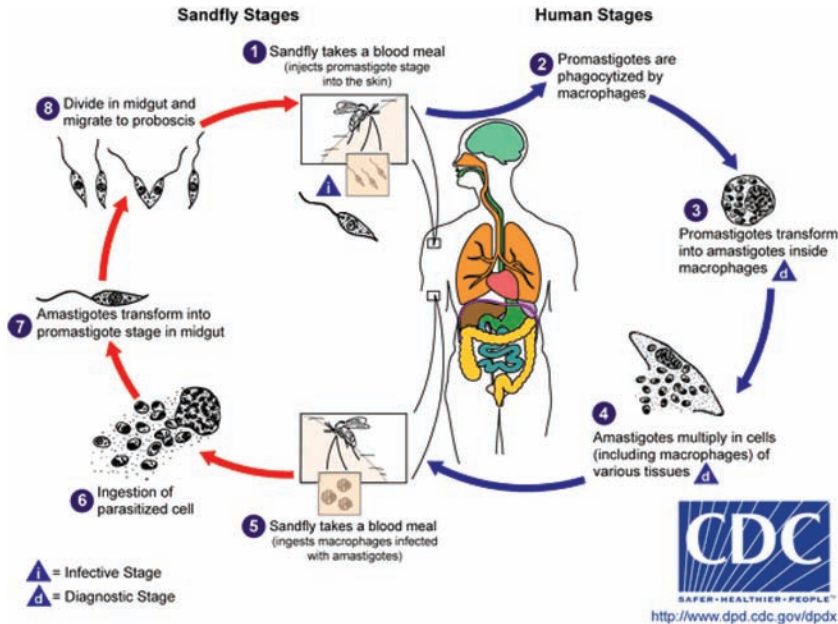
Figure 4.1.7.1. *Leishmania* spp. life cycle (with permission from CDC, Atlanta, USA- http://www.dpd.cdc.gov/dpdx/HTML/Leishmaniasis.htm)

4.1.7.1 *LEISHMANIA* SPP. AND ENVIRONMENT

According to our knowledge of *Leishmania* transmission, there are two main parameters that can be affected by various environmental factors: the vector (sand fly) and animals hosts (different mammals including feral cats) (Diniz *et al.*, 2008; McCown and Grzeszak, 2010). Among these factors one can find: animals (e.g., stray and pet dogs) in the neighborhood, strongly associated with visceral leishmaniasis (Oliviera *et al.*, 2006; Romero *et al.*, 2009; Diniz *et al.*, 2008; Baldi *et al.*, 2004); landless workers movement and ecological tourism in preserved forest (Alessi *et al.*, 2009), socio-economic status, densely populated urban area and as a result anthropogenic environmental changes (deforestation and urbanization) that impact new vectors and reservoirs/hosts at humans interface (synantropic zoonoses) creating new pathogenic complexes (Rotureau, 2006; Werneck *et al.*, 2007; Saraiva *et al.*, 2011; Tarallo *et al.*, 2010; Zeilhofer *et al.*, 2008); eclectic feed of sand flies in anthropic environments (de C Dias-Sversutti *et al.*, 2007); climatic conditions (wet forested areas and altitude, both connected with vector abundance and interannual climatic events related to El Niño Southern Oscillation, ENSO) (Chaves and Pascual, 2006; Ozbel *et al.*, 2011); irrigated habitats such as gardens and orchards (Kravchenko *et al.*, 2004); anthropogenic domestic activities (hen-houses, pig-pens, deforestation and human dwellings) (da Silva *et al.*, 2008; *Rotureau, 2006; Patz *et al.*, 2000); intensive use of insecticides and emergence of arthropod resistance (Alexander and Maroli, 2003; Kishore *et al.*, 2006); emergence of newly discovered

vectors (Savani *et al.*, 2009); changes in human population (increased number of immunodepressed and old people) (Baldi *et al.*, 2004); and increasing global tourism (Zamarrón Fuertes *et al.*, 2010) (Figure 4.1.7.2).
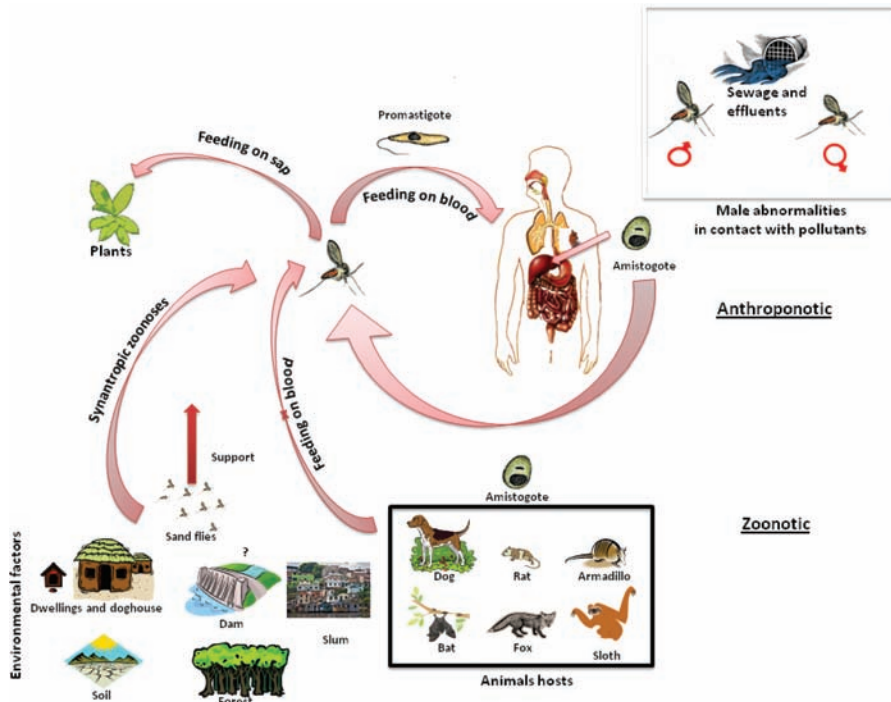
Figure 4.1.7.2. Some environmental factors involved in *Leishmania* and sand fly vector distribution

In relation to the sand fly vector, Kasili *et al.* (2009) found in Kenya, that goats significantly attract sand flies (*P. duboscqi* sp.) among several mammals used as traps, and equally successful is the CO_2-baited CDC light trap. Their study raises an interesting question related to CO_2 local concentration in endemic areas: Does car and industrial pollution encourage sand flies to move in those areas? A Brazilian sand fly (*Lutzomyia whitmani* sp. an important vector of *Leishmania*) distribution related to vegetation has been studied in different geographic areas of the Maranhao state, from the dense and moist forest of the Amazon to the more xeric areas (semi-moist and semiarid), including mixed vegetation types areas (Rebêlo *et al.*, 2009). The authors found an equal distribution between the geographic areas, with greater abundance in areas surrounding human dwellings (91.6%) than inside the dwellings (8.4%). They also suggested that following intensive deforestation the vector dispersion possibly reversed from west to east (from jungle towards urban area) to east to west (from peri- and urban areas to Amazonian forest). In urban areas of Teresina, Brazil, the highest incidence rates of leishmaniasis were mostly located in the city's peripheral areas. Multivariate analysis identified an interaction between population growth and the vegetation index, so that areas with high population growth and abundant vegetation showed the highest incidence rates (Cerbino *et al.*, 2009). Again, heavy vegetation in endemic urban areas can provide an excellent growth and hide site for sand flies.

In Argentina, phlebotomine sand flies were studied for abundance in primary vegetation-crop interface areas (Quintana *et al.*, 2010). The highest prevalence was found in modified primary vegetation (crop areas) and the lowest near primary vegetation, supporting the Brazilian study's conclusions. In addition, these authors found a strong negative correlation between sand fly abundance and precipitation, a fact supported by the distribution of Leishmaniasis in arid zones (Middle East, Africa, etc.). In these areas, land cover has been shown to be the most influential parameter in a distribution model applied for two sand fly types: *Phlebotomus papatasi* (Scopoli) and *P. alexandri* (Sinton) (Colacicco-Mayhugh *et al.*, 2010). In relation to vegetation, Elnaiem *et al.* (2003) found that increased densities of certain tree species (*Acacia* and *Balanites*) is well correlated to *Leishmania* prevalence. It is not clear if these tree species are biochemically favored by the sand fly vector or they just supply better hiding sites.

Other models in a more remote area (Brazil), applied to predict the distribution and potential risk of visceral leishmaniasis (VL) in this area, revealed similar results demonstrating good correlation between sand fly prevalence and a semi-arid and hot climate (Nieto *et al.*, 2006). In contrast, in a province of Iran known for its dry and cold climate, a positive correlation was found between canine laishmaniasis seroprevalence (*L. infantum*) and rainfall but also low temperatures (<0°C) (Salahi-Moghaddam *et al.*, 2010). To explain this inconsistency, the authors suggested that dog shelters (called "galakhs" in the local language) for cold days provide also an excellent refuge site for sand flies, consequently increasing the contact time between the vector and host. Similar results concerning animal' shelters were confirmed in a study performed in Brazil (Saraiva *et al.*, 2006).

Soil has been shown also to play a significant role in leishmaniasis prevalence being concurrently linked to vector and host (Bhunia *et al.*, 2010). In Israel's Western Negev Desert, rodents and sand flies have been trapped in this arid zone within three soil ecotones: loess, sand and sand-loess. The rodent *Psammomys obesus* was found to have the highest prevalence of Leishmania among other rodents, being soil dependent: loess (65%), sandy-loess (20%) and sandy habitat (0%). The vector sand fly was positively correlated to soil moisture in this order: loess> sandy-loess> sandy (Wasserberg *et al.*, 2002).

In France, researchers found two sand flies spp. (*P. ariasi* and *P. perniciosus*) that are distributed accordingly into two endemic different climatic zones: 1) located 200–1000 m above sea level characterized by relatively low winter temperatures (1.9°C average), 1042 mm average annual rainfall and much forest cover (vector *Phlebotomus ariasi*) and 2) located on the Mediterranean coastal plain, characterized by higher temperatures, lower rainfall and less forest cover (vector *Phlebotomus perniciosus*) (Chamaille *et al.*, 2010).

In an interesting overview, Coutinho-Abreu and Ramalho-Ortigao (2011) described phenotypic plasticity in sand fly (as an expression of the environmental effects on genes expression) (Gibson, 2008). Sand fly saliva contains bioactive molecules involved in blood feeding and vertebrate host immune response. These molecules seem to be modulated by environmental factors (biotic and abiotic) that dictate saliva quality and quantity and further drive the outcome of *Leishmania* infection. Such factors are water and sap content of vegetation from arid or irrigated areas. Based on these factors, plant carbohydrate content depends on water availability and therefore sand flies have to adjust biochemically in order to obtain more nutrients even in arid zones. Schlein and Jacobson (2002) supported these findings experimentally by showing that desert sand flies exposed to excess sugar became progressively resistant to infection. Those authors also reported that ∼85% of first-generation sand flies colonized from desert habitat retained experimental *Leishmania* infection versus 25% of sand fly offspring originating from irrigated sites.

In this context, Amóra *et al.* (2010) reported on the monitoring of the sand fly species *Lutzomyia longipalpis* (one of the main vectors of visceral leishmaniasis (VL)) in Brazil. The authors found that monthly analysis of different environmental variables, such as temperature, relative humidity and

rainfall, did not show a significant influence on sand fly population density. However, seasonal differences were found when ~70% of the arthropods were captured during the rainy season. As mentioned above, it can be speculated that this event is linked to vegetation's higher sugar content correlated to water availability.

Also in Brazil, Rezende *et al.* (2009) surveyed the population of different arthropods vectors, among them phlebotomine, in an area where a hydroelectric power station was built and compared it with that of previous years. The results showed a record of 2,979 phlebotominae before the power station installation and 912 afterwards, revealing a reduction in this arthropod population. Their conclusion was that "environmental changes did not increase the risk in the occurrence of these diseases" including leishmaniasis. In this special case, two speculations can be brought up: 1) deforestation caused by dam construction could take away the moist, organic, dark environment required by sand fly to rest and breed and 2) the tumultuous water movement around the dam does not favor their life cycle, including egg deposition. Another possibility, based on the above study, could be that phlebotominae flies moved to another ecosystem, but this assumption should be verified further. In Tunisia, an arid country, Salah *et al.* (2007) found that in the contrary, a dam built in the eighties was the major factor in a leishmaniasis outbreak, revealing high incidence rates spatially clustered in regions located close to this dam. Here it can be hypothesized, based on other studies, that a certain level of moisture is required to bolster sand fly development. Again, in the Brazilian case in which moisture is much more elevated, there are no highly distinctive humid regions as in the Tunisian case (dam versus desert) or in Israel (Müller, *et al.*, 2011). Milleron *et al.* (2008) presented results obtained in the process of sand fly rearing optimization by raising moisture levels that in turn increased adult blood-fed female egg development, whereas sand fly longevity decreased.

Related to sand fly gender, Guernaoui *et al.* (2010) surveyed *P. papatasi* males for genital malformation. They found that 20.4% of males tested have abnormal genitalia probably associated with environmental interference being prevalent in sewage dumps (e.g., mutagenic and teratogenic chemicals).

The host population in endemic areas has been shown also to contribute to leishmaniasis prevalence. In Israel, two clear peaks were recorded (between 1967–69 and 1980–82) (Anis *et al.*, 2001), reflecting environmental changes such as the introduction of a new non-immune population and close extensive urbanization in areas of endemic foci, agricultural/industrial projects and also global warming. The same trends were observed also in Palestinian Authority areas and countries in Mediterranean basin.

In Ethiopia, an outbreak of *L. aethiopica* in a non-endemic area was linked to several environmental factors such as: house proximity to a gorge where hyraxes reside, presence of certain plants (*Adhatoda schimperiana* and *Acacia* spp.) in the compound and sharing the same room with domestic animals, which were significantly associated with developing cutaneous leishmaniasis (Negera *et al.*, 2008).

Among the many animals (mainly mammals, e.g., dogs) reported to exercise as *Leishmania* spp. host, a recent report from Brazil showed seroprevalence and PCR positive results in Brazilian bats (Savani *et al.*, 2010) suggesting their potential reservoir role. In the state of Rio de Janeiro, Brazil, researchers found that sloths are also potentially involved in transmittance of *L.* (*Viannia*) *braziliensis* between sylvatic and extradomiciliary areas by the sand fly *Lutzomyia intermedia* (Meneses *et al.*, 2002; Herrer and Christensen, 1980).

Galvez *et al.* (2010) surveyed the canine population in the area of Madrid for leishmaniasis seroprevalence. They reported a higher prevalence trend that could not be explained solely by the socio-demographic characteristics of dog populations. One possible explanation could be the canines' increased outdoor abode due to warmer climate in the area, consequently exposing the host to increased contact with sand flies. Another aspect related to dogs was presented by Moreira *et al.* (2003) using a multivariate analysis, revealing canine short fur to be the strongest predictor of canine Leishmania

infection in Brazil. Taking into account that many canine owners cut their dogs' fur short in summer time it is possible that in this way they increase exposure to sand fly bites.

A strong link between domestic animals and seroprevalence has been also shown in Iran (Oshaghi *et al.*, 2010). The authors reported on *Phlebotomus papatasi* as the most dominant species infected with *L. major* in suburb an areas of a city. Patients living in these areas keep normally domestic animals in their houses which provide the appropriate environment to complete the sand fly life cycle in order to spread cutaneous leishmaniasis.

In human hosts in Central America, Convit *et al.* (2006) observed an interesting phenomenon linked to a frequent atypical form of cutaneous leishmaniasis (AtypCL) in patients. This atypical benign form is characterized by nonulcerating nodules containing a few parasites (*L. chagasi*, *L. mexicana*). Skin biopsies of lesions and nodules revealed numerous inorganic particles that seem to originate from volcanic ash prevalent in these areas (silica and aluminum being the most abundant components). The authors suggested that inorganic particles may play a significant immunomodulatory role in these infections, a fact that needs to be studied further.

In experimental mice infected with *L. major*, it was shown that exposure to subtoxic doses of mercury ($HgCl_2$) exacerbates the disease outcome and its effects on resistance are time-dependent. Pretreatment with mercury (at least 1 week before infection) induced autoimmune syndrome characterized by the induction of T-helper type 2 (Th2) cells, resulting in a higher susceptibility to parasite, footpad swelling and increased parasite burden. The authors raised the possible implication of mercury contamination in endemic areas of leishsmaniasis (Bagenstose *et al.*, 2001).

Interaction between human population and forest was associated with the emergence of American cutaneous leishmaniasis (ACL). As a conclusion of this postulate, people living close to forest are significantly more exposed to vector and parasite, and therefore deforestation of primary forests will eradicate the infectious cycle! Chaves *et al.* (2008) raised an interesting issue related to the complex nature of the deforestation process in Costa Rica, finding, on the contrary, that living close to the forest edge diminishes the infection risk. They suggested that landscape alone does not explain the spatial distribution of ACL, but socioeconomic inequities (e.g., socially excluded populations or so called marginality index), climate variability caused by the El Niño Southern Oscillation (ENSO) and biodiversity changes should be considered in any model. Indeed, this topic was later supported by another study performed in Brazil, in which, using a multivariate analysis, it was shown that ACL is associated with: gas stove absence, forest distance <200 m, low income families, low education ≤4 years, indoor birds and animals, forest-related leisure activities, rural work or school activities, absence of dogs and cats around the house and at the community level non-durable house' wall material (Pedrosa and Ximenes, 2009).

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Chapter 4.1.8

Microsporidiosis

[*MICROSPORIDIA* SPP.]

The phylum Microspora that contains the Microsporidea class is a phylum of spore-forming monocellular parasites that now are classified under the kingdom of Fungi. The known families are: *Encephalitozoonidea*, *Enterocytozoonidea*, *Microsporidea*, *Nosematidea*, *Pleistophoridea*, *Pleistophoridea*, *Nosematidea*. Microsporidia are restricted to animal hosts (including many mammals) and the distribution is worldwide. Among other animals: insects, crustaceans and fishes are also infected. Some species are opportunistic infecting humans through contact with polluted water or aerosols inhalation, possibly food-borne, but sexual contact have been also considered as a possible transmission way (Li *et al.*, 2003; Shaw and Kent, 1999; Hutin *et al.*, 1998). The life cycle of microsporidia is composed of two stages: external or environmental and obligatory intracellular. The environmental stage harboring infectious spores entering hosts goes through an extrusion process that forms a tubular polar filament. Once close to intestinal cells, this "syringe" form penetrates the cell membrane injecting the sporoplasm form. The intracellular sporoplasm goes through asexual (merogony) and sexual (sporogony) paths with final disruption of the host cell and release of new infectious spores into the intestinal lumen (Figure 4.1.8.1). Infection in humans and animals occurs horizontally (feces and urine) with many organs involved, while microsporidia vertical transmission (transovarial) was observed in insect hosts. Spores are highly resistant to environmental conditions, making them a good candidate for continuous and persistent infection. The parasite infection manifests itself in immunocompetent individuals as keratoconjuctivitis, diarrhea (self-limiting), nausea, abdominal pain and occasional fever. In immunocompromised individuals, the parasite affects different organs such as: respiratory tract, kidney, prostate, liver, peritoneum and brain.

4.1.8.1 ENVIRONMENT AND MICROSPORIDIA

In relation to human zoonotic infections, Microsporidia are a relative new comer in the group of zoonoses. These intracellular protists (now belonging to the fungi kingdom) were formerly discovered in insects and fish. However, this pathogen emerged dramatically due to increasing numbers of immunosupressed AIDS

patients or individuals under immunosuppressive drug treatment. Gradually it was realized that some species can cross borders from fish or insects into other animals, including humans (Cali and Takvorian, 2003; Mathis *et al.*, 1999; Lono *et al.*, 2010). For example, *Brachiola algerae* a parasite from invertebrates, was initially isolated from human surface infections, skin and eye, but later on it was isolated from human skeletal muscle, too (Coyle *et al.*, 2004; Cali *et al.*, 2005).
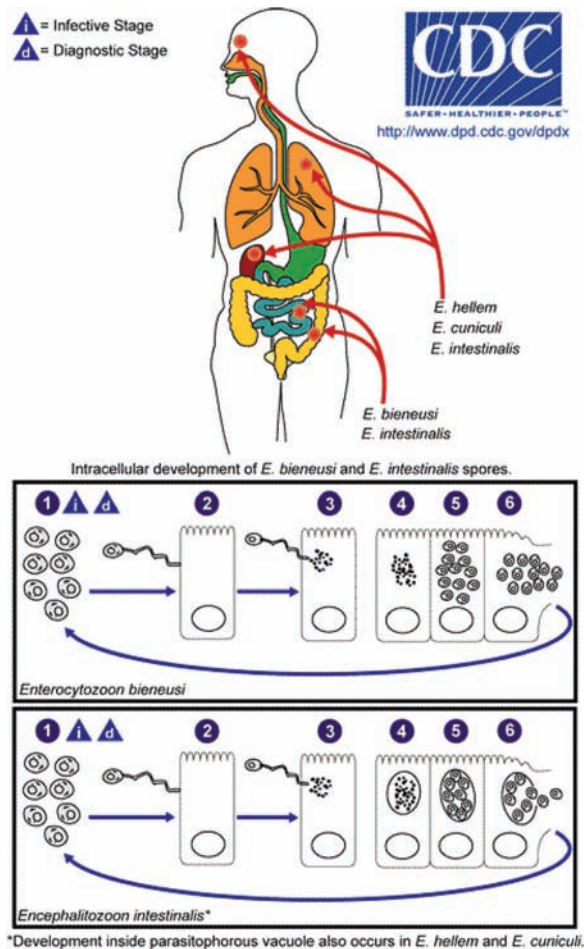
Figure 4.1.8.1. Microsporidia spp. life cycle and human infection sites. (with permission from CDC, Atlanta, USA. http://www.dpd.cdc.gov/dpdx/HTML/Microsporidiosis.html)

Slodkowicz-Kowalska (2009), using staining molecular techniques (FISH and multiplex FISH techniques) surveyed feces from 178 animal species for distribution of microsporidian species (*Encephalitozoon intestinalis*, *E. hellem*, *E. cuniculi*, and *Enterocytozoon bieneusi*) in Poland. The results showed higher distribution in birds than mammals, with preference in water fowls, possibly suggesting water as an infection source (Sparfel *et al.*, 1997; Graczyk *et al.*, 2008). Among the mammals, various lemur species and warty pigs were found positive for *E. bieneusi* and *E. intestinalis*. Thus, together with

Table 4.1.8.1 Human Microsporidia spp. and their environmental sources known thus far.

Species	Infection target	Nonhuman hosts	Environmental source
Enterocytozoon bieneusi	Small intestine, gall bladder, live	Pigs, nonhuman primates	Surface water, swimming pool, Seine River
Encephalitozoon spp.			
E. intestinalis	Disseminated	Ducks, pigs, dogs, cattle, goats, donkeys, gorilla	Ground and surface water, and sewage effluent Irrigation water used for crops
E. hellem	Disseminated	Psittacines	
E. caniculi (Strains I, II, III)	Disseminated	Mammals (wide range)	
Nosema spp.			
N. connori	Disseminated	Rabbits, mice	Ditch water
N. ocularum	Corneal stroma	Mice, arctic foxes	
Nosema-like spp.	Corneal stroma	Domestic dogs	
Trachipleistophora spp.		Unknown	
T.hominis	Skeletal muscle, nasal sinuses	Unknown	
T. anthropophthera	Disseminated	Unknown	
Pleistophora spp.	Skeletal muscle	Fishes and insects	Ditch water, Irrigation water used for crops, Rivers Seine and Loire, Food (fish)
Vittaforma corneae	Corneal stroma	Unknown	River Seine, Tertiary effluent
Microsporidium spp.	Disseminated	Unknown	Species of microsporidia that infect humans which have not been identified in water or potential sources of food include *Brachiola connori*, *Brachiolavesicularum*, *Nosema occularum*, *Pleistophora ronneafiei*, *Trachipleistophora anthropophthera*, *Trachipleistophorahominis*, and the unclassified species, *Microsporidium africanum*, and *Microsporidium ceylonensis*

different bird species that live in various environments, the epidemiological aspect points to the ubiquity of infection with these pathogens. Water fowl in particular, have unlimited access to surface waters, including waters intended for human consumption, and therefore, as shown by Slodkowicz-Kowalska *et al.* (2006), a single visit of a waterfowl flock can introduce into the surface water approx. 9.1×10^8 microsporidian spores of species known to infect humans.

In Spain, microsporidia species *Enterocytozoon bieneusi* (9.7%), *Encephalitozoon intestinalis* (4%), and *Encephalitozoon hellem* (0.8%) have been identified in urban park pigeons (Haro *et al.*, 2005). These findings are important from the epidemiological and sanitary points of view as children and elderly people are the main visitors of these urban parks. Similar results were presented by Graczyk *et al.* (2007) on urban feral pigeons, emphasizing that exposure to those birds excrement, through cleaning of contaminated surfaces, could expose a person through direct inhalation to 3.5×10^3 *Enterocytozoon bieneusi* spores and 1.3×10^3 spores to a nearby person. In Portugal, a group studied various bird orders (Columbiformes, Passeriformes and Psittaciformes) as hosts for microsporidia. *Enterocytozoon bieneusi* have been identified in 28.9% of these birds, while the isolated species was closely related to the Peru6 genotype previously found in humans, in Peru (Lobo *et al.*, 2006), emphasizing a worldwide distribution and the potential transmission through wild animal commerce.

In the recreational water environment, together with other protozoan parasites (*Giardia* and *Cryptosporidium*), microsporidia was isolated from bottom sediments disturbed by bathers or by their direct contribution, revealing two aspects: 1) this pathogen can survive in marine environment and 2) bathers are exposed to parasitic infections from recreational waters (the authors mentioned in their study, that these isolates were found on days deemed acceptable for bathing by fecal bacteria standards) (Graczyk *et al.*, 2007).
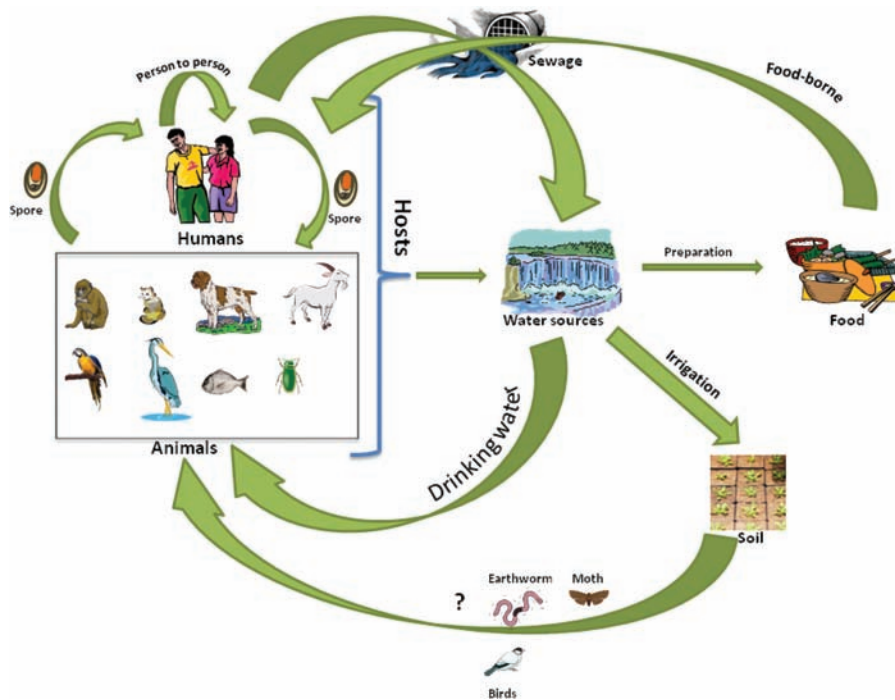
Figure 4.1.8.2. Microsporidia confirmed and potential environmental routes of infection

Finally, it can be stated that we are only in the first stages of understanding the ecology and epidemiology of these pathogens. The fact that once this parasitic group was classified as protists then later as fungi reveals the confusion of the scientific community; however, along with a substantial advance in molecular biology, the picture became brighter. Two recent manuscripts raised several issues about the parasite-host relation, infectivity and inter-species transmission of microsporidia. Goertz and Hoch (2008) presented vertical transmission of its microsporidia *E. schubergi* in gypsy moth and survival for eight months in moth' cadavers on the ground. In addition, two other species' spores (*N. lymantriae* and *V. disparis*) remained infective in overwintered cadavers only when they were snow covered for four months (Goertz and Hoch, 2008). The second manuscript described a new microsporidia as a common parasite infecting the classic nematode *Caenorhabditis elegans* in the wild by horizontal transmission (Troemel *et al.*, 2008). As already pointed out by Cali *et al.* (2005), invertebrate and cold-blooded microsporidia can develop in human pathogens, so it can be very possible that this parasite is found also in soil and consequently infects ground and underground organisms that further transmit it to higher organisms (e.g., birds, earthworms and different mammals including humans) (Figure 4.1.8.2). These inter-connections need additional scientific evidence but they cannot be ruled out as already proved with other microorganisms. In humans, questions still exist about the persistence of microsporidia in healthy individuals, vertical transmission (pregnancy or transplantation) and possible infection with temporary unrelated microsporidia species ? (Didier and Weiss, 2006).

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Chapter 4.1.9

Monkey Malaria (Simian Malaria)

[*PLASMODIUM* SPP.]

Genus *Plasmodium* causes the important disease malaria that infects humans and animals, with an estimated death toll of 1.5 to 2.7 million people each year. The parasite is vector-borne by a variety of mosquitoes from the genus *Anopheles*. Simian malaria like its relative human malaria (*Plasmodium* spp.) is also transmitted by *Anopheles* spp. mosquitos. In general, these *Anopheles* species differ from the common *Anopheles* spp. by their feeding habit: they feed on treetops (acrodendrophilic) while others feed at ground level (Tan *et al.*, 2008)(Table 4.1.9.2). This feeding pattern explains the rarity of human infection with simian plasmodia. *Plasmodium* distribution is mainly in the tropic and sub-tropic regions (the Americas, Asia and sub-Saharan Africa). The genus *Plasmodium* (class: Aconoidasida and order: Haemosporida) multiply in the mosquito gut and is transmitted to animals and humans through infected female mosquito bite. Plasmodium includes at least 172 species with a wide variety of infected animals (mammals, birds, reptiles and amphibians) with *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* as human parasites. *P. malariae* (synonym, *P. rodhaini*) can occur in chimpanzees too. Among different endemic zoonotic *Plasmodia* that infect primates and non-human primates, several are also transmitted to humans, and therefore are defined as travelers health risk (Table 4.1.9.1). *Plasmodium* species are intraerythrocytic parasites with life cycle that extends in two organisms: invertebrate (the mosquito vector) and vertebrate (mammals and other animals' hosts). The complex life cycle of *Plasmodium* spp. that includes asexual and sexual reproduction is shown in Figure 4.1.9.1. The clinical manifestations of simian malaria are: headache, fever, fatigue, anorexia, nausea, hepatosplenomegaly.

4.1.9.1 SIMIAN MALARIA AND ENVIRONMENT

Since mosquitoes are the main vectors of malaria transmission, it is important to understand their environmental behavior. Female mosquitoes are those that feed on blood while males feed on plants nectar, so one gender is highly connected to animals while the other is connected to plants (angiosperms). The diurnal activity of female mosquitoes is between dusk throughout the night. Water is a prerequisite for a successful reproduction, consequently most of the mosquitoes involved in simian

Table 4.1.9.1 *Plasmodium* spp. infecting primates and non-human primates and their relation to human infection (adapted from Baird, 2009).

Species	Microscopic diagnosis similarity	Sylvatic reservoir	Geography	Human infection	Vector	Zoonotic odds
P. falciparum	*P. falciparum*	Gorillas	Africa	Yes	[*Anopheles gambiae, A. funestus, A. coustani, A. squamosus, A. nili, A. pharoensis*] ?	Unknown
P. malariae (synonym *P. rodhaini*)	*P. malariae*	Chimpanzees	Africa	No	*A. gambiae*	Low
P. cynomolgi	*P. vivax*	Macaques (*Macacus cynomolgus*) and leaf monkeys	Southeast Asia	Yes	*A. hackeri, A. dirus, A. elegans, A. introlatus, A. balabacensis*	High
P. knowlesi	*P. malariae*	Macaques (*Macaca fascicularis*)	South East Asia	Yes	*A. dirus*	Confirmed
P. inui	*P. malariae*	Macques (*Macaca assamensis, Macaca fascicularis*)	China	No	*A. dirus, A. hackeri*	High
P. eylesi	*P. vivax*	Gibbon (*Hylobates lar*)	Malaysia	Yes	*Anopheles b. introlatus, Anopheles kochi, Anopheles lesteri, Anopheles letifer, Anopheles leucosphyrus, Anopheles maculatus, Anopheles roperi, Anopheles riparis macarthuri, Anopheles sinensis, Anopheles sundaicus, Anopheles umbrosus, Anopheles vagus*	Low
P. schwetzi	*P. vivax*	Chimpanzees and gorillas	Africa	No	*Anopheles balabacensis balabacensis*	Moderate

(Continued)

Table 4.1.9.1 (*Continued*)

Species	Microscopic diagnosis similarity	Sylvatic reservoir	Geography	Human infection	Vector	Zoonotic odds
P. simium (High similarity to *P. vivax*)	*P. vivax*	Black howler monkeys (*Alouatta fusca*) and wooly spider monkeys (*Brachyteles arachnoides*)	Southeast Brazil	No	*Anopheles cruzii*	High
P. brasilianum	*P. malariae*	Monkeys from *Alouatta, Ateles, Callicebus, Cebus, Chiropotes, Lagothrix, Pithecia, Saguinus, Saimiri* spp.	Central and South Americas	No	*A. freeborni, A. maculates, A. quadrimaculatus, A. aztecus*	High
P. pitechi	*P. falciparum*	Orangutan	Indonesia	No	*Anopheles balabacensis ?*	Low
P. reichenowi	*P. falciparum*	Chimpanzees and Gorillas	Africa	No	Unknown	Low
P. gonderi	*P. vivax*	Mangabeys and Mandrills	Africa	No	Unknown	Low
P. gaboni	*P. malariae*	Chimpanzees	Africa	No	Unknown	?

P. ovale	Plasmodium falciparum, Plasmodium vivax	Humans, Chimpanzees	Africa	Yes	Unknown	High
P. hylobati	P. falciparum	Gibbon	Southeast Asia	No	Anopheles balabacensis ?	Low
P. jefferyi	P. falciparum	Gibbon	Southeast Asia	No	?	Low
P. silvaticum	P. vivax	Orangutan	Southeast Asia	No	Anopheles balabacensis, Anopheles maculatus, Anopheles sundaicus	Low
P. youngi	P. vivax	Gibbon	Southeast Asia	No	? Anopheles maculatus, A. sundaicus, A. b. introlatus (succeptable)	Low
P. fieldi	P. vivax	Macaques	Southeast Asia	No	Anopheles hackeri, Anopheles introlatus ?	Moderate
P. simiovale	P. vivax	Macaques	Southeast Asia	No	Anopheles maculates, Anopheles vagus ?	Moderate
P. coatneyi	P. falciparum	Kra Macaque	Southeast Asia	No	Anopheles hackeri	Low
P. fragile	P. falciparum	Macaques	Indian subcontinent	No	Anopheles dirus	Moderate

Table 4.1.9.2 *Anopheles* spp. involved in simian malaria and some environmental characteristics.

Mosquito spp.	Water source	Breeding site	Vegetation	Distance from human habitat
A. gambiae	Sunny and clear water	Wells dug for mud bricks, cattle footprints or vehicle wheel grooves	No	~300m (maximum recorded flight distance was 661 m)
A. dirus	Wells, holes	Puddles on footpaths and turbulence pits at the heads of drainage gullies	Yes (Bushes, tree holes thick grass, under the surfaces of leaves, branches near the ground and under the trees' foots.	~3 km (effective flight range in forest was deduced to be about 1.5 km)
A. hackeri	Water puddles	Jungle and Nipa palm leaf bases	Yes (Jungle canopy, Nipa Palm leaves)	?
A. cruzii	Water puddles	Forests canopy along the Brazilian coast, Bromelias spp. in peri- and urban environment	Yes (Forest and Bromelias flowerpots)	Various
A. balabacensis	Domestic wells, puddles on footpaths and turbulence pits at the heads of drainage gullies	Forest	Yes (Forest)	~3 km (effective flight range in forest was deduced to be about 1.5 km)
A. maculates	Clear water pockets formed on the bank of rivers and waterfalls	Shallow pools 5.0–15.0 cm deep with clear water, mud substrate and plants or floatage	Yes (Deep forest and plantations) The mosquito also preferred open or partially shaded habitats	~400 m (longest detected flight was 1.6 km)

Species				
A. introlatus	Fresh water stagnant	Tropical rain forest in a mountainous area (jungle-breeding). Rocky stream pools, aqueducts shaded seeps and pools and animal wallows	Yes Hilly forest regions of central Malaysia in partial shade, at elevations varying from 244 to 610 m above sea level.	?
A. freeborni	Clear seepage water	Roadside pools, in rice fields, and in other similar habitats. Pools that are sunlit, at least part of the day, seem to be preferred, although larvae are occasionally found in rather densely shaded pools. Heavy production of larvae often occurs in matted algal growths in water along the margins of rice fields	Wooded area The females enter houses readily and feed on man. They are more active at dusk and during the night but occasionally do attack man during the daylight hours in dense shade or on cloudy days	Vertical distribution (up to 6 m)
A. aztecus	All sorts of ground water habitats including lakes, ponds, and canals. Also found in gutters, ditches and pools of clear water	Commonly found at altitudes of 1,500 to 2,200 m. A variety of breeding sites are used, from pools, ponds and lakes, to canals containing polluted water	Present in Chiapas; rural areas in the states of Nayarit and other tropical rainforest in Mexico	? This species will bite man indoors and outdoors.
A. latens	Fresh water, stagnant or standing, turbid or clear, sometimes with some degree of pollution and decaying leaves	Dense jungle and forest fringes, farms	Yes (Forest, partially or heavily shaded in primary and secondary rain forests either in mountains or valleys at elevations ranging from 76 m to 520 m above sea level	Close to forest fringe

malaria live and multiply near water sources, but any puddles format is also suitable (Suwonkerd *et al.*, 2004). Mosquitoes from the family Culicidae like the genus *Anopheles*, have been shown to survive well during dry seasons in Kenya, an endemic region of malaria (Minakawa *et al.*, 2001). In this case, *A. gambiae* sp. practices two survival strategies during the dry season: 1) continuous reproduction throughout the year and 2) embryo dormancy in moist soil for at least several days. In South America, anophelidae were found to multiply close to certain plants (the epiphytic subfamily *Bromelioideae*) that are also currently grown in periurban and urban regions, therefore bringing these vectors close to human habitats (Marques and Forattini, 2009; Molez, 1999). In general, it can be stated with a high degree of confidence that malaria-carrying mosquitoes (both human and simian) are highly adapted to survive in various extreme environments (Budiansky, 2002) including human made ones, such as orchards, used tire depots, fish farms, etc. (Obsomer *et al.*, 2007; Budiansky, 2002). In a study performed in the Peruvian Amazon region, Vittor *et al.* (2006) clearly showed that deforestation increased *Anopheles darlingi's* human-biting rate compared to forested areas. The behavior of these mosquitoes can be explained by landscape alteration via deforestation that reduces previous vertebrate host diversity, leaving humans as the main target in the absence of other warm-blooded animals in the open area, fewer sources of nutrients (plants and their flowers) and purely adaptation to new environment (Figure 4.1.9.2).
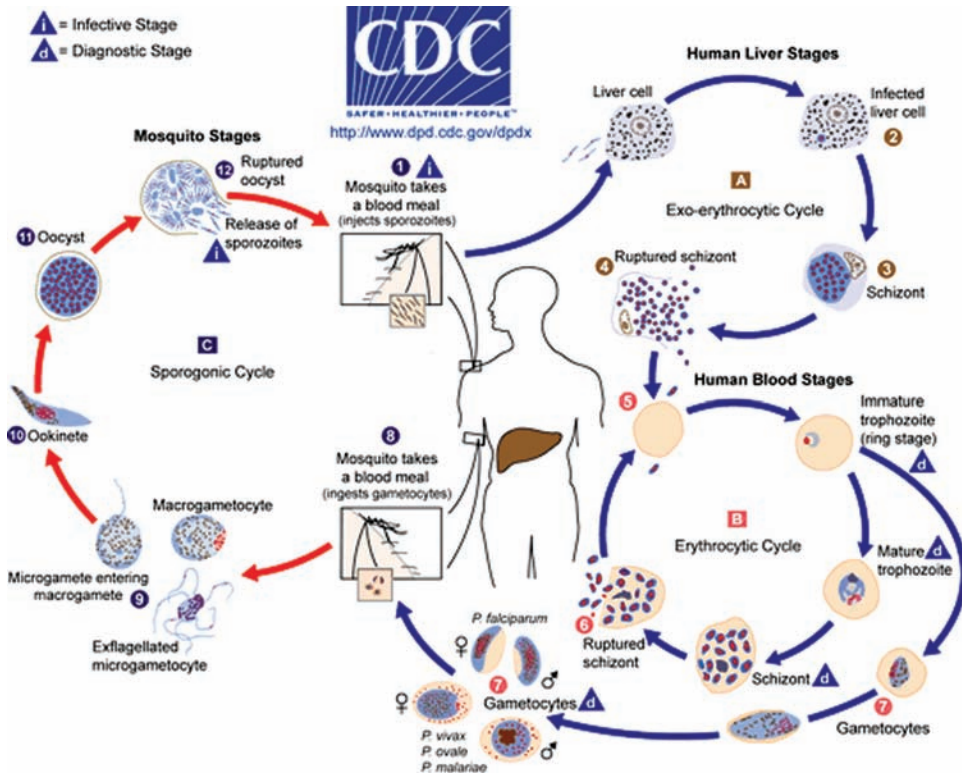
Figure 4.1.9.1. Life cycle of *Plasmodium* spp. in humans (with permission from CDC, Atlanta, USA; http://www.cdc.gov/malaria/about/biology/)

The second factor involved in this malaria type is the various primates and non-human primate species spread over tropical and sub-tropical regions. Global tourism into these regions may convey human' infection (Lee *et al.*, 2009). Primates are also kept in reservations, parks and zoological gardens around the globe. In the wilderness, primates', and especially non-human primates', infection can be controlled only by reducing the vector habitats, which seems an enormous task. In zoological gardens, water sources should be kept under continuous control to prevent mosquito reproduction and infection with *Plasmodium* spp.
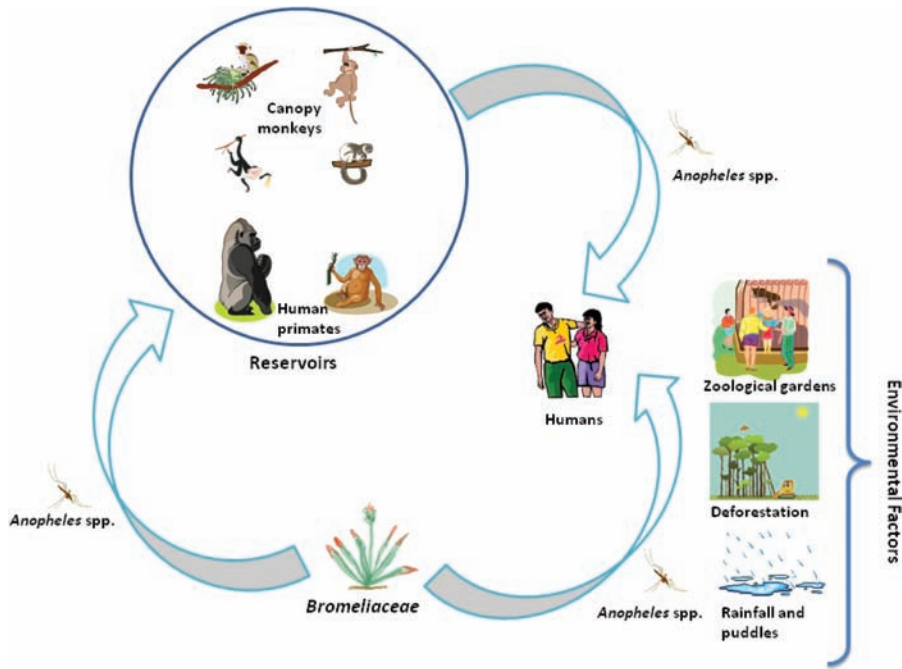
Figure 4.1.9.2. *Anopheles* vector transmission of simian malaria to primates (including humans) and potential environmental factors involved

Recently, it was shown that wild-living and captive gorillas in Central Africa (Cameroon and Gabon) and chimpanzees can harbor through infection two related lineages of parasites (*Plasmodium GorA* and *P. GorB* in gorillas) not yet described previously and also *P. falciparum*, *P. ovale* and *P. malariae*, species previously considered as strictly human specific (Prugnolle *et al.*, 2010; Duval *et al.*, 2010). These findings reveal that close contact between closely related species can transfer different diseases and in this particular case a very versatile parasite (with an unexpected genetic diversity). In addition, it is continuously evident that African apes and other primates can serve as reservoirs of different *Plasmodium* species able to infect humans (Duval *et al.*, 2010).

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Chapter 4.1.10

Sarcosporidiosis

[*SARCOCYSTIS* SPP. FORMERLY *ISOSPORA HOMINIS*]

Sarcocystis species belong to a genus of cyst-forming coccidia protozoan of the order Eucoccidiorida, all parasites of a large variety of animals. *Sarcocystis bovihominis* (synonim *S. hominis*) and *S. suihominis* are the two species infecting humans (as final host). The distribution is worldwide, where intermediate hosts are cattle and pigs (Sager *et al.*, 2006). Infection rate in cattle and pigs depends on hygienic conditions, e.g. in Europe *S. bovihominis* prevalence rate is 21 to 64%, while infested pigs in Germany carry *S. suihominis* at a rate of 30 to 40%. There are ~130 recognized species in this genus (*Sarcocystis*) with various intermediate or final hosts (Dubey, 1980; Kubo *et al.*, 2009; Cummings *et al.*, 2000). Transmission to humans occurs through consumption of raw or undercooked contaminated meat (pork and beef) with bradyzoites containing cysts. Human incidental contamination (as intermediate host) can occur also through ingestion of water or food contaminated with fecal sporocytes. The life cycle of *Sarcocystis* in humans as final host starts by sexual reproduction (gamogony) in intestinal epithelium following ingestion of infested meat. Briefly, the ruptured cysts release bardyziotes that in turn enter intestinal cells to multiply and form microgametes and macrogametes. In intestinal ephitelial cells, fertilization between the micro- and macrogametes forms immature cysts (zygote) followed by sporogony (formation of sporocystes). After ~7 days post infection, oocysts containing two sporocytes are formed (each sporocyst containing four sporozoites) and further released in the environment through feces (Figure 4.1.10.1). There are two major clinical manifestations of human sarcosporidiosis: intestinal infection with oocysts and sporocysts excretion in feces and a rare extraintestinal infection of striated muscles with cysts (cardiac and skeletal muscles). The immediate symptoms are self-limiting or severe diarrhea (depending on infection magnitude), nausea, abdominal pain, dizziness, muscle swelling, myalgias and fever (Li *et al.*, 2007).

4.1.10.1 *SARCOCYSTIS* AND ENVIRONMENT

Prevalence of *Sarcocystis cruzi* in Australian cattle has been studied in Western Australia. Savini *et al.* (1992) reported a high prevalence of this parasite in cattle from this area (52%). In relation to

environment, arid and semiarid zones had a lower prevalence (9% and 31% respectively) compared to tropical (87%) and temperate (60%) areas. The authors proposed reduced resistance of *S. cruzii* sporocysts under dry conditions as a factor in the epidemiology of this parasite (Fayer, 1980). Livestock manure (as wet condition) have been already shown in Germany to support presence of different parasites including *Sarcocystis* species (Hiepe and Buchwalder, 1991).
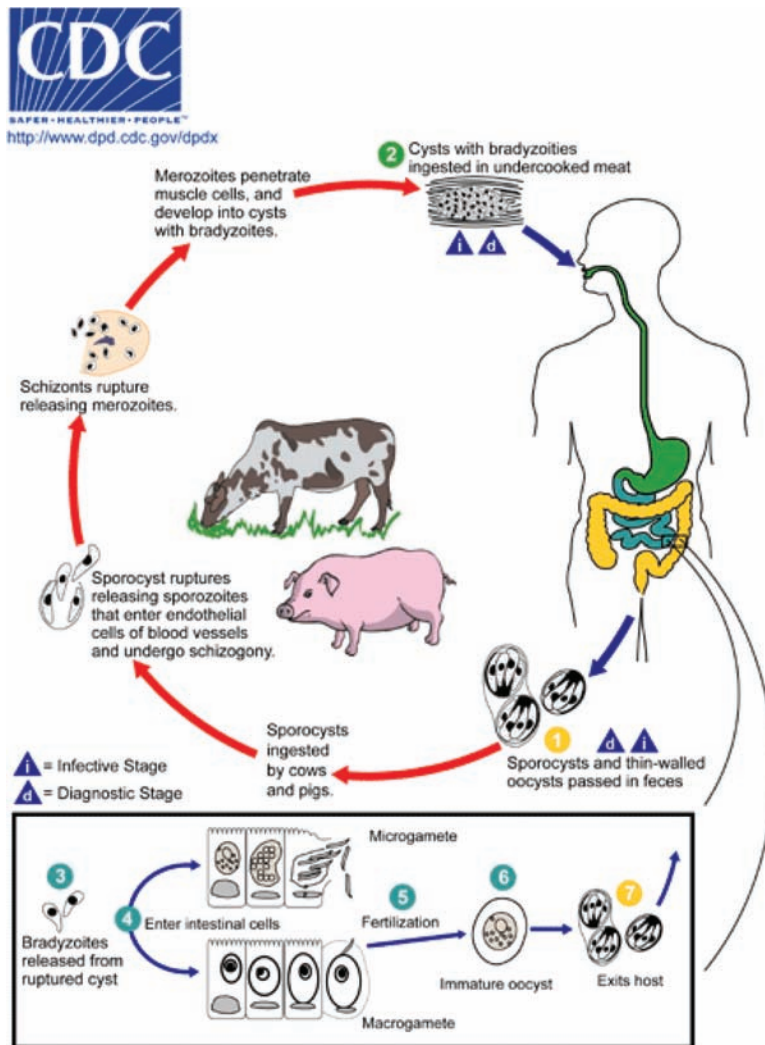
Figure 4.1.10.1. Life cycle of *Sarcocystis* spp. (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/HTML/Sarcocystosis.htm)

A reverse result was presented by Kalubowila *et al.* (2004) with cattle and buffaloes from Sri Lanka where higher *S. cruzi* infection prevalence was observed in dry zone. It should be mentioned that Sri

Lanka is defined as a tropical region; therefore dryness is relative compared to Australian desert. Cattle are an intermediate host of *S. cruzi* while infected dogs excreting them in feces are the final host. Humans are not affected by this Sarcocysytis species; however, Xiang *et al.* (2009) using PCR–RFLP and DNA sequencing of a partial 18S rDNA gene (ssrRNA) sequence were able to distinguish successfully among three taxa: *Sarcocystis hominis*, *Sarcocystis fusiformis* and a species of *Sarcocystis* from water buffalo that could not be distinguished from *Sarcocystis cruzi*. Interestingly, among four restriction enzymes used in their analysis, three showed identical restriction digestion patterns for *S. hominis* and the *Sarcocystis* from water buffalo (similar to *S. cruzi*). Future studies will possibly clarify the potential infection of humans by this highly prevalent cattle parasitic species.

Dubey *et al.* (2002) investigated the effect of heat and several disinfectants on *S. neurona*'s sporocysts. *Sarcocystis neurona* is the most common cause of equine protozoal myeloencephalitis (EPM) in America, with opossums as the definitive hosts and a variety of mammals as aberrant or natural intermediate hosts. Various disinfectants at various concentrations did not inactivate sporocytes while thermal treatment for 1 minute at 60°C did inactivate them, indicating that heat treatment was the most effective treatment. Supposing that oocysts of *S. neurona* are as closely resistant as *S. bovihominis* and *S. suihominis*, they will be also susceptible to heat treatment; for this reason well cooked meat is the best measure to prevent humans infection (Pozio, 2008).

A study performed in Argentina, on children ages 2–14 years old, for presence of intestinal parasites (frequency 50.7% of different parasites and 0% with *Sarcocystis* spp.) revealed a significant correlation to family hygiene level (Soriano *et al.*, 2001). The patients were from lower middle class with adequate public services. Environmental data were continental semiarid climate and sandy soil (with low organics content). Soil samples revealed 14.6% *Sarcocystis* spp., the highest frequency among the intestinal parasites. The prevalence discrepancy between human cases and soil presence was explained as a result of the frequent irrigation of the region's soil (twice a day, to avoid dust in the air) with possibly sewage contaminated water.

Finally, a new coccidian was reported in the renal infection of big brown bat. Analysis of the 18S rRNA gene sequence put the parasite in the Sarcocystidae family. Whether it is related to cattle, pigs and humans infection is yet unknown.

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Chapter 4.1.11

Sleeping sickness (African Trypanosomiasis)

[*TRYPANOSOMA BRUCEI GAMBIENSE, TRYPANOSOMA BRUCEI RHODESIENSE*]

African trypaniosomiasis is a human disease caused by a flagellated parasite that belongs to phylum Euglenozoa and genus Trypanosomatida. The parasite is vector-borne, transmitted by bites of the tsetse flies (order: Diptera, family: Glossinidae). Tsetse flies are multivoltine (organisms that have more than two broods or generations per year) with four generation/year and up to 31generations in their entire life span. There are two zoonotic parasites involved in human sleeping sickness: *T. brucei gambiense* and *T. brucei rhodesiense* (the *T. brucei* group) both with identical morphology. A third subspecies, *T. brucei brucei* infects horses (lethally) and other ruminants. Animals reservoir of the human trypanosomae are: 1) domestic: pigs, cattle, dogs, sheep (*T. b. gambiense*) and cattle, dogs, sheep, goats (*T. b. rhodesiense*) 2) wild: monkeys, antelopes (*T. b. gambiense*) and bushbucks, reedbucks, waterbucks, lions, hyenas (*T. b. rhodesiense*) (Cordon-Obras *et al.*, 2010). The disease is endemic in a large area of Central Africa (with >40 countries in sub-Saharan region) between the Atlantic and Indian Oceans. Transmission is linked to the presence of the tsetse fly, the only vector and host of these parasites. Among the *Glossina* genus (tsetse taxonomy): *Glossina palpalis*, *G. tachinoides* and *G. fuscipes* transmit *T. b. gambiense* while *G. morsitans*, *G. pallidipes* and *G. swynnertoni* transmit *T. b. rhodesiense*. The life cycle consists of two stages according to host: in tsetse fly trypomastigotes from human blood reach the insect stomach and crop and then form epimastigotes by binary fission in intestine and later form the metacyclic infectious forms (trypomastigote) in salivary glands. The trypomastigotes are then injected into human blood during fly blood meal. In humans, trypomastigotes multiply in the peripherial blood infecting the CNS (central nervous system) (Figure 4.1.11.1).

Clinical manifestations are lymphoadenopathy, splenomegaly, increased vascular permebility, edema, hemostasis, intravascular coagulophaty, anemia, tissue hypoxia and immunosupression. The infected person suffers from persistent headache, memory loss, apathy, sleep disturbances (daytime somnolence and nocturnal insomnia), impaired vision (even blindness), coma and death. In humans, without

treatment *T. b. rhodesiense* is more severe with fatal results in 6 to 9 months while *T. b. gambiense* fatality is longer, 2 to 6 years. Animal hosts infected with these parasites express subclinical symptoms.
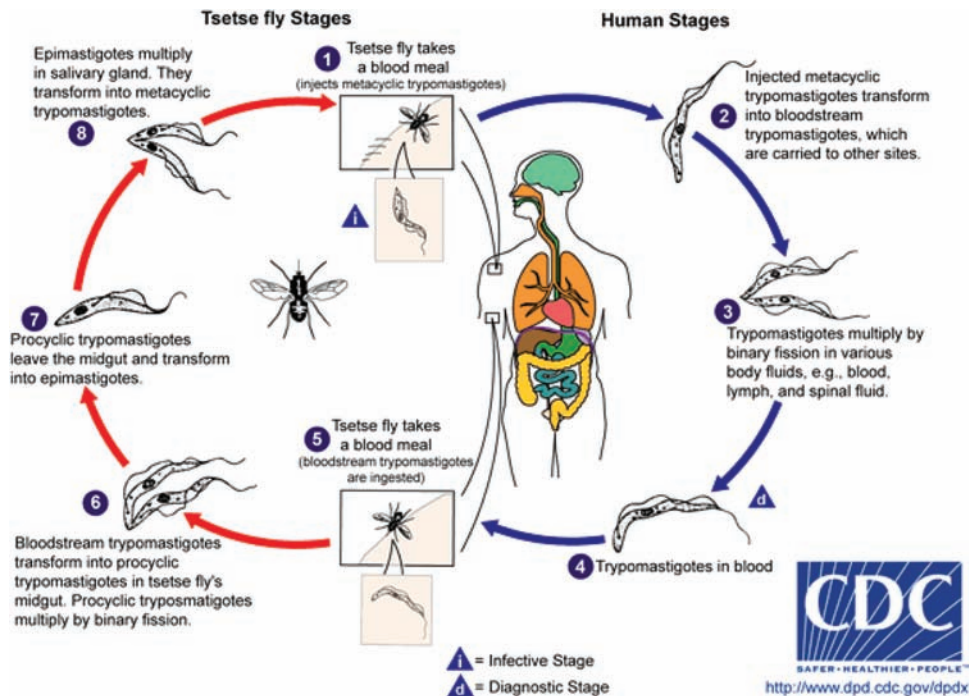
Figure 4.1.11.1. Life cycle of *Trypanosoma* spp. (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/DPDx/HTML/TrypanosomiasisAfrican.htm)

4.1.11.1 AFRICAN TRYPANOSOMIASIS AND ENVIRONMENT

In the Sub-Saharan Africa, tsetse flies occupy an area between 10 and 12 million km^2 in this region, of which some 7 million km^2 could potentially be used by livestock. Raising African cattle on large areas is hampered by the high pervasiveness of tsetse flies and altered agro-forestry practices (Ilemobade, 2009; Molyneux, 1998). Consequently, African farmers grow their cattle herds in arid (dry) zones to avoid tsetse flies that cannot survive well under these conditions. In this context, it should be mentioned that endemic human African trypanosomiasis (HAT) currently appears to be limited to areas where annual rainfall is higher than 1,200 mm, although the reasons for this remain unknown (Courtain *et al.*, 2008). New evidence was recently published possibly explaining this phenomenon based on tsetse fly water balance. Kleynhans and Terblanche (2009) showed that water loss rates (WLR) were significantly positively related to precipitation in pupa but not adults, suggesting that a reduction in WLR and increased size probably developed from a transitional ancestral state that may have facilitated survival in xeric environments. However, in recent times the water balance of pupae is critical, consequently limiting tsetse survival in dry environments (Opinel, 2008; Roubaud, 1957).

Thus, the overstocking in a limited area results in land degradation as well as poor cattle quality. If these areas are close to forests that undergo deforestation, the new land will be further cultivated by planting cocoa, coffee, oil palms, mangoes, etc., a vegetation that favor tsetse colonization (Hausner, 1992). In the African Sahel, tsetse flies inhabit river banks and forest while in rainy season they move to drier areas (Patz *et al.*, 2000; Jobin, 1999). Interestingly, Petney (1997) raised an arguable ecological point related to tsetse eradication. The author indicated that successful eradication programs against tsetse in Africa would remove a natural barrier that prevented in the past over exploitation of these environments by humans (overgrazing, increased desertification, vegetation changes and possibly climate change). In addition, he also pointed out the short and long terms effects of pesticides use to control this arthropod pest. One is the family of avermectins and their semi-synthetic derivatives (broad-spectrum pesticides) active against a wide range of nematodes, mites, ticks and insects (including helminthes), some with agricultural importance, and the second DDT (now banned in most world countries, although still used in developing countries). Both, beside their veterinary benefit, can alter the natural ecology, especially when they are sprayed from air (by helicopters) over a large area. For example, based on a vast published literature, DDT that has a half life of about 100 years, and is therefore highly persistent, can be further biodegraded into more toxic compounds. In turn, these compounds affect many vertebrates through hormonal unbalance and abnormality induction (Guillette *et al.* 1994). Other aspects of such insecticides (i.e., pyrethroids) were presented by Eisler *et al.* (2003) revealing that uncontrolled use may lead to environmental damage such as acaricide resistance in tick populations and a possible exacerbation of tick-borne diseases. However, as presented by Langley (1994), a brighter prospect should be mentioned here concerning the management of tsetse vector at local size. In spite of many insecticides' ban over the years, due to their environmental hazards, new trap devices, based on the role of olfactory and visual stimuli of tsetse fly found in research, have been implemented in endemic areas. Such devices treated locally with insecticides have been successful, being able to sustain fly population at manageable levels.

Wardrop *et al.* (2010) used spatial analysis (a generalized linear geostatistical model in a Bayesian framework) to verify the causes of increased numbers of *T. b. rhodesiense* in an endemic area of *T. b. gambiense* in Uganda. Their analysis significantly indicates that Rhodesian HAT (human African trypanosiomias) was introduced into Uganda by the movement and introduction of infected livestock (untreated from endemic areas) (Batchelor *et al.*, 2009). Another environmental aspect was highlighted by Turner (1986) in the Kenyan Lambwe Valley, a region shared by wild animals in a national park adjoining human settlements with domestic livestock. Lambwe Valley is characterized by thicket and woodland heavily infested with *Glossina pallidipes*. The author reported that trypanosomiasis' aggravated situation in recent years was attributable to the extension of tsetse habitat and encroachment of settlements into the national park.

Trypanosomiasis is essentially a rural disease; however recent urban reemergence was observed in Kinshasa, in the Democratic Republic of Congo, from 254 cases in 1996 and about 500 cases/year from then on (Louis *et al.*, 2003; Tshimungu *et al.*, 2010). The increase in case prevalence has been explained by refugee inflow into town outskirts from endemic areas and by the movement of urban populations towards the suburbs due to economic crisis. Both processes resulted in humans living on a subsistence micro-agriculture that establishes a trypanosiomiasis belt around the urban area (Fournet *et al.*, 2000). Grebaut *et al.* (2009) studied transmission sites of African trypanosomiasis in Kinshasa too. They found that dry season, argillaceous soil, raised ground cover, pig breeding, forest residues and rivers are important factors in disease transmission (Figure 4.1.11.2).

In Burkina Faso, using Landsat 7 thematic mapper (TM) images as remote sensing, similar human activities have been shown to have a high impact on riverine vegetation; the closer to the river the crops

were, the greater the observed impact on riverine vegetation and the degree of contact with riparian tsetse flies (Roumba *et al.*, 2009; De La Rocque *et al.*, 2001). This and other studies confirmed that peri-riverine human activities have a negative impact on gallery forest vegetation, in contrast to "protective" swamp forests, in consequence increasing trypanosomiasis risk (De Deken *et al.*, 2005).
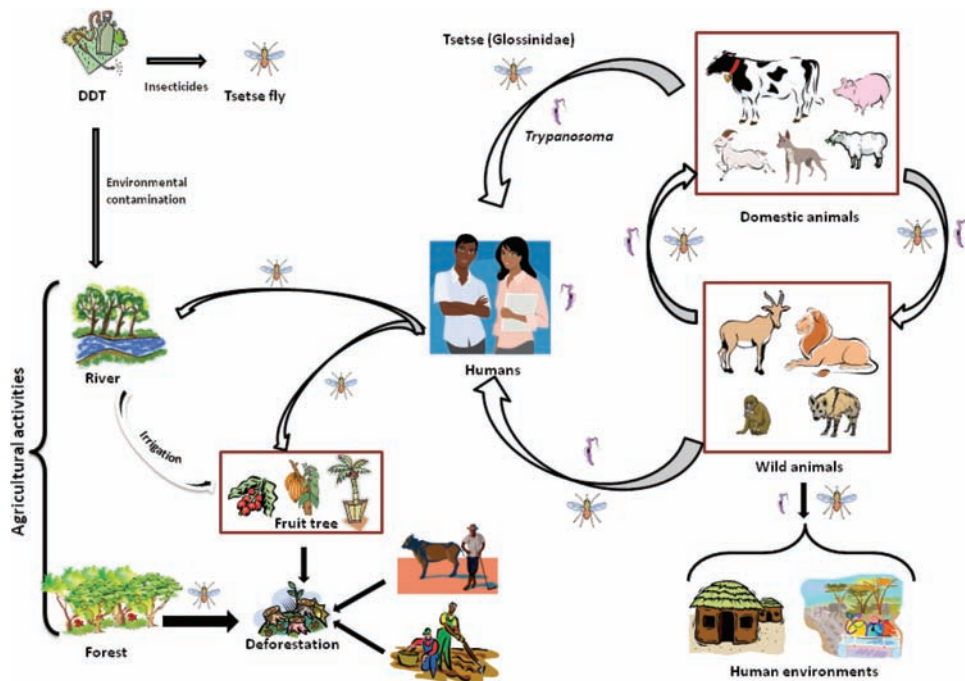
Figure 4.1.11.2. Environmental and man-made factors affecting tsetse fly and *Trypanosoma* spp. spread

Courtin *et al.* (2005) using spatial analysis studied HAT in Bonon city, a focus located in the mesophile forest of the Ivory Coast. Besides Bonon city, the authors looked at two areas connected to the town: the northern rural one with low prevalence and the southern rural one with high prevalence. The southern area was found to comprise patches of both relict forest and uncultivated lowland remains, and was therefore a favorable breeding area for tsetse flies. However, tsetse density is not utterly correlated to transmission rate and other contextual factors seem to be related. Such a factor emphasized by the authors is human mobility and presence in risky areas. Thus, the southern part's track to town crosses a tsetse favorable environment (edges of uncultivated lowland, plantations and forest) while the northern tracks do not. Courtin *et al.* (2010) showed in Guinea that people with more diverse spatial occupation that involves longer walking distance including mangrove forests, rice culture and attendance of pirogue jetties have higher risk of infection.

Finally, Kodama *et al.* (2008) reported that humic substances extract protects experimental mice against infection with *T. brucei* (cattle African trypanosomiasis agent). It will be interesting to find out if these results are true also for larger animals, such as cattle, thus suggesting a possible natural "external immunization" against this animal parasite!

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Chapter 4.1.12

Toxoplasmosis

[*TOXOPLASMA GONDII*]

Toxoplasma gondii is a protozoan parasite that infects a large variety of animals (felidae family are the primary host) including humans (as intermediary host) (Dubey *et al.* 2009). Transmission occurs through ingestion of contaminated water, milk, vegetables or consumption of undercooked meat (domestic or game), iatrogenically (blood and tissue donation) and congenitally (transplacentally from mother to fetus) (Cook *et al.*, 2000; Aramini *et al.*, 1999; Radon *et al.*, 2004; Dawson, 2005; Avelino *et al.*, 2004). The parasite is distributed worldwide; however certain countries have higher prevalence due to several factors: 1) consumption of raw or lightly cooked meat (France, serologic prevalence 88%); 2) stray cats and favorable climatic conditions that extend oocysts' soil survival (Central and Latin America, 51 to 72%); 3) animal' skinning and eating raw seal meat and liver (Canadian Inuit population, 59.8%). The life cycle includes asexual and sexual reproduction of this parasite. Briefly, unsporulated oocysts are shed in cat's feces, then in environment after 1 to 5 days they become infective when oocysts sporulate. The sporulated oocysts are further ingested by intermediate hosts (birds, rodents, pigs, etc.) through contaminated soil, plant debris and water (Smith *et al.*, 1992; Asgari *et al.*, 2009; Dubey *et al.*, 2003b). Inside intermediate hosts, oocysts transform in tachozoytes that are confined in neural and muscle tissue to develop into tissue bradyzoites (Figure 4.1.12.1). Cats as primary host become infected (but also other felids such as cougar and lynx) by devouring these intermediate hosts or by direct ingestion of sporulated oocysts (Aramini *et al.*, 1998). In immunocompetent individuals infection is mostly asymptomatic; however, 10 to 20% of the cases develop a flu-like illness, fever, weakness and debilitation, ophthalmitis, severe multisystemic infections and lymphadenopathy (McAllister, 2005), while in immunocompromised individuals clinical manifestations are encephalitis and neurologic diseases, lesions in different organs (heart, liver, inner ears), dyspnea, diarrhea, and chorioretinitis (visual disorders). In the context of neurological disorders, there is accumulating evidence from many studies that *T. gondii* is somehow related, together with other pathogens (i.e., rubella, influenza and herpes simplex virus type 2) to schizophrenia. The exact path and biological interaction is not clear at the moment, as most data are based mainly on human epidemiological studies and animal models, including maternal immune activation, that have yielded evidence suggesting that these exposures may cause brain

and behavioral phenotypes that are analogous to findings observed in patients with schizophrenia (Brown, 2011). While reflecting on such a possibility, the *H. pylori* scientific history instantly leaps into one's mind, where so many in the past believed that the etiology of peptic (gastric) ulcer is purely physiologic stress!
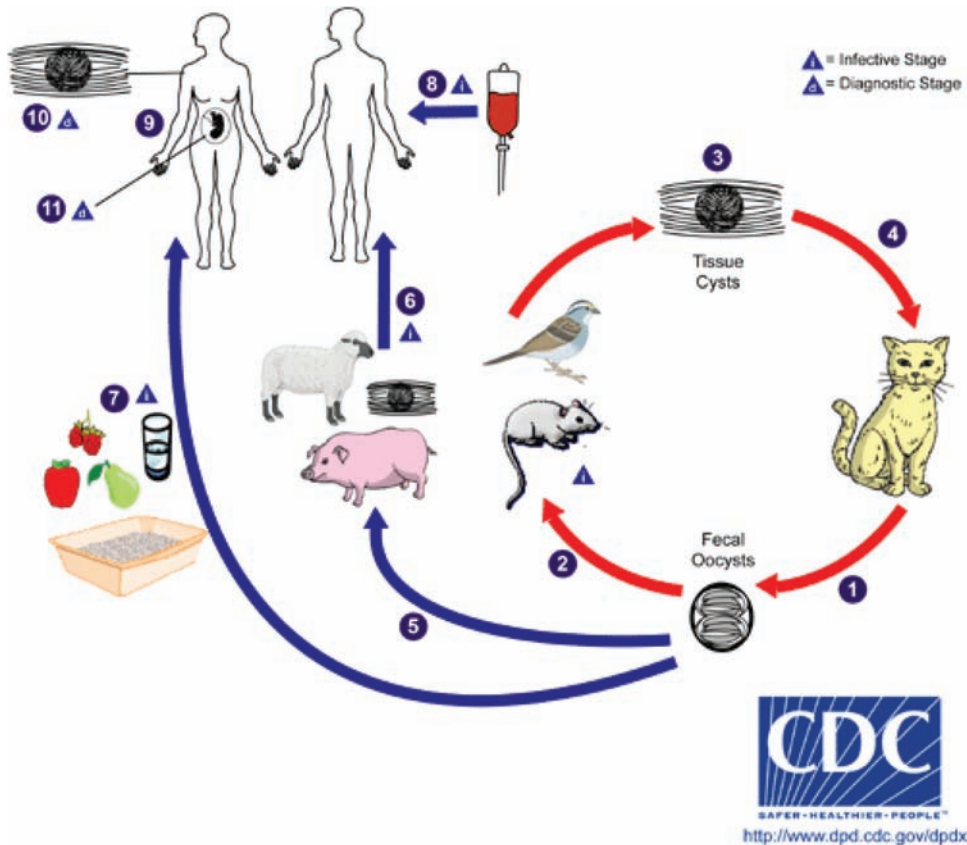
Figure 4.1.12.1. *Toxoplasma gondii* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/HTML/Toxoplasmosis.htm)

4.1.12.1 *TOXOPLASMA GONDII* AND ENVIRONMENT

Wild animals seem to carry *T. gondii* as intermediary or final hosts (Figure 4.1.12.2). In Israel, a seroprevalence survey for anti-*Toxoplasma gondii* antibodies has been conducted in wild pigeons. The prevalence was 4% with inverse proportionality for two environmental parameters: human proximity and water sources (Salant *et al.*, 2009). Spanish researchers looked for seroprevalence in wild rabbits from different geographical regions. Whole country prevalence was 14.2%; however, in the Catalonia region (northeast part of Spain) with rabbits living in forests the prevalence was much higher (53.8%), while in the Cadiz region with its dry habitat it reached only 6.1%. According to the authors, the parasite cycle includes infected feral cats that excrete oocysts further ingested by wild rabbits via water or soiled food

contact and then back to carnivores (specifically felidae, but also foxes) (Aramini *et al.*, 1999). Interestingly, Gauss *et al.* (2003) substantiated the high seropositivity prevalence (45%) in domestic cats in Barcelona (Catalonia) and even higher in feral cats (51.9%), possibly explaining the elevated seroprevalence of wild rabbits. In Holland, wild boar (game) have been also examined for *T. gondii* seroprevalence revealing an estimated 24.4%, suggesting that eating undercooked wild boar meat may pose a risk of infection with this parasite. Sheep and goats as grazing domestic animals have been also shown to be affected, especially in the form of primary infection of pregnant sheep and goats, resulting in fetal death and resorption, abortion or stillbirth that have an economic consequence (Buxton, 1998). Lindsay *et al.* (2003) clearly showed that *T. gondii* oocysts can sporulate and remain viable in seawater for several months. Using oocysts exposed to various conditions (salinity and temperature), they were able to show mice infectivity through inoculation with oocysts kept for six months at 4 and 22°C. This study emphasized the sturdy characteristic of this parasite in environment and its potential to infect a large variety of animals (both terrestrial and aquatic). Low temperature resistance (refrigeration) should be considered as an important environmental attribute especially in cold regions of the globe and its potential distribution in these areas (Lindsay *et al.*, 2002). Miller *et al.* (2002) examined live and dead southern sea otters (*Enhydra lutris nereis*) along the California coast for seroprevalence against *T. gondii*. They reported a seroprevalence of 42% in live otters, and 62% in the dead ones. Importantly, using spatial analysis, these authors found that otters in proximity to areas with maximal freshwater runoff were three times more likely to be seropositive. However, human population density and sewage exposure were not associated with high seropositivity, therefore pointing at freshwater runoff (that may carry felids' excreta deposited on soil surface) as a source of infection for marine animals with *T. gondii*. In the same geographic area, Shapiro *et al.* (2010) evaluated how human coastal landscape alteration impacts the transport of *T. gondii* oocysts to estuarine waters, consequently affecting sea otters. Using surrogate particles that mimic the parasite's behavior in water, they found that increasing surrogates were recovered from unvegetated mudflat habitats (degraded wetlands) compared to vegetated ones. They also found on a certain location, known for its high otter infection rate, that 36% erosion of the vegetated wetlands may increase oocysts flux by 2 orders of magnitude, while complete degradation may increase the flux by 6 orders of magnitude.

Another possible source of otters' infection in marine environment has been studied by Arkush *et al.* (2003) with marine bivalve shellfish (*Mytilus galloprovincialis* mussels) as paratenic hosts. Experimental infection of these mussels with *T. gondii* oocysts revealed a persistence of up to 21 days post-exposure mainly in digestive glands (89%). Assuming that otters do feed on this type of mussel, these bivalves can concentrate and preserve oocysts hence serving as an intermediate host. The same results have been obtained for Eastern oysters (*Crassostrea virginica*) exposed to *T. gondii* oocysts. The oysters removed successfully oocysts from seawater (thus being used as intermediate concentrators), keeping them infectious for several months (Lindsay *et al.*, 2004). In marine environments, dolphins, walruses and sea otters are infected by *T.gondii*. Shellfish species have been shown to be excellent filters and concentrators of this parasite' oocysts. Recently new marine participants in the infection cycle have been shown: northern anchovies (*Engraulis mordax*) and Pacific sardines (*Sardinops sagax*) both filter-feeding fishes (Massie *et al.*, 2010). Experimentally, those filter-feeding fishes exposed to *T. gondii* oocysts (even at low concentrations 1197 oocysts/L) tested positive for their alimentary canal. The authors showed in laboratory experiments that oocysts can persist and remain infective in filter-feeding fishes for 8 hours post-exposure, hypothetically serving as biotic vectors of the parasite from nearshore to pelagic environments.

A recent publication related to aquatic life of *T. gondii* showed an interesting interaction between amoebae and oocysts (Winiecka-Krusnell *et al.*, 2009). Free-living protozoa such as *Acanthamoeba*

castellanii in direct contact with oocysts were found to be capable of internalizing those cysts by active uptake. Most of the internalized oocysts kept their infectivity for long periods of time inside amoebae continuous cultivation and in addition amoebae uptake did not restrain infection transmission to murine model.
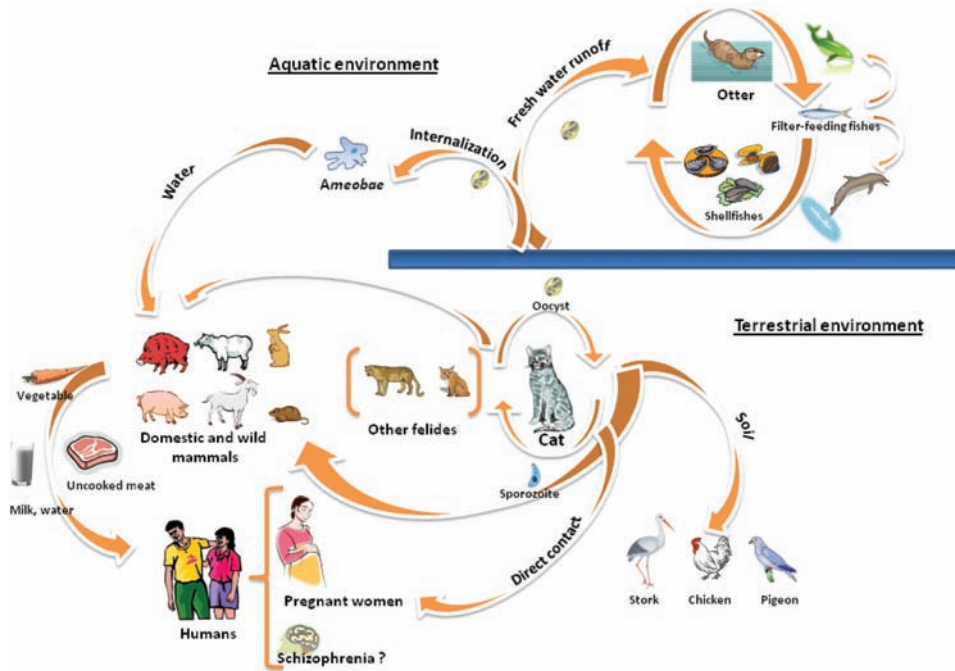
Figure 4.1.12.2. *T. gondii* interactions with various animals in aquatic and terrestrial environments

In Poland, Lass *et al.* (2009) examined different soil samples (sand-pits, farm and soil close to rubbish dumps) for the presence of *T. gondii* oocysts. Using PCR amplification of a repetitive element (REP) sequence methods, they found 17.8% samples positive for this parasite, suggesting that contaminated soil could be a risk factor for human toxoplasmosis. In relation to soil, de Thosy *et al.* (2003) looked for *T. gondii* seroprevalence in free ranging forest mammal species from different orders in French Guiana and found the following seroprevalence percentages: Marsupiala (various opossums, 8-20%), Xenarthra (sloth-0%, anteater-39% and armadillo-44%), Rodentia (paca-60%, porcupine-0%, acouchy-4% and gouti-18%), Carnivora (kinkajou-10%, tayra and coati-72%), Artiodactyla (deer-40% and peccary-68%) and Primate (red howler monkey-4% and tamarin-0%). These authors concluded that terrestrial animals are more exposed to *T. gondii* infections. In this context, Dubey *et al.* (2003) examined prevalence of *Toxoplasma gondii* in free range chickens and ducks in Egypt, based on their ground feeding behavior. The seroprevalence was 40.4% in chickens, and 15.7% in ducks. The lower ducks' antibodies prevalence against *T. gondii* could be attributed to their more aquatic life habits compared to chickens that are mainly terrestrial, but this assumption should be further investigated. Similar results have been obtained in Brasil, where free range chickens where analysed for *T. gondii* in a known endemic area (da Silva *et al.*, 2003). Chicken seroprevalence was 65.1% and more importantly viable parasites were isolated

from 70.9% of the chickens tested, demonstrating high environmental contamination. Wild birds have a real potential of spreading zoonoses attributable to their global migratory routes. Andrzejewska *et al.* (2004) surveyed wild and captive white storks for *T. gondii* seroprevalence and reported 5.8% in chicks and 13.5% in adults. From the point of view of human health risk, adult storks usually nest close to human habitat with a predilection for high voltage poles and roof' chimneys, therefore posing an environmental risk.

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Chapter 4.2

Helminthic zoonoses

Chapter 4.2.1

Trematodes

[*SCHISTOSOMATIDAE*]

In spite of their limited fatality towards humans, helminths in general represent a steady burden with many side effects and economic outcome. At the beginning of each helminths group description (trematodes, cestodes and nematodes), we decided to summarize some environmental parameters to emphasize the importance of zoonotic helminthology. Trematodes global infection burden is presented in Table 4.2.1.1.

4.2.1.1 CERCARIAL DERMATITIS

Swimmer's itch or cercarial dermatitis is caused by repeated infection with cercariae (larvae of trematodes, a free-living form of the schistosome worm infective to humans) released from an intermediate host (snails from different water types: ponds, lakes and oceans) able to penetrate human skin or other animals (mainly waterfowl) (Hoeffler, 1977). Cercariae are not able to propagate in humans, but due to their antigenic activity, they can cause allergenic reaction. The final hosts are waterfowls and different mammals. Among cercariae releasing trematodes, the following schistosomatids can be found: *Austrobilharzia*, *Trichobilharzia*, *Gigantobilharzia* and *Schistosomaticum*. The swimmer's itch is distributed worldwide affecting swimmers, rice field workers, marine mollusc collectors and tourists. For instance: in Central Europe it is caused by duck bilharziosis agent *Trichobilharzia szidati* (or *Trichobilharzia ocellata*), in United States coastal areas including Hawai, *Austrobilharzia variglandis* (sea swallows and ducks final hosts), in Southeast Asia and Iran ruminant schistosomiasis in rice fields by *Schistosoma spindale* and *Orientobilharzia turkestanica*. Transmission occurs when trematodes eggs from infected final hosts reach water through defecation. Hatched miracidia invade snails (intermediate hosts) and propagate asexually to produce cercariae that are mass released in water in search for a final host (waterfowl, etc.) (Figure 4.2.1.1). If humans are exposed to these waters, their skin is accidently penetrated by free cercariae whose survival is however, short with surface symptoms only. The clinical manifestation is primary itch (depending on past exposure), papules and lumps development, erythema, fever, malaise and if repeated infections anaphylactic reaction can occur.

Table 4.2.1.1 Trematodes as a global threat for humans.

Trematodes (between 18,000 to 24,000 species)	Global infection	Geographic distribution	Disease
Schistosomatidae *Austrobilharzia, Trichobilharzia, Gigantobilharzia* and *Schistosomaticum*	Thousands (?)	Worldwide	Cercarial dermatitis
Clonorchis sinensis	Estimated: 35 million people	Japan, China, Korea, Taiwan, and Southeast Asia,	Clonorchiasis
Dicrocoelium denditricum, D. hospes	Estimated: Hundreds	Europe (former U.S.S.R., Switzerland, Italy, Germany, Spain, Turkey), the Middle East (Iran), Asia (China, Japan, Vietnam), Africa (Ghana, Nigeria, Sierra Leone), North and South America and Australia	Dicrocoeliasis
Echinostomatidae family: e.g. *Echinostoma hortense, E. revolatum, E. lindoense, E. cinetorchis, Echinochasmus perfoliatus, Hypoderaeum conoideum, Euparyphium melis*	Thousands (?)	East Asian countries such as Korea, China, and Japan	Echinostomiasis
Fasciola hepatica and *Fasciola gigantic,*	2.4 million people are infected, 180 million are at risk	Europe, North and South America, Oceania, Africa and Asia	Fascioliasis

(Continued)

Table 4.2.1.1 (*Continued*)

Trematodes (between 18,000 to 24,000 species)	Global infection	Geographic distribution	Disease
Fasciolopsis buski	10 million people infected in East Asia	Asia	Fasciolopsiasis
Heterophydae *Heterophyes heterophyes, Metagonimus yokogawai, Haplorchis* spp. and *Procerovum* spp.	10,000 to >500,000/year	Southeast and East Asia, Northern Africa, Middle East	Heterophyiasis
Opisthorchis felineus, Opisthorchis viverrini	Worldwide number of cases ~9 million. In Thailand alone ~7.3 million. Worldwide 67.3 million of people are at risk of infection	Eastern and Southern Europe, Asiatic regions of former USSR (including Siberia, along big rivers) while *O. viverrini* in Southeast and East Asia (Laos, Thailand, Vietnam and Cambodia).	Opisthorchiasis
Paragonimus genus (*Paragonimus africanus, Paragonimus westermani, Paragonimus mexicani, ecuadorensis*)	Rare Estimated to infects 22 million people worldwide	*Paragonimus westermani* is distributed in southeast Asia and Japan. Other species of Paragonimus are common in parts of Asia, Africa and South and Central America. P. westermani has been increasingly recognized in the United States during the past 15 years because of the increase of immigrants from endemic areas such as Southeast Asia, Asia and South America	Paragonimiasis (Pulmonary distomatosis)

	Estimated infections	Geographic distribution	Disease
Schistosoma (*Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. mattheei*, *S. intercalatum*, *S. bovis* and to lesser extent *S. magrebowiei*, *S. rodhaini*]	Estimated infections: *S. mansoni*-57 millions *S. haematobium*-78 millions *S. japonicum*-69 millions *S. mekongi*-thousands *S. intercalatum*-thousands Approximately 20,000 deaths/year	Asia, Africa, and South America, especially the disease is found in tropical countries in Africa, the Caribbean, eastern South America, Southeast Asia and in the Middle East. *Schistosoma mansoni* is found in parts of South America and the Caribbean, Africa, and the Middle East; *S. haematobium* in Africa and the Middle East; and *S. japonicum* in the Far East. *S. mekongi* and *S. intercalatum* are found locally in Southeast Asia and central West Africa, respectively.	Schistosomiasis (Bilharziosis)
Other human miscellaneous trematodes infections *Eurytrema pancreaticum*, *Gastrodiscoides hominis*, *Nanophyetes salmonicola*	Rare: between tens to hundreds infections	*E. pancreaticum*: Southeast Asia and China but also Russian Far East. *G. hominis*: India, Southeast Asia including China. *Nanophyetes salmonicola*: USA Pacific area (Northwest USA) and northern California	Pancreatic inflammation Enteritis Hemorrhagic enteritis
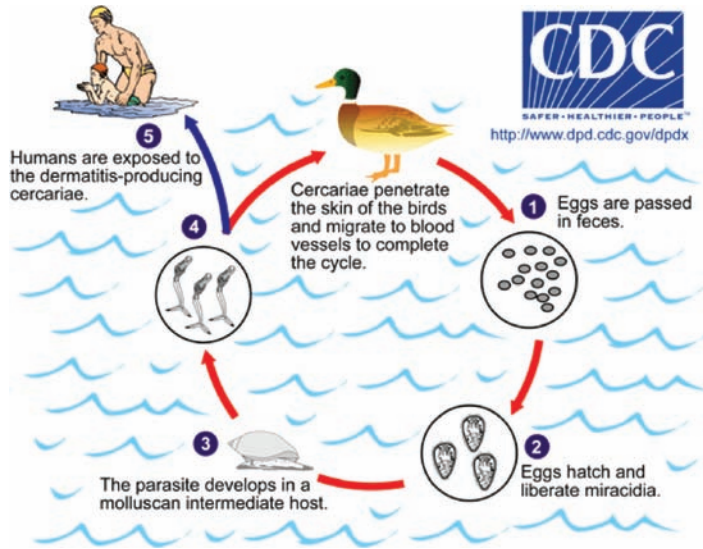
Figure 4.2.1.1. Cercarial dermatitis (swimmer's itch) life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/HTML/CercarialDermatitis.htm)
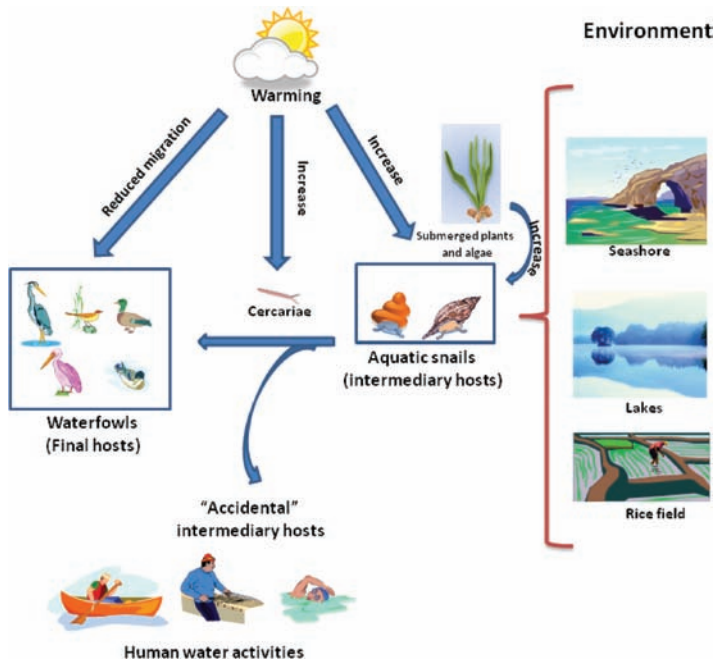
Figure 4.2.1.2. Potential impact of global warming chain on cercarial dermatitis

4.2.1.1.1 Cercarial dermatitis and environment

Increased cases of cercarial dermatitis are directly linked to ecological and environmental factors. Because the life cycle of these trematodes is linked to water (water snails and waterfowl), any disturbance of the ecological balance can directly or indirectly impact one of those bioparameters. For example if dense mats of algae develop under anthropogenic pressure, large numbers of plant-grazing anatids and cyprinids will be attracted and in turn trematode transmission is facilitated. Anthropogenic pressure found expression in the construction of large freshwater impounds (e.g., dams) that dramatically affects both the natural environment and wildlife parasite fauna (Soldánová *et al.*, 2010). Parasites' responses are diverse; however, in the longrun such anthropogenic alterations result in the facilitation of trematodes life cycle and Swimmer's itch has become an increasing problem in Europe and North America lake regions (Kolárová *et al.*, 2010; De Gentile *et al.*, 1996). Perhaps the most striking evidence came from Israel, where fishponds and other fresh or brackish water impounds were built for economic development of aquatic agriculture and water collection. These impounds are visited each year by thousands of migratory birds, many of them belo waterfowl (Frumkin *et al.*, 1995). Another important factor is climate change impact on biosphere in spite of reports on cercarial dermatitis from relatively cold climate areas such as lakes in French Alps, Savoy district, Iceland and Canada (Dubois *et al.*, 2001; Jouet *et al.*, 2008; Kolárová *et al.*, 1999; Levesque *et al.*, 2002). Thus, warmer temperature may increase algal development and consequently snail population and accelerate trematode development within snails. In addition, from the ornithological point of view, migrating birds under global warming conditions do change their habits, becoming sedentary in central European lakes (Cotton, 2003). When the final host remains in place, the life cycle of bird schostosomiasis will expand increasing cercarial numbers with higher risk for humans (Figure 4.2.1.2).

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Chapter 4.2.1.2

Clonorchiasis

[*CLONORCHIS SINENSIS*]

Clonorchiasis is a disease caused by infection with eggs of the trematode *Clonorchiasis sinensis. C. sinensis* is a hermaphroditic fluke that requires two intermediate hosts (snails and fish) before establishing itself in a final host (humans, cats, dogs, pigs and small carnivores). Fluke eggs are excreted in feces of infected human, feces that may reach water sources and there further ingested by various mollusks (snails of *Balinus* and *Parafossarulus* species). In snails, as the first intermediary host, the parasite goes through asexual multiplication and growth (miracidium, sporocyst and redia stages). Snails release active cercariae that can swim and penetrate the second intermediary host (small fresh water fishes mostly from Cyprinidae family). Then cercariae encyst in fish muscles to develop in metacercariae to be further transferred to the final host (humans) by ingestion of raw or inadequately cooked fish meat. Young flukes hatch in humans' duodenum and migrate to distal bile ducts via bile duct to adhere to epithelial cells (Figure 4.2.1.2.1). Following maturation, flukes produce constantly eggs excreted in human feces. The life span of this parasite (without treatment) within final host can reach 20 to 25 years. Endemic areas of clonorchiasis are found in the Far East (Korea, Japan, Vietnam, Taiwan and Southern China) where raw fish consumption is a common practice (e.g., sashimi in Japan). Clinical manifestations are acute or chronic cholangitis (depending on initial fluke load intake) with potential risk to of developing cholangiocarcinoma (biliary system neoplasm), periportal cirrhotic lesions, upper abdomen pain, jaundice, hepatosplenomegaly, flatulence, changeable diarrhea and constipation, dizziness, vomiting, headache and anemia.

4.2.1.2.1 CLONORCHIASIS AND ENVIRONMENT

As previously mentioned, *C. sinensis* is wide -spread in the Far East. Tang and Luo (2003) looked for prevalence of different parasitic infections (including *C. sinensis*) in rural areas of West China. They attributed the high parasitic prevalence (51.7%) and persistence to the following factors: moist and remote environment, traditional life, education, contaminated potable water and inadequate health services. *Clonorchis sinensis* prevalence was low (0.04%) in comparison with other parasites (e.g.,
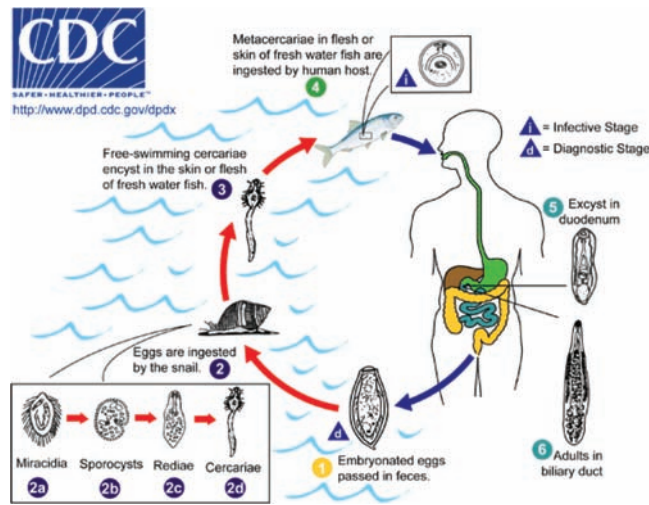
Figure 4.2.1.2.1. *Clonorchis sinensis* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/HTML/Clonorchiasis.htm)

41.4% for *Ascaris lumbricoides*), which may be explained by low fish consumption in these rural areas. In another study performed in China, stool examinations have been performed on people suspected of *C. sinensis infection*. Among these individuals, 21.43% were infected with *C. sinensis* exclusively and 34.64% were co-infected with *C. sinensis* and other pathogens. These authors also attributed this helmintic infection prevalence to living environment, occupations and hygienic and eating habits. In South China, Yu *et al.* (2003) examined stools for presence of eggs among 1,552 individuals and found a 31.6% *C. sinensis* prevalence. Their study showed higher prevalence in adults than children and adolescents. The authors emphasized that while endemicity of soil-transmitted helminths is in decline in the region surveyed, *C. sinensis* cases have increased. They attributed this increase to possible socioeconomic conditions, fish pond contamination with contaminated feces, fishery practices and farm animals' infection, which need further verification. In a related studied, Zhang *et al.* (2007) studied *C. sinensis* infection prevalence in 1,473 individuals in the Shenzhen area (located in Zhujiang delta of Guangdong province), one of cities in the south of China with the largest number of immigrants. In addition, various snails and fishes (intermediate hosts) from this area were also tested for the presence of cercariae and metacercariae. Infection prevalence in humans was 4.75%, in snails 1.15% and fishes 16.97% (carps leading with 40.74%). A distributed epidemiologic questionnaire among the study group participants revealed the following: 1) 27% had raw fish meat one or two times a month; 2) 5% used for raw fish and cooked food the same appliances; 3) among fishpond owners, 40% fed fishes with human and animal excrements. Indeed, all these factors are plausible explanation for continuous infection of *C. sinensis* in humans in this area. A very similar study in another area of China has been conducted by Lin *et al.* (2005) emphasizing the same points: 51% of participants ate raw fish at least 1 to 2 times a month; utensils were used for both raw fish and other foods; and fish ponds were "fertilized" with excreta. In addition these authors also reported the prevalence of *C. sinensis* in other final hosts: cats (70%), dogs (50%) and pigs (27%). In this study, fish (as intermediary host) had an infection rate of 40%. In another highly endemic area, Vietnam, Nguyen *et al.* (2009) looked for fish borne zoonotic trematodes (including *C. sinensis*) in different domestic animals. In spite of low prevalence of *C. sinensis* in these

animals, other trematodes revealed a similar infection prevalence pattern as presented by Lin *et al.* (2005): cats (70.2%), dogs (56.9%) and pigs (7.7%), all affected by risky husbandry practice of feeding animals with raw fish.

In summary, it should be stated that infections with *C. sinensis* can be controlled by education, higher hygienic standards and possible elimination of one of the "players" involved in *C.sinensis* life cycle. Molluscides use is still questionable due to their hazardous environmental effects on other aquatic fauna, and therefore Pokora (2001) suggested biological control (predators, parasites or pathogens and intra-species competition) as a more "green" solution for snail population reduction.

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Chapter 4.2.1.3

Dicrocoeliasis (Distomatosis)

[*DICROCOELIM DENDRITICUM, DICROCOELIM HOSPES*]

Dicrocoelium dendriticum and *Dicrocoelium hospes* are trematodes that rarely infect the bile ducts of humans (Ashrafi, 2010). Other reservoirs and final hosts are mammals, such as sheep, cows, goats, pigs, llamas, rabbits, alpacas and water buffaloes (Rinaldi *et al.*, 2009). Land snails (e.g., in USA, *Cochlicopa lubrica*; in Spain, *Helicella itala*) and specific ant species (e.g., in USA, *Formica fusca*; in Spain, *Formica rufibarbis*) are intermediate hosts. In infected land snails, ingested eggs release miracidia that transform into sporocytes and finally into cercariae. Land snails deposit slimeballs containing cercariae via respiratory pore. The slimeballs are further consumed by ants that become infected with cercariae that will further transform inside the ant into metacercariae. Accidental intake of infected ants (*Formica* spp. for *D. dendriticum* and *Camponotus* spp. for *D. hospes*) transport these parasites into the digestive system of an intermediary or final host and further to bile ducts (Figure 4.2.1.3.1). The newly infected host, sheds eggs containing larvae within feces into environment. Dicrocoeliasis is thought to be endemic worldwide, being found in Europe, the Middle East, Asia, Africa, North and South Americas, Australia, but mostly reported in more temperate zones of the Northern Hemisphere. This trematode tends to be present in intermediate hosts that favor dry, chalky and alkaline soils. Clinical symptoms are relatively mild; however, if a high infective dose is ingested, fatigue, anorexia, diarrhea, abdominal pain, fever, hepatomegaly, cirrhosis, edema, ascites, icterus and urticaria may develop.

4.2.1.3.1 DICROCOELIASIS AND ENVIRONMENT

The environmental cycle of *Dicrocoelim dendriticum* and *Dicrocoelim hospes* is somehow peculiar requiring two intermediary terrestrial hosts. In ants, infectious cercariae can cross the insect craw and one to three will settle in the suboesophageal ganglion of the ant, called "brainworm", while the rest will develop into metacercariae. The brain-infected ants are influenced by temperature and when temperature falls, ants enter a state of tetania of mandibular muscles causing them to remain temporarily attached to grass tips for the period when solar intensity and temperature decrease (until the next morning). This behavior increase fluke' odds of being ingested by a transitory grazing host together with the tetanic

intermediary host. From the epidemiological point of view, ants are important in the dissemination of dicrocoeliosis due to their abundance, wide distribution and "brainworm" dependent behavior alteration (Manga-González *et al.*, 2001). Finally it will be interesting to find out whether other ant' species can be infected and whether in turn ant predators such as bears and anteaters, may also be infected with this trematode. While anteaters are primarily distributed in Central to South America, bears live also in the northern hemisphere where these flukes are more common, hence additional studies may clarify this interesting question.
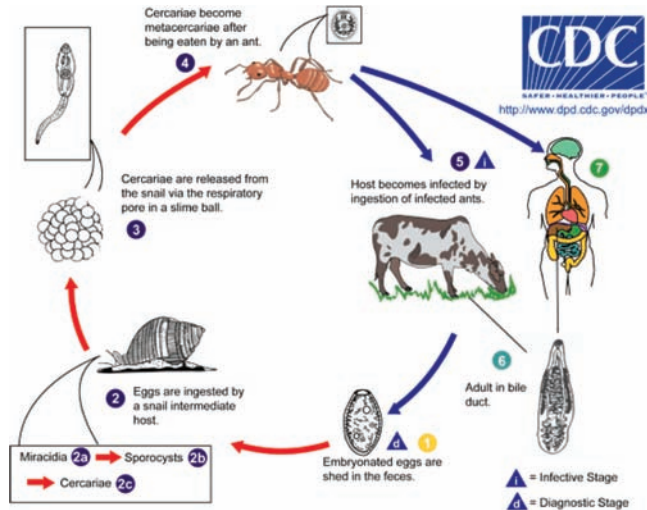
Figure 4.2.1.3.1. Life cycle of *Dicrocoelium denditricum* (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/html/ImageLibrary/Dicrocoeliasis_il.htm)

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Chapter 4.2.1.4

Echinostomiasis (Intestinal Fluke Infection)

[*ECHINOSTOMATIDAE FAMILY:* E.G. *ECHINOSTOMA HORTENSE, E. REVOLATUM, E. LINDOENSE, E. CINETORCHIS, ECHINOCHASMUS PERFOLIATUS, HYPODERAEUM CONOIDEUM, EUPARYPHIUM MELIS*]

The Echinostomiatidae family are parasitic flukes infecting humans, birds (mainly waterfowl), rats, dogs, cats and pigs. Like other trematodes, they have an intermediary host (snails, mussels and possibly freshwater fishes) with definitive hosts (humans, birds and other mammals). In humans, it is usually seen as a rare intestinal parasite of little clinical importance except in heavy infections. Humans are infected through consumption of contaminated undercooked meat, freshwater snails and mussels. Echinostomiasis is endemic in Far East and Southeast Asia; however, due to the ecological shift in the snail population composition it lost its zoonotic importance (nonetheless in certain areas its prevalence can range from 1% in Indonesia to up to 44% in the Philippines). It seems that echinostomiasis is underreported, being most prevalent in remote rural areas with malnutrition, poverty and poor sanitation. The life cycle starts from excretion of unembryonated eggs excreted by final hosts (e.g., humans or waterfowl) into feces that contaminate water sources. In water, unembryonated eggs embryonate and release developed miracidia that in turn infect snails (as an intermediate host). Hooked on snail tissue, the miracidia will develop into a final cercariae (via sporocytes and rediae stages) that can freely swim and infect other water snail (a second intermediary host). When these second intermediary hosts are consumed raw by humans or waterfowl, the metacercariae formed in this second intermediary host will excyst in the gastrointestinal system to form adult flukes colonizing the small intestine (in humans) (Figure 4.2.1.4.1). Clinical manifestation are mostly imperceptible however, heavy infestation with this fluke may cause diarrhea, anorexia, flatulence, abdominal pain and even ulcerative lesions (Miyamoto *et al.*, 1984; Chai *et al.*, 1994).

4.2.1.4.1 ECHINOSTOMIASIS AND ENVIRONMENT

In endemic areas, echinostomiasis express two separate life cycles: human and sylvatic. According to its intermediary hosts (primary and secondary), the parasite can be found in both fresh and brackish water

sources. Consistent with the ecological aspects it should be mentioned here that due to their extremely broad specificity for secondary intermediate hosts, these flukes do not need humans to complete their life cycle (Chung *et al.*, 2001) (Figure 4.2.1.4.1). As mentioned earlier, with other flukes, echinostomiasis can also spread through fishpond fertilization with "night soil" (human excreta collected from latrines). The disease is most prevalent in remote rural areas in South-East Asia at low socioeconomic status, growing free-food markets with poor sanitation and lack of food inspection (Graczyk and Fried, 1998). Some encouraging reports on possible prevention routes of this parasite have been demonstrated by the introduction of fish which prey on the larval stages of the essential molluscan hosts (Carney, 1999).

Davis (2005) showed in his study that eggs of *E. revolatum*, a human pathogen, recovered from Canada geese and stored at 4°C for up to 72 weeks hatched successfully following incubation at optimum temperature (25–30°C). Experimentally, snails have been also successfully infected at a temperature range from 10 to 25°C, demonstrating that an environmental water temperature of 10°C could support infections in nature. An interesting question that should be asked in relation to almost all flukes is: What is the impact of different anthropogenic chemical pollutants on the interaction between flukes and their hosts (mainly primary and secondary ones)? Recently Rohr *et al.* (2008) studied the net effect of common pesticides (atrazine, glyphosate, carbaryl and malathion) on *Echinostoma trivolvis* (albeit not a human parasite) in relation to its first and second hosts (the snail *Planorbella trivolvis* and the green frog tadpoles *Rana clamitans* respectively). In general pesticides caused significant greater mortality of *E. trivolvis* cercariae, with atrazine having a significant effect but at much more elevated concentrations than commonly found in aquatic environments ($\geq 200\,\mu g/L$). None of the pesticides had significantly enhanced *E. trivolvis* virulence, decreased tadpole survival, or reduced snail survival, growth, or fecundity. According to these authors, their results suggested that the net effect of exposure to environmentally realistic levels of pesticides could be elevated infections of amphibians with this trematode type. Implementation of additional studies are necessary with human *Echinostoma* species in order to understand the impact of anthropogenic pollution.
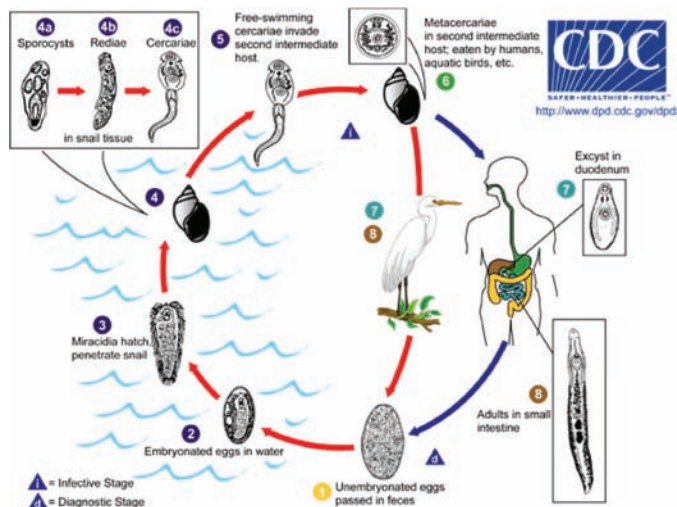
Figure 4.2.1.4.1. Echinostomidae family life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/html/ImageLibrary/Echinostomiasis_il.htm)

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Chapter 4.2.1.5

Fasciolasis

[*FASCIOLA HEPATICA* AND *FASCIOLA GIGANTICA*]

In humans, fasciolasis is caused by hepatic infection with two flukes: *Fasciola hepatica* and *Fasciola gigantica.* Human infection occurs mainly through ingestion of plants (e.g., raw cress) or vegetables harboring active metacercariae, but a water route is also possible (Pozio, 2008; Blaise and Raccurt, 2007). While human infections with *Fasciola hepatica* are common, infection with *Fasciola gigantica* are scarce and found only in certain geographic regions (Mas-Coma *et al.*, 2009). Beside humans, *Fasciola hepatica* infects other final host such as cattle, sheep, water buffaloes, lagomorphs, rodents and pigs, causing major economic damages in endemic areas (Pritchard *et al.*, 2005; Rinaldi *et al.*, 2009; Menard *et al.*, 2000). The fluke is present in moist areas (increased rainfall), lakes, canals, pools, secondary of tertiary irrigation canals and ditches obstructed by plants and much less in rice fields, all favoring the growth of the intermediate hosts: primarily snails of the genus *Lymnea* but also others genera (in Egypt a planorbid species named *Biomphalaria alexandrina*) (Wibaux-Charlois *et al.*, 1982). *Fasciola gigantica* is found in tropical areas of Asia (mainly Southeast Asia), Africa, countries in the Pacific area and in secluded areas of Near East and southern republics of former USSR with a similar host spectrum as *F. hepatica*. The life cycle includes excretion of fluke eggs (containing a zygote and nutrition cells) from humans or other mammals through excreta, that contaminate water sources. In water a miracidium hatched from the egg will swim to find a molluscan intermediate host (e.g., *Lymnea trunculata* snail). While inside the intermediate host, the miracidium develops into sporocyst and further into redia forms. The cercarial form leaves the snail and in the water phase transforms into metacercariae (encysted cercariae) that adhere to aquatic plants where they can survive for several months in humid conditions. Consumption of these plants by animals and humans introduce metacercariae into the gastrointestinal system of these final hosts. Metacercariae develop into adult flukes through hatching into the duodenum that crosses the intestinal wall into the peritoneal cavity and finally locate themselves in the liver. After 6 to 8 weeks a fluke will reach its final destination the bile ducts (Figure 4.2.1.5.1). Clinical manifestations of fascioliasis are fever, fatigue, anemia, anorexia, weight loss, pruritus, pain and icteric episodes. It should be mentioned, that in long term of liver damage by fasciolasis the anaerobe *Clostridium novy* (type B) spores can be activated (under anoxic conditions) leading to the so called

"black disease" (an immune-mediated haemolytic anaemia-IMHA) expressed as haemoglobinuria. Some recent commentaries have been published, possibly linking cholangiocarcinoma (hepatobiliary carcinoma) in Thailand to chronic exposure to *F. hepatica* infection, nitrosamine and fish contaminated with nitrosamine as potentially underlying factors leading to this carcinogenesis (Wiwanitkit, 2009).
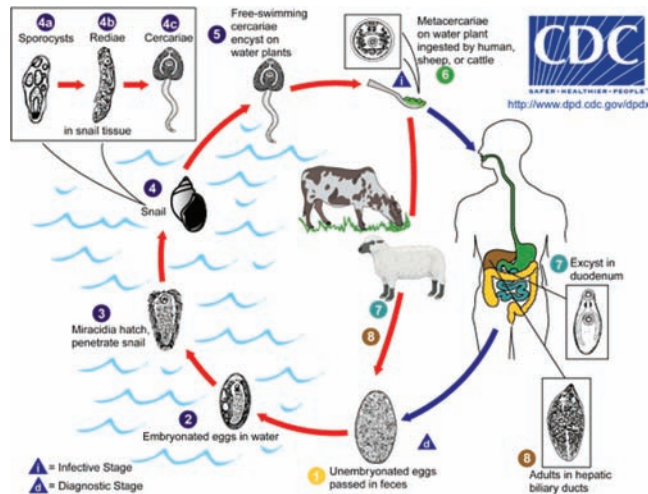
Figure 4.2.1.5.1. *Fasciola hepatica* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/html/ImageLibrary/Fascioliasis_il.htm)

4.2.1.5.1 FASCIOLASIS AND ENVIRONMENT

The reemergence of fasciolasis around the globe since the mid-1990s, revealed the successful expansion of this vector-borne parasite. If Europe was the original geographic area, now fasciolasis is spread over five continents despite some biological restrictions (intermediate host presence and prevalence). Environmental and human activities such as global transport of domestic animals, agriculture including irrigation and pastures, pollution, parasite genetic and newly discovered intermediary hosts are important factors in the expansion of fasciolasis (Mas-Coma, 2005; Relf *et al.*, 2009; Mas-Coma *et al.*, 2005; Walker *et al.*, 2011; Canete *et al.*, 2004; Bennema *et al.*, 2011). Although not in the scope of this book, it should be mentioned that only 62.5% of the *F. hepatica* eggs subjected for 90 days to activated sludge processes were damaged, revealing high environmental resistance (Guenthoer, 1971).

Walker *et al.* (2008) studied *F. hepatica* and *F. gigantica* in East Africa (Kitulo Plateau of the Southern Highlands, Tanzania) and sustainable conditions for lymnaeid species joint existence (*L. truncatula* and *L. natalensis*) and possible hybrids creation. At high altitude, molecular methods confirmed the match of *L. trancatula* and *F. hepatica* while at lower altitudes *L. natalensis* and *F. gigantica* was the preferred match with no hybrid fasciolids in either region.

Bovine fasciolasis prevalence in Switzerland ranges from 8.4 to 21.4%. Rapsch *et al.* (2008) created an interactive map based on different environmental factors in order to chart the relative risk of bovine *F. hepatica* transmission. These factors were temperature, rainfall (moisture), soil and forest, all connected to the snail *Lymnaea truncatula* (the intermediary host) life cycle. In Switzerland, snail

susceptibility to *F. hepatica* infection is closely linked to: 1) temperature- snails can survive and rein a temperature range from 10 to 25°C; 2) moisture- a rainfall in the range of ~90 to 210 mm/month is optimal while higher values probably can wash parasite larvae and snails, consequently decreasing the potential contact between these two organisms; 3) soil- clay soils are more water-retentive than sandy soils and facilitate the creation of small water courses (pools, drainage ditches, puddles, etc.); 4) forest-shady areas do not allow much solar radiation penetration into water pools, and therefore snails that feed on algal growth are inhibited. Fuentes *et al.* (1999) pointed to similar climatic and environmental factors involved in human *F. hepatica* prevalence in Bolivia (northern Bolivian Altiplano, a highly endemic area). *Lymnaea truncatula* snails occur in this region at altitudes of 3800–4100 m, exposed to various conditions: large variations in daily temperature, long dry and wet seasons (with high evapotranspiration) and intense solar radiation (due to altitude and lack of shade). Consistent with these conditions, the optimal transmission time is between December and March (summer in southern hemisphere). In this particular case, involving high altitude regions, several studies emphasized the longer shedding period and a higher production of cercariae of the Altipano lymnaeid snails than of the European ones, and hence a better environmental strategic adaptation (Mas-Coma *et al.*, 2001; Vignoles *et al.*, 2002). In another South American area (Andean Patagonian valleys of Argentina), Kleiman *et al.* (2007) studied the prevalence, egg output and the dynamics of bovine fasciolasis. In this case, the specific intermediate host snails (*Lymnea viatrix*) that is able to live for ~15 months, have been isolated mostly from standing and temporary water sources (e.g. lagoons) and rarely from streams. *L. viatrix* snails carried a relatively low burden of infection with bovine fasciola (overall 0.67%) primarily in summer and autumn when infection transmission to heifers was highest (heifers-80% and cows-60%) with the exception of suckling calves (15%), suggesting highly effective transmission. Besides high summer rainfall and increased prevalence of *L. trunculata*, emergence of bovine fasciolasis in East England has been also attributed to sheep influx from endemic areas for seasonal grazing into nonendemic ones (Pritchard *et al.*, 2005).

In South Africa, de Kock *et al.* (2003) studied the geographic distribution and habitats of *Lymnaea truncatula*. As environmental habitats, swamps seem to be the most populated (42% recovery), with most samples obtained from slow-flowing water (45.8%), muddy substratum (62.5%), mean annual temperature and rainfall of 10–20°C (86.3%) and 600–900 mm (69%) respectivelly. The authors concluded that in this geographic area temperature and water bodies' type plays a major role in the occurrence of this snail. In relation to temperature, Thomas *et al.* (2007) described a heat wave in 2003 that affected bovine helminthosis (including *F. hepatica*) in Southern France through lower prevalence of parasite eggs in feces followed by a quick increase in the following year. The authors attributed this phenomenon to several factors: helminths' adaptation, spread of new intermediate hosts and movement of hosts and reservoirs. When dealing with *F. hepatica* it should be remembered that an intermediate host is involved in the life cycle of this fluke, and thus a heat wave can strongly affect the wet and moist habitats of snails, cutting short the spread of this fluke.

Overall climate changes have been shown to influence trematodiases akin to the Ecuadorial experience, where human fasciolasis incidences increased significantly for the 2 years following the occurrence of El Niño Southern Oscillation (Fuentes, 2007). Rainfall and temperature are central climatic factors that affect fluke free-living stages, vegetation occurrence and population dynamics of hosts (snails and mammals) (Mas-Coma *et al.*, 2007).

Environmental pollution with a large variety of anthropogenic chemicals undoubtedly impacts the life of different parasites and their hosts in different directions, yet to be identified. Krupicer *et al.* (1996) studied the effect of sub-chronic heavy metal (Hg, Pb, Cu, Zn, Cr and Cd) intoxication in lambs also infected with ovine' fasciolasis. In intoxicated animals, the authors reported exceeding mercury and partially exceeding copper values in parenchymatous organs and muscles. In addition, the mean intensity of *F. hepatica*

infection also strongly increased, suggesting the negative effect of metal emission on infected animals in that they impair their immune system. Sures *et al.* (1998) looked for cadmium and lead concentrations in *F. hepatica* infecting cattle and found a lead concentration 172, 53 and 115 times higher than in the surrounding organ (muscle, kidney and liver, respectively).

Soliman (2009) looked for pre- and post-exposure of *Lymnea natalensis* snail (the intermediate host of *F. gigantic*a) to cadmium at different concentrations (0.1 to 100 µg/L) and studied the infection rates with *F. giagantica*, cercariae shedding and pattern. Cadmium pre-exposure decreased infection rates (temperature dependent) while post-exposure increased cercariae shedding (temperature independent) altering also the cercarial output pattern (higher proportion of floating and dead cercariae that might reduce transmission). The author attributed cadmium effect on both mollusk and fluke to the trematode's location within the mollusks; digestive gland, which is also the main organ for metal accumulation. The effect of temperature on cadmium could be explained based on a study performed with other aquatic mollusc (the oyster *Crassostrea virginica*) and one of its mitochondrial functions such as oxidative capacity (Cherkasov *et al.*, 2006). These authors reported that oysters exposed to cadmium at 12°C revealed elevated intrinsic rates of mitochondrial oxidation while at 28°C a pronounced decline was observed including mitochondrial membrane potential, with increased sensitivity of this oyster to extramitochondrial cadmium. Consequently, cadmium toxicity at elevated temperatures may occur simultaneously in both organisms (snail and fluke) resulting in a more complex reaction.

Igbinosa and Okafor (1988) studied the effects of two commonly used chemicals, Gramoxone (an herbicide) and Hexadrin (an insecticide), on *F. gigantica*'s miracidia. Eggs of *F. gigantica* were 99% inactivated at 4 to 5.5 ppm (twice of LC_{50}), for both pesticides, with enhanced synergism between the two pesticides (Okafor and Igbinosa, 1988). Additionally, sublethal concentrations of these chemicals prolonged the embryonic process and inhibited miriacidia hatching.

Lastly, Kiziewicz (2006) studied different water fungi and fungus-like organisms (known as decomposers and facultative parasites) able to grow on *F. hepatica* eggs. Seventeen species of fungi and straminipiles (a kingdom comprising diatoms, brown algae, chrysophites and some protozoa) have been found to be able to colonize this fluke's eggs, where most of colonizing fungi are known also as animal parasites or necrotrophs.

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Chapter 4.2.1.6

Fasciolopsiasis

[*FASCIOLOPSIS BUSKI*]

Fasciolopsis buski is a large fluke with a life cycle similar to that of his relatives *F. hepatica* and *F. gigantica*, but differing in the final animal host (instead of ruminants such as cattle and sheep it infects pigs) and final colonization site in animals and humans (duodenum instead of hepatobiliary system). The distribution of this fluke is in Southern and Eastern Asia (China, Taiwan, India, Bangladesh, Indonesia, Thailand and Vietnam). Transmission is very similar to fasciolasis, i.e., adherence of metacercariae to water plants and their fruits *Trapa natans*, *Trapa bicornis* and *Elocharis tuberose*, so called water nuts and used as food in China and other Asian countries. Cultivation ponds for water nuts are commonly fertilized with manure and water nuts are also used to feed pigs. Miracidia hatch from eggs in moist environment to further penetrate water snails (e.g., *Planorbis* and *Segmentia* genera) in order to multiply asexually. Developed cercariae from the intermediary host are released and than encyst, while adhering to water plants, to form metacercariae. Metacercariae are rapidly inactivated when exposed to desiccation, and therefore the disease spreads when fresh fruits are consumed. Swallowed metacercariae reach the gastrointestinal track and develop into young flukes in humans or pigs duodenum while attached to mucosa. Adult flukes will produce unembryonated eggs excreted in feces and further disseminated into water (Figure 4.2.1.6.1). The disease manifests itself as duodenal inflammatory and ulcerative lesions, epi- and hypogastric pain, altering diarrhea and constipation with whitish feces. In severe cases, anorexia, anemia, edema, cachexia and ascites may develop.

4.2.1.6.1 FASCIOLOPSIASIS AND ENVIRONMENT

Like its other relatives, *F. buski* is linked to freshwater habitats (stagnant or slow moving in order to allow good contact between cercariae and snails) and aquatic agriculture contaminated with feces (Mas-Coma *et al.*, 2005). Consumption of raw aquatic plants by humans and pigs and drinking from these water sources, as well handling of water plants and their fresh fruits are the main epidemiological factors involved in the endemic persistence of fasciolopsiasis. Increased prevalence has been observed among people living close to water caltrop plantations, where in order to keep plants fresh the grower dips water

caltrops in already contaminated water (Hsieh, 1960; Mas-Coma *et al.*, 2005). An important factor that emerges all through the present book, related to the majority of the described pathogens and not solely to *F. buski*, is the low social and economic level of infected people. Poverty, malnutrition, poor sanitation and uncontrolled food market, all fit in the definition of a low socio-economic population (Anonymous, 1995). Quang *et al.* (2008) studied the prevalence of *Fasciola* spp. and *Fasciolopsis buski* in southern Laos. Prevalence of human fasciolopsiasis in Laotian villages because of *F. buski* infection was 33.7%. In pigs, the main reservoir, grown with contaminated fresh aquatic fodder and water various infection prevalences have been reported from different countries: India (30%), China (10%) and Taiwan (52%) (Mas-Coma et al, 2005). Seasonal variations had also affected prevalence reports (Roy and Tandon, 1992). For example, in India, the prevalence of *F. buski* -induced infection in cattle increased from June to September followed by a decline to low levels from November to March, corresponding to human cases (highest in late summer and early autumn).
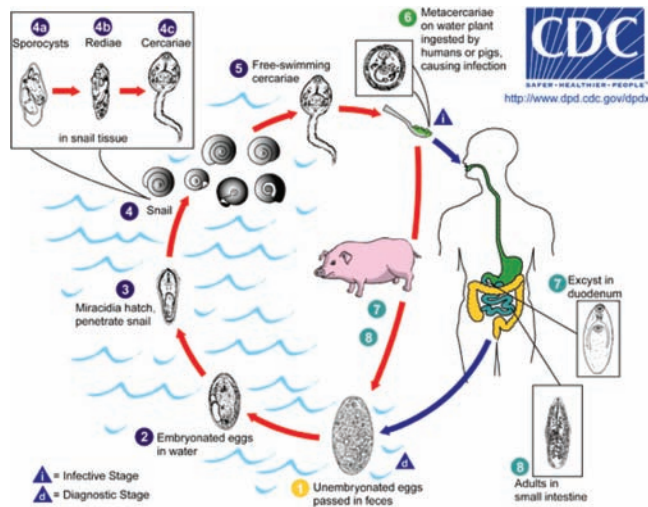
Figure 4.2.1.6.1. *Fasciolopsis buski* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/html/ImageLibrary/Fasciolopsiasis_il.htm)

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Chapter 4.2.1.7

Heterophyiasis

[*HETEROPHYDAE FAMILY*]

This group of small size flukes comprises ~30 species of the Heterophyidae family (e.g. *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Haplorchis* spp. and *Procerovum* spp.) distributed worldwide in brackish and fresh water (Elsheikha and Elshazly, 2008). Human infections occur in tropical areas such as Southeast and East Asia but also in Northern Africa (Egypt), the Middle East and Europe (Duong *et al.*, 1979; Taraschewski and Nicolaidou, 1987; Bastien *et al.*, 1995; Guk *et al.*, 2006). At high risk are fishermen and other people that consume raw fish and live near faecally contaminated sources, such as lakes of brackish and fresh water, river banks, bay waters and estuaries (Adams *et al.*, 1986; Elshazly *et al.*, 2007). Human prevalence in endemic areas ranges from <10 to >50% (Belizario *et al.*, 2001). Foreign residents and tourists in endemic areas are also at high risk (Hamada *et al.*, 1998). Flukes from the Heterophyidae family have a similar life cycle to that of *Clonorchis sinensis* that has two intermediate hosts: snails and fishes. Final hosts are fish-eating mammals, waterfowls and humans (Schuster *et al.*, 2009; Taraschewski, 1985b). Briefly, final hosts excrete embryonated eggs that are introduced into water sources and there are ingested by snails. Miracidia hatch from ingested eggs into snail intestine to form cercariae to be later released into the surrounding water. Released cercariae penetrate a second intermediary host (freshwater fish from salmonids and cyprinids families) to develop into metacercariae inside fish tissue. Final hosts, such as as cats, dogs, waterfowl and humans that consume raw fish infected tissue, become infected with metacercariae that will excyst into their small intestine to form mature flukes (Figure 4.2.1.7.1). Clinical manifestations are mostly very mild with intestinal symptoms such as diarrhea, abdominal pain and very rare heart (causing infarcts) and CNS conditions when hematogenous eggs spread to other organs (Bastien *et al.*, 1995).

4.2.1.7.1 HETEROPHYIASIS AND ENVIRONMENT

Elsheikha and Elshazly (2008) studied the seasonality of heterophyid flukes' infection of brackish water fish in Egypt. All heterophyids revealed seasonality (38.2% in summer, >26.6% in spring, >19.3% in autumn >and 8.7% in winter) and also dependence on host type. These results were explained based on various

factors, such as host type, climate and biotic conditions. Flukes' seasonality correlated well with the fish breeding season (summer and spring) and the release of cercariae from snails, which is highly temperature-dependent. A significantly higher prevalence of heterophyid encysted metacercariae in mullet (*Mugil* spp.) than in "St. Peter's fish" (*Tilapia* spp.) indicated species preference and susceptibility to this trematode. Environmental fecal contamination in this area has been reported to be very high during rainy season and coincided with the rapid snail population raise. The rapid development of intensive aquaculture in Egypt (Lake Manzala) for the last three decades, in order to supply fish meat as an important protein source, raises serious health risk problems related to this pathogen in spite of its mild symptoms. Lobna *et al.* (2010) described an interesting association related to *Tilapia* spp. infection by heterophyids encysted metacercariae in Northern Egypt: infection prevalence declined as fish size increased. According to these authors there are two potential explanations for this phenomenon: a) the fish stunted growth by infecting parasite that increases the prevalence of small-sized fish or b) the small-sized fish have a thinner skin that is much more easily penetrated by cercariae (Paperna, 1980).
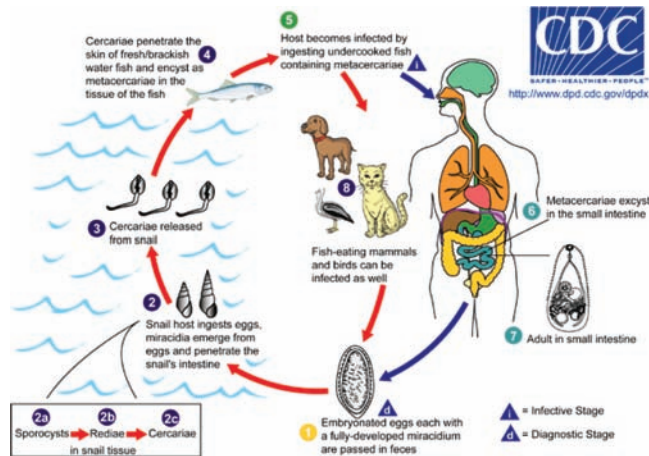
Figure 4.2.1.7.1. Heterophydae family life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/html/ImageLibrary/Heterophyiasis_il.htm)

In contrast to Lake Manzala (Egypt), Taraschewski (1985a) reported that in comparison with 1950s abundance of this fluke, a decline in *Heterophyes heterophyes* the most prevalent heterophyid in the Nile Delta, was observed. It is very possible that the following factors were involved in this regression: intermediate hosts shift (both snail and fish) as a result of pollution and intensive drainage for intensive farming activity.

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Chapter 4.2.1.8
Optisthorchiasis

[*OPISTHORCHIS FELINEUS, OPISTHORCHIS VIVERRINI*]

Opisthorchis felineus is a cat liver fluke able to infect additional mammals (including humans). The distribution of *O. felineus* is Eastern and Southern Europe, Asiatic regions of former USSR (including Siberia, along big rivers) while *O. viverrini* is distributed in Southeast and East Asia (Laos, Thailand, Vietnam and Cambodia). In endemic areas, 80% of the population is infected with these flukes; for example, it is estimated that 1.5 million people in Russia (mainly inhabitants of Siberia) are infected via consumption of raw, slightly salted and frozen fish. Transmission occurs through ingestion of raw freshwater fish infected with metacercariae (mainly cyprinids that are used as intermediary hosts). Briefly, the life cycle route is through fecally excreted eggs from infected hosts into water sources. In water, eggs are ingested by water snails (genera *Codiella* for *O. felineus* and *Bythinia* for *O. viverrini*) to develop internally into cercariae and once more released in water. The next encounter of cercariae is with the second intermediate hosts (freshwater fishes). In fish, encystation takes place to form metacercariae ingested by a final host feeding on raw fish meat. In cats, humans and other mammals the metacercariae develop into young flukes in the duodenum, followed by migration to small bile ducts (Figure 4.2.1.8.1). Infected humans without treatment can carry this fluke for >10 years. Clinical symptoms are cholangitis, cirrhosis, pancreatitis and in hyperendemic areas (e.g., Thailand). *O. viverrini* is highly suspected of being involved in human cholangiocarcinoma.

4.2.1.8.1 OPISTHORCHIASIS AND ENVIRONMENT

In an interesting study on *O. viverrini's* comprehensive genetic characterization from different geographical localities in Thailand and Laos, Saijuntha *et al.* (2007) found a possible co-evolution between *O.viverrini* species and their first intermediate hosts (snails). They detected significant genetic variations among *O. viverrini* from different wetlands and among *B.s. goniomphlos* (a snail species) from different geographical areas, raising the important question about the relative importance of the biotic and abiotic features of different wetlands on the ecology and genetic variation of trematodes and their intermediate

hosts. In this context, human migration and extensive floods seem to be highly important factors in the co-evolution of these organisms and the dissemination of closely related species.
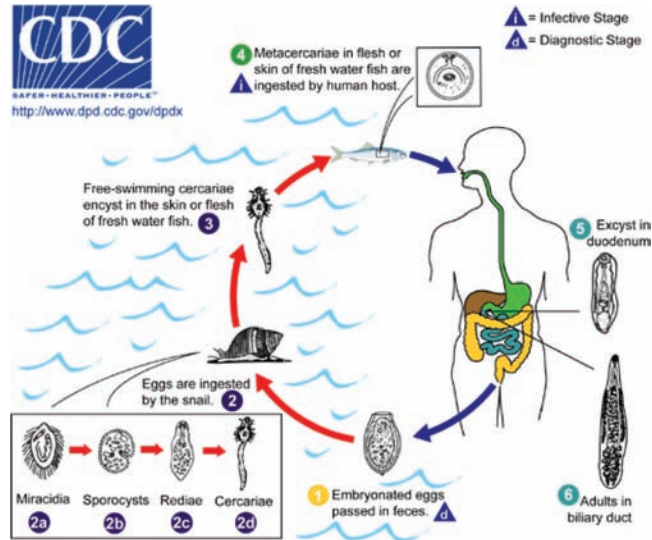
Figure 4.2.1.8.1. *Opistorchis felineus* and *viverrini* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/DPDx/html/ImageLibrary/Opisthorchiasis_il.htm)

Among mammals infected by *O. viverrini*, next to humans cats are the most prevalent (Enes *et al.*, 2010). In northeastern Thailand, the reported infection prevalence in dogs with *O. viverrini* was 3.8% while in cats was 36.4%. As cholangiocarcinoma is possible the most serious complication of infections with *O. viverrini*, studies carried out in this specific region of Thailand, found a positive correlation between the endemicity of opisthorchiasis and the frequency of cholangiocarcinoma (Watanapa and Watanapa, 2002; Abdel-Rahim, 2001). For example in Sakol Nakhon (upper Northeast Thailand) owing the highest national mortality rate due to liver and bile duct cancer, a mortality rate of 61.4 per 100,000 people had been reported (Sripa and Pairojkul, 2008).

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Chapter 4.2.1.9

Paragonimiasis (Pulmonary distomatisis)

[GENUS *PARAGONIMUS, PARAGONIMUS WESTERMANI*]

Lung flukes of the genus *Paragonimu*s that comprises 30 species infecting different animals (cats, dogs, pigs, and mustelidae family) cause Paragonimiasis while in humans as much; >10 species have been proved to be pathogenic. The most notorious species of Paragonimus is *Paragonimus westermani*. Other *Paragonimus* flukes are presented in Table 4.2.1.9.1. In humans infection results from ingestion of raw or insufficiently cooked crayfish or fresh-water crab' meat that are infected with the metacercarial stage, but infected uncooked pork meat may also lead to infection. The life cycle includes expectorated eggs from infected animal lungs that are again swallowed and reach the feces through digestive system. Eggs from feces are released in water and transform in miracidia that hatch and penetrate the intermediate host: snails (e.g., *Melania* spp., *Ampullaria* spp., etc.). Following asexual reproduction in snails, the developed cercariae leave snails to enter a second intermediary host, crustaceans. Crustaceans consumed as raw, uncooked meat initiates infection of final hosts with metacercariae that will develop further into young flukes that migrate from duodenum into lung tissue (Figure 4.2.1.9.1). As a cosequence of pulmonary colonization (parenchyma) ectopic invasion of other organs may occur (liver, CNS, etc.). In humans, infections may persist for 20 years. Clinical manifestations of paragonimiasis are: inflammation, chest pain, cough with brown sputum, asthma, fever, and if the brain is colonized: cramps, headaches, dizziness, vomiting and fever.

Table 4.2.1.9.1 *Paragonimus* spp. geographic distribution and their intermediate and final hosts.

Species	Main geographic distribution	Intermediate hosts	Final hosts
Paragonimus westermani	Central, Southeast and East Asia, South America	Snails (*Semisulcospira* spp., *Paludomus* genus, etc.), fresh water crabs	Animals such as pigs, dogs, and a variety of feline species and humans

(Continued)

Table 4.2.1.9.1 (*Continued*)

Species	Main geographic distribution	Intermediate hosts	Final hosts
P. ohirai	Central, Southeast and East Asia	Snails (*Oncomelania* sp.), fresh and brackish water crabs	Cats, dogs, rats and humans
P. skrjabini	China (mountain regions)	Snail (*Tricula cristella*), freshwater crab host	Cats, dogs and humans
P. miyazakii	Japan	Snails, fresh water crabs (*Geothelphusa dehaani*)	Cats, dogs and humans
P. heterotremus	Southern China, Thailand, Laos	Snails (*Tricula wumingensis, Tricula aperta*), crabs (*Samanniathelphusa nanningensis, Tiwaripotamon beusekomae*)	Dogs, cats and humans
P. kellicotti	North America	Snails and crustaceans (crabs or crayfishs)	Bobcats, dogs, gray and red foxes, raccoons, mink, skunk, coyote and humans
P. mexicana	Central and South America	Snails, fresh water crabs	Cats, dogs and humans
P. africanus	Tropical Africa	Snails, fresh water crabs (*Sudanonautes africanus*, land crab *S. aubryi* and *S. granulates*)	Cats, dogs and humans
P. uterobilateralis	Tropical Africa	Snails, fresh water crab (*Sudanonautes africanus* and the land crab *S. aubryi*)	Cats, dogs and humans
P. heterotremus	India	Snails, freshwater crab	Cats, dogs and humans

4.2.1.9.1 PARAGONIMIASIS AND ENVIRONMENT

A Chinese researcher investigated *P. westermani* infection in intermediate hosts (snails, crabs) and animal reservoirs (civet, wild and domestic cats, dogs, etc.) in forest farms and town. Infection in snail species *Semisulcospira libertina* and *Sinopotamon* spp. ranged from 0.21% to 54.3% respectively, and 5.6% in reservoir hosts. The authors attributed the reduction in infection rates in comparison to data obtained 20 years ago to environmental changes (Yan *et al.*, 2004). Another *Paragonimus* species, *Paragonimus skrjabini*, has been studied in an endemic region in China (Xu *et al.*, 2008). Intermediate hosts such as freshwater crabs (*Sinopotamon denticulatum*) and definitive hosts (cats and dogs) were found to be infected with metacercariae at the following rates: 20.5% and 25%, respectively. The high infection rate was attributed to animals foraging and defecating close to water routes and the children's habits of consuming raw under-cooked crabs. In Western Africa, human paragonimiasis is present with a prevalence ranging from 2 to 31% (Pozio, 1991). Macpherson (2005) emphasized the increasing proclivity for eating meat, fish, crabs, shrimp, molluscs raw, undercooked, smoked, pickled or dried that facilitates human infections with flukes, beside other environmental alterations.
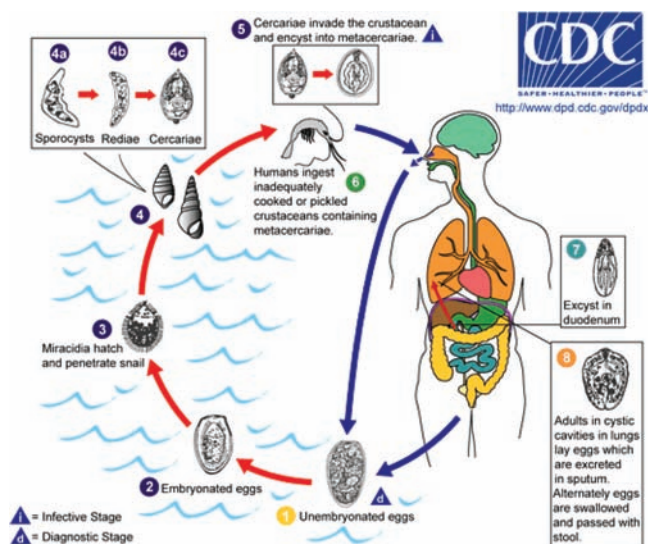
Figure 4.2.1.9.1. *Paragonimus* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc. gov/DPDx/html/ImageLibrary/Paragonimiasis_il.htm)

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Chapter 4.2.1.10

Schistosomiasis (Bilharziosis)

[*SCHISTOSOMA MANSONI, S. HAEMATOBIUM, S. JAPONICUM, S. MEKONGI, S. MATTHEEI, S. INTERCALATUM, S. BOVIS* **AND TO LESSER EXTENT** *S. MAGREBOWIEI, S. RODHAINI*]

Schistosomiasis is one of the most significant trematodic diseases in humans in the tropical and subtropical areas of the globe, parasitizing intestinal tract, liver and urogenital tract. For example, *Schistosoma mansoni* occurs in Egypt, East, Central and West Africa, Middle East, Northern and Western South America and Caribbean Island; *S. japonicum* is limited to Southeast and East Asia; *S. mekongi* in Cambodia and Vietnam; *S. haematobium* in Middle East, India, Portugal and Africa; *S. mattheei* in Central Africa; *S. intercalatum* and *S. bovis* in Africa; *S. margrebowiei* in Southern and Central Africa and *S. rodhaini* in parts of Central Africa. Additionally, *Schistosoma* genus comprises atypical trematodes with two sexes (dioecious) versus most other trematodes that are hermaphroditic. Humans are the main host of *S. mansoni*, *S haematobium*, *S. japonicum* while other schistosoma infect mainly animals including humans. Infections are percutaneously by free-swimming cercariae. The life cycle of this parasite includes fecal or urine eggs excretion from the final infected host into water sources. Miracidia hatch from eggs to invade water snails as an intermediate host in shallow aquatic areas. Each *Schistosoma* spp. has a specific intermediary snail (e.g. *S. mansoni* invades *Biomphalaria* spp.; *S. haematobium* invades *Bulinus* spp. and *S. japonicum* invades *Oncomelania* spp.). The snail developed cercariae actively exit into water milieu where they can reside for ∼2 days in order to meet their final host, close to the water's surface. Cercarie in close encounter with their final host, rapidly penetrate loosing the tail so as to transform into a schistosomula form. Inside the lungs, after blood circulation transport, the schistosomula will develop in mature trematodes (male and female). Both male and female migrate to their final destination organ (mesenterial veins-*S. mansoni* and *S. japonicum* and urinary bladder veins-*S. haematobium*). In infected persons, *Schistosoma* sexual reproduction produces between hundreds to thousands of eggs/pair/day that can persist for 30 years, if untreated (Figure 4.2.1.10.1). Clinical manifestations are linked to the invaded organ; in bladder, the following symptoms are seen: pruritius, fever, headache, general pain, vertigo, vomiting and hematuria; in intestinal tract: fever, headache, hepatosplenomegaly, lymphadenopathy, colicky pain, alternating bloody diarrhea or constipation,

cirrhosis and encephalopathy. Increased urinary bladder cancer rate has been linked to *S. haematobium* infections (Mara and Clapham, 1997).
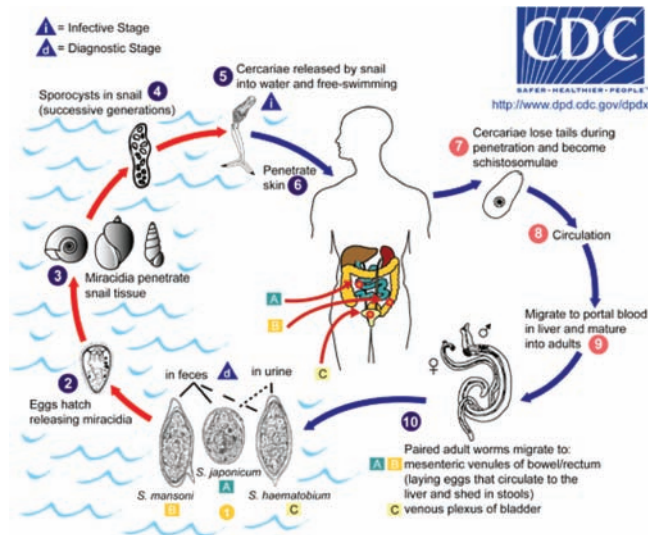
Figure 4.2.1.10.1. *Schistosoma* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/DPDx/html/Schistosomiasis.htm)

4.2.1.10.1 SCHISTOSOMIASIS AND ENVIRONMENT

Resembling other trematodes that rely on one or two intermediary hosts, *Schistosoma* species also depend on their specific hosts in order to accomplish their life cycle. In turn, the intermediary hosts might be influenced by modulation of environmental factors: water quality and flow dynamics, geography, human population, vegetation, climate, agricultural practices and irrigation, etc. (Klumpp and Chu, 1977; Webster *et al.*, 2001; Ernould *et al.*, 1999; Saliba *et al.*, 1997). In a study carried out at Kigungu canoe landing and recreational sites on Lake Victoria (Uganda), the researchers found that, in spite of bush clearing (papyrus swamps that were the major habitats for snails species such as *B. choanomphala*, *B. pfeifferi* and *Bulinus* spp., the intermediate host) snails did prevail and their population density dynamic and infection rate were inversely proportional to rainfall outline. An explanation of these results can be deduced from other trematodes' behavior in which snails do survive in smaller water patches increasing the contact likelihood with miracidia, hence the infection rates. In the same way, it has been shown that rapid water flow as well high rainfall do dilute the aquatic stage of these parasites, reducing contact odds and the consequent infection (Odongo-Aginya *et al.*, 2008). Also in Uganda, Rubaihayo *et al.* (2008) reported on a *Schistosoma mansoni* infection prevalence of 27.8% in schoolchildren living around 13 high altitude crater lakes (range of 1487 to1682 m) in Western Uganda. Using a multivariate logistic regression model these authors found that altitude and water source (crater lakes) were significantly associated with infection. The main question related to this study that needs further scientific evidence is: Does this parasite change its altidinal threshold as a result of climate change or other factors?

In a more mathematical approach, Liang *et al.* (2007) have showed that, in spite of regional and country campaigns to eradicate schistosomiasis by use of antihelminthic drugs (praziquantel) and molluscicides

(niclosamide), after a sharp decline the disease reemerged in Western China. These authors attributed the parasite's reemergence on environmental factors, in particular those related to snail habitat and human agricultural and sanitation practices at village level. While preparing this chapter, we came across many studies emphasizing poverty (low income), low education and sanitation as well inhabiting waterways as the main factors of schistomiasis endemicity, besides other environmental factors (Ugbomoiko *et al.*, 2010; Ernould *et al.*, 2004; Ollivier *et al.*, 1999; Matthys *et al.*, 2007; Pereira *et al.*, 2010; Kazibwe *et al.*, 2010).

Yang *et al.* (2005) assessed the impact of climate change and water resources development on *Oncomelania hupenis* in China (the intermediate host snail susceptible to *Schistosoma japonicum*). Two databases covering a 30 year period have been analyzed for temperature change during the month of January (the critical month for survival of *O. hupensis*). Interestingly, China's average January temperature rose by 0.96°C, consequently lifting up the northern temperature limit of *S. japonicum* transmission, increasing the potential transmission area by~41.000 km^2 and potentially exposing 20.7 million people to the risk of Schistosomiasis. In addition, the authors also mentioned that under the new isotherm, two lakes are now included, both a part of a proposed South-North water transfer project. These results have been also stoutly supported by a biology-driven model developed to assess the potential impact of rising temperature on the transmission of schistosomiasis in China (Zhou *et al.*, 2008). Alternatively, Mangal *et al.* (2008), also using a mathematical model, explored the effects of long-term mean ambient temperature increase on *Schistosoma mansoni*'s prevalence and abundance. In this case they looked at the upper temperature limits (30 to 35°C) where this parasite can still proliferate and infect humans. These authors, based on their model, found that at temperatures of up to 30°C the mean infection burden in humans increased while at 35°C it crashed, primarily due to increased mortality among the snail population (the intermediate host). Based on these studies it can be concluded that a more extended approach to the effect of temperature on parasites life-cycle stages and their intermediary hosts can provide a better evaluation of schistosomiasis risk in humans.

Watts and El Katsha (1995) pointed also at man-made environmental changes that affected the Nile Delta in Egypt. For 30 years, the population and the domestic water use increased significantly in parallel with canal water pollution, lack of suitable drainage for sewage and sanitary effluent disposal, all contributing to an increase in *S. mansoni* infection cases. el-Sayed *et al.* (1995) looked for prevalence and epidemiology of *S. mansoni* and *S. haematobium* in two areas reclaimed from the desert for agricultural use (near Ismailia, Egypt). In comparison to a previous study performed ten years previously, they reported that *S. mansoni* infection prevalence had increased from 21.7% to 42.1% among settlers, while that of *S. haematobium* had decreased from 7.8% to 1.7%. These authors suggested that increased infection prevalence was a result of irrigation practices and local environment alterations that may change the intermediate host accommodation and species competition. A similar situation was described in Brazil (Pernambuco state), where uncontrolled peripheral urbanization and environmental deprivation increased schistosomiasis cases (Barbosa *et al.*, 2010). As a result of shortage in basic infrastructures of sanitation and health, fecal contamination perpetuates *Schistosoma* spp. transmission and the emergence of newly contaminated sources (Rollemberg *et al.*, 2011).

In relation to animals as schistomiasis reservoirs, Fernandez *et al.* (2007) screened 50 villages in the Philippines for *Schistosoma japonicum* infection prevalence in different animals, as possible reservoirs of this parasite. In contrast to Chinese studies that showed cattle and water buffaloes as the primary animals infected with *S. japonicum*, in the Philippines rats and dogs showed high prevalence proportions of infection while water buffaloes showed very low. This disparity between countries might be explained by potential genotype clustering between humans and some other animal species including potential strain substructuring of *S. japonicum*, similar to clustering of human and bovine genotypes (strain

substructuring) as suggested by Wang *et al.* (2006), in China. However, in order to elucidate these assumptions, further molecular epidemiological studies are requested.

Elsheika *et al.* (2008) looked at the clinico-pathological effects of simultaneous exposure to malathion (an insecticide) and *S. mansoni* in Swiss outbred albino mice. This double exposure can occur realistically, as malathion has been regularly used in the past to combat various insects (e.g., mosquitoes) that breed closely to *Schistosom*a spp. habitat (slow and shallow waters). Indeed, their results revealed statistically that affected hepatic functions are correlated with increasing *S. mansoni* cercariae infection and malathion dose. The affected hepatic functions were: 1) liver and spleen pathologies; 2) increased serum bilirubin and decline in hemoglobin content; 3) increase in certain enzymatic activities; 4) reduction in serum albumin and proteins; 5) reduced count of white and red blood cells and 6) higher mortality rate of the exposed group. To sum up, their data indicated that malathion exposure exacerbates schistosomiasis as well its clinical and pathological outcome.

Regarding organophosphate insecticides, Ibrahim *et al.* (1992) studied the effect of chlorpyrifos (trade name Dursban) on the snail intermediate host *Biomphalaria alexandrina* and hence on its parasite *Schistosoma mansoni*. First, they reported that snails' exposure to 0.5 ppm chlorpyrifos for 20 days revealed high fatality (therefore reducing cercariae development and excretion) and secondly *S. mansoni*-infected snails exposed for different time periods to lower chlorpyrifos concentrations (0.125 and 0.25 ppm) revealed blockage of cercarial shedding. Interestingly from the evolutionary/ environmentally point of view, both insecticides (malathion and chlorpyrifos) do have much more impact on primary and intermediate hosts than on the parasite itself (mostly an indirect effect).

Being aware of the vast amounts of anthropogenic chemicals released or accidentally introduced into aquatic environment in developing countries, it is very possible that parasitic organisms are also affected directly or indirectly. For example, MacInnis *et al.* (1974) already showed that *Biomphalaria glabrata*, the intermediary host of *S. mansoni*, excretes certain amino acids that elicit chemotactic and chemokinetic responses by miracidia. How chemical contaminants impact this process is unknown and still a waits elucidation.

Cosseau *et al.* (2010) raised an interesting point concerning epigenetics (the science of heritable but modifiable information) on *Schistosoma mansoni* epigenotype modulation that increases the phenotypic variability of this parasite, allowing it to survive and spread into new landscapes without genotypic modification. These authors showed that treatment of *S. mansoni* larvae with an inhibitor (Trichostatin A) of histone deacetylase resulted in an increase in the phenotypic variability of this trematode. In view of the large number of environmental pollutants, it is not an overstatement to suggest that such scenarios are feasible in multi-components milieu such as contaminted environment.

Finally, several other aspects of schistosomiasis have been published revealing some interesting characteristics of this parasite. Stienstra *et al.* (2001) in their review on *Mycobacterium ulcerans* (also known as Buruli ulcer, a bacterium prevalent in riverine and swamp areas of tropical zones in Africa, Asia, South America, and much less in Australia-see *Mycobacterium* Chapter 2.15), mentioned that this bacterial pathogen transmission mode had not been fully elucidated, and is believed probably to occur through skin trauma penetration. These authors observed that high *Schistosoma haematobium* endemicity was found in many *M. ulcerans* foci in West Africa, with a striking increase in transmission following river dams construction. Based on this observation and other interactions between schistosomiasis and tuberculosis, they hypothesized that schistosomiasis is a risk factor for Buruli ulcer through altering the host's immune response in such a way that it debilitates the host's protection against mycobacterial infection. Further studies will be needed to confirm this interesting hypothesis through experimenting with schistosomiasis control and its impact on Buruli ulcer prevalence.

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Chapter 4.2.1.11

Other Trematodal Infections

[*EURYTREMA PANCREATICUM, GASTRODISCOIDES HOMINI, NANOPHYETES SALMINCOLA*]

Eurytrema pancreaticum is a relatively rare fluke infection in humans, hogs, monkeys but mostly in ruminants (cattle, water buffaloes, sheep, camels and goats). Its main distribution is Southeast Asia and China but also in Russian Far East (Dvoriadkin, 1976). Interestingly, in spite of the aquatic phase that includes snails, it has also an arthropod terrestrial second intermediary host (grasshopper), which may explain its lower prevalence in humans and higher in ruminants. Briefly, the *E. pancreaticum* life cycle consists of adult flukes in infected mammals that produce eggs in the pancreatic duct. Eggs are excreted with feces and if in contact with water sources, they will be ingested by snails as first intermediary host. In snails, it reproduces asexually to produce cercariae that are shed on grass (mainly near the shore of aquatic bodies). Cercariae adhere to grass stems to be further ingested by grasshoppers (the second intermediary host). Into grasshopper, cercariae will encyst and develop into metacercariae; then accidental ingestion of this arthropod by mammals consuming grass (mainly ruminants) will produce infection and development of adult flukes (in pancreatic duct) (Figure 4.2.1.11.1). Clinical manifestation is inflammation of the pancreatic duct that may lead to pancreatitis (Ishii *et al.*, 1983).

Gastrodiscoides hominis is also a rare fluke infection in humans and more frequent in pigs (final hosts). Its main distribution is India, Southeast Asia including China. Snails (Planorbids, *Heliocorbus* spp.) and water plants and fruits are the first and second intermediate hosts, respectively. Briefly, pigs and humans excreta containing eggs reach water bodies where eggs mature. Eggs hatch to release miracidia into water where they penetrate snails (the first intermediary host). Inside snails, miracidia develop into rediae that further multiply and then form cercariae. Cercariae are again released into water where they encyst and form metacercariae that adhere to aquatic plants (the second intermediary host). Final hosts, pigs, humans or other animals that ingest aquatic plants carrying metacercariae, become infected. Inside the final host, metacercariae will migrate to cecum (humans) and colon (pigs) to mature in a final fluke that will further release eggs (Figure 4.2.1.11.2). Clinical manifestations are enteritis with continuous thick diarrhea.

Nanophyetes salmonicola is a rare fluke infection of humans and more often in of animals with a broader spectrum among carnivores, such as canids, felids, mustelids, procyonidae, mephitidae, pinnipeds and

piscivorous birds, transmitted by ingestion of raw fish meat (salmon and trout) (Kistner *et al.*, 1979; Stroud, 1978; Foreyt *et al.*, 1987). *N. salmonicola* distribution is mainly in the Pacific area (Northwest USA), but stream snails are also found west of the Cascade Mountains in Oregon, north to the Olympic Peninsula in Washington and in part of northern California. Human infection by direct contact (handling of fresh-killed salmon) was also reported; however, it is very possible that oral contact was involved (hand-to-mouth route) (Harrell and Deardorff, 1990). Among the infected animals, raccoons, skunks, and other fish-eating animals are used as reservoirs of this pathogenic fluke without pathological symptoms, by continuous eggs excretion in or near water sources. Similar to that of its other trematodes relatives, *N. salmonicola's* life cycle goes through two intermediate hosts: snails (e.g., *Oxytrema silicula*) and both fresh and ocean water fishes (e.g., Salmonidae, Cottidae, Cyprinidae). Briefly, excreted eggs reach water sources where miracidia hatch and swim away to infect the first intermediary host: snails. Inside snails, miracidia develop into rediae and further into cercariae, which emerge into water to encounter the second intermediate host: fish. In fish, cercariae develop into metacercariae and encyst within muscles, kidney and fins waiting for its final host. Ingestion of infected raw fish meat transfers metacercariae into the final host (humans and animals) to develop into flukes that colonize intestinal mucosa (Figure 4.2.1.11.3). In severe human infection, clinical manifestations are hemorrhagic enteritis, unexplained peripheral blood eosinophilia, abdominal discomfort, nausea and vomiting, weight loss and fatigue (Eastburn *et al.*, 1987). It should be mentioned here that from the veterinary point of view, this parasite transfers *Neorickettsia helminthoeca* (a gram negative bacterium and obligate intracellular parasite, belonging to Proteobacteria family) a rickettsial agent that causes a fatal disease called "Salmon poisoning" in canines (Farrell *et al.*, 1964; Foreyt *et al.*, 1987).
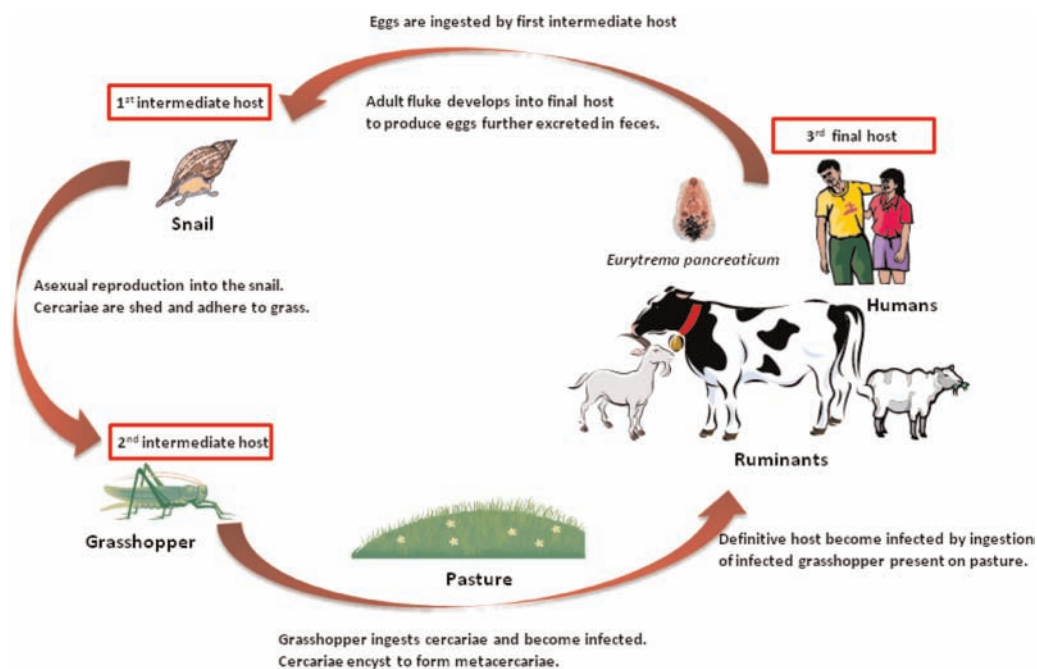
Figure 4.2.1.11.1. *Eurytrema pancreaticum* life cycle

Cercariae encyst and adhere to aquatic plants to develop into metacercariae.

2nd intermediary host

Water

Cercariae released in water.

Definitive host ingest metacercariae by consuming aquatic plants.

Metacercariae migrate to cecum and colon of the final host and mature into adult flukes.

1st intermediary host

Rediae reproduce and further produce cercariae.

Miracidia transform in rediae.

Gastrodiscoides hominis

Final host

Eggs maturation

Water

Mature flukes produce eggs to be further excreted in feces.

Eggs hatch and miracidia enter Planorbid snails.

Figure 4.2.1.11.2. *Gastrodiscoides hominis* life cycle

Ingestion of infected raw fish meat transfers metacercariae into the final host (humans and animals) to develop into flukes that colonize intestinal mucosa.

Neorickettsia helminthoeca

N. Salmincola parasite transmit *N. helminthoeca* rickettsial agent, fatal to canines.

2nd intermediary host

Fish

Cercariae penetrate fish and transform into metacercariae that encyst colonizing fish tissues.

Inside snail, miracidia develop into rediae and then into cercariae to be further released in water.

Final hosts

Canids

Nanophyetes salmincola

Animals

1st intermediary host

Snail

Humans

Eggs excreted in feces.

Miracidia hatch from eggs and infect snails.

Water

Figure 4.2.1.11.3. *Nanophyetes salmincola* life cycle

4.2.1.11.1 *EURYTREMA PANCREATICUM, GASTRODISCOIDES HOMINI, NANOPHYETES SALMINCOLA* AND ENVIRONMENT

Roy and Tandon (1992) studied the effect of seasonal variations on the prevalence of cattle's *F. giantica* and *E. pancreaticum* in India. The study area was located in a humid, subtropical north-east hilly region of India with an average temperature of 25°C in summer and 10°C in winter. The overall prevalence rate was 53.02%, with continuous annual presence and high peaks during cold winter months and lower in summer and autumn. The high winter prevalence of *E. pancreaticum* had been attributed to the increased availability of the second intermediary host, the grasshopper, already infected from the preceding summer and early autumn. Among other parasitic worms, they also looked for *Gastrodiscoides hominis* in pigs and reported an overall prevalence of ~13%. In pigs, *G. hominis* revealed a high peak during June-September, dropping to low levels during winter months. The overall high prevalence had been attributed to the presence/absence of the intermediate host species (snails). It is known that larval stages of flukes and the snail intermediate hosts are influenced by environmental factors such as temperature, water quality and flow intensity. These conclusions had been confirmed from another climatic area, the Far East of Russia, where Dvoriadkin (1976) reported an *E. pancreaticum* peak between May and October, a period coinciding with the active life of its intermediate host. When ambient temperature dropped to 8°C, fluke growth slowed down to a final halt in October. According to this author, in contrast to tropical and subtropical zones, the extended pause in flukes' development during cold months (October to April) caused a longer development period. Parenthetically, under laboratory conditions (temperatures between 8 and 22°C) this fluke development proceeded without intervals within 6.5 months. This is one of the classic examples where potential climate change may affect parasite development.

As already mentioned, *Gastrodiscoides hominis* distribution is in India, Southeast Asia including China. However, in 2004 Dad-Adegbola *et al.* (2004) reported a case of a seven year old Nigerian child who suffered from malnutrition and anemia, who was found to harbor both *Gastrodiscoides hominis* and *Ascaris lumbricoides*. These authors did not give detail of the epidemiology and environmental factors involved on the "appearance" of *G. hominis* in Africa. However, there is increasing evidence related to *G. hominis* infections in non-human primates that may allude to their possible origin in humans (Herman, 1967; Fox and Hall, 1970). *G. hominis* presence has been also reported in endemic foci in Russia revealing some "geographic movement" toward northern part of Asia (e.g., Siberia) (Sergiev *et al.*, 2008).

Rodnick *et al.* (2008), while studying the effect of water temperature on juvenile coho salmon *Oncorhynchus kisutchi*, among the various parameters they tested, they also looked for parasite load (*Nanophyetus salmincola* and *Apophallus* spp.). Here it should be mentioned that a gender distribution variation has been found related to temperature: more females occupied the cooler upper reach of the river compared to males' predominance in the warmer lower reach. Parasite prevalence was higher in the warmer, lower reach (with males' predominance) and the degree of parasitism was not correlated with swimming ability or gender. Based on these results, it can be suggested that in this ecosystem, the primary intermediate hosts (snails) are the ones impacted by higher temperature, allowing better development and release of cercariae into fish environment.

Gai and Marks (2008) reported on salmon poisoning in captive Malayan sun bears. These results show that *N. salmincola* infection is not restricted to endemic areas but may be transplanted to new environments through imported infected fish for sport fishing.

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Chapter 4.2.2

Cestodes

A summary of cestodes global infection burden is presented in Table 4.2.2.1.

Table 4.2.2.1 Cestodes as a global threat for humans.

Cestodes (> 1000 species)	Global infection	Geographic distribution	Diseases
Taenia multiceps, *T. brauni*, *T. serialis*, *T. glomerata*	~150 (hundreds)	*Taenia multiceps* (France, Africa, England, Brazil, Israel, United States and more), *T. serialis* (Canada and United States, T. brauni* (North Africa, Rwanda, and the Democratic Republic of Congo) and *T. glomerata* (Nigeria and the Democratic Republic of Congo)	Coenurosis
Diphyllobothrium latum, *D. denditricum*, *D. pacificum*, *D. nihonkaiense*	Estimated 20 million infections	Scandinavia, western Russia, China, Japan, the Baltics, Ireland, Italy, France, Romania, North America, especially the Pacific Northwest	Diphyllobothriasis

(*Continued*)

Table 4.2.2.1 (*Continued*)

Cestodes (> 1000 species)	Global infection	Geographic distribution	Diseases
Dipylidium caninum	Hundreds ?	Worldwide (Europe, Philippines, China, Japan, Argentina, and United States)	Dipylidosis
Echinococcus genus *E. multilocularis E. granulosus E. vogeliE. oligarthus*	Estimated2-3 million infections	Worldwide (all)(except Iceland and Greenland)	Echinococcosis
Hymenolepis nana, *H. fraterna*, *H. diminuta*	Millions	Worldwide(Higher prevalence in Southern Europe, North and Central Africa, Near and Far East, Central America)	Hymenolepiasis
Spirometra genus (*S. mansoni, S. ranarum, S. mansonoides* and *S. erinacei*, and the aberrant *Sparganum proliferum*)	Rare (hundreds to>thousand) infections	Southeast Asia (China, Vietnam, Korea, Japan) other but rare Europe, USA, Australia, Africa and South America	Sparganosis
Taenia saginata saginata, *Taenia saginata asiatica*	~50 million people worldwide are infected by *T saginata* or *T solium*.	Africa, Eastern Europe, Southeast Asia, South America	Taeniasis Saginata (Including Taeniasis Asiatica)
Taenia solium	~50 million people worldwide are infected by *T saginata* or *T solium*. ~50,000 people die annually of cysticercosis.	Asia, Africa, South America, Southern Europe and partially USA	Taeniasis solium and cysticercosis
Other miscellaneous cestodes infecting humans(*Bertiella* spp., *Raillientina* spp., *Inermicapsifer madagascariensis*, *I. cubensis*, *Mesocestoides varibilis*, *M. lineatus*, *Diplogonoporus grandis*	Estimated infections: hundreds	South America, Africa, Asia, Madagascar and Caribbean area	Abdominal discomfort, ?

Chapter 4.2.2.1

Coenurosis

[*TAENIA MULTICEPS, T. BRAUNI, T. SERIALIS, T. GLOMERATA*]

Coenurosis is a rare disease (∼150 cases) caused by the ingestion of eggs of dog tapeworm species *Taenia multiceps* (France, Africa, England, Brazil, Israel, United States and more), *T. serialis* (Canada and United States, *T. brauni* (North Africa, Rwanda, and the Democratic Republic of Congo) and *T. glomerata* (Nigeria and the Democratic Republic of Congo). The definitive hosts of these parasites are canids (e.g., dogs, foxes, wolves and coyotes), and humans are only accidental hosts with incomplete life cycle of these tapeworms. In humans, ingested eggs will develop into mature larvae but not into mature worms (see below life cycle). The intermediate stage of these tapeworms, the larvae, group within cysts called coenuri formed in the central nervous system (CNS), subcutaneous tissues and muscles. Intermediate hosts do exist such as goats, sheep, horses, cattle and rabbits that are infected by ingestion of dog contaminated feces with gravid proglottids (tapeworm segments, containing both male and female reproductive organs) but they do not transmit the parasite from one to another. In herbivores, the intermediate hosts, the infection is an economic problem. Briefly, the life cycle consists of adult tapeworm infecting canids and eggs production, excreted in feces as gravid proglodits containing eggs or already embryonated eggs. Ingestion of contaminated feces by an intermediate host (mainly herbivores) or an accidental host (humans) causes oncospheres to hatch from eggs into the intestine and further into a blood vessel of the intestinal wall.

Blood stream carries the embryo to various body parts with preference for the CNS. The oncospheres develop into coenuri in intermediate hosts and further into adult tapeworms in the final host. Ingestion of intermediary host meat by a final host (canids) introduces the coenuri in their digestive system with further development towards a complete adult tapeworm (Figure 4.2.2.1.1). Clinical manifestations are: in case of heavy infestation, acute meningoencephalitis develops, while in light infestation cases, a pressure-induced atrophy of surrounding CNS tissue will develop causing headaches, nausea, vomiting, paraplegia, seizure, hemiplagia aphasia, eye symptoms and death.

4.2.2.1.1 COENUROSIS AND ENVIRONMENT

Based on the life cycle of these parasites, it is obvious that low hygienic standards, poverty and low education are the main factors contributing to their successful infections in herbivores. However, in the

published literature, human infections seem to be mostly related to accidental ingestion of contaminated canids' feces such as in close encounter with pets (El-On *et al.*, 2008). Dalimi *et al.* (2006) surveying stray dogs, red foxes and golden Jackals in Iran found a *Taenia multiceps* prevalence of 4.82% but only in stray dogs. Beside direct contact with canids feces, other sources such as contaminated water and food with canids excreta may also infect humans, consequently creating the need for the following preventive measures: education, farming conditions improvement, improved detection methods, control of sewage sludge application on pasture and prevention of close contact between wild (the main reservoir) and domestic animals (Pazio, 2008).
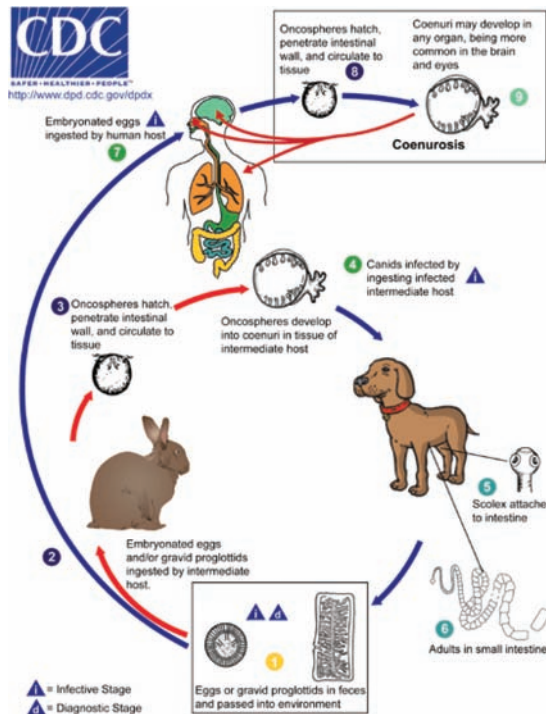
Figure 4.2.2.1.1. *Taenia multiceps*, *T. brauni*, *T. serialis*, *T. glomerata* (Coenurosis) life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/Coenurosis.htm)

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Chapter 4.2.2.2
Diphyllobothriasis

[*DIPHYLLOBOTHRIUM LATUM, D. DENDITRICUM, D. PACIFICUM, D. NIHONKAIENSE*]

Tapeworms of the *Diphyllobothrium* genus infect humans through their consumption of raw or undercooked fish. The *D. latum* species that infects fish and mammals (cats, dogs, pigs, bears, etc.) is found in temperate and subarctic regions such as Scandinavia, western Russia, the Baltics, Asia (Japan), Uganda, Peru, Chile and now also present in North America (especially the Pacific Northwest). In the United States, the disease is now rare; however, once it was more common and ethnically was referred to as "Jewish housewife's disease", as a Jewish traditional food specialty "gefilte fish" had been frequently tested by housewives before cooking. Briefly, the life cycle starts from an infected final hosts (animal or human) shedding eggs via their feces and contact with water sources. In water, the larva develops into a swimming coracidium ingested by small copepods (*Cyclops* and *Diaptomus* spp.) where it develops into a procercoid form. Fishes consuming copepods become infected with procercoids that settle into their tissue to become plerocercoids (the infective stage of final host). Here there are two possibilities: 1) plerocercoids ingestion by a bigger fish as a paratenic host (intermediary host) waiting to enter the final host or 2) direct ingestion by the final host. If the second scenario takes place, plerocercoids hatch in small intestine to develop into a mature tapeworm to produce new eggs (Figure 4.2.2.2.1). Clinical manifestations are mostly asymptomatic, but some people might develop megaloblastic anemia (a result of parasite vitamin B_{12} seizure), weakness, abdominal pain, intestinal obstruction, diarrhea and rarely paresthesia and ataxia.

4.2.2.2.1 DIPHYLLOBOTHRIASIS AND ENVIRONMENT

It is clear that continuous pollution of water sources (including estuaries) with sewage carrying fecal content from infected humans is the vicious cycle of continuous reports of this disease. Infected human migration or emigration and fecal contact with water may also cause a heavy infection load of plerocercoids in fish populations, even in lakes of considerable size. Because of *Diphyllobothrium* species' general broad host specificity, their life cycles are maintained in nature independently of humans (Scholz *et al.*, 2009).

Wardle (1933) a long time ago reported that the plerocercoid larval stage is highly tolerant to temperatures (between −2.8 to 38°C), HCl (from 0.001 to 0.3 M in the presence of 0.5% pepsin) and different salts (from 0.001 to 0.2 M) possibly explaining successful infections through marinated fish. Dupouy-Camet and Peduzzi (2004) reported infection levels of secondary hosts with *D. latum* as high as 33.3% in Western Europe, some lakes in Italy, Switzerland and France. These physiological characteristics make this tapeworm an excellent environmental survivor, explaining the high numbers of infections. However, eradication in humans can be easily implemented by changing their praxis to eat raw fish. In wild life pescivorous (birds, whales, seals and bears) this task would be much more difficult to accomplish, therefore a wild reservoir will always prevail.
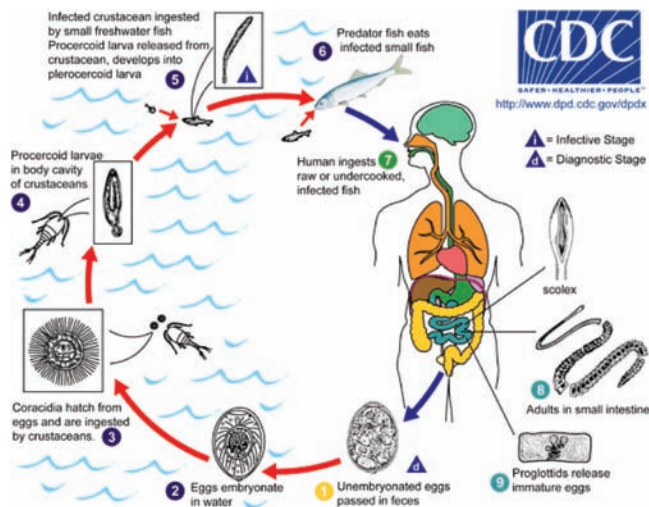
Figure 4.2.2.2.1. *Diphyllobothrium latum* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/dpdx/HTML/Diphyllobothriasis.htm)

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Chapter 4.2.2.3

Dipylidosis

[*DIPYLIDIUM CANINUM*]

Dipylidiosis is a rare disease caused by the tapeworm *Dipylidium caninum* found in dogs and cats. *D. caninum* is distributed worldwide, with its highest prevalence in European dogs. In humans infection occurs through ingestion of cystercoids or infected fleas. The life cycle of *D. caninum* includes release of gravid proglottides found in dogs or cats small intestine. Eggs containing larvae are hosted in proglottids in egg capsules. The egg capsules are spread around by feces and by ingestion by the larval stage of fleas (e.g., *Ctenochephalides canis* and *Ctenochephalides felis* species) (Guzman, 1984). Further, the ingested eggs hatch inside the flea's body cavity (during its pupal stage) to form oncosphere larvae that further develop to become cysticercoid larvae. This is the intermediate stage of *D. caninum* infective toward mammal hosts. Ingestion of a whole flea or direct contact with infected dogs (e.g. hand licking) that carry the infectious stage in its oral cavity will introduce the cystcercoids into the digestive system where they will reach their final stage of adult tapeworm development (Figure 4.2.2.3.1). In humans clinical manifestations are mostly asymptomatic but heavy burden infection can cause abdominal discomfort, bloody and mucous diarrhea, anal pruritus and weight loss.

4.2.2.3.1 DIPYLIDOSIS AND ENVIRONMENT

Overgaauw and Boersema (1998) surveyed 25 cat kennels in Holland for presence of the nematode Toxocara's eggs in feces, soil and dust samples. The nematode's eggs were not present in soil and dust samples, however *Dipylidium caninum* eggs were present in two household dust samples. Based on *D. caninum's* life cycle, their results indicate the presence of fleas as an infective source. Flea larvae of *Ctenocephalides felis* have been experimentally infected with *D. caninum* eggs at different temperatures (20 to 35°C) (Pugh and Moorhouse, 1985). Parasite development and host reaction were found to be independent of host maturation, although the host's marked and prolonged reactions between 20 and 25°C were observed. *D. caninum* growth did not occur at 20°C while faster growth was observed at higher temperatures (highest growth rate occurred at 35°C) in spite of the host's death at 35°C (at pupation stage). The effect of elevated temperature on *D. caninum* development in the flea host may possibly

explain its successful infection of mammals through ingestion and exposure at these temperatures inside a mammal's body!
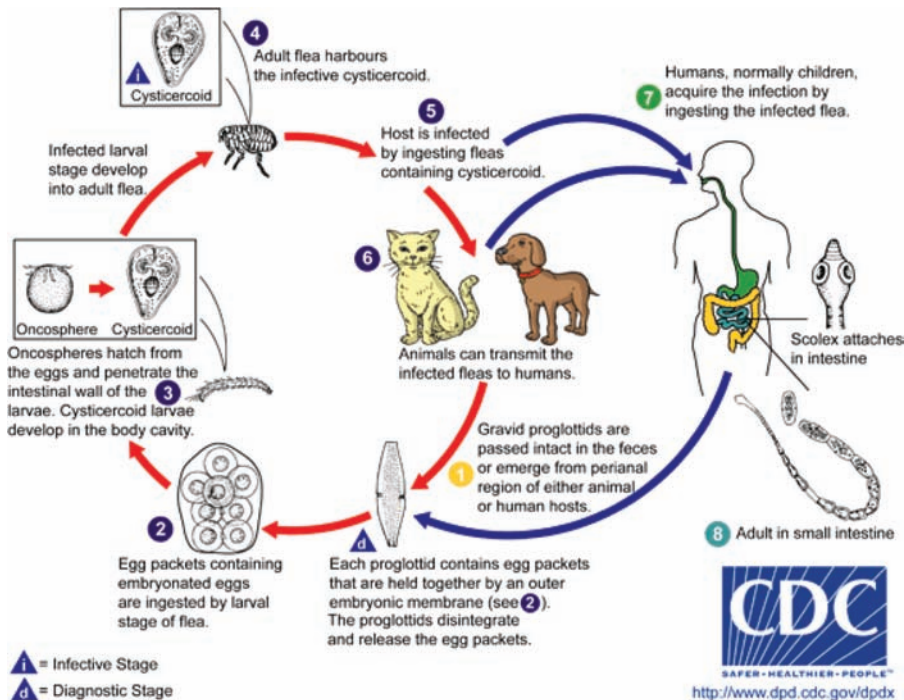
Figure 4.2.2.3.1. *Dipylidium caninum* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/Dipylidium.htm)

An Argentinean group studied ∼2000 urban and rural dogs for the presence of diverse parasites in their stool samples, in areas of the province of Neuquen, Patagonia (Soriano *et al.*, 2010). Among the large diversity of parasites present, *Dipylidium caninum* had a prevalence of 0.31%, with an incidence in urban dogs slightly higher than in rural ones. The authors mentioned that rural dogs in this study area were under periodic treatment with antihelmintic drugs, while the urban dog population increased as a result of a proliferation of stray dogs. Another important environmental fact brought up in relation to *D. caninum* prevalence, is the absence of regulations against canine fecal deposits in public areas, increasing contamination and favorable parasite transmission in such places.

In Poland, Okulewicz *et al.* (1994), surveying intestinal parasites among rural and urban dogs, reported on an overall higher infection prevalence in rural dogs (100%) than in urban dogs (40–48%) including *D. caninum*. This difference was attributed to different breeding conditions and to rural dogs recurrent contact with additional wild canids (wolves, foxes, etc.).

In another geographical area, Kainji Lake area in Nigeria, Okaeme (1985) investigated gastrointestinal helmithiasis in human and dog population taking into consideration inadequate health, lack of veterinary facilities, crowdedness and squalid environment. Indeed, among other helminths, *Dipylidium caninum* had a prevalence of 28.26%. (second in magnitude) in dogs. Also in Nigeria, Maikai *et al.* (2008)

surveyed soil samples for different helminth eggs in Kaduna metropolis in 14 various environmental and socio-cultural playgrounds. *D. caninum* overall prevalence was 26.3% with a higher prevalence during the dry harmattan period (a dry and dusty West African trade wind) revealing dust carriage. Interestingly, highly rated sites (socio-economically) were more contaminated. From the statistical point of view, parameters revealing significantly high odds ratio were: rain, dogs' presence and grass (vegetation) while insignificant parameters (low odds ratio) were: dry periods, refuse presence on playground and their topography, soil depth and socio-economic status of people attending those playgrounds.

In Wales, a foxhound pack and gundog kennel were surveyed for infestation with various cestodes. *D. caninum* was found in hounds but only related to diet. Owing to foxhounds' activity, they play an important role in environmental dissemination of cestodes' eggs (including *D. caninum*) because of frequent diet on uncooked carcasses and large hunting areas coverage (Stallbaumer, 1987).

Finally, Orta-de Velasquez *et al.* (2002) studied the inactivation of different helminth eggs by ozone. At 18.4 mg/L/min ozone exposure it took 3 hours to inactivate *D. caninum* eggs completely, which reveals an increased resistance of this parasite to disinfection and explicate its environmental persistance.

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Chapter 4.2.2.4

Echinococcosis

[*ECHINOCOCCUS MULTILOCULARIS*, *E. GRANULOSUS*, *E.VOGELI*, *E. OLIGARTHUS*]

Human echinococcosis is a disease that affects both humans and animals with severe outcome. Depending on *Echniococcus* species there are three forms of echinococcosis in humans: 1) alveolar echinococcosis caused by the tapeworm *Echinococcus multilocularis*; 2) cystic echinococcosis caused by the tapeworm *Echinococcus granulosus* and 3) polycystic echinococcus caused by the tapeworms *E. vogeli* or *E. oligarthus*. The life cycle of Echinococci consists of eggs or proglottids containing eggs excretion in feces and spread by canids in environment (water, soil, plants, etc.). An intermediate host (sheep, goats, camels or pigs) ingests/inhales these embryonated eggs; thenafter the oncospheres hatch and penetrate the intestinal wall and are transported to the liver or other organs through blood or lymph vessels. The larval stage (oncospheres) further develops into metacestode through tissue infiltration and formation of hydatid cysts in different organs (e.g. liver, lungs, brain, etc.). The final host's ingestion of meat containing hydatid cysts releases protoscoleces that adhere to the intestine to develop into scoleces and eggs producing mature tapeworms (Figure 4.2.2.4.1). Humans are dead-end hosts and their main infection occurs through contact with canids' feces or ingestion of contaminated meat from an intermediary host.

Alveolar echinococcosis, caused by *E. multilocularis*, has a Northern Hemisphere distribution that includes: North America (including Alaska), Southwestern Asia (including Turkey), Northeastern Asia (including Siberia, northern China and Japan) and Central Europe (France, Germany, Austria, Czech Republic and Poland), with a tendency to spread northward (Vuitton *et al.*, 2003). Natural final hosts are red and arctic foxes but other canids (dogs, wolves, raccoon dogs and coyotes) and cats may be infected (Thompson *et al.*, 2006). Intermediate hosts include a large variety of rodents, such as voles spp., muskrats, lemmings and deer mice. Transmission to humans occurs through egg ingestion or inhalation (aerosolized eggs) from feces of a final infected host or direct contact with food (e.g., forest berries and mushrooms), soil, dirt or animal hair that holds eggs; coprophagic flies, carrion birds and arthropods may also act as mechanical vectors of this parasites eggs. *Echniococcus* eggs are highly resistant under moist and cold conditions and can survive for months (8 months viability after storage at $-18°C$),

possibly explaining its northern extension simultaneously with growing fox population in European urban areas (Deplazes, 2006; Veit *et al.*, 1995). Its clinical manifestations may be divided in three phases: 1st phase-incubation that may last between <5 to 15 years, where abortive infections may occur (no infection); 2nd phase mainly in mature humans (average 50 years old), infection progresses with liver affected but with a variable advance (slow or rapid with metastases); 3rd phase hepatomegaly develops, infiltration and congestion of bile ducts and blood vessels with icterus and ascites development. Lungs and brain metastases are fatal.
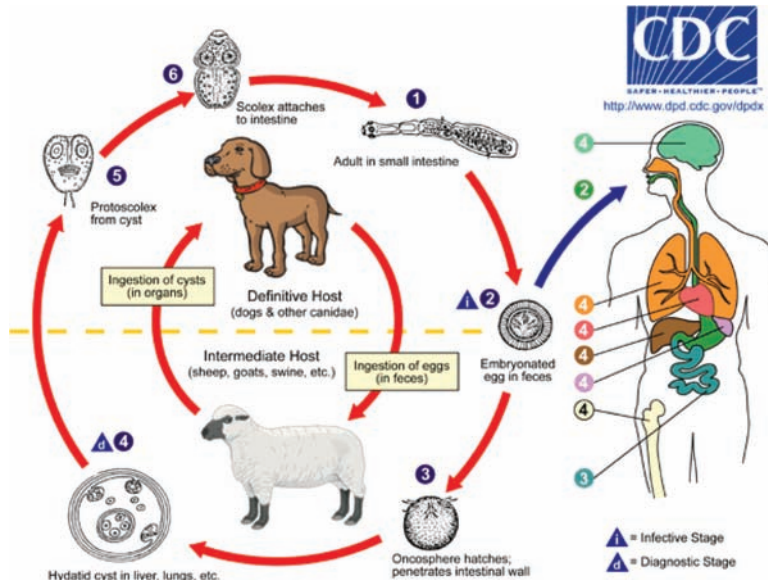
Figure 4.2.2.4.1. *Echinococcus* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/dpdx/HTML/Echinococcosis.htm)

Cystic echinococcosis, caused by *E. granulosus* is distributed in Mediterranean countries, the Middle East, China (Northwest), India, southern Australia, South America (mainly Chile and Uruguay), Africa (central, southern and east, mainly Ethiopia and Kenya), North America (USA partially) and Central America. Final hosts of *E. granulosus* are also canids (dogs and wild dogs, foxes, dingoes and wolves) but lions have been also described as a potential host (Jenkins *et al.*, 2000). Clinical manifestations are slower in comparison with alveolar echinococcosis; cyst growth is slower but to a much bigger extension (fist or bigger size of cyst) without tissue infiltration and metastases (except when cyst is cracked), affected organs are in decreasing order of prevalence: liver > lungs > spleen and peritoneum ≫ brain. When growing, cysts impact blood vessels or bile ducts, icterus and ascites are present and any abrupt rupture of big cysts can cause sudden death by anaphylactic shock caused by the internal large amount of released antigen. Nonspecific signs may include anorexia, weight loss and weakness.

Polycystic hydatidosis distribution, caused by *E. vogeli* and *E. oligarthrus*, occurs in Central and South America, but is a rare infection in humans. The definitive hosts for *E. vogeli* are bush dogs (*Speothos venaticus*) and the intermediate hosts are South American rodents, especially pacas (*Cuniculus paca*) and the definitive hosts for *E. oligarthrus* are wild felids and the intermediate hosts are rodents too.

E. vogeli tends to occur initially in the liver; however, the cysts are invasive and can spread to nearby organs and tissues. The clinical signs resemble alveolar echinococcosis with common symptoms, such as hepatomegaly, abdominal distension, weight loss, abdominal pain, jaundice, anemia (when liver is involved), coughing, chest pain and hemoptysis (if the lungs are involved). Rupture of esophageal varices can result in hematemesis (esophageal involvement), congestive heart failure and acute pulmonary edema (when heart is involved). Calcified metacestodes have been also reported.

E. oligarthrus is confined to internal organs of its normal intermediate hosts: subcutaneous tissues and muscles. Little is known about this organism in humans; only rare cases have been described (two cases): 1) a single cyst behind the eye caused irritation of the eye, exophthalmia and blindness, and 2) cysts found in a person heart that died of tetanus. The lesions included an enlarged heart, myocarditis, and excess pericardial fluid.

4.2.2.4.1 ECHINOCOCCOSIS AND ENVIRONMENT

Larrieu *et al.* (2002) performed a case-control study on the risk factors for cystic echinococcosis in children of the province of Rio Negro, Argentina. According to univariate and multivariate analyses, the significant calculated odds ratios for cystic echinococcosis in children were: 1) having a relative with this disease, 2) first years of life in contact with a large number of dogs and 3) having a father who slaughtered sheep at his workplace. Clean tap water was a protective factor on the disease spread.

In Africa (Kenya), a slaughter survey has been carried out in order to establish an accurate prevalence status of cystic echinococcosis in infected intermediate hosts (cattle, goats, sheep and camels). Animals originating from a certain area were reported to have a higher echinococcosis prevalence. This particular area had a higher mean annual rainfall (430 mm) than other areas (380 mm and 19 mm) and also a lower environmental temperature, two climatic factors that support *E. granulosus*'s egg survival. Additional factors attributed to high prevalence in this specific area were: 1) constant migration of infected livestock across the border, 2) high prevalence of infected final host (dogs) and 3) higher stocking rate (Njoroge *et al.*, 2002). Interestingly, among the surveyed post-mortem animals, camels had the highest prevalence (61.4%) but the authors did not elucidate this divergence. It can only be speculated that camels' closeness to humans' dwellings in these areas enhances their direct contact with canids feces in comparison with cattle, sheep and goats that migrate to more distant regions as large areas free-grazers.

During a five years span, in a temperate climatic environment (Slovakia), red foxes have been studied for *E. multilocularis* infection throughout the country (Miterpakova *et al.* 2006). Northern and central parts of the country had high-endemicity foci with an estimated prevalence of 30% (Hurnikova *et al.*, 2009). Climatic conditions, such as low temperature annual mean, high rainfall and soil humidity, in addition to the ecological condition for instance small mammals' density, all supported *E. multilocularis's* distribution and consequently tapeworm' prevalence. Similar results related to climate have been supported by other experimental studies from Japan and Germany (Matsumoto and Yagi, 2008; Hansen *et al.*, 2004). However, an interesting study performed in an inferior arid climate (Patagonia, Argentina) revealed *E. multilocularis* egg survival under highly different climatic conditions (Thevenet *et al.*, 2005). The authors exposed *E. multilocularis* eggs for 41 months to the external climate within a temperature range from 37 to −3°C (mean annual temperature of 10°C) that might support viability but against a very low level of precipitation <300 mm/year! Experimental infection of ovines (at 1200 eggs/ovine challenge) with these "old" eggs resulted in a successful infection of 100% (4/4) showing their capacity to penetrate, settle down and start the post-oncospheral development, as proved by hepatic and pulmonary cyst formation. The authors explained this extended infectivity of "old"eggs as related to

their semi-senescent stage. Indeed, Gemmel *et al.* (1987) proposed that the processes of egg maturation and ageing of taeniids (in his case *T. hydatigena*) continue in the environment and that the eggs might go through different stages during those processes: immature, mature, semi-senescent, senescent, and dead. An egg in semisenescent stage overcomes the pre-cystic immunity, settling down in the target organ and starting the development of the larva stage, though later, when larva is formed, it is destroyed by the post-cystic immunity, which was not the case in the *E. multilocularis* ovine infection.

In Austria, a study revealed that pet animals are also a suitable risk factor of *E. multicularis* in humans but not consuming mushrooms or certain wild berries growing near the ground. Cat ownership (OR = 6.47) and hunting (OR = 7.83) were the sole risk factors reported by this study (Kreidl *et al.*, 1998). A study performed on red foxes' infection with *Echinococcus multlocularis* in Switzerland, an area defined as the core of the endemic range of this zoonotic cestode in Central Europe, revealed that intensity of infection in these animals decreased from rural and residential areas to the city (as densely urban area), suggesting a lower contamination of the urban environment (Fischer *et al.*, 2005; Reperant *et al.*, 2007). Similar results have been also reported in a northeast endemic area of France, suggesting that the decreasing gradient observed from rural to urban area is linked to behavior and feeding habits of the host (Robardet *et al.*, 2008).

In more extreme environments such as arctic and sub-arctic regions, Rausch (2003) proposed four predator/prey patterns in relation to *E. granulosus* occurrence and infection. In these areas, the final and intermediary hosts are less diverse than in other climatic regions (wolves, dogs, elks, moose and reindeer). The four patterns are illustrated in Figure 4.2.2.4.2 and include wild transmission through predation, synanthropic and semi-synanthropic pathways related to humans' sedentary life (including consumption of cervid viscera by free-roaming canids) (Himsworth *et al.*, 2010). Indeed, in Alaska, all cystic echinococcosis cases have been related to indigenous people and significantly decreased when herding and sled dogs were replaced by machines. In Russia, in the northeastern Siberia, an increase in cystic echinococcosis has been reported, suggesting the use of herding-dogs by the locals.

Romig *et al.* (2006) raised the issue of an anthropogenic factor involved in human alveolar echinococcosis. According to epidemiological studies, endemic regions have been linked to deforestation and agricultural practices that created favorable conditions for parasite's animal hosts (e.g., arvicolid rodents), for example: conversion of forests or crop fields into meadows/pastures in Europe, China and North America and overgrazing of natural grassland in central Asia. An excellent example has been provided by a study performed in Kazakhstan where a substantial increase in cystic echinococcosis was observed in recent years (Shaikenov *et al.*, 2003). The primary factors involved in this process were: the degradation of the traditional nomadic system of livestock breeding, closing of large collective farms and establishment of small private farms (with year round stock) in closer proximity to human habitation, deserting routine anthelmintic prophylaxis of dogs; and inadequate control over the use and disposal of animal carcasses; and finally an increase in people and dog population per 1000 sheep. As a result, the prevalence of *E. granulosus* in village dogs was 5.8% while in shepherd dogs it reached 23.2% representing a high-risk source of human infection.

Additionally, wildlife disease control by interference with host population densities through hunting pressure shift and provision of new habitats (e.g., in urban areas) are anthropogenic too. Other possible anthropogenic involvements are: growth in domestic dog population (as pet animals) and their involvement in wildlife (contact with urban foxes); introduction of neozootic host species (red foxes in northern Japan to control voles); and accidental introduction of the parasite in non-endemic regions may also increase human transmission (Eckert and Deplazes, 2004; Jenkins *et al.*, 2005). In relation to urban areas, a recent study showed that, besides red fox distribution, rodent intermediary hosts, such as *Microtus* spp. and *Arvicola terrestris*, are also dominant factors in spreading *Echinococcus multilocularis*

in urbanized areas. Since *Microtus* spp. have been found to have an increased population density in urban wasteland and their red fox diet predominance, these rodents definitely play a key role in *E. multilocularis* transmission (Robardet, *et al.*, 2011; Reperant *et al.*, 2009).
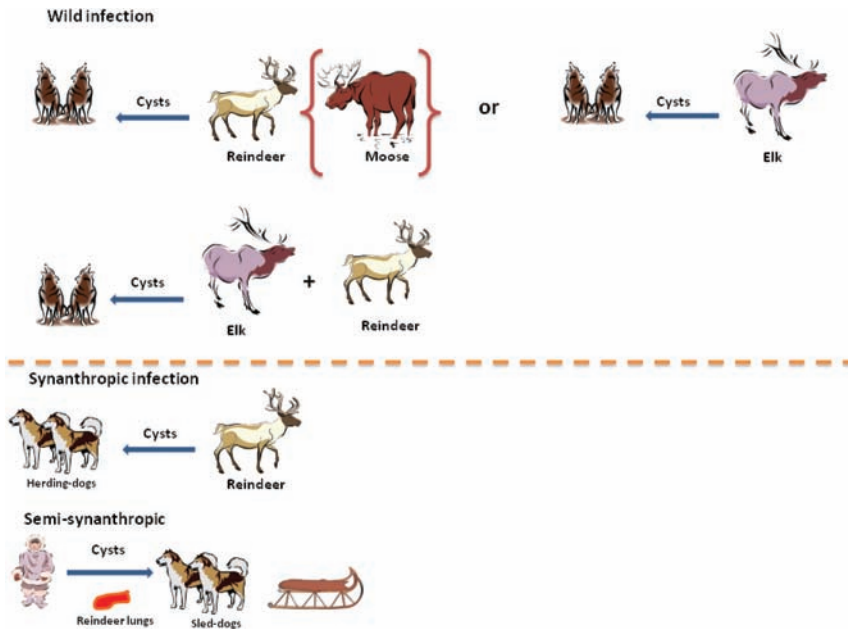
Figure 4.2.2.4.2. Cystic echinococcosis infection patterns in the Arctic and Sub-Arctic environments

A Japanese study targeted the *Echinococcus multilocularis* environmental contamination prevalence in an urban fringe-the Nopporo forest park of Sapporo city, Hokkaido, Japan (Lagapa *et al.*, 2009). Their results of this study showed that Nopporo forest park in the urban fringe of Sapporo City, is a reservoir site with a high prevalence of zoonotic infective agents for alveolar echinococcosis, suggesting that interface habitats between forests or woodlands and open fields are indispensable for continued maintenance of *E. multilocularis* life-cycle.

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Chapter 4.2.2.5

Hymenolepiasis

[*HYMENOLEPSIS NANA* (*H. FRATERNA*), *H. DIMINUTA*]

Hymenolepsias is an intestinal infection caused by one of the universally most common cestode: *Hymenolepsis nana. H. fraterna* is present in rodents and seems to be a different strain of the same species as *H. nana. H. diminuta* that is mainly found in rats and rarely causes human infections. Among tapeworms, *H. nana* is the smallest, also nicknamed "dwarf tapeworm" and has another unique attribute: it is the only cestode that does not need an intermediate host. Transmission occurs by eggs oral ingestion (including autoinfection and person to person) or rarely by intake of insects which contain the cysticercoid infective stage in their body. The life cycle of *H. nana* includes egg ingestion followed by oncospheres hatching in the villi of the host small intestine. Oncospheres penetrate the villi to develop into cysticercoides that later emerge from tissue to develop further into adult tapeworms (adult life span ~2 months) (Figure 4.2.2.5.1). Eggs containing progloditts are released in feces; however, if constipation occurs, larvae can hatch in intestine to repeat the infectious cycle again without leaving the host. Clinical manifestations depend on infection load: infections with low number of worms are mostly asymptomatic while heavy infestation (>100 worms) cause villi atrophy with mucosal inflammation, abdominal pain, spongy feces, diarrhea, weakness, anxiety, headache and weight loss.

4.2.2.5.1 HYMENOLEPIASIS AND ENVIRONMENT

According to its life cycle, *H. nana* is mostly prevalent in places with low hygienic standards. *H. nana* eggs are not resistant to environmental stresses, and therefore most infections will frequently occur from soiling, for instance in nurseries as a result of direct contact with contaminated feces. Infections, yet rare, with *H. diminuta* (a rat and mice parasite) occur by accidental ingestion of arthropods (e.g., beetles and myriapods) that may carry cystercoides. A study of intestinal parasitic infections was carried out in two rural communities of Nigeria (Agi, 1997). *Hymenolepsis nana* had a low prevalence (2.9%) compared to other parasitic helminths. An important feature presented by the author was the relation between toilet facilities and parasitic infections in decreasing order (from the most promiscuous to the safest): bush, shoreline, pit, bucket and water closet. In relation to drinking water, the safest water was piped water

versus well, while other differences between the two communities were also linked to climatic and edaphic factors (soil salinity) that impact the parasites' survival (even if this is not the case of *H. nana*).
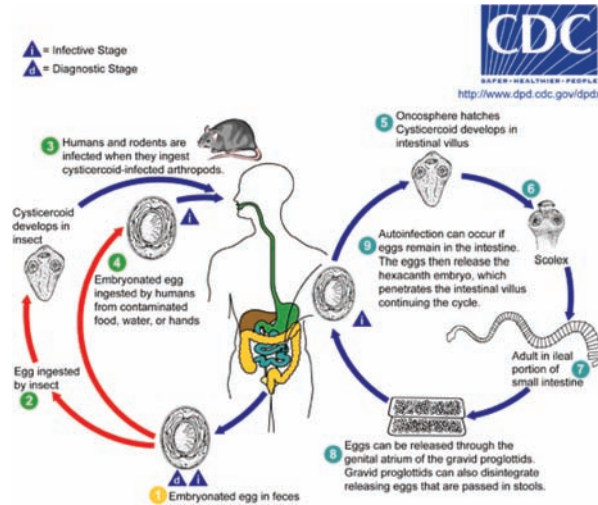
Figure 4.2.2.5.1. *Hymenolepis nana* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc. gov/dpdx/HTML/Hymenolepiasis.htm)

Gamboa *et al.* (2009) compared urban, suburban and rural areas of Buenos Aires (Argentina) for geohelminths species and socioenvironmental conditions. *H. nana* was found to have the highest frequency in one of the suburbs. The authors, once more emphasized that "poor hygiene habits in food preparation and toileting, overcrowding, walking barefoot, and dwellings with dirt floors were significantly associated with a higher frequency of intestinal geohelminths and pseudogeohelminths". Perhaps, here is the place to mention that poverty (not a rare attribute around the globe) is one of the main reasons for parasitic infections, diarrhea and premature death. In Honduras, Kaminsky (1991) performed a longitudinal study of children from rural and marginal barrio (city suburb-Sp.) linked to diarrheotic episodes. *Hymenolepis nana* was found in 8% of the children most often in combination with protozoan or other helminthic parasite. Again, the recognized risk factors were environmental high fecal contamination, lack of potable water, education and poor housing. The marginal barrio was more affected than the rural villages, likely to a large extent due to overcrowding.

Finally, a study performed in Aden indicated the increased numbers of Indian house crows (*Corvus splendens*) as a possible source of environmental contamination with bacterial pathogens, parasitic protozoa and helminths. Among the parasites isolated, *Hymenolepis nana* ova were recovered too (el Salami, 1991). These findings are not surprising in light of the fact that crows do feed on carcasses of rodents and other animals, spreading their droppings close to human habitats.

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Chapter 4.2.2.6

Sparganosis

[*SPIROMETRA* GENUS, *S. MANSONI, S. RANARUM, S. MANSONOIDES* AND *S. ERINACEI,* AND THE ABERRANT *SPARGANUM PROLIFERUM*]

In humans, sparaganosis is an accidental rare disease caused by *Spirometra* genus tapeworms. Cases have been reported around the globe, however China, Japan and other Southeast Asian countries have higher prevalence (Nithiuthai *et al.*, 2004; Park *et al.*, 2001). Transmission occurs by ingestion of larvae found in the first or second intermediate host (via water or consumption of an undercooked second intermediate host) (Yoshimura, 1989). In the Far East, an extra infection route has been observed linked to traditional healing: application of raw, chopped frog meat on wounds, ulcers or eyes, all allowing active invasion of the patient. The life cycle of these parasites is linked to water sources and two intermediary hosts related to parasite. Predators (cats, dogs, birds, wild carnivores, etc.) harboring adult tapeworms excrete unembryonated eggs into water. In water eggs embryonate and coracidia hatch to be further ingested by crustaceans. In the crustaceans' body cavity, procercoid larvae develop and when ingested together with their hosts (crustaceans, e.g., *Cyclops* spp.) by a second intermediary host (fish, reptile or amphibian) they are released and further develop into plerocercoid larvae. When a predator (including humans accidentally) ingests the first or the second intermediate host, the plerocircoides larvae develop into mature tapeworms in a subcutaneous tissue following passage through intestinal wall (Figure 4.2.2.6.1). Clinical manifestations are subcutaneous painful migratory nodules, inflammation, edema, ophtalmodynia, lacrimation, ptosis (in eye infection), headache, seizures, etc. (in brain infection).

4.2.2.6.1 SPARGANOSIS AND ENVIRONMENT

In China, sparganosis is an emerging helmithiasis due to food consumption habits as well treatment of wounds and lesions with frog and snake flesh poultices (Li *et al.*, 2011; Wiwanitkit, 2005). A Chinese study on the frog *Rana nigromaculata* infection with tapeworms reported a 16% prevalence of *Spirometra mansoni* (the tapeworm causing spaganosis in humans) in these amphibians (Mao *et al.*, 2009). Lin *et al.* (2010) looked experimentally at several parameters involved in sparganosis cycle. Cyclops, tadpoles and mature frogs have been infected with spargana revealing an infection rate of 3.5%, 35.9% and 16.8%, respectively. In

predators (cats but mostly dogs), the *Spirometra mansoni* experimental infection rate was ~20% with fecal eggs excretion, revealing a high prevalence of this tapeworm in intermediate and final hosts. The authors concluded that local residents' eating habits include tadpoles, thereby increasing human infection risk. In Australia, free-ranging amphibians of the group tree frogs (e.g., *Litoria caerulea*, *L. aurea*, *L. gracilenta* and *L. peronii*) have been found to be heavily infected with spargana of *Spirometra erinacei* (Berger *et al.*, 2009). According to these authors, the infection of tree frogs with spargana is now a serious recognized threat for free-ranging frogs, with unknown consequences to other wildlife.
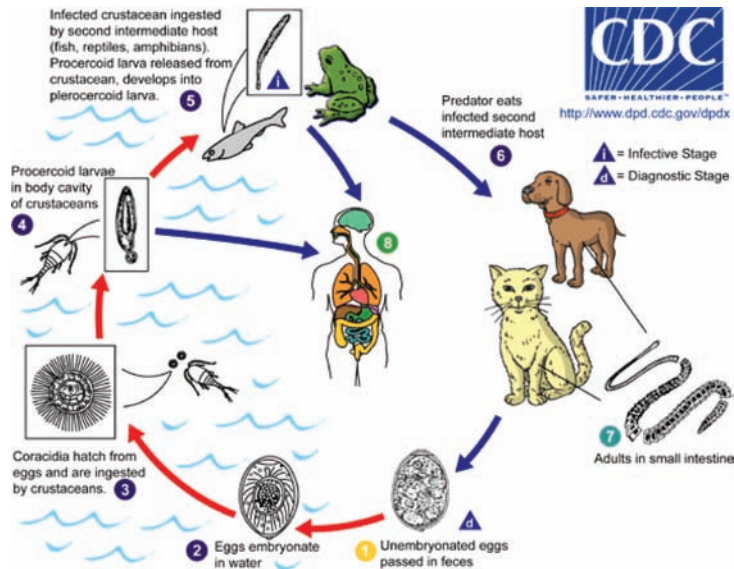
Figure 4.2.2.6.1 *Spirometra* genus life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc. gov/dpdx/HTML/Sparganosis.htm)

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Chapter 4.2.2.7

Taeniasis Saginata (Including Taeniasis Asiatica)

[*TAENIA SAGINATA SAGINATA, TAENIA SAGINATA ASIATICA*]

Taeniasis saginata is a disease caused by infection with the beef tapeworm *Taenia saginata saginata* that parasitizes mainly cattle. Humans are the final and exclusive hosts. *T. saginata asiatica* is a subspecies that is outspread in Southeast Asia. Transmission in humans occurs by consumption of raw or undercooked beef or contaminated reindeer meat containing cysterci. The life cycle of this tapeworm is too linked to its transmission. *T. saginata/asiatica* eggs are shed in mature proglottids within feces. In different environments where they can survive for months, an intermediary host (cattle or other ruminants) ingests eggs that hatch to produce larvae (oncospheres) in the intestine. Oncospheres penetrate the intestine wall and are spread hematogenously into striated musculature (diaphragm, heart, masseters) to develop further into cysterci in the infected animal muscles. After human ingestion of cysterci by consumption of infected beef or pork (see further on *T. solium*), cysterci avert their scoleces to attach to upper small intestine mucosa and initiate formation of proglottids (Figure 4.2.2.7.1). In humans as final host, *T. saginata* can survive between 30 to 40 years. *T. saginata asiatica* in an intermediary host is primarily found in liver and other peritoneal organs.

Clinical manifestations do not show clear symptoms (beside discomfort and embarrassment due to proglottids anal crawling), nevertheless obstruction, diarrhea, hunger pain and weight loss have been already reported.

4.2.2.7.1 TAENIASIS SAGINATA AND ENVIRONMENT

From the environmental aspect, improper excrement disposal is the main cause of these infections. Pastures treated with feces or irrigated with inadequately treated wastewater or exposed to incidental floods are the major focuses of cattle infestation. *Taenia saginata* eggs are able to pass sewage treatment thereby spreading them through irrigation or sludge application on soil and vegetation it allows their persistence and survival for months (5 to 6 months in Denmark) (Ilsoe *et al.*, 1991). Arthropods and birds have been

also suggested as vectors of *T. saginata* eggs dissemination. In an intensive epidemiological study, Ilsoe *et al.* (1990) identified the main sources of herd infection in Denmark. The most frequent sources of infection were reported to be sludge from septic tanks illegally applied on pasture or crops, in some cases after having been mixed with animal slurry! In contrast to earlier observations, application on farmland of sewage sludge from municipal treatment plants was not involved in any of the reported outbreaks, due to a more effective egg oval treatment. Animals in permanently housed herds were infected through fodder or by contamination of the indoor environment by slurry containing *Taenia* eggs. Other herds were infected by grazing on pastures located in close proximity to municipal sewage treatment plants. Sludge application on grazing pastures and short retention time proved to increase infection risk of cattle and humans (Cabaret *et al.*, 2002).
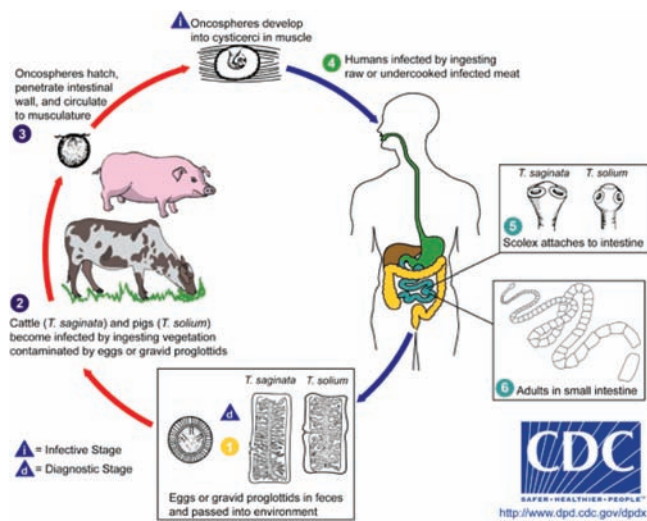
Figure 4.2.2.7.1. *Taenia saginata* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/Taeniasis.htm)

Pastures also harbor earthworms and insects (dung beetles). Lonc (1980a; 1980b) inoculated earthworms and dung beetles with *T. saginata* eggs and followed their intestinal transmission in these organisms. He concluded that tapeworms' eggs may be transmitted by the digestive tracts of earthworms (contributing to eggs' horizontal and vertical spreading in soil) and dung beetles (through passive transmission for long distances), consequently playing an important role in the epizootiology of cysticercosis in cattle.

Slaughterhouses are also to be blamed for the spread of this parasite, mainly due to poor veterinary inspection and even the illegal action of selling infested beef (Ring, 1992). In Poland, a study has been carried out to find taeniasis' prevalence rate in two environments: urban and rural. According to this study results, the incidence rate was higher in urban than in rural residents (Krol-Pakulska *et al.*, 2000). It is very possible that higher urban incidences are linked to some slaughter houses described above that may spread contaminated meat to a larger number of people in comparison with rural areas where slaughtering is on a smaller scale and usually performed locally.

Finally, Dutto *et al.* (2009) have described recently an interesting aspect linked to infection sources. These authors described a case of teniasis in a child attending primary school (Piedmont Region, Italy)

where several proglottids had been repeatedly found by cleaners on the book box of several school desks in the same classroom. Laboratory tests showed that the isolated proglottids belonged to *T. saginata* tapeworm. In the subsequent year, the same authors reported another case of teniasis caused by *Taenia saginata* (tapeworm) in a pediatric patient with done-on-purpose dispersion of proglottids (by another child carrier) that happened in an elementary school (also in Piedmont Region, Italy) (Dutto *et al.*, 2010). Immigrant workers who carry these parasites and employed in developed countries are also a source of transmission and infection, in spite of general first-rate environmental management in these countries (Vogtle-Junkert, 1975).

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Chapter 4.2.2.8
Taeniasis Solium and Cysticercosis

[*TAENIA SOLIUM*]

Another human taeniasis is caused by the pork tapeworm *Taenia solium*. This cestode uses pigs as predominant intermediate hosts and humans as the definitive ones. The geographic distribution is linked to pigs growing farms or other pig related facilities. Endemicity of taeniasis can be found in Central and South America, Southern and Eastern Europe, Africa and Southeast Asia. Industrial countries have a much lower rate of infections due to regular controlled inspection of pork meat and advanced pig farming. Main human transmission occurs when infected pork meat is ingested; however, autoinfections as a consequence of insufficient personal hygiene have been also reported, as well as infections through close contact with infected persons (Somers *et al.*, 2006; Flisser, 1991). The life cycle of this tapeworm is as follows. *T. solium* eggs are shed in mature proglottids within pig feces. In different environments, where they can survive for months, eggs are ingested by an intermediary host (pigs) and hatch to produce larvae (oncospheres) in the intestine. Oncospheres penetrate the intestine wall and hematogenously are spread in the striated musculature (diaphragm, heart, masseters) to develop further into cysterci in the animal's muscles. Subsequent to human ingestion of cysterci via consumption of infected pork, they avert their scoleces to attach to upper small intestine mucosa and start formation of proglottids (Figure 4.2.2.8.1). It should be mentioned that oncospheres reveal distinct neurotropism especially toward brain hemispheres but also eyes. In humans as final host, *T. solium* can survive up to 25 years. Clinical manifestations with adult worms do not show severe symptoms besides abdominal discomfort, indigestion or constipation, diarrhea, and hunger pain. The severity of the disease (cystercosis) differs with infection site: cerebral cysterci may cause reduced consciousness and personality changes, while multiple infections is fatal; intraventricular cysts may cause intracerebral pressure, nausea, vomiting, up to occlusive hydrocephalus, chronic meningitis, cerebral infarcts, neuritis; spinal cord cystercosis may cause paresis; heart cystercosis may cause myocarditis, etc.

4.2.2.8.1 TAENIASIS SOLIUM AND CYSTICERCOSIS AND ENVIRONMENT

T. solium should be rigorously prevented in comparison with *T. saginata* in consequence of infections that are more serious including their outcome in humans (neurocysticercosis, etc.). Epidemiologically, it is clear

that *T. solium* can be easily prevented by adequate control of the environmental and social behavior factors (Joshi *et al.*, 2001). Environmental factors found to be involved in the spread of this parasite are water, sludge application onto pastures, soil, lack of sanitary toilet, earthen floor, pigs scavenging freely and contact with pigs' feces (Gweba *et al.*, 2010; Sanchez *et al.*, 1998). In Mexico, a study carried out in a rural community showed a high pig cysticercosis (32.8%) and even higher human seroprevalence (43.8%) (Fleury *et al.*, 2006). Among the seroprevalent persons, 9.1% were diagnosed with neurocysticercosis but mostly asymptomatic. Also in Mexico, another study showed that the main risk factor identified for porcine cysticercosis was free-range husbandry that enables access to human faeces (Rodriguez-Canul *et al.*, 1999). In Nepal, another endemic area of taeniasis, epidemiological assessment showed that *T. solium* infections are highly linked to: 1) meat producers and sellers; 2) animals' access to human feces in pig husbandry; and 3) nonhygienic slaughterhouses (Joshi *et al.*, 2001).
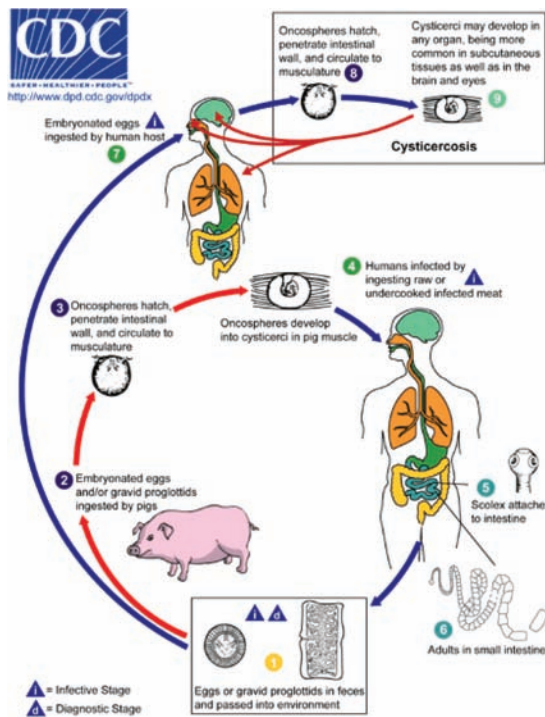
Figure 4.2.2.8.1. *Taenia solium* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Cysticercosis_il.htm)

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Chapter 4.2.2.9

Other Intestinal Cestode Infections

[*BERTIELLA* SPP., *RAILLIENTINA* SPP., *INERMICAPSIFER MADAGASCARIENSIS*, *I. CUBENSIS*, *MESOCESTOIDES VARIBILIS*, *M. LINEATUS*, *DIPLOGONOPORUS GRANDIS*]

Bertiella spp. (*studeri* and *mucromata*) were isolated from non-human primates; therefore, infections in humans (mostly in children) occur in South America, Africa and Asia where these animals are distributed (Howells *et al.*, 2011). Transmission is by accidental ingestion of the intermediate hosts (free-living mites-Oribatidae). *Raillientina* spp. (*dermerariensis*, *asiatica*, etc.) are tapeworms distributed in rodents, primates and humans (Figure 4.2.2.9.1). Intermediated hosts are insects (flour and corn beetles and cockroaches). *Inermicapsifer madagascariensis* (*I. cubensis*) cestodes are found in small rodents of tropical areas (Africa, Asia, Madagascar and Caribbean area) (Goldsmid and Muir, 1972; Gonzalez *et al.*, 1996; Hira, 1978). Free-living mites seem to be the intermediary hosts. *Mesocestoides varibilis* and *M. lineatus* are found in Africa, Asia, North and South America infecting humans, and yet other carnivores are their final hosts whereas free-living oribatid mites are the first intermediary hosts and various mammals (rodents), birds, amphibians and reptiles are the secondary intermediate hosts (Figure 4.2.2.9.2). Ingestion of undercooked viscera or the whole animal infected with these tapeworms induce infection in humans. Among this group of miscellaneous cestodes, *Diplogonoporus grandis* is the only one attributed to marine mammals such as whales (as final host). The intermediate hosts are saltwater fishes that also infect humans via uncooked or raw fish ingestion (most reports coming from Japan) with a life cycle similar to *D. latum* (see Figure 4.2.2.9.3). Clinical symptoms in humans are rare but massive cestodal intestinal colonization can cause abdominal pain, obstruction, diarrhea and nausea.

4.2.2.9.1 OTHER INTESTINAL CESTODES AND ENVIRONMENT

In the western part of Iran, different carnivores (dogs, foxes and jackals) have been studied for their intestinal helminthic content (Dalimi *et al.*, 2006). Among human infectious miscellaneous cestodes, *Mesocestoides lineatus*, were isolated at high frequency; in stray dogs (26.5%), red foxes (81.82%) and jackals (70%). In Costa Rica, Rodriguez *et al.* (2000) surveyed the helimithological fauna of cotton rat (*Sigmodon hispidus*)
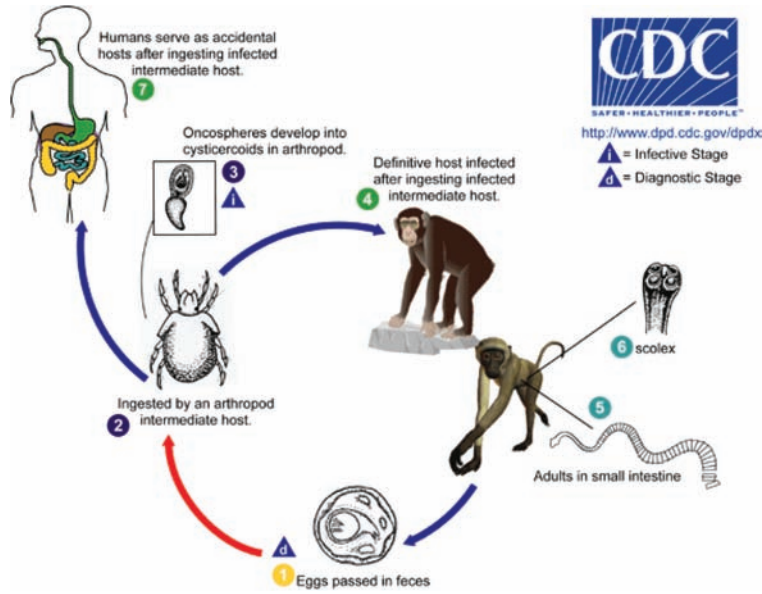
Figure 4.2.2.9.1 *Bertiella* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc. gov/dpdx/HTML/ImageLibrary/Bertiella_il.htm)
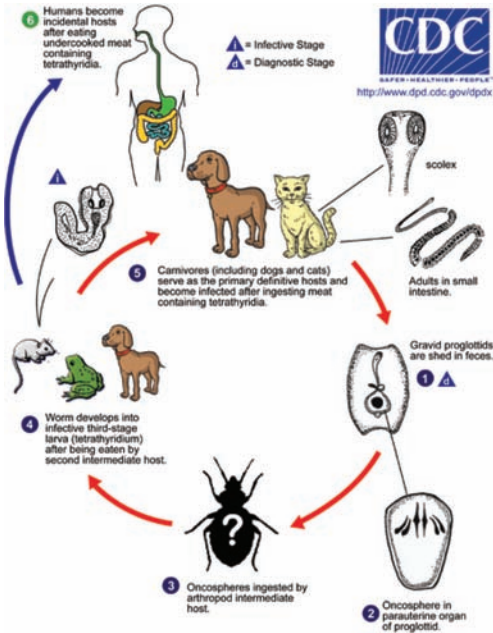
Figure 4.2.2.9.2. *Mesocestoides* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/dpdx/HTML/ImageLibrary/Mesocestoidiasis_il.htm)

known to be related to the habitat and feeding behavior of this rodent. Indeed, *Raillietina* spp. colonized cotton rats from extensive dry lands with high temperatures offering a heterogeneous diet, but not those cotton rat species originating from pineapple plantation with humid and mild temperatures.
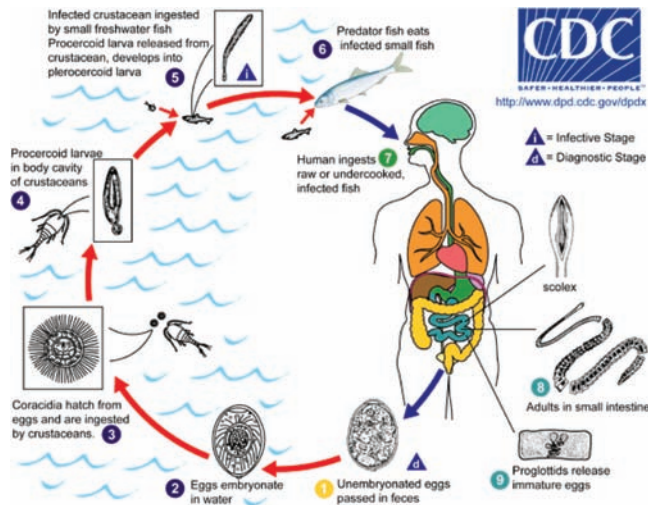
Figure 4.2.2.9.3. *Diphyllobothrium latum* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/dpdx/HTML/ImageLibrary/Diphyllobothriasis_il.htm)

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Chapter 4.2.3

Nematodes

A summary of nematodes global infection burden is presented in Table 4.2.3.1.

Table 4.2.3.1 Trematodes as a global threat for humans.

Nematodes	Global infection	Geographic distribution	Disease
Angiostrongylus cantonensis, Angiostrongylus costaricensis	Thousands	Australia, China, India, Southeast Asia and Pacific, Egypt, southern USA and Carribean Islands	Angiostrogyliasis (Cerebral angiostrongyliasis-easinophilic meningoencephalitis or eainophilic meningitis Intestinal Angiostrongyliasis
Anisakis simplex, Pseudoterranova decipiens	Rare, in Japan >1000 cases/year	France, Spain, Japan, The Netherlands, North America, Pacific coast of South America	Anisakiasis (Herring worm disease)
Capillaria hepatica, C. philippinensis, C. aerophila	Rare, 10 to >1000	North and South America, Africa, Asia and Central Europe, Philippines, Southeast Asia, Western Pacific, [Iran, Egypt, India and South America (sporadic)]	Capillariasis (hepatic, intestinal and pulmonary
Dioctophyma renale	Rare, tens of cases	Worldwide, North and South America, Asia, Europe	Dioctophymiasis
Dracunculus medinensis	In the past an estimated 3.5 million infections, since 1986 in decline, 2007 <10,000, 2011 on <1000	Africa, Asia (India)	Dracunculiasis (Guinea Worm Infection)
Ancylostoma caninum	Rare, hundreds	Northeastern Australia	Eosinophilic enetertis
Brugia malayi, B. timori	Estimated 120 million infection, one billion at risk	Asia, Africa, Central, South America and Pacific Island nations	Brugia filariasis (lymphatic filariasis)
Dirofilaria immitis, D. tenuis, D. ursi, D. striata	Rare, Approx. 800 reported cases, with increasing emergence	Worldwide (particularly in southern USA, Central and South America) and Spain, Italy, East Asia, Mediterenean area	Dirofilariasis
Gnathostoma spinigerum, G. hispidium,	Rare, but with high prevalence countries: 9000 cases in Mexico	Southeast Asia, Central America, Australia, Europe, Japan	Gnathostomiasis

(*Continued*)

Table 4.2.3.1 (*Continued*)

Nematodes	Global infection	Geographic distribution	Disease
G. doloresi, G. nipponicum	and Thailand; 4000 cases in Japan; 600 cases in Vietnam		
Gongylonema pulchrum	Rare, approx. 50 reported cases	Worldwide: United States, Germany, Iran, Japan, Laos, Morocco, China, Italy, New Zealand, and Egypt	Gongylonemiasis
Lagochilascariasis minor	Very rare, ~70 reported cases	Tropical areas (Central and South America, Carribean countries)	Lagochilascariasis
Ancylostoma braziliense, Uncinaria stenocephala, A. caninum, A. tubaeformae, Bunostomum phlebotonum	Reported 17,353 cases, estimated between 10,000 to 100,000	Subtropical and tropical climates: Southeastern United States, Caribbean, Africa, Central and South America, Southeast Asia, sub-Saharan Africa, Australia.	Cutaneous Larva Migrans (CLM)
Toxocara canis, Toxocara mystax, Baylisascaris procyonis	Estimated: Millions around the world; ~100,000 clinical cases in U.S., ~10,000 (OLM)	Worldwide	Visceral Larva migrans (VLM)
Oesophagostomum bifurcum, O. aculeatum, O. stephanostomum	~250,000 people are infected worldwide, with 1 million more at risk.	Oesophagostomum spp. are widely distributed wherever livestock is raised, but more common in the tropics and subtropics. The highest incidence in humans is in the northern regions of Togo and Ghana, where O. bifurcum (primarily a monkey parasite) appears to cycle naturally in the human populations. Sporadic cases in humans have also been recorded in Brazil, Malaysia,	Oesopahgostomiasis

Organism	Prevalence	Geographic distribution	Disease
Strongyloides stercoralis, S. fuelleborni, S. fuelleborni kellyi	An estimated 100–200 million persons are infected worldwide in 70 countries.	Indonesia, French Guiana, and West Africa. Strongyloides is endemic in tropical and subtropical areas with sporadic occurrence in temperate areas. Sub-Saharan Africa, South and Southeast Asia, Central America, and South America, and parts of Eastern Europe are considered endemic areas of *Strongyloides* infection. Worldwide prevalence is estimated as 2–20% in endemic areas.	Strongyloidiasis
Mammomonogamus laryngeus	Very rare: ~100 cases	Southeast Asia, India, Central and South America, Caribbean	Syngamiasis
Thelazia callipaeda, T. californiensis	Very rare: ~257 cases	Asia, Europe, western North America	Thelaziasis
Trichinella spiralis, T. native, T. brivoti, T. pseudospiralis, T. murrelli, T. nelson, T. papue and T. zimbabwensis	~27 million people infected	Worldwide, except Australia. Higher prevalence in temperate climates	Trichinosis
Genera *Trichostrongylus, Marshallgia, Mecistocirrus* Other Nematodes: *Ancylostyoma ceylonicum, Physaloptera caucasica, Temidens deminatus*	Estimated prevalence of 5.5 million people	Worldwide, in Middle East and Asia higher prevalence.	Trichostrongylidiasis (Trichostrongylosis)

Chapter 4.2.3.1

Angiostrongyliasis

Cerebral Angiostrongyliasis (Eosinophilic Meningoencephalitis or Eosinophilic Meningitis) Intestinal Angistrongyliasis

[*ANGIOSTRONGYLUS CANTONENSIS*, *ANGIOSTRONGYLUS COSTARICENSIS*]

The nematode (roundworm) *Angiostrongylus cantonensis* is a parasite that infects humans and causes eosinophilic meningitis. Rodents are the definitive hosts while snails are intermediary hosts. Human infections have been reported from Southeast Asia and the Pacific Basin but recently *Angiostrongylus costaricensis* a closely related worm that causes intestinal Angiostrongyliasis in Central and South America has been also reported. Transmission in humans as accidental final host occurs by ingestion of larvae from raw or undercooked infected snails or contaminated water and vegetables. Other paratenic hosts that humans may consume raw are crabs, crayfishes, freshwater fishes. Life cycle consists of mature roundworms leaiving in rats' pulmonary arteries where eggs are deposited. Larvae hatch from eggs and penetrate alveoli to be coughed up, swallowed and excreted through feces. In water, larvae invade snails to develop internally into infective larvae. Ingestion of an intermediate or paratenic host by the final host (rodents and accidentally humans) produces infection. In final host, *A. cantonensis* larvae invade the intestinal wall to further spread hematogenously to the brain and further migrate via the venous system to the pulmonary artery and heart (right side) (Ramirez-Avila *et al.*, 2009) (Figure 4.2.3.1). In humans, brain larvae are enclosed in granulomas where they end their lives. Clinical manifestations depend on infection burden but major symptoms are severe headaches, vomiting, fever, convulsions, facial paralysis, sleep disturbances, paresthesia, spech impediment, hearing disorders, paresis and death. In *A. costaricensis* infections, ingested larvae migrate to intestinal arteries and small blood vessels where they mature into sexually productive roundworms to produce eggs. Released eggs accumulate in intestinal blood capillaries (ileum) to develop into first-stage larvae that are subsequently excreted in feces. Clinical manifestations are induction of necrotic alterations in appendix, cecum and ascending colon, abdominal pain, fever, anorexia, vomiting, diarrhea or constipation and death in heavy infections.
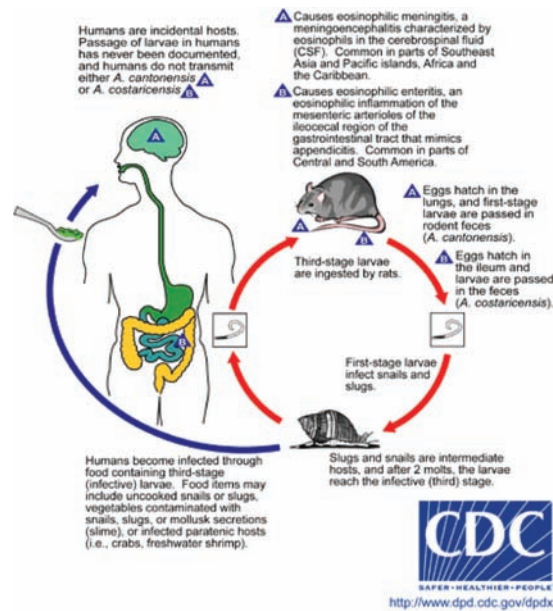
Figure 4.2.3.1. *Angiostrongylus cantonensis* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Angiostrongyliasis_il.htm)

4.2.3.1.1 ANGIOSTRONGYLIASIS AND ENVIRONMENT

In recent years, population shifts and rapid transport have facilitated the spread of certain helminthic diseases from endemic to non-endemic areas (Polley, 2005). For example, with the increasing maritime world trade have dispersed this parasite in many continents, mainly carried by cargo ship rats (Pien and Pien, 1999). Among the many helminthic parasites known to cause various human diseases affecting millions of people worldwide, neurohelminthiases (e.g. *A. cantonensis*) are often undiagnosed because of clinicians' limited knowledge of exotic parasites or unavailable confirmatory diagnostic tests (Nishimura and Hung, 1997). Diaz (2008) described recently that *Angiostrongylus cantonensis* became an endemic eosinophilic meningitis in the US, following the importation of infected rats and African land snails on container ships, as biological controls and exotic pets. *A. cantonensis* is now enzootic in Louisiana and has caused eosinophilic meningitis in humans. Chikweto *et al.* (2009) described a prevalence of 23.4% of *A. cantonensis* in rats in Granada, revealing geographical expansion. Prociv and Carlisle (2001) in Australia have described a real evidence of the rapid expansion of this parasite. Since the 70s, an increasing numbers of autochthonous infections have been reported in Australia (southeast Queensland and northern New South Wales) in a variety of animals including humans, rats, dogs, horses, flying foxes and marsupials. In the 90s, the parasite was isolated from a dog in Sydney (∼1000 km south) and since then increasing numbers of infections were reported in dogs, flying foxes, marsupials and zoo primates. The authors suggested that ingestion of native or exotic snails and other molluscs as the intermediate hosts helped to spread this parasite. In contrast, an almost identical native species, *A. mackerrasae* was not identified as being competent to infect people (or other non-rodent hosts) as all roundworms recovered from a fatal human case and from many animal infections, have been confirmed as

A. cantonensis. Finally, exotic food preparation with raw meat from snails or slugs, prawns, frogs, fish or planarians is also a source of *A. cantonensis* infection, especially for tourists (Wang *et al.*, 2008; Malvy *et al.*, 2008). Indeed, Eamsobhana *et al.* (2009) showed that, in spite of the addition of flavoring spices and alcohol to a certain Thai speciality dish, a complete inactivation of *A. cantonensis* larvae found in snail meat has not been accomplished. Kramer *et al.* (1998) were the first to report an intestinal (abdominal) angiostrongyliasis (*A. costaricensis* parasite) outbreak attributed to consumption of raw mint in Guatemala. The authors speculated that the mint leaves were contaminated with the mucous secretions of infected mollusks or that infected mollusks or planarians remained adherent onto the mint leaves even after wash.

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Chapter 4.2.3.2

Anisakiasis (Herring Worm Disease)

[*ANISAKIS SIMPLEX, PSEUDOTERRANOVA DECIPIENS*]

The parasitic nematodes *Anisakis simplex* and *Pseudoterranova decipiens* (now including ∼5 sibling species) cause human infection following consumption of raw or undercooked saltwater fishes (e.g., salmon, sardine, mackerel, cod, flounder, etc.) and squids (Repiso *et al.*, 2003; Kleter *et al.*, 2009; Arizono *et al.*, 2011; Skirnisson, 2006). This marine fauna carry the parasite in their body cavities, muscles and tissue with high prevalence (up to 100%), and consequently raw consumption of their meat has a high risk of humans' infection. The parasite distribution is worldwide except in marine areas where cetaceans are rare. Human cases involving *Anisakis simplex* have been reported from Japan (>1000 cases/year), Spain, France, The Netherlands and North America and cases involving *Pseudoterranova* spp. from the northern Pacific area (Arizono *et al.*, 2011). Life cycle of these parasites is mainly aquatic (marine environment) with humans as accidental hosts. Infected marine mammals with adult roundworms excrete unembryonated eggs into sea or ocean water (Aspholm *et al.*, 1995). Eggs embryonation occurs in water and L2 type larvae develop. L2 larvae hatch, swim freely and are ingested by a first intermediary host (mostly crustaceans) where they mature into L3 larvae. Squids and various saltwater fishes ingest L3 larvae where upon the host's death, the L3 larvae migrate to muscle tissue (Muenker & Karl, 2001). Predation by other fishes or marine mammals (whales, seals and dolphins) transfers L3 larvae to a second intermediary host or in the case of marine mammals to the final host. At this point, humans enter the life cycle of this parasite as an accidental player by consuming raw infected fish and turn into final hosts (in humans *Anisakis simplex* larvae invade intestinal tract walls-from the esophagus to the rectum while *Pseudoterranova* spp. favour stomach wall) (McClelland, 2002). In marine mammals, the L3 larvae stage molts twice to develop into an egg producing adult roundworm (Figure 4.2.3.2.1). Clinical signs are severe epigastric pains, nausea, vomiting, blood in gastric juice and feces an outcome of ulcerative lesions, stenosis, intestinal perforation and pruritis, urticaria and anaphylaxis as instant-type hypersensitivity. Since larvae do not survive longer that one week in humans, the disease is self-limiting.
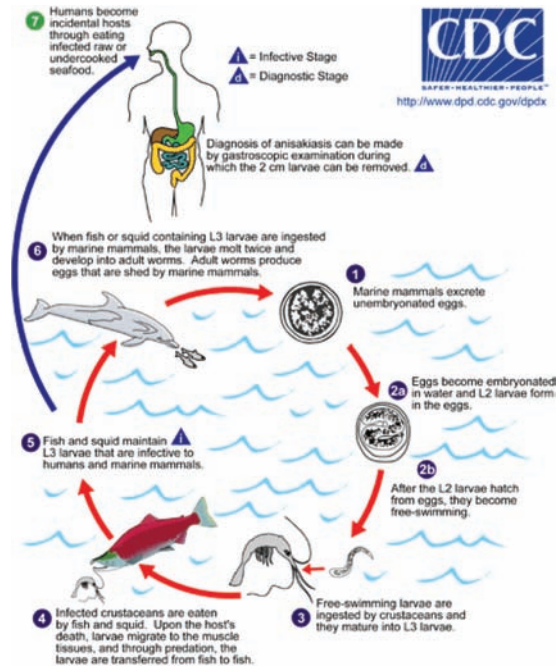
Figure 4.2.3.2.1. *Anisakis simplex* life cycle (with permission from CDC, Atlanta, USA: http://dpd.cdc.gov/dpdx/HTML/Anisakiasis.htm)

4.2.3.2.1 ANISAKIASIS AND ENVIRONMENT

Besprozvannykh *et al.* (2003) studied various fish species found in the Gulf of Peter The Great (the largest gulf of the Sea of Japan) for infection with these nematodes. The highest infection rates with *Anisakis simplex* have been found in chum salmon, masked greenling and Alaska pollock species. The high infection rate has been explained based on the fish seasonal migration into open marine space and back into the gulf waters while feeding on deep-sea (herrings) and bottom-dwelling (bases) fishes infected by other bottom-dwelling invertebrates (the first intermediate hosts).

Noguera *et al.* (2009) reported on a frequent encounter of the "red vent syndrome" of wild Atlantic salmon (*Salmo salar*) returning to various rivers in Scotland. The syndrome revealed itself as swollen, hemorrhagic (sometimes severe bleeding), skin erosion and scale loss of the vent (the anus adjacent to the opening of fish from where it excretes sperms or eggs), predominantly found in 1-sea-winter grilse but also in 2-sea winter salmons, without any apparent health deterioration, mortality or spawning inhibition. The researcher isolated high numbers of *A. simplex* from the infected vents, an unusual anatomic site for this parasite and hypothesized that this is a new parasite species. However, molecular studies did not confirm this hypothesis and therefore another theory has been proposed. The new assumption has been that ocean-scale environmental changes are involved in this syndrome's intensity. The most logical factor suspected at ocean scale (and above), is water temperature that can impact both parasite cycles and its host. North Atlantic sea water warming has been recorded, with Scottish sea-surface temperatures rising 0.2 to 0.6°C per decade including the foraging areas exploited by salmon (with seven out of ten warmest years occurring in the last decade, with 2006 being the second warmest

year in UK coastal waters since records began in 1870). In addition, these authors mentioned that the 15°C summer isotherm has moved 330 km north in the last 17 yr. Therefore, temperature increase in the eastern North Atlantic pelagic ecosystem might favor different parasites, as already has been shown in terrestrial ecosystems (Kutz *et al.*, 2004). Due to spatial and temporal differences, salmon might face reduced availability in prey, and the need to compete (temporary absence of a given prey), or variability (different feeding options, e.g., pelagic swarmfish), leading to a shift in their diet hence exposing them to increased *Anisakis* spp. loads (Klimpel *et al.*, 2008). Klimpel *et al.'s* (2004) study supports these findings by demonstrating that this nematode is able to select its definite host species depending on the locality, apparently having a very low level of host specificity. From the epidemiological point of view, an increase in human allergic and gastro-allergic reactions to *A. simplex* has been reported in Spain, possibly reflecting changes in culinary and feeding habits or improved diagnosis, but also an actual general increase in anisakid parasite prevalence in a wide range of fish hosts, again related to low host specificity (Valero *et al.*, 2006). In Peru, Cabrera *et al.* (2004), suggested concerning a current epidemy and the probable emergence of anisakidosis in this country that besides increased raw fish consumption the "El Niño" weather phenomenon might also be involved in the elevated prevalence of this nematode.

In relation to environmental pollution, evidence has started to pile up of an interesting feature of *A. simplex* related to this external pollution (Sures, 2004). Fish exposure to heavy metals revealed a much higher concentration of heavey metals in the colonizing parasite in comparison with the surrounding fish tissues (Pascual and Abollo, 2003; Jankovska *et al.*, 2011). These authors by means of atomic adsorption spectrometry found that L3 larvae and adult infra-populations of the whaleworm *Anisakis simplex* from their respective fish, cephalopod and cetacean hosts caught or stranded in the Galician coast (north-west Spain) accumulated lead and copper to levels far in excess of their host tissues. The immediate question should be: Why do these parasites concentrate heavy metals (a real biohazard) within their tissues? Screening the published literature on this subject, we were not able to find or this type of query, or answer it either. However, several explanatory directions can be reasonable speculated: 1) the parasite tissue is by definition a good milieu for host molecule adsorption (including pollutants) in order to accomplish its parasitic functions without much energy investment, leading to heavy metals extensive accumulation; 2) by accumulating higher heavy metals concentrations (actively or passively) the parasite defends itself against the host's immunological system. Kolinkoeva *et al.* (1988) showed that lead and cadmium were the leading heavy metals that inhibited spermatozoa agglutination by antiserum. Karmaus *et al.* (2001) also looked at exposure to heavy metals and their effect on immunoglobulin levels in children. These authors identified a linear, statistically significant positive association between blood lead concentrations and IgE levels.

In environments where saltwater (sea) and freshwater (rivers) meet (mainly estuaries), anisakids (e.g., *Pseudoterranova decipiens*) transfer have been reported (Rausch and Adams, 2000). Burbot, a freshwater fish, could become infected (as paratenic host) by three interactions: devouring marine fishes in brackish waters at river mouths, consuming marine fishes that enter the lower reaches of rivers and by preying on anadromous fishes on their up -river migration.

Deep oceans present high pressures and cold temperatures as additional harsh environmental factors faced by anisakids. *P. decipiens* has been isolated from 23 fish species at two different sites in the Antarctic (Weddell Sea and South Shetland Islands) (Palm, 1999). The Weddell Sea is estimated to have the clearest water of any sea while South Shetland Islands harbor cold climate vegetation (mosses, lichens and algae) where seabirds, penguins and seals feed in the surrounding waters. *P. decipiens* occurred at water depths of 80 to 820 m, with high prevalence in transport host fishes that feed mostly on benthic nototheniid fish species (as nematode accumulators) surrounding the South Shetland area.

Bathypelagic, pelagic, or mainly euphausid feeding fish species were only lightly infested, thus demonstrating the benthic life cycle of *P. decipiens* in the Arctic. This author also reported on the parasite's preference to infest fish body cavity and liver and not the usual fish musculature, possibly explained by low temperature effect. Overall, this roundworm seems to be well established in the extreme Antarctic environment (with subzero temperatures).

The parasite's successful survival and distribution under extreme cold conditions has been attributed to trehalose (a biological cryptobioprotectant) accumulation as a general response to thermal stress (Stormo *et al.*, 2009). Interestingly, acclimation to high temperatures triggered trehalose accumulation to an even greater extent than cold acclimation, a process that can be viewed evolutionarily as adaptation to heat rather than coldness, thus providing these nematodes with a moderate freeze tolerance.

Infection of secondary intermediate host such as smelts (*Osmerus eperlanus*) with *P. decipiens* has been studied in connection with their swimming capabilities and anthropogenic interventions (Rohlwing *et al.*, 1998). Infection of fish musculature with larval stage 3 induced reduced swimming speed as observed by frequent collisions of infected fishes with the screen system of the cooling water inflow of a power plant. The authors emphasized the vulnerability of speed-limited infested fishes to anthropogenic activities such as suction by power plant cooling systems and/or enhanced removal by trawl fisheries, both inducing removal of infested fishes, thus in turn reducing the parasitic third-stage larvae in the local smelt population.

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Chapter 4.2.3.3

Capillariasis (hepatic, intestinal and pulmonary)

[*CAPILLARIA HEPATICA, C. PHILIPPINENSIS, C.AEROPHILA*]

Among capillariasis forms, hepatic capillariasis in spite of its rarity is the most life threatening one. The disease is caused by *Capillaria hepatica*, a nematode that invades liver parenchyma in many mammals including humans, without intermediary hosts (so called a one -host life-cycle parasite). Cases (~50) have been reported from North and South America, Africa, Asia and Central Europe. Transmission occurs in humans (as accidental hosts) through embryonated eggs ingestion from soil contaminated by carcasses of dead rodents (prevalence in wild rats can reach 100%) (Conlogue *et al.*, 1979; Farhang-Azad, 1977) (Figure 4.2.3.3.1). Infected rodents do not excrete eggs in feces as the eggs are enclosed within their host in granulomas and can be released only after tissue decomposition. Soil contamination with unembryonated eggs starts with the final predominant host, rat (but also other rodents, monkeys and predators), death (caused by disease or cannibalism), decomposition or ingestion by a predator/scavenger and direct infection with tissue containing eggs. After weeks in soil environment, eggs embryonate and become infective. If a human by accident ingests contaminated soil or something else, larvae hatch from eggs to invade the intestinal wall to further reach the liver via the portal system. Clinical manifestations depend on the infection burden; light infections are mostly asymptomatic while heavy infection will result in extended liver damage, hepatitis, extrahepatic symptoms (lung diseases) and death (primarily in children). In moderate infections, epigastric complaints, hepatoslenomegaly, eosinophilia, leukocytosis were reported.

Capillaria philippinensis infections are also severe with lethal outcome if untreated (el-Karaksy *et al.*, 2004). *C. philippinensis* was primarily reported from the Philippines but now it has been reported from other geographic areas, such as Southeast Asia, Western Pacific, and sporadic cases from Iran, Egypt, South America and India (Hoghooghi-Rad *et al.*, 1987). Different piscivorous birds are the natural hosts and reservoirs of this nematode (Cross and Basaca-Sevilla, 1991) (Figure 4.2.3.3.2). Infection occurs in humans through the ingestion of raw/undercooked infected fishes with larvae caught from fresh or brackish-water or via the unusual autoinfection through larviparous females producing larvae without

leaving human body. In nature, unembryonated eggs reach water from infected birds or humans, to further embryonate and be ingested by present fishes. In fishes, embryonated eggs hatch to realease larvae that infect fishes as intermediary/paratenic hosts. The cycle is completed when pescivorous birds or humans ingest the infective larval content of fishes. Clinical manifestations also depend on the larvae number ingested or developed through autoinfection. Slight infection shows abdominal pain, diarrhea, anorexia, and weakness, while more severe infections result in weight loss, vomiting, maladsorption and diarrhea, muscle wasting, pneumonia and death.
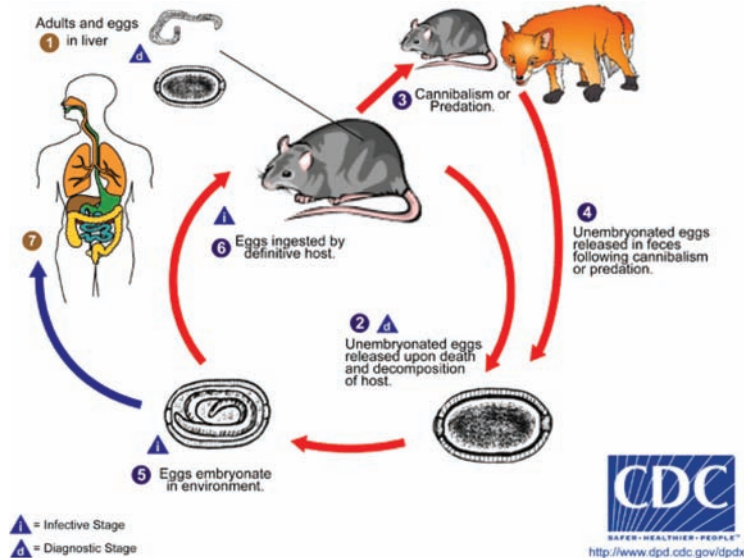
Figure 4.2.3.3.1. *Capillaria hepatica* life cycle (with permission from CDC, Atlanta, USA; http://dpd.cdc. gov/dpdx/HTML/Capillariasis.htm)

C. aerophila is perhaps the rarest human infection (so far~20 cases) which causes pulmonary capillariasis. This nematode can be found in a large list of predatory mammals (wild and domestic) such as: cats, dogs, foxes, mongooses, bears, marten spp., etc., and other animals such as hedgehogs and opossums (Henke *et al.*, 2002; Magi *et al.*, 2009; Beck, 2007; Thiess *et al.*, 2001; Yasuda *et al.*, 1994; Fiorello *et al.*, 2006). The life cycle is similar to that of *C. hepatica*, meaning that it can be completed in one host. In an infected host, the adult nematode lays eggs in the lungs from where they are coughed up and swallowed to reach feces and are excreted on to soil. In soil, infective larvae develop within excreted eggs to remain infective for up to one year. Contaminated soil ingestion by a suitable host (in humans, children are the ones frequently in contact with soil) releases the infective larvae from eggs in the intestine which migrate further to the lungs. Clinical manifestations are therefore bronchitis, coughing, bloody sputum, eosynophilia and dyspenia.

4.2.3.3.1 CAPILLARIASIS AND ENVIRONMENT

The long-term exposure of parasite eggs in soil to changing environmental conditions and their persistence may increase the reemergences of these parasites (Traversa *et al.*, 2010; Traversa *et al.*, 2009). Lee *et al.*

(2008) reported that among a large variety of bacterial, protozoan and helminthic pathogens in large-billed crow feces (from an urban setting in Kuala Lumpur, Malaysia), *C. hepatica* ova have been detected. Crows are known to feed on carcasses and therefore should be included as possible biovectors. In relation to birds, Cross (1990) described how, from an isolated report of a *C. philippinensis* infection (in 1963), a few years later epidemics of intestinal capillariasis have been reported from other Philippines areas, Thailand, Japan, Iran, Middle East (Egypt) and recently from Romania (Aftandelians *et al.*, 1977; Mircean *et al.*, 2010). He considered that, based on the life cycle of this parasite (small fresh and brackish water fishes as intermediate hosts), it might definitely be feasible that migratory piscivorous birds that feeds on these fishes may spread the parasite globally.
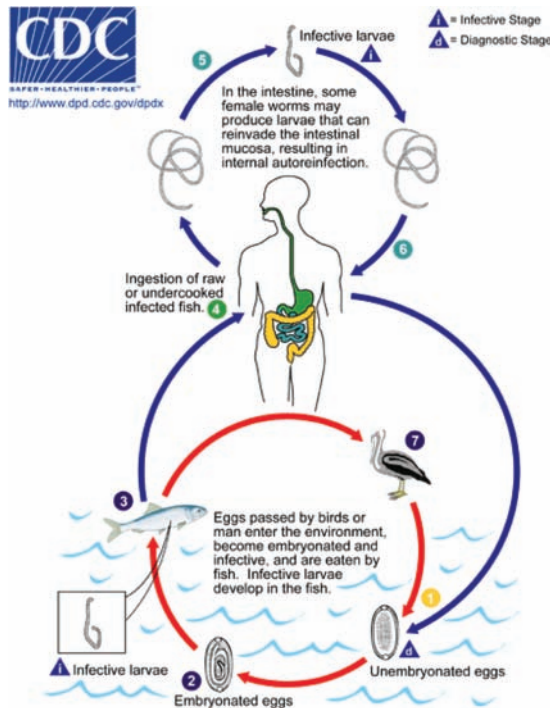
Figure 4.2.3.3.2. *Capillaria philippinensis* and *aerophila* life cycle (with permission from CDC, Atlanta, USA; http://dpd.cdc.gov/dpdx/HTML/Capillariasis.htm)

Barutzki *et al.* (1987) reported that among European hedgehogs' *C. aerophila* prevalence was 15.1 to 40.7% and concluded that these parasites are able to survive the winter in the host during the hibernation period. Here it should be mentioned that earthworms might be involved in transmission (even not as a necessary intermediate host, similar to the case of *T. saginata* eggs) since hedgehogs do feed on earthworms in addition to other soil insects, but further research is needed to substantiate this hypothesis. In Iceland, a cold climate area, arctic foxes have been screened for different parasites and *C. aerophila* has been found, a new record for this big island (Skirnisson *et al.*, 1993), as well demonstrating this parasite's excellent survival in warm as well very cold climates.

In Hungary, an extraintestinal nematode survey in red foxes revealed a high prevalence of *C. aerophila* (66%) suggesting that dogs and cats may be infected due to fox colonization of urban areas (Sreter *et al.*, 2003). Similar high prevalences of *C. aerophila* in wild foxes has been reported from Holland (46.8%), Denmark (74.1%) and Norway (88%) (Borgsteede, 1984; Saeed *et al.*, 2006; Davidson *et al.*, 2006).

Finally, a recent report on pseudoparasitism involving *C. hepatica* in Brazil has been published (Carvalho-Costa *et al.*, 2009). The authors described the isolation of *C. hepatica* eggs in a fecal sample of an old woman in a riverine community. The egg source was probably consumption of an infected liver of a tapir, but in this particular case they passed through the gut and eliminated without hepatic symptoms. The authors suggested that the evolution of these eggs to infective stages in environment and the poor sanitation of such communities could enable a high occurrence risk of hepatic disease in humans caused by this nematode.

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Chapter 4.2.3.4

Dioctophymiasis

[*DIOCTOPHYMA RENALE*]

Dioctophymiasis is caused by infection with the "giant kidney worm" *Dioctophyma renale*. Humans are infected by consumption of raw fish or frog meat or by accidental ingestion of aquatic oligochaetes.

The distribution is worldwide but with a very low number of clinical cases reported. This nematode infects dogs, cats, mustelids, foxes, raccoons and pigs. The life cycle consists of *D. renale* eggs being shed by the parasitized animal (or human) in urine that contaminate water sources. In water, the first-stage larvae develop in eggs that are ingested by intermediate hosts such as aquatic oligochaetes (annelid worms, e.g. *Lumbriculus variegatus*). In oligochaetes intermediate hosts, larvae develop further to reach third-stage larvae in their blood vessels. At this stage ingestion by a paratenic host that feed on aquatic worms (e.g., fish or frogs) can take place. If not passed, in paratenic hosts the larvae encyst and stop their adult roundworms maturation. Proviso the paratenic hosts are ingested by carnivores or humans, larvae from the paratenic host migrate through the peritoneal cavity to kidney (to settle in parenchyma or renal pelvis) (Figure 4.2.3.4.1). Clinical manifestations are renal colic, haematuria and hydronephrosis (due to renal obstruction).

4.2.3.4.1 DIOCTOPHYMIASIS AND ENVIRONMENT

In spite of many known carnivores reported as major final hosts of *D. renale*, Ishizaki *et al.* (2010) recently reported on a capuchin monkey infected with this nematode. Capuchin monkeys are known to feed on fish among others things and therefore in nature it is very possible that these infections are more prevalent among primates feeding on fish then thought before (Hellner-Burris *et al.*, 2010).

Among fishes infected with *D. renale*, Measures and Anderson (1985) reported a 5 to 23% prevalence in pumpkinseed sunfish (*Lepomis gibbosus*) in three Canadian lakes. It should be mentioned that sunfishes are subject to sport fishing, therefore have been intentionally introduced in many areas outside their original ranges to turn out to be an invasive species. Whether these ecological changes in the paratenic host distribution might increase the risk of infection in humans should be further studied.
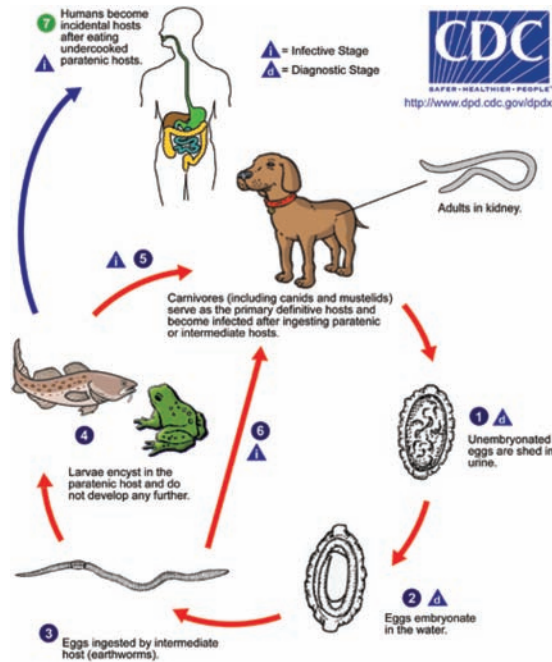
Figure 4.2.3.4.1. *Dioctophyma renale* life cycle (with permission from CDC, Atlanta, USA; http://dpd.cdc.gov/dpdx/HTML/Dioctophymiasis.htm)

An interesting issue raised in connection to another environmental contaminant: mercury (Klenavic *et al.*, 2008). These authors measured mercury concentrations in different organs of two semi-aquatic fish-eating mammals: wild mink and river otter. Notably, they reported that mink infected by *D. renale* and exposed to mercury had higher mercury levels compared to the uninfected. As the kidney is the main organ involved in the removal of mercury in urine (Baselt, 2008), parasitic infection of this organ and serious damage will affect excretion and cause further damage to other internal organs in mammals.

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Chapter 4.2.3.5

Dracunculiasis (Guinea worm infection)

[*DRACUNCULUS MEDINENSIS*]

Dracunculiasis is a disease caused by one of the longest nematodes: *Dracunculus medinensis* also known as Guinea worm. Humans are the final hosts. Prior to 1986, the disease affected millions of people with a high distribution in Africa and Asia (India), mainly in arid zones, while now is in a very steep decline and expected to be eradicated almost completely. Transmission occurs through accidental ingestion of unclean water containing small crustaceans (*Cyclops* spp.) that exercise as intermediate hosts. The life cycle of this parasite consists of rhabtidiform larvae that penetrate the digestive tract wall and locate in the connective tissues. The adult worms (a very elongated female and a short male morphology) reproduce and gravid females migrate to subcutaneous tissue of human organs (feet) that have contact with water and discharge larvae (L1 stage) through a skin blister into surrounding water. Larvae (L1 stage) in water are consumed by crustaceans that become infected (as intermediary host). Inside crustaceans, the larvae pass two molts to form the final infective L3 stage larvae. Final ingestion of water containing infected crustaceans transfers the infective larvae (L3 stage) to humans (Figure 4.2.3.5.1). Clinical manifestations are primarily asymptomatic but migration of the adult gravid female to her final destination (skin) causes: local pain, pruritis, fever, nausea, ulcerative blister and abscesses due to secondary infection.

4.2.3.5.1 DRACUNCULIASIS AND ENVIRONMENT

During the preparation of this book, we were happy to realize that probably this disease has been practically erradicated completely through education and water sources protection. However, remote rural areas still serve as potential foci for reemergence, especially in low socio-economic societies in developing countries. It can be only hypothesized that if a future peril exists it may result from an evolutionary process, i.e., a similar mammal parasite will adapt through a common intermediary host to humans! Bimi *et al.* (2005), sequencing the genes coding for the small-subunit rRNA (18S rRNA) gene, have been able to provide a tool to differentiate between two Dracunculus species: *D. medinensis* (human host) and

D. insignis (dog host). Morphologically, those two guinea worms are undistinguishable but valid identification using molecular tools is now possible.
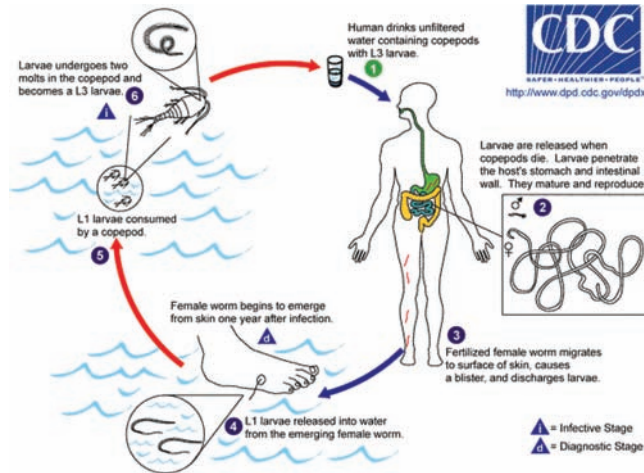
Figure 4.2.3.5.1. *Dracunculus medinensis* life cycle (with permission from CDC, Atlanta, USA; http://dpd. cdc.gov/dpdx/HTML/Dracunculiasis.htm)

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Chapter 4.2.3.6

Eosinophilic enteritis

[*ANCYLOSTOMA CANINUM*]

Ancylostoma caninum is the etiological factor that causes eosinophilic enteritis in humans. The disease has been reported mainly from Australia, where infected patients revealed hypersensitivity to this nematode (Prociv and Croese, 1990). Transmission occurs from infected young dogs and is mainly limited to northeastern regions of Australia with a few hundred cases reported, but it has also a worldwide distribution: North and South America, Asia and Europe. The parasite colonizes human gut, but at low numbers with reduced size and mostly infertile matures.

The life cycle of *A. caninum* consists of third-stage larvae from dog feces that are infective stage of both dogs and humans. In humans, larvae invasion is through skin while in dogs via root sheaths and carried through the blood vessels to heart and lungs. Afterward, larvae penetrate pulmonary alveoli to further ascend the bronchial tree to pharynx and finally are swallowed. Once swallowed, they move into the small intestine to mature and produce eggs (as mentioned earlier, in humans they are infertile). Adult worms living attached to the intestinal wall suck mucosal plugs to access the host's blood (Figure 4.2.3.6.1). Clinical manifestations are mostly focal reaction to infection expressed as inflammation, ulceration, epigastric colic, nausea, vomiting, and diarrhea, and in severe cases acute abdomen.

4.2.3.6.1 EOSINOPHILIC ENTERITIS AND ENVIRONMENT

Tarsitano *et al.* (2010) surveyed dog feces and bird guano in the city of Bari (Apulia region, Italy) for parasites on various sites, such as park green areas, playgrounds, public housing, parkways and school. Among the different parasites, this group reported *Ancyllostoma caninum* eggs in 9.2% of the canine fecal samples. Therefore this parasite is present in European dogs; however, due to its specific water related transmission, it seems that human infection is nadir in the semi-arid areas of Italy. Croese (1995) in Australia has underlined favorable conditions for transmission of this hookworm. In his retrospective study, the number of eosinophilic enteritis patients decreased in June and remained at this level for the rest of the winter. An infection rate boost was observed in spring proceeding the wet season (monsoon). The author obviously linked the infection prevalence with climatic conditions and also suggested that

"extraneous infections are promoted by the irrigation of domestic gardens and possibly endogenous reinfection of the gut by hypobiotic larvae". In Nigeria, Onwuliri *et al.* (1993) reported also on the fecal isolation of *A. caninum* from dogs and pigs and found a direct relation between ecological factors (rainfall and temperature) and the parasites' prevalence. In a more extensive study in Kaduna metropolis (Nigeria), Maikai *et al.* (2008) surveyed soil samples for the presence of helminth eggs. *Ancylostoma* spp. had a prevalence of 9% and as for other screened parasites, a significant association with rainy periods was revealed, as well as the impact of the presence of dogs and vegetation in the studied area. It is also clear that dog ownership is also a significant factor especially since dogs in this area revealed a high infestation prevalence. Perec-Matysiak *et al.* (2008) in a much more northern area (Wroclaw, Poland) also reported on *A. caninum* egg presence in soil samples (from parks, backyards, playgrounds and city squares) at a prevalence of 4.9% (city squares having the highest rate of contamination). Also in Poland in a recreational area (Wolin Island), soil samples and feces of boars and foxes have been surveyed for helminth eggs. *Ancylostoma caninum* has been recovered only from fox feces (18.2%) and is absent in soil samples, in spite of the fact that these animals are free ranging in the area. The authors suggested that soil characteristics (loose sand soil) as well the behavior of the parasite hosts (boars and foxes) do not favor transmission.

Granzer and Haas (1991) studied the response of *A. caninum* larvae to environmental and host stimuli in order to facilitate infection. They indicated four behavioral phases: 1) larvae snake-like movement stimulated by warmth and subject to defined vibrations; 2) waving behavior as a prerequisite for passive change-over to dog hairs, stimulated by heat radiation and CO_2 concentration, warmth and humidity; 3) creeping direction, where larvae are attracted by very low temperature gradients and also by skin hydrophilic compounds (but not lipids or serum); and 4) penetration stimulated by heat and dog's hydrophilic skin compound and serum. All these factors are micro-environmental factors affecting dogs' infection when in close proximity to larvae in water milieu. These results may help in the development of a potential trap to clean *A. caninum* larvae infested water.
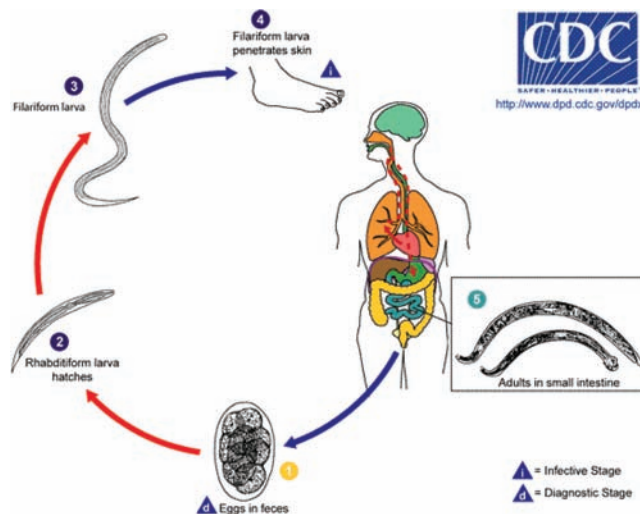
Figure 4.2.3.6.1. *Ancylostoma caninum* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/dpdx/HTML/Hookworm.htm)

Finally, Sciacca *et al.* (2002) presented results of a study carried out on the response of helminths larvae to CO_2 concentrations, among them *A. caninum* third-stage larvae (L3). They found that at exposure to 5% CO_2, *A. caninum* L3 larvae stopped crawling for 1 minute, becaming active again 9 to14 minutes after CO_2 (5%) exposure was terminated. In the same experimental set-up, activity resumed more rapidly (10 to15 sesonds) if L3 larvae were subsequently exposed to breath or breath through the scrubber (for CO_2 removal) (CO_2 concentrations in human breath is 3.3 to 4%). This study also suggests that humans breath out additional volatile organic compounds that may attract larvae, beside the recognized gases.

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Chapter 4.2.3.7

Filariasis

[*Brugia malayi*, *B. timori*, *Dorofilaria immitis*- real zoonoses] [*Onchocerca volvulus*, *Wuchereria bancrofti*, *Dipetalonema perstans*, *D. streptocerca*, *Mansonella ozzardi*, *Loa loa*, *Onchocerca lienalis*, *O. gutturosa*, etc.-pseuodo zoonoses]

4.2.3.7.1 GENERAL

The human is the definitive host of nine known filarial nematodes, divided in three groups according to their infection niche: lymphatic (*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*) (Okon *et al.*, 2010), subcutaneous (*Loa loa*, *Mansonella streptocerca*, *Onchocerca volvulus*, and *Dracunculus medinensis*-see chapter 4.2.3.5) and serous cavity filariases *(Mansonella perstans* and *Mansonella ozzardi)*. All these nematodic parasites are vectorally transmitted by insects (mosquitoes or flies) or by copepods (see *Dracunculus medinensis*) (Figure 4.2.3.5.1).

4.2.3.7.2 BRUGIA FILARIASIS (LYMPHATIC FILARIASIS)

Brugia filariasis is a disease caused by the nematodes *Brugia malayi* and *Brugia timori*, often asymptomatic but also with severe outcomes. *B. malayi* is mostly distributed in South, Southeast and East Asia (India, Thailand, Vietnam, Burma, Philippines) while *B. timori* has been reported from Lesser Sunda Islands of Indonesia, all characterized by presence in humid tropical and subtropical regions. *B. malayi* has two strains with different animal reservoirs significance: 1) the nocturnal periodic strain (found in peripheral blood during the night and in the lung capillaries during the day) found also in various animals but usually transmitted from human to human by night-active mosquitoes (*Aedes* and *Mansonia* spp.) in areas of open plains with intensive farming and 2) a sub-periodic strain (found in peripheral blood without notable periodicity) transmitted in rainforest areas by *Mansonia* spp. mosquitoes, active day and night with dogs, cats and wildfelids as reservoirs. In Indonesia, the location of *B. timori*, felids and pangolins use as reservoirs. Transmission occurs through bites of L3 larvae infected mosquitoes. The life cycle of the parasites includes a mosquito infected with L3 larvae that takes a blood meal from humans or animals, introducing the L3 larvae into the skin through the bite wound. The introduced larvae

develop into adult worms that further locate themselves in the lymphatic system (vessels and nodes). Inside the infected animals, viviparous females release first-stage (L1) larvae called microfilaria into the blood stream. When a mosquito takes his blood meal, microfilaria are ingested and transferred into the arthropod to develop into L3 larvae, the infective form for mammals (Figure 4.2.3.7.1). Clinical manifestations can remain asymptomatic in spite of microfilaria presence in blood circulation, but in severe cases lymphangytis and lymphadenitis are observed (legs and groin), fever, headache, backache, edemas, pruritis and elephanthiasis that develops in the later stage of the disease.

4.2.3.7.3 FILARIASIS AND ENVIRONMENT

Since filariasis is a vector-borne (arthropods) disease, external temperature plays an important epidemiological role. Lardeux and Cheffort (2001) studied the ambient climatic conditions on *Wuchereria bancrofti* transmission by *Aedes polynesesiensis* Marks (Diptera: Culicidae) in the French Polynesian archipelagos. Using a statistical model of the extrinsic cycle duration as a function of temperature, the authors distinguished three patterns of *W. bancrofti* transmission dynamics: continuous, fluctuating and discontinuous, occurring geographically from north to south. Clearly, these patterns were linked to temperature, man-vector contact and seasonal agricultural activities (Sattenspiel, 2000). In Africa, bancroftian filariasis is endemic in many countries these parasites were also found to be related to rainy seasons, vector habitats and abundance (Rwegoshora *et al.*, 2005; de Souza *et al.*, 2010). Hassan *et al.* (1998) using Geographic Information System (GIS) analyzed the spatial distribution of filariasis and its mosquito vector in the Nile Delta, Egypt. The spatial pattern of microfilariasis prevalence was shown to be negatively associated with annual rainfall and relative humidity, while it was positively associated with annual daily temperature. The same significant parameter (weekly mean temperature) has been shown with *C. pipiens* mosquitoes in Buenos Aires city, Argentina (Fischer and Schweigmann, 2004). Mosquitoes spp. have been already shown to have different preferences for breeding sites, such as salinity, pH, organic load, temperature, etc. but with large preferential brackets, explaining some of the published contradictory results (Petersen and Chapman, 1969).

Apiwathnasorn *et al.* (2006) presented a study on the bionomics of *Mansonia* spp. mosquitoes as the nocturnal vector of *B. malayi*. They reported forty-four new species belonging to 12 genera isolated from the peat swamp forest in Thailand. Among these mosquitoes, some had both day and night activity, with high biting density and various animal hosts: cats, cows, monkeys and humans. The authors concluded that biting activity patterns corresponded with the periodicity found in man and domestic cats and may play an important role in either transmission or maintenance of the filarial parasites in the peat swamp forest.

In connection with breeding sites of mosquitoes, Oemijati *et al.* (1978) showed an interesting environmental issue related to human activity. Six villiges in West Java (Indonesia) have been examined for microfilaremia (*B. malayi*) resulting in a very low number of infections in comparison with a ten year old survey in the same localities. According to these authors, without drug treatment, insecticides application or any drastic population migration the observed decrease could be attributed solely to the development of a well-organized irrigation system in parallel to swamps conversion into rice-fields, consequently diminishing *Mansonia* mosquitoes breeding sites! This improvement may be attributed to water level and flow rates in irrigation channels as already shown (Mogi and Motomura, 1996). Flow rates of >20 cm/s (the flow speed required for mosquito control) seem to be the significant factor and not water levels (>15 cm$\pm$<5 cm), and hence high-quality engineering planning is required to prevent stagnancy in irrigation channels.

Erlanger *et al.* (2005) in a extensive literature review on lymphatic filariasis in Africa (*W. bancrofti*) tried to establish global and regional estimates of populations at risk, with particular consideration of water
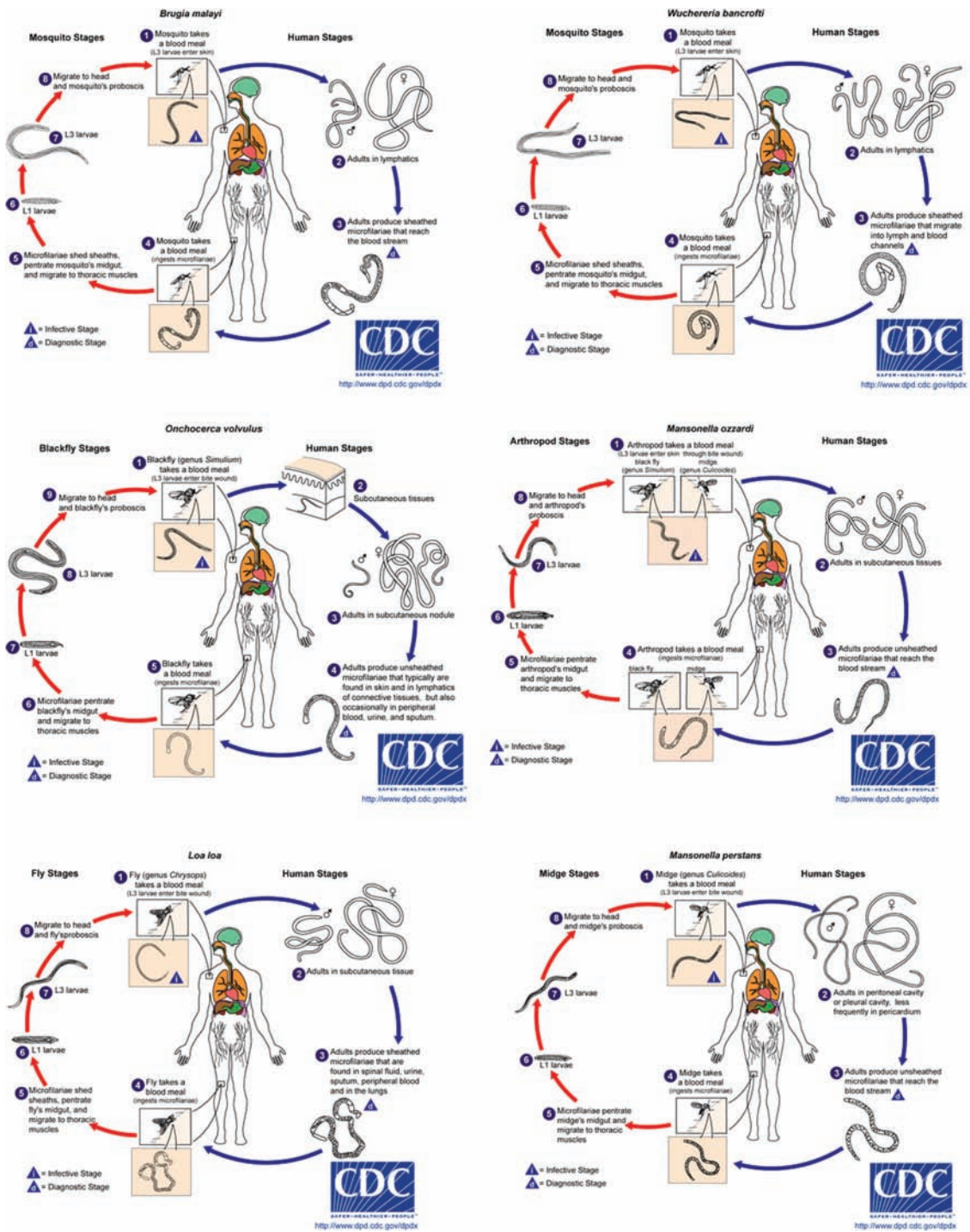
Figure 4.2.3.7.1. *Brugia malayi* (first raw, left), *Wuchereria bancrofti* (first raw, right), *Onchocerca volvulus* (send raw left), *Mansonella ozzardi* (second raw, right), *Loa Loa* (third raw, left) and *Mansonella perstans* (third raw, right) life cycles (with permission from CDC, Atlanta, USA; http://dpd.cdc.gov/dpdx/HTML/Filariasis. htm)

resource development projects, and to assess the effects of water resource development and management on the frequency and transmission dynamics of the disease. After almost 30 years, these authors were unable to define decisively whether implementation of water projects (including irrigation) are indeed associated with clinical symptoms. Patz *et al.* (2000) underlined the main environmental factors involved in filariasis expansion: rice cultivation and flooding, changes in soil moisture, polluted standing water and free-ranging domestic animals (pigs and cows) destroying the banks of unlined drains (thus creating shallow larval habitats). However, it should be noted that well designed irrigation without organic pollution does not encourage mosquitoes' proliferation resulting in minor filariasis is spread.

Adeleke *et al.* (2008) surveyed different environmental habitats (ground pools/ponds, gutters/open drains, tires, domestic containers and treeholes/leaf axils) for their mosquito growth support in the city of Abeokuta, Nigeria. Among the ten species encountered, *Aedes aegypti* bred in all the above habitats, while other species differed in their preferences. Tree holes/leaf axils was least preferred by all the surveyed mosquitoes species, emphasizing that man-made environments in contrast to natural ones are important habitats for these vectors. In Burdwan district, West Bengal (India), Adhikari and Haldar (1995) showed that preferentially *C. quinquefasciatus* spp. mosquitoes inhabit more colliery than non-colliery areas and are well correlated to microfilariae infection and infectivity rates. Nonetheless, they pointed out that the colliery environment can also affect, besides mosquito vectors, also the hosts, therefore influencing the filariasis outcome. These results are supported by another Culex species (*Culex fatigans*) that has been shown to breed preferentially in coalmine underground pits in India (Dutta, 1977).

In relation to mosquito vectors, Fonseca *et al.* (2006) studying worldwide populations of *Cx. quinquefasciatus* and the southern house mosquito (in Hawaii) defined also as *Cx. quinquefasciatus*, reported that they are significantly genetically differentiated and well correlated to known *W. bancrofti* vector competence, and their pathways of expansion are remarkably similar to those inferred for *Ae. aegypti*, another vector species associated with humans parasites.

Another environmental problem has been reported by Poopathi *et al.* (2001). These authors found that *Culex quinquefasciatus* developed resistance to the insecticide malathion, possibly affecting filariasis persistence in spite of the mosquito control campaign in India. Malathion resistance in mosquitoes had been reported to be related to aliesterase malathion-degrading activity and not to acetylcholinesterase sensitivity to malaoxon (Miyata *et al.*, 1984).

As a final point, Cho-Ngwa *et al.* (2009) studied endemic elephantiasis distribution and found evidence that podoconiosis is exacerbated by onchocerciasis (lymphatic filariasis). Podoconisis is a geochemical lymphedema seen in geographic areas of red clay soils around volcanoes (fertile volcanic highland) in Africa, Indonesia, Central and South America where susceptible families of bare-footed farmers absorb soil silica particles into their bodies. Absorption causes the patient's lymphatics to fibrose and further lymphatic ducts obstruction that finally cause enlargement of femoral nodes. As a result of both conditions it is obvious that this exacerbation does occur and it requires special physician attention to environmental conditions.

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Chapter 4.2.3.8

Dirofilariasis

[*DIROFILARIA IMMITIS, D. REPENS, D. TENUIS, D. URSI, AND D. STRIATA*]

Dirofilariasis is caused by several species of the so-called heartworm of dogs: *Dirofilaria. Dirofilaria immitis* has a worldwide distribution preferably in warm climates (Ng and Kelly, 1975). *D. immitis* and *D. repens* have dogs and other canids as final hosts, while *D. tenuis* develops in raccoons, *D. ursi* in bears and lynxes and *D. striata* in felids (mainly North and South America) (Deem and Emmons, 2005; Anyanwu *et al.*, 2000). Transmission occurs by blood-sucking infected mosquitoes or blackflies (simulia) that transfer L3 larvae. The *D. immitis* adult parasite can be found in the right heart and pulmonary artery of canids (final hosts). Blood circulating microfilariae are sucked into the intermediate host (for the duration of blood meal), develop into L3 infective larvae and are further transmitted to humans by additional insects' bites. In humans, *D. immitis* inhabits lung' parenchyma, as does *D. repens* (occasionally), and other species settle in subcutaneous tissues (Figure 4.2.3.8.1). Clinical manifestations are mostly asymptomatic; however, vasculitis and pulmonary infarcts may occur with granulomas, expressed as pain, coughing, hemoptyasis and painful subcutaneous and conjunctiva nodules (Otranto and Eberhard, 2011).

4.2.3.8.1 DIROFILARIASIS AND ENVIRONMENT

Resembling lymphatic filariasis, transmission main vectors of dirofilaria nematode species are mosquitoes and biting flies. An investigation carried out in Padova (Italy) tried to assess the actual role of *Aedes albopictus*, a very aggressive anthropophylic mosquito (30–48 bites/h) in the transmission of dirofilariasis. Indeed, *A. albopictus* was found to harbor *D. immitis* (highest prevalence 27.5%) therefore presenting a high-risk source for human and canid' infection (Cancrini *et al.*, 2003; Pietrobelli, 2008). Recently, Miller and Crosbie (2011) surveyed canine heartworm prevalence in California (Fresno and Madera Counties) where to their surprise found only a low prevalence (3.5 to 3.8%). They attributed this low prevalence to vector distribution and density that seem to be impacted by three years drought in central California. It has been suggested that *Aedes sierrensis*, the main putative vector species that uses tree holes to breed is affected by lack of rain and low humidity. Other factors suggested to affect the low

prevalence were time spent outdoor, lower number of susceptible mosquito vectors (three in Madera County vs. seventeen in Florida) and elevations (from previous studies: significantly higher in foothill habitats than in valley habitats). Dogs' outdoor activity has also been shown to be a significant factor in dirofilarisis prevalence in Korea. In rural hunting dogs, *D. immitis* prevalence was 22.3% and 14.6% among stray dogs found within the city limits (Lim *et al.*, 2010).
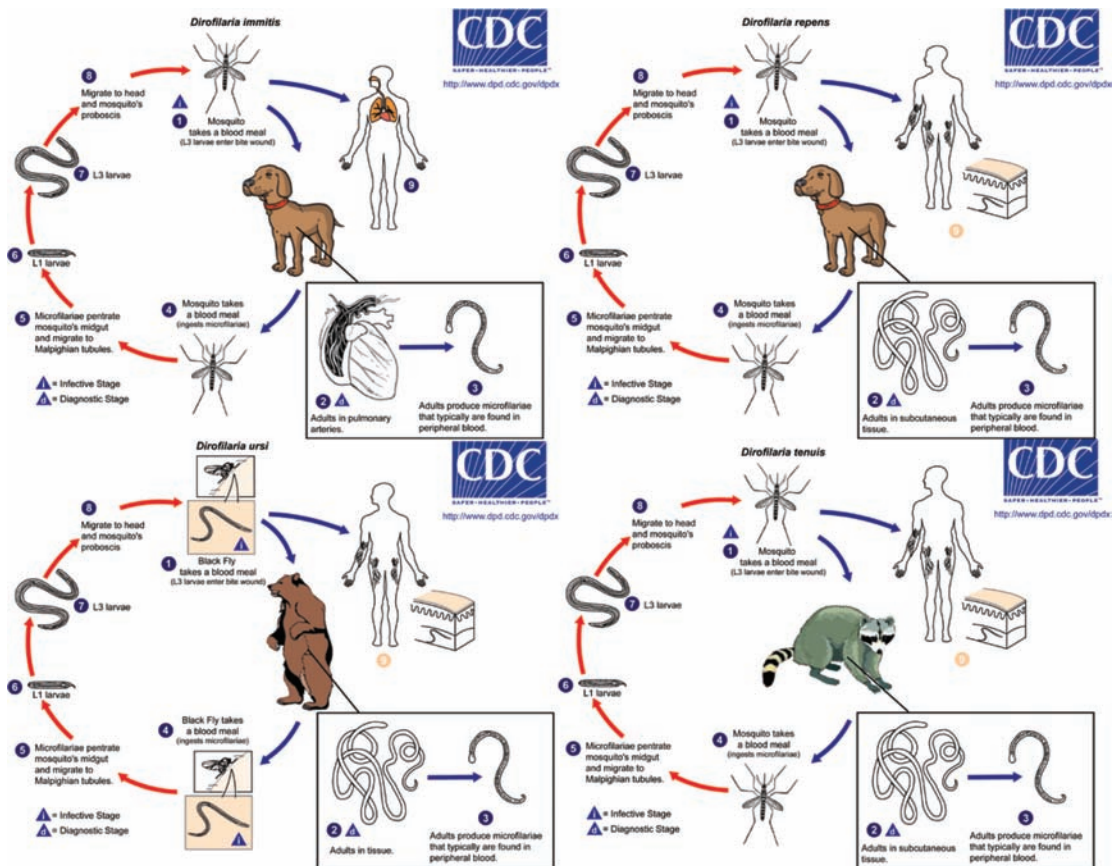
Figure 4.2.3.8.1. *Dirofilaria immitis* (first raw left), *D. repens* (first raw, right), *D. ursi* (send raw, left) and *D. tenuis* (second raw, right) life cycles. (with permission from CDC, Atlanta, USA; http://www.dpd.cdc. gov/dpdx/html/ImageLibrary/A-F/Filariasis/body_Filariasis_il22.htm)

In California, a seropositivity survey in coyotes (*Canis latrans*) for the bacteria *Bartonella vinsonii* subsp. *berkhoffii* has been carried out to find out the environmental factors affecting the seroprevalence in these canids. Geographic clusters overlap between *Bartonella* sp. and *Dirofilaria immitis* had been reported in association with higher precipitation (with season variability) and proximity to coast. It may be very possible that proliferation of the two vectors (sandflies and mosquitoes) in these areas was well supported under these conditions. In Europe, especially in Italy, which is located at the subtropical and temperate climates boundary, a permanent rise in dirofilariasis has been observed during the last decades

(Genchi *et al.*, 2009). Various models are based on three limiting factors such as: 1) a threshold of 14°C below which *Dirofilaria* development will not proceed; 2) a requirement of 130 GDD (growing degree day) for parasite larvae to reach infectivity and 3) a maximum life expectancy of 30 days for a vector mosquito. They, predict that the summer temperatures (with peaks in July) are sufficient to facilitate extrinsic incubation of *Dirofilaria* even at high latitudes, into previously infection-free areas, including mosquito species new distribution along the new climate zone (Rinaldi *et al.*, 2011).

In Argentina, a survey on canine heartworm epidemiology was carried out in urban areas of Buenos Aires Province (Vezzani *et al.*, 2011). According to these authors, the best model predicted maximum heartworm prevalences around middle values of bare soil cover. It seems that this predictor is linked somehow to mosquito breeding sites and the available humidity, but the authors did not comment extensively on these findings.

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Chapter 4.2.3.9

Gnathostomiasis

[*GNATHOSTOMA SPINIGERUM, G. HISPIDUM, G. DOLORESI, G. NIPPONICUM*]

Gnathostomiasis (or under its other synonyms: creeping eruption, wandering swelling, larva migrans profundus, Yangtze edema, physaloptera and Choko-Fuschu Tua chid) is caused in humans by the nematode *Gnathostoma spinigerum* and related species. *G. spinigerum* distribution is predominantly in Southeast Asia, Central America, and Australia with lower prevalence in Europe and Japan. In animals, the infection is severe with fatal outcome. Transmission occurs by consumption of raw or undercooked fish and meat. The life cycle of this parasite is linked to several intermediary and final hosts, including humans as accidental hosts. Unembryonated eggs excreted from infected hosts (dogs, cats, wild boars or pigs) contaminate water sources where they embryonate releasing L1 larvae. Copepods or Cyclops (water fleas) spp. ingest L1 larvae that develop into L2 larvae, inside these intermediary hosts. When a second intermediary host (e.g. fish, frog or snail) consumes copepods or cyclops, it becomes infected with L2 larvae that further develop into the L3 larvae infective for a final host. A paratenic host such as waterfowl or chickens can feed on a second intermediary host, thus becoming infected with L3 larvae to be further transmitted to a final host that consume the paratenic host. Humans are dead-end host that may ingest L2 or L3 larvae (individuals who are exposed to contaminated meat or water, larvae may rarely penetrate their skin) but they do not develop into adult worms to produce eggs (as in cats, dogs, pigs, etc). Inside humans, larvae penetrate the stomach wall and migrate to various inner organs or to subcutaneous tissue (Figure 4.2.3.9.1). Clinical manifestations are: epigastric pain, malaise, fever, urticaria, anorexia, nausea, vomiting, diarrhea, itching, painful skin erythematous migrating swellings including lesions, invasion of inner organs and inflammation (including eyes and central nervous system).

4.2.3.9.1 GNATHOSTOMIASIS AND ENVIRONMENT

Gnathostomiasis has been reported in Mexico since 1970, continuously increasing up to several thousand cases. Interestingly, the spread of this parasitic disease in Mexico is a lucid example of how environmental changes can affect the distribution of a zoonotic disease. Numerous gnathostomiasis cases

have occurred near a dam (named Presidente Miguel Aleman), whose water has been also used to grow imported tilapia fishes, concurrently with the rise in the popularity of these fishes in dishes such as ceviche or callos (both popular seafood dishes made from freshly marinated raw fish). During the same time period, part of the tilapia harvest (probably already infected with *G. spinigerum*) had been shipped to other aquacultures areas of Mexico, therefore transferring the infective agent around the country (McCarthy and Moore, 2000; Martínez-Cruz *et al.*, 1989). Following these emerging cases, studies have been carried out in an attempt to identify the source of infection in freshwater fishes near Culiacan, the capital of Sinaloa, a state on the north Pacific coast of Mexico. No larvae were found in the examined fishes and the authors attributed the negative results to sample size; however, larvae were found in the second intermediary hosts such as pescivorous birds (four species), thereby corroborating the presence of this parasite in the aquatic fauna (Ogata *et al.*, 1998; Diaz Camacho *et al.*, 1998). Humans, as dead-end hosts, resulting in non-productive adult worms, can not transmit the parasite while migrating or traveling to other geographical areas, but can be infected only in those areas, thus requiring clinicians' awareness of tropical infections (Charoenratanakul, 1997).
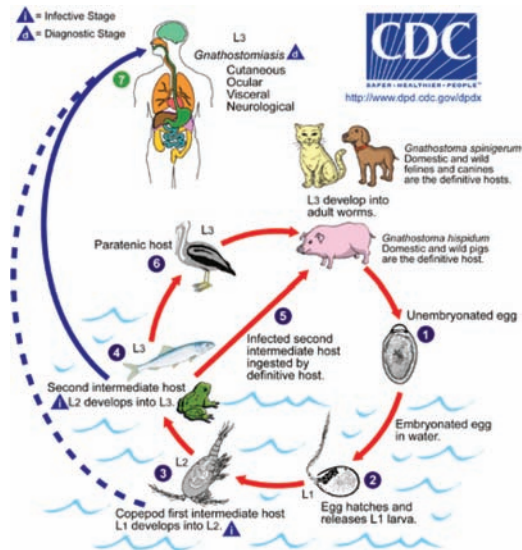
Figure 4.2.3.9.1. *Gnathostoma* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.cdc.gov/parasites/gnathostoma/biology.html)

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Chapter 4.2.3.10

Gongylonemiasis

[GENUS *GONGYLONEMA*, *GONGYLONEMA PULCHRUM*]

Gonglylonema pulchrum parasitic nematode belongs to the genus *Gongylonema* and is the only one capable of infecting humans. Humans are accidental hosts who, due to poor sanitary conditions, become infected by direct ingestion of coprophagous insects, or food and water contaminated with these insects (e.g., dung beetles and cockroaches). The distribution is worldwide (Germany, United States, Iran, Laos, Japan, Morocco, China, Italy, New Zealand and Egypt) with very low numbers of reported cases (∼50 cases). Buccal mucosa is the ideal infection site of the parasite. The final hosts include cattle, hedgehogs, dogs, cats, ruminants, rabbits, skunks, etc. while dung beetles and cockroaches are both vectors and intermediate hosts. Besides insect ingestion that transfers the infective larvae, direct ingestion of water contaminated with free-larvae (from an intermediary host) can also infect humans. Humans can support the growth of adult worms. Clinical manifestations are mainly expressed in mucosa of the oral cavity where the parasite finally migrates with local inflammation and bleeding with moderate pain.

4.2.3.10.1 GONGYLONEMIASIS AND ENVIRONMENT

Corn *et al.* (1985) studied the helminth community of collared peccaries of the southern plains of Texas (a known arid habitat) in order to compare it to collared peccaries from Gulf coastal prairies. Collared peccaries are mammals resembling hogs (also called musk hog and Mexican hog) that feed on fruits, roots (and bulbs), tubers, palm nuts, grasses, invertebrates and small vertebrates. The authors reported the presence of *G. pulchrum* in collared peccaries from drier habitats compared to none in those from the coastal plains. It seems that infection (without clinical symptoms in these animals) occurs by ingestion (as one of nutrition components) of dung beetles known as intermediate hosts. The highest densities of these coprophagous insects have been reported to be during the spring and early summer (June) with fully correlated *G. pulchrum* presence in its final host during and after periods of utmost intermediate host density.

Another infection with *G. pulchrum* has been reported in Bolivian squirrel monkeys (*Saimiri boliviensis*) from a zoological garden in Kyushu, Japan (Sato *et al.*, 2005). The prevalence of eggs containing larvae was between 25.5 to 55.5% revealing a continuous infection of these monkeys and possible other zoo animals

through the presence of dung beetles and cockroaches as intermediate hosts in this particular close environment.

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Chapter 4.2.3.11

Lagochilascariasis

[*LAGOCHILASCARIS MINOR*]

Lagochilascariasis is a very rare disease caused by the ascarid nematode *Lagochilascaris minor*, a parasite with felids as probable final hosts (Sakamoto and Cabrera, 2002). The distribution of this parasite is linked to remote jungle forest areas in the tropics. Humans are infected by the ingestion of intermediate hosts from rural sylvatic environments (e.g., mice, agoutis, paca, capibara and other rodents) but direct embryonated egg contact can be also a possible path of infection (including autoinfection from eggs and larvae containing nodules) (Vieira *et al.*, 2000; Moncada *et al.*, 1998; Paco *et al.*, 1999). The life cycle consists of intermediate hosts (mainly rodents) infected with embryonated eggs from feces of felids that develop into larvae that migrate to inner organs and subcutaneous fatty tissue to become encapsulated. When infected rodents are preyed on by felids, the ingested larvae develop into adult worms in the esophagus, pharynx and cervical lymph nodes of this final host. Clinical manifestations are chronic nodular swellings in the neck, throat, nasal sinuses, tonsils, brain and lungs. Puss discharge from lesions contains various stages of the parasite (larvae and adults) suggesting possible autoinfections. Patients' general conditions is severe and in some cases fatal.

4.2.3.11.1 LAGOCHILASCARIASIS AND ENVIRONMENT

There are very few publications on this parasitic nematode and its environmental behavior, however, some clues can be drawn from a specific case report of the natural infection of a bush dog (found killed) on a rural road in the Northeast of Bolivar State, a locality where a patient with lagochilascariasis had lived for the past 16 years (Volcan and Medrano, 1991). Bush dog is a gregarious, diurnal carnivore (preying on Paca), mainly in wet savannahs and tropical and equatorial forests of South America. Bush dogs use hollow logs and cavities (e.g., armadillo burrows) for shelter. It is very possible that infection with *Lagochilascaris minor* will take place in such environments where infected rodents reside underground.

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Chapter 4.2.3.12

Larva migrans cutanea

[*ANCYLOSTOMA BRAZILIENSE, UNCINARIA STENOCEPHALA, A. CANINUM, A. TUBAEFORMAE, BUNOSTOMUM PHLEBOTONUM*]

Larva migrans cutanea (also known as creeping eruption or cutaneous larva migrans-CLM) is mainly an acute syndrome of the skin caused by the penetration of parasitic nematode larvae, mostly by *Ancylostoma braziliense* and *Uncinaria stenocephala* that are dogs' hookworms, and occasionally by other hookworms *A. caninum* (from dogs), *A. tubaeformae* (from cats) and *Bunostomum phlebotonum* (from cattle). The distribution is worldwide with preference in moist tropical and subtropical areas but also with *U. stenocephala* in moderate climates (Mukaratirwa and Taruvinga, 1999; Malgor *et al.*, 1981). Humans are accidental hosts (tourism near warm climate beaches) by percutaneous infection with larvae in moist and warm environments while dogs, cats and cattle are the definitive hosts (Edelglass *et al.*, 1982; Scaini *et al.*, 2003; Watson *et al.*, 1981). In these definitive hosts, the filariform larvae develop into adult worms to produce eggs. Rhabditiform larvae hatch from eggs excreted by the definitive hosts (cats, dogs, etc.) and further develop in moist environments (beaches and playground) into infective filariform larvae that in turn infect again its final hosts, including accidental hosts such as humans. The parasite does not develop into adult worms in humans, but can migrate subcutaneously as filariform larvae (Figure 4.2.3.12.1). Clinical manifestations are: development of papules at entrance sites of filariform larvae and burrows between skin's corium and stratum granulosum generating erythema, edema, crusts, itching and pricking pain with possible secondary infections if scratching is present.

4.2.3.12.1 LARVA MIGRANS CUTANEA AND ENVIRONMENT

Michaud *et al.* (2003) surveyed parasitic helmith presence in feral non-human primates' feces and compared it to that in the human population from the same area. The authors found that in Peru, non-human primates and that in the humans from the same area harbor *Ancylostoma braziliensis* besides other parasitic helminths.

It seems that filariform larvae of *Strongyloides* spp. are resistant to a variety of chemicals, have chemoattraction to plasma and NaCl salt and persist in moist fresh excreta, which may explain their

infective successful routes when present on sand beaches where dogs or cats have defecated and people come in direct contact with it (Blank *et al.*, 1959; Ma, 1987; Mukaratirwa and Taruvinga, 1999). Zhu *et al.* (2011) performed a model study to understand the impact of non-point pollution (from bather shedding, animals' feces, near shore sand and other environmental conditions) on the transport of bioindicators at a subtropical recreational marine beach in South Florida. These authors clearly showed that dog fecal events had the major transient impact (in this case with enterococci as indicators) in a limited area within several hours while other non-point sources had a limited impact on bacterial contamination. This model might also be applied to dog' feces containing filiform migrating larvae that under moist conditions can survive for a prolonged time. Similar persistence and resistance have been reported for *Strongyloides* spp. in soil samples contaminated with dog' feces in Kuala Lumpur-an urban area and Kula Lipis-a rural area (Malaysia) (Azian *et al.*, 2008).
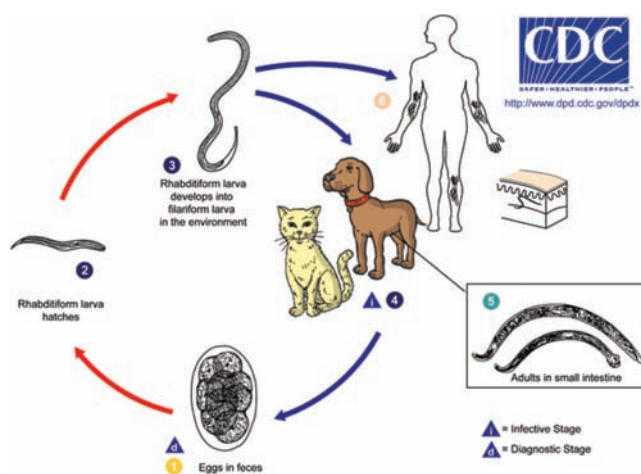
Figure 4.2.3.12.1. *Ancylostoma braziliense* and other *Strongyloides* spp. causing cutaneous larva migrans life cycle (with permission from CDC, Atlanta, USA; http://dpd.cdc.gov/dpdx/HTML/Hookworm.htm)

Reperant *et al.* (2007) studied the prevalence of dixenous helminths in red foxes from rural and urban area expecting to find a declining effect as a consequence of rodent' reduction in urban area. In relation to *Uncinaria stenocephala* (overall prevalence 78.2%) the authors did not find any significant variation between the two environments. This result may be explained by the transmission route of this hookworm that is mainly dependent upon the abundance and survival of the free-living stages along host density on which urbanization has no significant effect (in contrast to increasing numbers of pets and stray dogs that may cause constant persistance).

In relation to domestic animals, a study from Poland showed that dogs from the Warsaw region (an area with high *U. stenocephala* prevalence) differ in their hookworm prevalence (Gorski *et al.*, 1996). Housedogs' infection prevalence was between 2.7 to and 4.1%, while stray dogs or dogs living in shelters had a much higher infection prevalence: from 10.1 to 47.7%. Importantly, some locations with high numbers of *U. stenocephala* infections were in proximity to or surrounded by forest. The authors suggested that forest might support a better survival of hookworm larvae than open spaces and provide close contact with other infected animals (e.g., foxes) for constant transmission.

Also in Poland, a recent study of a recreational area was carried out in order to find out the relation between wild animals' helminths infestation (red foxes and wild boars) and soil contamination

(Mizgajska-Wiktor and Jarosz, 2010). The authors reported *Strongyloides* spp. prevalence in positive feces of 36.6% in wild boar and 45.5% (mainly *U. stenocephala*) in foxes; however, no soil contamination was found. Possible explanations have been suggested based on soil characteristics (loose sand soil) that do not support the persistence and survival of larvae or eggs and wild animals' behavior in human crowded areas related to defecation.

Finally, Perec-Matysiak *et al.* (2008) evaluated the contamination of various urban areas with geohelminth eggs in Wroclaw, Poland. According to their results, the highest rate of contamination was observed in city squares (*U. stenocephala* 7% and *A. caninum* 4.9% among the positive samples). There are at least two supporting factors for this high prevalence: 1) city squares are one of the most crowded sites in terms of dog owners and 2) owing to municipal maintenance; street washing provides a moist milieu for these hookworms survival.

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Chapter 4.2.3.13

Larva migrans visceralis

[*TOXOCARA CANIS, TOXOCARA CATI*-SYN. *MYSTAX, BAYLISASCARIS PROCYONIS*]

Larva migrans visceralis (also known as visceral larva migrans -VLM) is a worldwide syndrome caused by nematode' larvae invasion of inner organs or the eye (mainly by larvae of the dog nematode -*Toxocara canis*, cat nematode -*Toxocara cati*, syn. *mystax* and rarely a raccoon nematode - *Baylisascaris procyonis*). Distribution is worldwide in carnivorous animals (dogs, cats, raccoons, etc.) (Himsworth *et al.*, 2010; Shalaby *et al.*, 2010). As a paratenic host, infections in humans may occur by accidental ingestion of contaminated soil, consumption of unwashed vegetables or meat (hunting) of another paratenic host contaminated with embryonated eggs of those nematodes (Glickman *et al.*, 1987; Borecka *et al.*, 2010). Most of the cases are asymptomatic, with immunological responses only: eosinophilia and positive serology. However, when heavy infection occurs, two main clinical presentations will take place: visceral larva migrans (VLM) and ocular larva migrans (OLM). In preschool children, visceral larva migrans occurs when larvae invade multiple tissues (liver, heart, lungs, brain and muscle). In the general population, ocular larvae migrans occurs when larvae or even one larva reach the eye to encyst within the orbit produce lesions. The life cycle of these nematodes includes excretion of eggs by infected dogs, cats or raccoons in their feces. Outside, in the environment eggs develop into highly resistant embryonated eggs that are set to be ingested by a new healthy dog/cat or other paratenic host. Following ingestion, the embryonated eggs hatch and release larvae that develop into mature roundworms (male and female) to complete the cycle. In paratenic hosts (birds, pigs, rodents, goats, monkeys, rabbits and humans) larvae will penetrate the intestinal wall, migrate to the lungs or are disseminated through the arterial system to many organs where they enter the parenchyma to be encapsulated by connective tissue, without further development in adult worms (Talvik *et al.*, 2006). Encapsulated larvae (L2 stage) can persist for years and even reactivate. In addition, humans may become infected by undercooked meat consumption of a paratenic host (e.g., rabbit) that will transfer its infectious larvae (Figure 4.2.3.13.1). Clinical manifestations are diverse and depend on the infestation load; most infections seem to be asymptomatic but when a serious infection occurs symptoms such as fever, abdominal pain, coughing, wheezing (asthmatic complaints) will present. Persistent reinfection will

cause more severe symptoms, such as lymphadenophaty, hepatosplenomegaly, urticaria, interstitial pneumonia, anorexia, neurotropism (CNS seizure, as in the cases of *B. procyonis* infection, regarded as highly pathogenic with fatal outcome), heart invasion and death. In OLM, visual acuity is reduced with manifestation of endophtalmitis, uveitis, and chorioretinitis or with intraretinal granulomas.
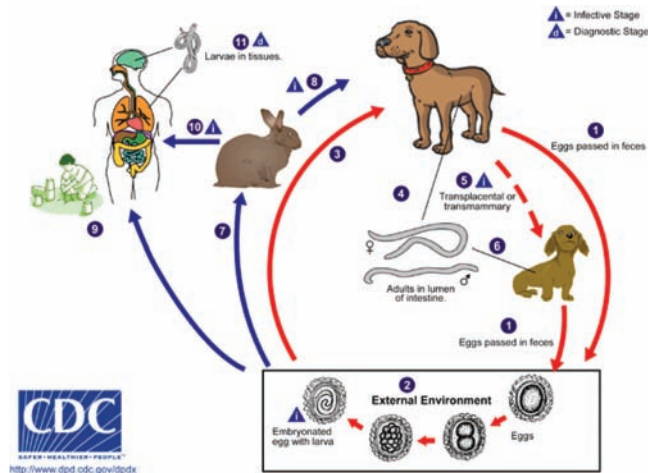
Figure 4.2.3.13.1. *Toxocara canis* life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Toxocariasis_il.htm)

4.2.3.13.1 LARVA MIGRANS VISCERALIS AND ENVIRONMENT

Direct human infection by ingestion of soil containing embryonated eggs occurs more often in crawling infants and preschool children that play with soil and sand in playgrounds, park green areas, public housing areas, parkways, and school yards (Tarsitano *et al.*, 2010; Grygierczyk *et al.*, 2003; Gillespie *et al.*, 1991). Therefore, soil contamination in urban and rural areas by infected dogs and cats is the most important environmental factor in the enhanced transmission of these parasites (Azian *et al.*, 2008; Mizgajska *et al.*, 2001; Castillo *et al.*, 2000; Ladczuk and Balicka-Ramisz, 2010). Mizgajska-Wiktor and Jarosz (2007) compared the degree of soil contamination with eggs of *T. cani* and *T. cati* in rural and urban areas of Poznan, Poland. Urban areas revealed 19.8% positive samples while rural areas 15.6%, with similar percentages in spring and autumn. *T. cati* eggs were more prevalent in urban areas (97% of all eggs recovered) and *T. canis* more prevalent in rural areas (84% of all eggs recovered). According to the defecation habits of these two animals, cats do cover their excreta while dogs do not, and therefore it can very possibly be that in areas where bare soil is scarcer (e.g., the urban area) cats' excreta will be found more often, while dogs can defecate on paved ground to be further washed or removed through municipal activity.

Another interesting aspect of eggs environmental distribution linked to dogs' behavior has been recently presented. Roddie *et al.* (2008) showed that dogs, in their case, stray dogs, carry *T. canis* eggs on their fur (67% positive) with a significantly higher prevalence in puppies. This valuable study indicates the significant epidemiological and environmental aspects of Toxocariosis: 1) dogs do shed fur in and around human dwellings; 2) infants are very fond of puppies, and therefore close contact and

transmission is imminent; 3) transmission occurs at long distances; 4) direct and early infection of puppies do occur and 5) potential transmission to many paratenic hosts without soil or meat ingestion.

In relation to climate, Jimenez *et al.* (1997) using a serological survey of human toxocariasis in the Canary Islands (Spain) reported variable exposure of the local population to those helminths. They explained this variability based on the different Canarian mesoclimates with significant differences being found between humid and arid islands (several reports revealed eggs excellent survival for several years in shaded area, appropriate humidity, temperature and oxygen) (Sommerfelt *et al.*, 2006; Uga, 1993; Maikai *et al.*, 2008). They found a significant positive correlation between De Martonne's humidity-aridity index (Iar-DM is the ratio between the mean annual values of precipitation (P) and temperature (T) +10°C (De Martonne, 1926) and the percentage of seropositive people on the different islands. Humidity and increased temperature (optimal at 19–24°C, but not at 34°C where infective eggs survival declined) have been shown to enhance survival and development of *Toxocara canis* eggs (Gamboa, 2005; Andresiuk *et al.*, 2007). In another study performed in rural areas in Argentina on toxocariasis-related factors, among the significantly related ones were floods and dog ownership (Chiodo *et al.*, 2006). Considering *T. canis's* biological cycle, flooding will favor the evolution and survival of eggs, extending their viability that increases contamination odds.

Finally, Papajova and Juris (2009) studied the effect of aerobic composting and anaerobic stabilization of organic wastes on the survival of parasitic germs, among them *T. canis*. According to their results, aerobic composting (>50°C) was much more efficient in this parasite's eggs inactivation (total devitalization after 48 hours) compared to anaerobic stabilization ("composting") of organic wastes from public areas (maximum temperature 35°C) (only 50% devitalization after 48 hours).

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Chapter 4.2.3.14

Oesophagostomiasis

[*OESOPHAGOSTOMUM BIFURCUM, O. ACULEATUM, O. STEPHANOSTOMUM*]

Oesophagostomiasis is a parasitic chronic disease of the colon, caused by a species of worms falling under the family Strongylidae definition. *Oesophagostomum* spp., in particular O. *bifurcum*, are common parasites of livestock and animals such as goats, pigs and primates, although it seems that humans are increasingly becoming complimentary hosts as well. Although the routes of human infection have not yet been adequately elucidated, it is believed that transmission occurs through oral-fecal means, with infected humans unknowingly ingesting soil or vegetables containing the infectious filariform larvae (stage L3). Recently, human-to-human transmission has been reported, questioning the classification of this disease as zoonosis! *Oesophagostomum* infections are largely localized to northern Togo and Ghana in western Africa, but reports have been published from other geographic regions (Brazil, Indonesia, and Malaysia).

The life cycle consists of fecal excretion of eggs produced by mature worms parasitizing its final host (goats, sheep, cows, wild boars, primates, humans, etc.). First-stage larvae (L1) develop inside the eggs and hatch as rhabdiform larva that will further develop into third-stage larvae (L3), the so called the infective filariform larvae. The filariform larvae when ingested, invade the large intestine walls to form small nodules. After additional development, including molting, the new fourth-stage (L4) migrates back to intestinal lumen to mature into adult worms (Figure 4.2.3.14.1). In some cases, the final development may be arrested following repeated infections, with formation of persistent and enlarging nodules. In high endemic areas like those in Togo and Ghana, the primate population is too small to impact the human population, and consequently it became obvious that person-to-person transmission is very possible, perhaps periodically with some animals as catalysts. Clinical manifestations range from asymptomatic to acute abdomen (mimicking appendicitis), fever (low grade), vomiting, anorexia, diarrhea, intestinal obstruction, purulent peritonitis, intestinal and even cutaneous nodules.
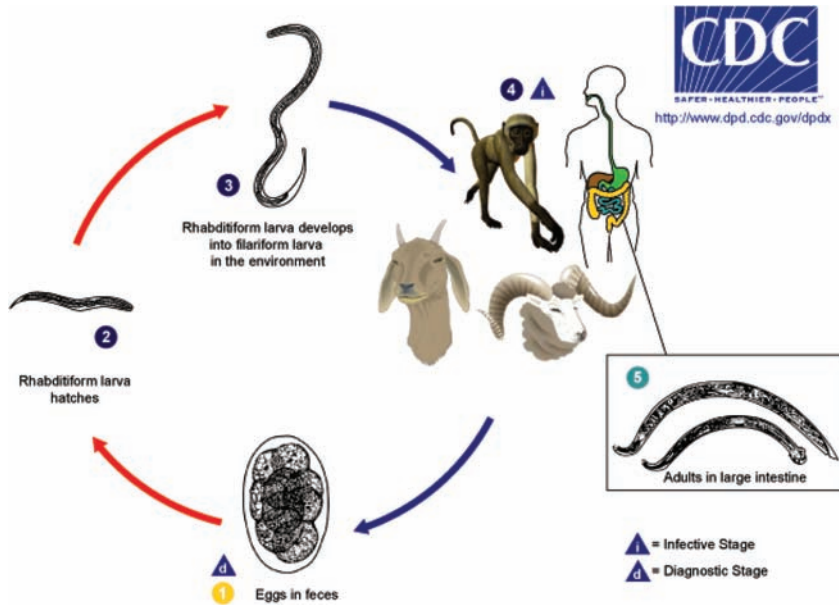
Figure 4.2.3.14.1. *Oesophagostomum* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/Oesophagostomiasis.htm)

4.2.3.14.1 OESOPHAGOSTOMIASIS AND ENVIRONMENT

There is accumulating evidence that the *O. bifurcum* isolated from humans is different from the ones those isolated from non-human primates (de Gruijter *et al.*, 2006; Eberhard *et al.*, 2001). Northern parts of Ghana and Togo are well known endemic foci (as a result of poor hygiene, agricultural practices and a relative lack of potable water) where at least a quarter of a million people are infected with this parasite reaching an ~70% prevalence in some villages. Researchers have screened fecal samples of various non-human primates (olive baboon, mona monkey and black and white colobus monkey) for the presence *O. bifurcum* eggs in two non-endemic areas in Ghana (Figure 4.2.3.14.2). Olive baboons and mona monkeys were found to have a high prevalence of eggs (75–99%) while humans and black and white colobus monkeys were absolutely negative (van Lieshout *et al.*, 2005). In spite of favorable conditions for zoonotic transmission (proximity to non-human primates, defecation habits and food consumption) no human infection with *O. bifurcum* has been observed. According to these authors, their results support the hypothesis that two distinct species of *O. bifircum* exist: one anthropogenic and one "cercopithecidaegenic", and consequently the high northern prevalence of infections is presumably human-to-human, with non-human primates as reservoirs (?) (Gasser *et al.*, 2006). In addition, Storey *et al.* (2000, 2001), studying a rural community with high endemicity in Ghana, reported that patients' infection and symptoms' appearance are linked to the rainy season (most commonly presented cases were during the late rains and early dry season) implying a connection to water as a possible infection route. In another report, reinfection and transmission appeared to be also limited to the rainy season, in spite of antihelminitic treatment in the middle of the dry or in the middle of the rainy season (Krepel *et al.*, 1995). Steenhard *et al.* (2000) linked rainy season and free-ranging pigs as a potential reservoir and

spreader of *O. bifurcum* among villages' residents based on their coprophilic habits. However, only 5% of the experimental eggs fed to pigs were found to be viable/infectious (retrieved as third-stage larvae after coproculture of the pigs' feces). It seems that pigs are able to inactivate almost 95% of ingested eggs. These authors raised an interesting ecological/environmental hypothesis: preventing pigs from consuming human feces during the rainy season (being tied up) is a factor that contributes to high transmission during this season, while during dry season, when pigs are released to free-range, they manage to remove human feces, removing/inactivating also *O. bifurcum* eggs. This theory seems to be supported by there being no transmission during the dry season. This is an interesting hypothesis, but further studies are needed for a final confirmation, especially that it can help as a biological mean in transmission reduction of this parasite. Irrigation of pasture plots contaminated with helminths eggs among them *Oesophagostomum columbianum* (carried by infected goats), have been also shown to provide favourable conditions for eggs to develop into larvae (Gruner *et al.*, 1989).
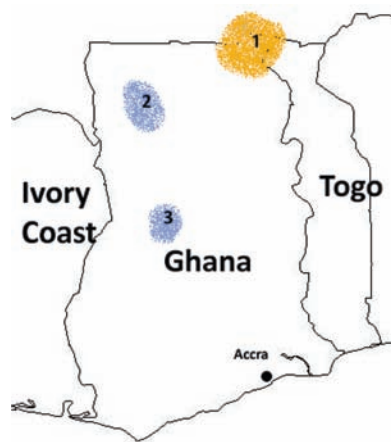
Figure 4.2.3.14.2. Ghana map including a highly endemic area (**1**-orange) and other two non-endemic sites (**2** and **3**-blue) (adapted from van Lieshout *et al.*, 2005)

African wild great apes (chimpanzees and gorillas) have a high prevalence of fecal' *O. stephanostomum*, another species that has been isolated very rarely in humans (Polderman and Blotkamp, 1995; Chabaud and Larivière, 1958). Krief *et al.* (2008) studied oesophagostomiasis in captive gorillas, free-ranging and ex-captive chimpanzees for clinical signs (granulomas and lesions/abscesses). Their analysis revealed that great apes kept in captivity suffer from severe clinical symptoms while free-ranging wild chimpanzees are not affected, showing good health. The authors suggested an environmental factor to be responsible for this difference: wild great apes swallowing rough leaves. According to this hypothesis, leaves swallowing leaves may contribute to two factors: antihelminthic activity by physical removal and possible plant pharmacological properties (Fowler *et al.*, 2007). The *O. stephanostomum* and *O. bifurcum* prevalent co-infections in chimpanzees raised the issue of a potential source for human infection, especially due to increasing contacts between humans and great apes through ecotourism, forest fragmentation in areas of high population density and crop-raiding by high-ranking males (Krief *et al.*, 2010).

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Chapter 4.2.3.15

Strongyloidiasis

[*STRONGYLOIDES STERCORALIS, S. FUELLEBORNI, S. FUELLEBORNI KELLY*]

Strongyloidiasis is caused by two nematode species: *Strongyloides stercoralis* and *S. fuelleborni* (*S. fuelleborni kelly* has been reported only from Papua New Guinea). Mammals such as dogs, cats, non-human and human primates have been reported to be infected by these nematodes (Bezjian *et al.*, 2008; Muehlenbein, 2005; Ashford *et al.*, 1990). The parasite is mostly distributed in endemic areas of tropical and sub-tropical countries (e.g. Vietnam, Cambodia, Laos, Brazil as well Central America and Sub-Saharan Africa) with poor hygiene (soil and water contaminated with feces), however some sporadic cases have been reported in temperate areas too (South America and Eastern Europe) (Kitvatanachai *et al.*, 2008; Sayasone *et al.*, 2009; Steinmann *et al.*, 2007; Widjana and Sutisna, 2000). Infection may occur percutaneously, through autoinfection or galactogenously by the filariform third stage larvae (L3). Following percutaneous invasion, L3 larvae migrate to the lungs (via lymph and blood vessels) to penetrate the alveolar walls and further reach the small intestine (via the trachea and esophagus). Located into intestinal mucosa, L3 larvae pass molting to produce adult parthogenetic females that produce eggs. Humans and other infected mammals excrete in their feces rhabditiform L1 larvae that in environment (e.g., moist soil) can follow two paths: 1) direct development into L3 filariform larvae that can again penetrate percutaneously the host and 2) development into free-living adult worms (male and female) that produce embryonated eggs from which rhabdiform larvae hatch. In the same environment, hatched rhabdiform larvae may develop into: free-living adult worms (to reproduce without host) or infective filariform larvae (to invade new host) (Figure 4.2.3.15.1). Beside this amazing plasticity (asexual and sexual reproduction including parthogenesis), *Strongyloides* spp., or in particular *S. stercoralis*, can produce autoinfection in immunocompromised patients (when L3 larvae develop from eggs persistent in the intestine) and galactogenous infections (when incoming larvae settle in the surrounding tissue of breast-hypobiotic larvae activated and shed during lactation). Clinical manifestations depend on infection burden; if light infection occurs, no symptoms will be present, but in chronic infections abdominal pain, nausea, intermittent diarrhea (for weeks), urticaria, dry cough, enerocolitis, damage to intestinal mucosa including sepsis, pulmonary hemorrhage, pneumonia and meningitis are found in patients.
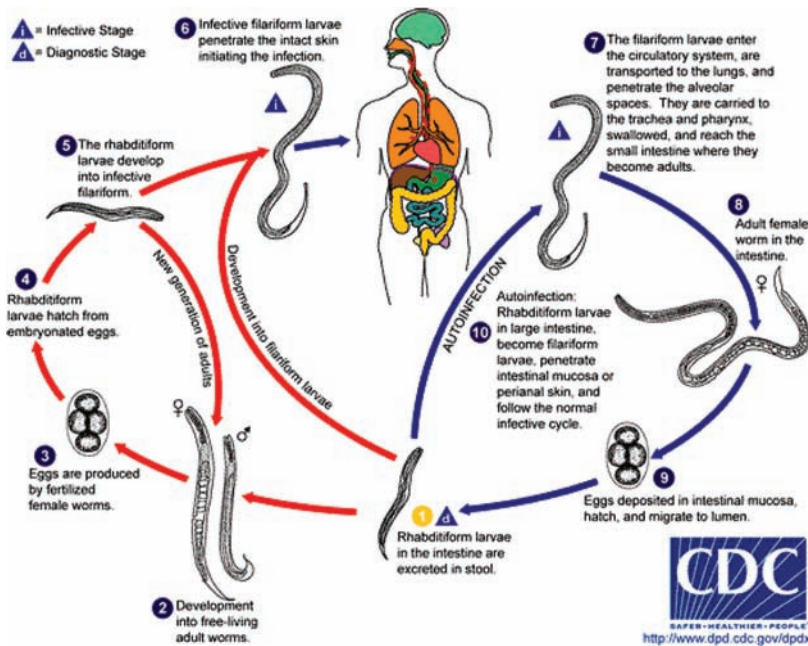
Figure 4.2.3.15.1. *Strongyloides* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/Strongyloidiasis.htm)

4.2.3.15.1 STRONGYLOIDIASIS AND ENVIRONMENT

Strongyloidiasis has been reported worldwide with a higher prevalence in tropical and subtropical countries (where temperatures over 20°C and humidity enhance soil development of the parasite next to deficient hygiene). In an epidemiological study performed in the province of Valencia, Spain (characterized by a Mediterrenian climate-dry and warm summers), researchers reported a connection between strogyloidosis cases and human occupation (Alcaraz *et al.*, 2004). In this area, the main occupation has been and continues to be agriculture. Until 1960, rice crops were the main agriculture activity while since then it changed to vegetables and citrus growth based on river irrigation or marshland draining ditches. Due to poor mechanization, irrigation activities have been performed manually, increasing the direct contact with water and mud. Indeed, the researchers found much lower strongyloidiosis cases in the interior of this area, characterized by calcareous elevations, steep slopes, hard limestone and dolomite rocks not favorable for the environmental cycle of these nematodes. In contrary st, higher numbers of cases were observed in populations inhabiting piedmonts, alluvial fans, close to marshlands areas and even in areas without stagnant or pooled waters but with intensive irrigation (comprising a wide network of irrigation ditches and canals). Barnish and Ashford (1990), in a different geographic area (Papua New Guinea), substantiated these findings, reporting on the absence of *Strongyloides* spp. from limestone and polygonal karst areas and altitudes >1,000 m above sea level (connected to lower temperature that does not favor L3 larvae development). Knopp *et al.* (2008) presented similar a high prevalence of infections with *S. stercoralis* in Zanzibar's areas characterized by alluvial clayey soils, moist forest regions and increased precipitations.

Gillespie and Chapman (2008) studied forest fragmentation impact upon host-parasite interactions in Uganda. Two populations of colobus monkeys have been studied: red colobus (*Procolobus rufomitratus*) and black-and-white colobus (*Colobus guereza*). These authors reported a significant decline in the red colobus population, an endangered species, in fragmented forest but no decline in black-and-white colobus in both fragmented and unfragmented forests. Infection prevalence and the magnitude of multiple infections (with *S. fulleborni* and *S. stercoralis* among other parasites) were greater for red colobus in fragmented than in unfragmented forest with no difference in black-and-white colobus in both ecosystems. The results suggest that forest fragmentation can alter host-parasite dynamics related to changes in host population size in forest fragments.

Tatfeng *et al.* (2005) studied the mechanical transmission of a large variety of pathogens (bacterial, parasitic and fungal) by cockroaches (*Diploptera punctata*), commonly found in the tropics. In their study, cockroaches trapped from different sites (toilets, parlours, kitchens and bedrooms) from houses with pit latrines and a water system, carried bacterial, fungal and parasitic isolates at identical magnitude irrespective of the sampling site, including *Strongyloides stercoralis* larvae. In the same geographical area, Nigeria, Ogbolu *et al.* (2009) carried out a study on parasitological contamination of vegetables sold at selected markets in the southwestern part of the country. 68.3% of the vegetables were contaminated with different parasites including *S. stercoralis* (prevalence 45.8%) emphasizing the high risk transmission of these parasites in poor hygienic environments. The high parasitic prevalence in this area has been attributed to soil fertilization with human excrements and irrigation with contaminated water.

Dogs in Japan reared indoors (Itoh *et al.*, 2003) presented another important source of transmission. Puppies from pet shops and breeding kennels revealed a higher prevalence of *Strongyloides* spp., consequently presenting a real threat to humans. Similar results have been reported from Finland in kennel dogs infected with this parasite (including puppies' death as a result of infection) in spite of the temperate climate (Dillard *et al.*, 2007). This study revealed that *S. stercoralis* can complete its life cycle in dogs also in Northern Europe and may become a chronic problem in kennel environments.

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Chapter 4.2.3.16

Syngamiasis

[*MAMMOMONOGAMUS LARYNGEUS*]

Syngamiasis is a factual rare disease (∼100 cases so far) caused by the nematode *Mammomonogamus laryngeus*, a parasite of the respiratory tract of many mammals (cattle, deer, sheep, goats, cats, buffaloes, orangutans and elephants) (Foitova *et al.*, 2008; Marques *et al.*, 2005). Based on the reported cases, its distribution seems to be in China, Korea, Thailand, Philippines and the Caribbean (Turner *et al.*, 2003). Humans are infected accidentally by ingestion of larvae or eggs (Sossai *et al.*, 2007). The life cycle is not completely known into detail, but following other members of Syngamidae (the family to which it belongs systematically) the ingested eggs or larvae seem to migrate to intestine in order to invade the intestinal walls. From the intestine, the larvae are disseminated via the liver or heart towards the lungs. In the lung, mature worms develop and reproduce to produce eggs that are expelled through sputum. If ingested by sputum re-swallowing, eggs will be excreted in feces to infect reservoir animals possibly through some vectors (beetles, snails or earthworms?) (Figure 4.2.3.16.1). Clinical manifestations are heavy, dry chronic cough, hemoptysis and asthma-like symptoms.

4.2.3.16.1 SYNGAMIASIS AND ENVIRONMENT

Our knowledge about the environmental transmission of this parasite is very limited, primarily because of the very low prevalence of infections. However, some clues can be drawn from case studies. For example, a young woman visiting a resort in Jamaica complained of headache, a sore throat and a dry cough following salad consumption at this resort (Nosanchuk *et al.*, 1995). If the life cycle of this parasite includes vectors, such as beetles, snails or earthworms (see Figure 4.2.3.16.1) that may come in contact with eggs containing feces from different mammals as final hosts, then it can be speculated that accidental ingestion of these vectors can introduce this parasite into humans' gastrointestinal system. It is somehow clear that the parasite requires warm and humid environments to survive and reproduce, conditions met in the distribution countries. Another clue allegedly connected with insects' consumption might be drawn from orangutan's infection with *M. laryngeus* (Foitova *et al.*, 2008). Orangutans are known as insectivores and also eat jungle fruits; their habits include the practice of eating soil or stones. More studies are required to clarify the most potent vector for human infection with this parasite, albeit its low infection risk.
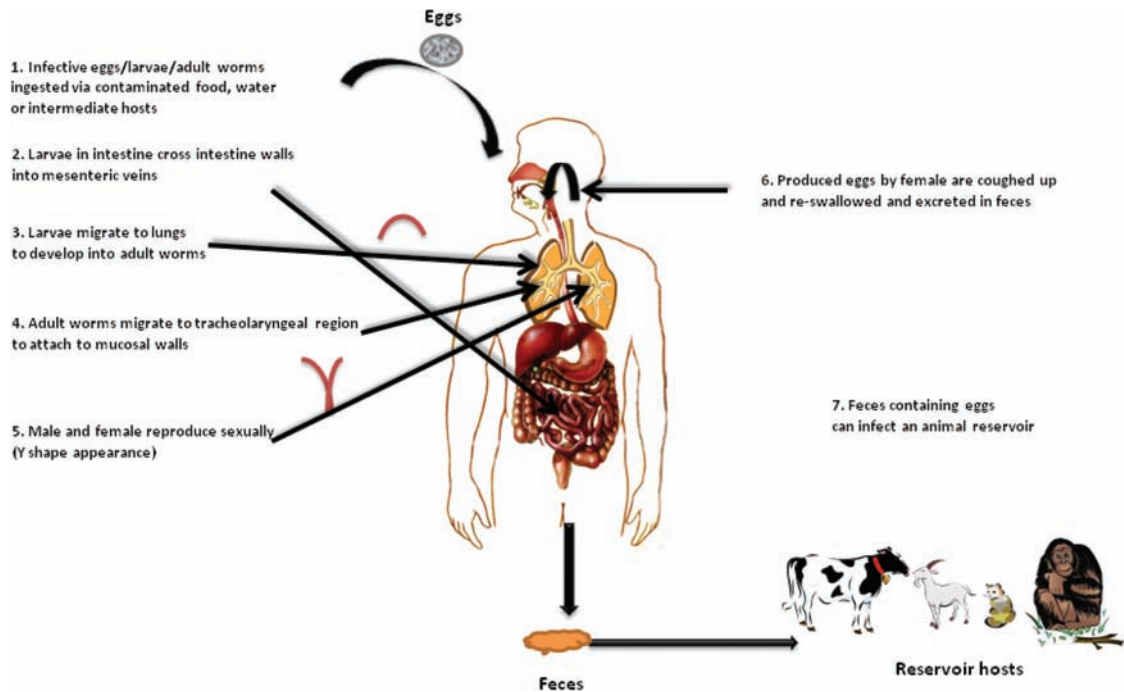
Figure 4.2.3.16.1. *Mammomonogamus laryngeus* presumed life cycle

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Chapter 4.2.3.17

Thelaziasis

[*THELAZIA CALLIPAEDA, T. CALIFORNIENSIS*]

Thelaziasis is also a very rare disease in humans caused by the nematodes *Thelazia callipaeda* and *T. californiensis*, affecting eyes and associated tissues resulting in inflammation. The parasite is distributed in Asia, Europe, and western North America. *T. callipaeda* occurs in Asia while *T. californiensis* in western United States with canids as main hosts (although birds, deer, cats can also be infected) (Dubay *et al.*, 2000). Transmission occurs through arthropod vectors such as flies (*Musca* and *Fannia* spp.) as intermediate hosts, that carry L1 stage larvae from the infected host eye following direct contact (via conjuctival secretions ingestion). Within the fly, L1 larvae develop into L3 larvae that leave the insect through mouthparts when flies feed on the final host's conjuctival fluid to develop into mature worms in the conjunctiva sac. Adult worms produce and shed larvae (ovoviviparity) into the conjunctiva (Figure 4.2.3.17.1). Clinical manifestations are usually mild, resembling conjunctivitis, watery eyes, corneal opacity and ulcerative keratitis.

4.2.3.17.1 THELAZIASIS AND ENVIRONMENT

Thelaziasis's hosts' diversity has been clarified by many studies but the role of different insect vectors on transmission is still debatable. In a recent meticulous study on *Musca domestica* (Diptera: Muscidae), it has been shown that this insect cannot act as a vector of *T. callipaeda* in southern Europe (Otranto *et al.*, 2005a) in contrast to a previous report (Shi *et al.*, 1988). Consequently, other insects have been studied for their vectorial potential. Otranto *et al.* (2005b) reported on *Phortica variegata* and *Phortica okadai* (both belonging to Drosophilidae family) as true vectors for *T. callipaeda* in southern Europe and in China. In addition, these authors revealed the overwintering capacity of these vectors (hibernation) infected with *T. callipaeda* that may explain the dissemination routes under various environmental conditions. The same group went a step further and found an interesting phenomenon, possibly unique in entomology: the fruitfly *Phortica variegate* (the drosophiloid vector) feeds on lachrymal secretions besides vegetable matter and under natural conditions *P. variegate* males are predominant in lacrymogenic feed in contrast to other arthropodborne diseases (see malaria, Kala-Azar, etc.) (Otranto

et al., 2006). The authors suggested that the relationship between *T. callipaeda* and the vector *P. variegate* is an example of convergent evolution in parasitology, requiring further examinations.
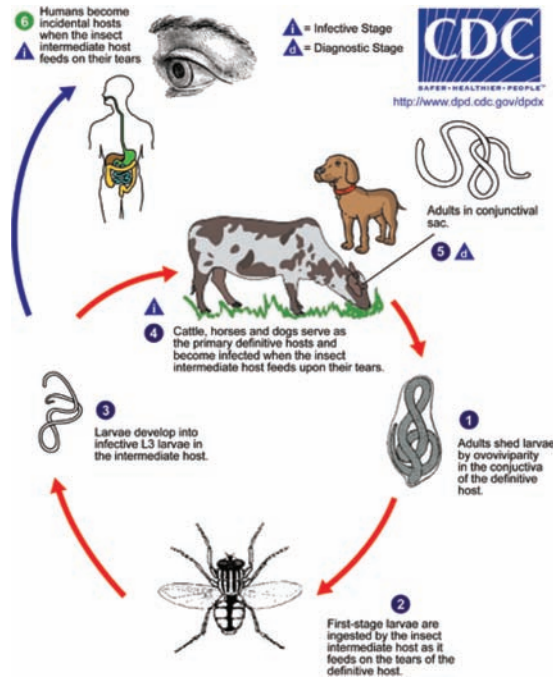
Figure 4.2.3.17.1. *Thelazia* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Thelaziasis_il.htm)

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Chapter 4.2.3.18

Trichinosis (Trichinellosis)

[*TRICHINELLA SPIRALIS*, *T. NATIVE*, *T. BRIVOTI*, *T. PSEUDOSPIRALIS*, *T. MURRELLI*, *T. NELSON*, *T. PAPUE* AND *T. ZIMBABWENSIS*]

Trichinosis is a disease caused by different species of *Trichinella* nematodes with a worldwide distribution in almost all climates, except desert (Kia *et al.*, 2009). Infection occurs when humans consume raw or undercooked infected meat of different animals infected with the parasite (mainly pigs, wild boars, horses, etc.) (Kia *et al.*, 2009; Haim *et al.*, 1997). Inspite of being the smallest nematode it may cause fatal disease in humans depending on infection burden. In spite of advanced veterinary control programs, there are still thousands of reported cases around the globe mainly due to ingestion of uncontrolled meat from a large variety of game (Kociecka *et al.*, 1992). *Trichinella* spp. have two infectious cycles: sylvatic and domestic where synanthropic animals (e.g. rats, foxes, mustelids, cats, dogs, wolves, etc.) contribute to sylvatic *Trichinella* genotypes' flow from wildlife to domestic animals and of *T. spiralis* from domestic to sylvatic animals (Pozio, 2000; Beck *et al.*, 2009). In this context, rats have been shown to play a significant role in the transmission and maintenance of *T. spiralis* in pig farms, even in the absence of infected meat, probably through cannibalism (Leiby *et al.*, 1990). In general, it can be stated that the *T. spiralis* genotype is mainly transmitted and maintained in a domestic cycle, although it can be present also in wildlife, while other genotypes (*T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. murrelli*, *T. nelsoni* and *T. papuae*, *Trichinella* T6, T8, and T9) are transmitted and maintained only in the sylvatic cycle (Masuoka *et al.*, 2009). More data on the various genotypes is presented in Table 4.2.3.18.1. Generally, *Trichinella* nematodes are divided into two groups according to larvae status in striated muscles: encapsulated (encysted) and nonencapsulated (unencysted) larvae. Their life cycle consists of ingestion of undercooked infected meat (that holds larvae). These larvae (encapsulated or unencapsulated) are then exposed to gastric acid and pepsin and are released to invade the small bowel mucosa to develop finally into adult worms (female and male). Females release larvae that migrate to striated muscles where they encapsulate (encyst), causing various clinical symptoms (Figure 4.2.3.18.1). Since the parasite is transmitted and carried by carnivores, it can be said that humans are dead-end hosts (in contrast, in pigs, where cannibalism is present, continuous infection takes place) (Pozio, 2000). Clinical manifestations are directly related to the larval content taken up by the patient. In the intestinal

Table 4.2.3.18.1 Some environmentally linked characteristics of *Trichinella* spp.

Larval status	Species or genotype	Geographic distribution	Host spectrum	Human infectivity	Human disease	Larval resistance in frozen muscles	Remarks
Encapsulated							
	T. spiralis	Worldwide	Pigs, wild boars, rats, horses, sheep, goats	+++	Myocarditis and encephalitis, can result in death	Yes in horse muscles	Slight cold resistance
	T. nativa	American arctic and subarctic regions, Asia, Europe	Polar bears and other carnivores (pigs rarely), black bear, walruses	+++	Fever, periorbital and limb edema, diffuse myalgia and rash	Yes in carnivore muscles	High cold resistance
	T. britovi	Temperate areas of Europe and Asia, Northern and Western Africa	Wild carnivores, pigs, horses	+	Myalgia, arthralgia, eyelid and facial edema	Yes in carnivore and horse muscles	Moderate cold resistance
	T. murrelli	United States and Southern Canada, Iran	Sylvatic carnivores	+	Provokes skin reactions and less facial edema. Long incubation period (post-Infection:10		Moderate cold resistance

Trichinela T6	Canada, Alaska, Rocky Mountains, and Appalachian Mountains in the United States	Wild carnivores	?	w-larvae encapsulation; 6 y-live encapsulated larvae; 14 y- larvae remnants). Moderate to severe pathogenicity Fever, diffuse erythematous papular rash, marked fatigue, chills, sweats, generalized headache, myalgia, pruritus, facial edema, anorexia.	Yes in carnivore muscles	High cold resistance, not in pigs
T. nelsoni	Eastern-Southern Africa	Wild carnivores (hyenas)	+	Myalgia, muscle weakness and damage, myositis, arthralgia. The arthralgia score was strictly related to myalgias-myositis.	No	Not resistant to cold, long survival in dead tissues
Trichinella T8	South Africa and Namibia	Wild carnivores	?	-	No	
Trichinella T9	Japan	Wild carnivores	?	-	No	
Trichinella T12	Argentina	Cougars	?	-	Unknown	

(*Continued*)

Table 4.2.3.18.1 (*Continued*)

Larval status	Species or genotype	Geographic distribution	Host spectrum	Human infectivity	Human disease	Larval resistance in frozen muscles	Remarks
Nonencapsulated							
	T. pseudospiralis	Worldwide	Sylvatic mammals and birds, domestic pigs (Tasmanian devil, birds of prey, tiger cat, brushtail possum)	+	Tiredness, muscle fatigue and pain, muscular swelling, myalgia, and asthenia persisting for >4 months	No	Not resistant to cold, unencapsulated larvae in muscle
	T. zimbabwensis	East Africa	Nile crocodiles, monitor lizards	?	-	No	Unencapsulated larvae in muscle, larvae size 1/3 of T. pseudospiralis's larvae
	T. papuae	Papua New Guinea	Wild pigs, saltwater crocodiles	?	-	No	

?- Not documented
a- Compiled from (Gottstein *et al.*, 2009; Krauss *et al.*, 2003).

phase (development into adult worms), nausea, vomiting, epigastric pain, diarrhea or constipation prevail while during the visceral phase (migration and colonization of striated muscle) headaches, fever, arthralgias, cough, myalgia, conjunctivitis, edema, hemorrhage, anorexia, neurological complications (deafness, encephalitis, and convulsions), myocarditis and death are the main clinical manifestations.
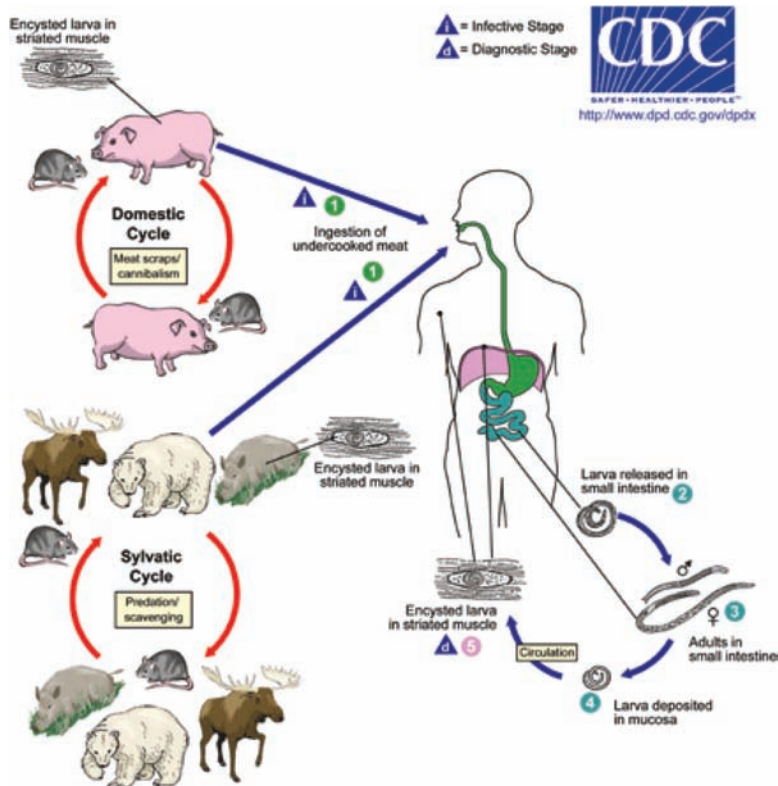
Figure 4.2.3.18.1. *Trichinella* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Trichostrongylosis_il.htm)

4.2.3.18.1 TRICHINOSIS AND ENVIRONMENT

Environmentally, it is clear that the prevalence of infections depends solely on human behavior. Commercial growth of pigs or fur animals (blue and silver foxes, minks and raccoon dogs) in farms can both contribute to the environmental spread of *Trichinella* spp. (Miller *et al.*, 2006; Capó and Despommier, 1996). Hill *et al.* (2010) described a pig farm infected with *T. spiralis* in Maryland, USA. Six months after, depopulation of the pig farm, surrounding scavenging animals were trapped and tested for infection after 6, 12 and 18 months. The peridomestic scavenging mammal' population revealed a drastic decline in infection: 41, 10 and 0% respectively, revealing that breaking the vicious cycle of rat-pig-human transmission by eliminating the pigs could put infection to an end. In relation to rats, Oivanen *et al.* (2002) showed that

Trichinella spiralis larvae in rat carcasses could survive for up to 6 weeks. Trichinosis transmission is based on two most important biological factors: 1) the muscle stage larvae's ability to survive in decaying carcasses (anaerobic metabolism in nurse cells) and 2) survival at low freezing temperatures (Owen and Reid, 2007). Both characteristics play a major role in the distribution of this parasite. For example, *T. papuae*, a nonencapsulated larvae, may retain infectivity in decaying pig tissue exposed at 35°C for nine days and for 4 months in heavily rotten meat at ambient temperature (Gottstein *et al.*, 2009). In the other extreme, *T. britovi* has been reported to survive in frozen carrion for up to one year, while the *T. nativa* and *Trichinella* genotype T6 for up to several years, all maintaining potential infectivity for future hosts (Dick *et al.*, 2001; Pozio *et al.*, 2006). Another excellent environmental adaptation of this parasite has been shown by the distribution of *Trichinella nativa* and *Trichinella britovi* in a northern European country (Estonia) according to its isotherms. In sylvatic animals, *T. nativa* was found mostly in central-eastern regions of the country (east of isotherm −5°C) while *T. britovi* was detected in western parts of the country (west of isotherm −4°C), both isotherms being measured in January (the winter coldest month) (Pozio *et al.*, 1998). Pozio *et al.* (1996) surveyed domestic and sylvatic animals for infection with *T. spiralis* and *T. britovi* in France, Italy, and in the Extremadura region of Spain. These authors showed that *Trichinella spiralis* and *Trichinella britovi* distribution is influenced by both environmental and human behavior factors. The results of this survey suggested the following issues: 1) domestic trichinellosis occurs only in rural areas of Western Europe in association with traditional swine-rearing practices, but not in industrialized pig farms; (2) sylvatic trichinellosis occurs only in natural habitats such as widespread and mountainous areas (in Western Europe); (3) vulpines are the primary reservoir of the parasite in a sylvatic cycle closed circuit and (4) among sylvatic animals, *T. spiralis* is present at a lower altitude than *T. britovi*. From another region on the globe (Argentina) a potential flow of the parasite from domestic to sylvatic swine in a trichinosis-free area has been reported (Cohen *et al.*, 2010). These authors based their speculation on the fact that the wild boar has a high degree of tolerance to human presence (pasture environments) and human improper handling of domestic and wild swine carcasses (scattering pork scraps in the environment, consequently spreading *Trichinella* infection among wild boars (Pozio and Murrell, 2006).

Birds have been studied for their potential infection with *Trichinella* spp., however only *T. pseudospiralis* has been reported in night-birds of prey (Pozio *et al.*, 1999). Birds have a real potential of spreading this parasite due to long distance journeys and also the potential to be preyed on by different carnivores. In a recent study, quails were exposed to larvae of the arctic strains of *T. native* in order to investigate their viability and preservation (Odevskaia, 2010). A rapid expulsion of bulk helminths was observed (due to abundant mucus excretion in response to infestation) with prolonged survival in environment (probably connected to the excess mucus as a protectant). The author suggested that according to these results it couldn't be excluded that birds could infest other marine mammals (e.g., pinnipeds). Cockroaches have been also suggested as a potential vector of *Trichinella* (based on preliminary results supporting these nematodes' persistence and transmissibility in the environment); however, more studies are needed to support this direction (Chan *et al.*, 2004).

Finally, Rosenthal *et al.* (2008) compared the genetic diversity of *T. spiralis* around the globe and found a remarkable uniformity across the continents. They concluded that European lineages originate from pig domestication (several thousand years ago) where swine husbandry maintained the infective cycle. In addition, these authors implied that the New World spread was unintentionally caused via infected pigs and/or rats introduction through maritime trade. Perhaps, here is the occasion to ask: whether these parasites did previously exist in the New World? and, if affirmative, what role did humans play in its further expansion through hunting before domestication?

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Chapter 4.2.3.19
Trichostrongylidiasis (Trichostrongylosis)

[*TRYCHOSTRONGYLUS, MARSHALLGIA, MECISTOCIRRUS* GENERA]

Trichostrongylosis is a mild disease caused by a group of ruminants' nematodes from the genera: *Trychostongylus*, *Marshallgia* and *Mecistocirrus*. The distribution of these nematodes is worldwide with some geographic differences, but herbivorous herds (cattle, sheep, donkeys, goats, deer, tapir and rabbits) are commonly infected, besides humans and primates (Freeman *et al.*, 2004; Cruz *et al.*, 2006). Higher prevalence has been reported from Middle East countries such as Iraq and Iran. Trichostrongyles are transmitted by ingestion of water, vegetables or other plants contaminated with third-stage larvae of the nematode and/or farmers utilizing dung for burning and fertilization. The life cycle consists of eggs excreted on pasture by infected ruminants. In environment, rabditiform larvae hatch and develop into filariform larvae (L3 stage) that are the infective to humans and animals. Once ingested, the filariform larvae move briefly into the crypts of the small intestine to develop further into eggs producer adult worms (Figure 4.2.3.19.1.1). Clinical manifestations are mostly asymptomatic but mild gastroenteritis may develop. In high infestation cases, persistent diarrhea, abdominal pain and anemia have been reported.

4.2.3.19.1 TRICHOSTRONGYLOSIS AND ENVIRONMENT

Consistent with their definitive environment, *Trichostongylus* spp. survival is linked to pasture moisture, temperature, migration and presence of available hosts (herbivorous herds) (Banks *et al.*, 1990; Almeida *et al.*, 2005; Nogareda *et al.*, 2006; O'Connor *et al.*, 2006). Lettini and Sukhdeo (2006) demonstrated that L3 larvae of *Trichostongylus colubriformis* could enter an anhydrobiotic stage following desiccation. These larvae were capable of resisting up to seven desiccation/rehydration cycles, revealing low metabolic activity during this process. Interestingly, in the anhydrobiosis state larvae could survive low temperatures (0°C), a feature that promotes their competence to persist under freezing temperatures (Garcia *et al.*, 1997). Desiccation resistance of L3 larvae has been also shown in Guadeloupe (a tropical region tempered by trade winds with moderately high humidity), where *Trichostongylus's* frequency was positively correlated to the dryness component (Simon *et al.*, 1996). These resistance characteristics

of the *Trichostrongylus* species may explain their persistence in pastures subjected to an intensive rotational grazing (IRG) regime in cold temperate regions in comparison to other gastrointestinal nematodes (Colvin *et al.*, 2008; Rossanigo and Gruner, 1995). Another well-known phenomenon of the superfamily Trichostrongyloidea is arrested development. In temperate regions, these nematodes usually inhibit their development before the winter season in areas where transmission and infection occur mainly in summer or summer inhibition in areas where transmission is mainly from autumn to spring. Langrova *et al.* (2008) studied this phenomenon and concluded that in temperate climate (e.g., Europe) the hypobiosis seems to be connected to the advent of changing photoperiod and cool temperatures. Thus, exposure to low temperature and changing photoperiod could triggers the arrested development of infective larvae in favor of more preferable conditions (coinciding with lambing). It can be speculated that under global warming effect, the hypobiosis will shorten hence increasing the parasite burden in those animals (including humans) (Hudson *et al.*, 2006).
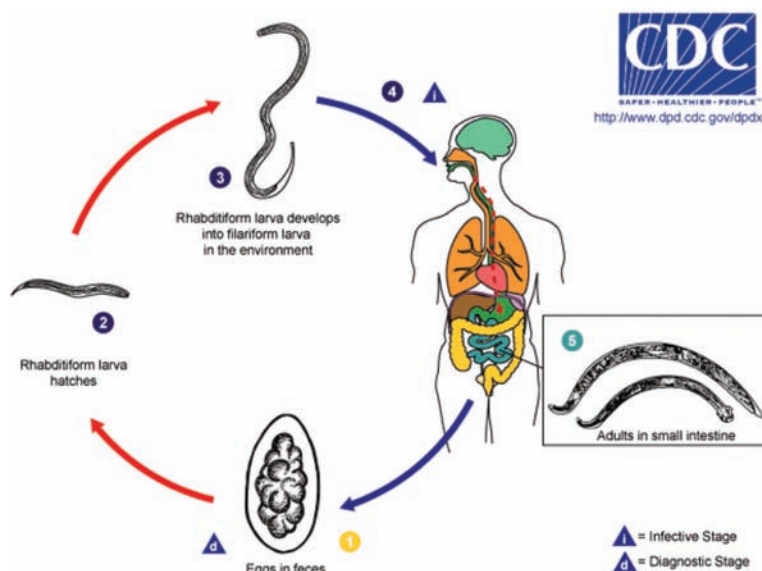
Figure 4.2.3.19.1. *Trichostrongylus* spp. life cycle (with permission from CDC, Atlanta, USA; http://www.dpd. cdc.gov/dpdx/HTML/ImageLibrary/Trichostrongylosis_il.htm)

Lacharme-Lora *et al.* (2009) studied the connection between parasitic helminths as possible vectors of pathogenic bacteria (e.g., *Salmonella* gp 3B -diarizonae) similar to well know free-living helminths (earthworms, see chapter 2.1 on Anthrax) in connection with soil environment (Tymczyna *et al.*, 2000). Using fluorogenic proteins, they were able to prove bacterial internalization inside these parasites. The remaining question is: How does this "collaboration" affect animals' and human health.

Uriarte and Gruner (1994) conduct an experiment on the nematodes eggs infestation degree of pasture plots by means of two irrigation methods: flooding and spraying. A significant difference was found between the two irrigation methods in relation to the vertical distribution of larvae: spraying affected grass migration while flooding affected soil migration. These authors concluded that spray irrigated pasture has a higher risk of helminthic infection for calves. These results have been supported by another

laboratory study carried out on simulated rain impact (closely resembling spraying irrigation) on L3 larvae (present on cowpats) translocation (Gronvold and Hogh-Schmidt, 1989). Pasture plants' composition had been shown also to play a significant role in the successful transmission of *Trichostrongylus tenuis* L3 larvae to a susceptible host. Saunders *et al.* (2001) demonstrated experimentally that L3 larvae exhibited selective migration towards heather, possibly reflecting a general response to plant cues which may be stronger for heather than for grass (used as the control in their experiments). It remains to be seen whether pasture without heather is less infectious for herbivores… Also related to pasture ecology and forested areas, Jackson *et al.* (1986) compared experimentally the survival of infective larvae on an open pastured block and an adjacent block forested with *Pinus radiata*. The results revealed a significantly higher recovery of *Trichostrongylus* spp. from the pine forested block, explained by these authors as most likely to be related to the more favourable climatic environment under the trees. Another bioenvironmental parameter, occasionally neglected, is the different insects involved in the ecology of herbivorous excretions. Chirico *et al.* (2003) studied the impact of dung beetles on the development of trichostrongylid eggs into larvae. Dung beetles (*Aphodius* spp.) were reported to optimize conditions in fresh dung to favor larval development of these nematodes under stable environmental conditions. An interesting observation made by the authors was that this synergistic effect is a result of the specific experimental dung beetles employed in their study: dung dwellers and not dung removers/burrowers like other dung beetle spp.

Hoste *et al.* (2001) compared nematode burden (including *Trichostrongylus* spp.) in two breeds of goats: Saanen dairy goats and Angora goats, both placed in the same rangeland environment. Angora goats repeatedly had higher nematode egg excretion levels compared to Saanen goats. The authors attributed this fact to different feeding behavior of the two goat species: Angora beeing mainly grazers whereas Saanen beeing mainly browsers. Assuming both breeds similar immunological response of toward helminthic infection, it can be positively suggested that browsing reduces infestation by means of statistical probability.

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Chapter 4.2.3.20

Other Nematodic Infections

[*ANCYLOSTOMA CEYLANICUM, PHYSALOPTERA CAUCASICA, TERNIDENS DEMINATUS*]

Ancylostoma ceylanicum parasitizes carnivores' small intestine (e.g. dogs, cats) and occasionally humans (Baker *et al.*, 1989; Chowdhury and Schad, 1972; Yoshida *et al.*, 1968). Distribution is mainly in Africa and Asia, but recently it was reported to be present in dogs from Australia (Rep and Heinemann, 1976; Scholz *et al.*, 2003; Setasuban *et al.*, 1976; Yoshida *et al.*, 1973; Palmer *et al.*, 2007). Transmission is percutaneous by L3 stage larvae or direct ingestion (via contaminated vegetables). *A. ceylanicum* mature into small intestine mucosa while sucking blood, to further produce eggs. Consequently, the direct clinical manifestation can be anemia.

Physaloptera caucasica parasitizes monkey's esophagus and small intestine (suborder Simiae). In the past (early 20th century) tropical Africa was the focus of humans infections, however additional human cases had been reported from South America (Liang-Sheng, 1957; Fain and Vandepitte, 1964; Apt *et al.*, 1965; Pettifer, 1984). The life cycle is not clear; however, possible transmission may occur through ingestion of arthropods (beetles, cockroaches and crickets) that harbor infectious larvae. In humans, it can be found along the digestive tract (from esophagus to small intestine). Clinical manifestations are vomiting, bloody diarrhea and abdominal pain.

Ternidens deminatus is another simian parasite (e.g. red-tailed monkeys, baboons) found in Africa and Asia (Kooriyama *et al.*, 2010; Bezjian *et al.*, 2008; Hope *et al.*, 2004; Hemsrichart, 2005). Humans' infections are prevalent in South Africa (Goldsmid, 1972). The life cycle is not well defined, but it seems that L3 stage larvae infect humans directly without an intermediate host, via ingestion. The parasite development in digestive tract is blood dependent; consequently, anemia has been reported in acute infections beside nodules formation.

4.2.3.20.1 *ANCYLOSTOMA CEYLANICUM, PHYSALOPTERA CAUCASICA, TERNIDENS DEMINATUS* AND ENVIRONMENT

Like most of the previously described nematodes, low socio-economic status, poverty, poor hygiene, ignorance and close animal contact (domestic and wildlife) are the main factors contributing to

helminthic infections, including rare ones. The rare parasitic nematodes described here seem to accidentally infect humans mostly in Africa and Asia where primates are in close contact with. Experimental studies on cross-infection of hamster with *A. ceylonum* isolated from a dog revealed that this nematode has a broader distribution possibly in rodents too, implying on a possible role of rodents in transmission (Ray *et al.*, 1972).

An interesting report came from United Arab Emirates, a desert area, revealing *A. ceylanicum* prevalence of 8.8% in domestic and stray cats (Schuster *et al.*, 2009). Indeed, oral and cutaneous larval infections had been proved to be a possible route of infection in animals and humans, but according to the authors transplacental and galactogene routes or involvement of paratenic hosts could not be ruled out in the present case (similar to other known hookworms). Another reasonable possibility is import of already infected animals from other countries where this parasite is endemic.

Goldsmid and Jablonski (1982, 1986) reported on specific granules present (with some inconsistency) in *T. deminutus* that contain ZnS. They suggested that these inorganic elements are a result of blood feeding (sulphur originating from sulphur-containing amino acids and zinc from host dietary intake). A brief glance in any elementary inorganic chemistry book shows that ZnS is phosphorescent on illumination with blue or ultraviolet light and can be used as infrared optical material. If ZnS accumulation is linked to these optical phenomena for parasite benefit in some ways or just a by-products result from host feeding is not clear. Finally, Cappelo *et al.* (2006) showed that a crystal protein from the bacterium *B. thuringiensis* (acknowledged for many years as an excellent insecticide) has antihelminthic activity against *Ancylostoma ceylonicum*. It will be interesting to find out if *Physaloptera caucasica* that is possibly transmitted by insects is also sensitive to the crystal protein of *B. thuringiensis*?

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Chapter 5

Fungal Zoonoses

Chapter 5.1

Microsporum spp.

**[*MICROSPORUM CANIS, M. NANUM, M. AUDOUINII, M. EQUINUM,
M. GALLINAE*]**

Microsporum is a genus of fungi that causes various dermatophytoses such as: tinea capitis, tinea corpus, ringworm, etc. *Microsporum* encompass seventeen species described till now, divided by their environment: zoophilic, geophilic and anthropophilic. *Microsporum* has the typical fungal forms macroconidia, microconidia and conidiophores. Macroconidia range from 7 to 20 by 30 to 160 μm while microconidia range from 2.5 to 3.5 by 4 to 7 μm in size. The shape, size and cell wall features are important characteristics for species identification. *Microsporum canis* is the most significant zoonotic fungi followed by *M. nanum* (from pigs), *M. audounii* (from dogs), *M. equinum* (from horses) and *M. gallinae* (from chickens) (Table 5.1.1). The distribution is worldwide and via numerous animals in close contact with humans: cats (largely), dogs, horses, pigs, sheep, rabbits, hamsters, rats and zoo animals (monkeys, tigers, jaguars, lynxes) (Cafarchia *et al.*, 2006; Cafarchia *et al.*, 2010; Sykes and Ramsay, 2007; Galo *et al.*, 2005; Favrot and Zaugg, 2005; Gonzalez *et al.*, 1995; Gallo *et al.*, 2005). Besides direct contact with infected animals, contact with arthropod carriers such as: lice, flies, fleas and mites may transmit these dermatophytes to humans. The main clinical symptoms are a result of keratolysis of skin, hair, and nails that include: rash, inflammation, lesion and secondary contamination as a result of area scratching. It can be stated that dermatophytes infection are chronic without any acute impact on human health; however, chronic infections cannot be ignored as described later on. In humans, internalization through inhalation has been suspected to cause more severe diseases (Nakachi *et al*, 1999). Fatal cases were only reported in pigs (Gonzalez *et al*, 1995).

5.1.1 *MICROSPORUM* SPP. AND ENVIRONMENT (FIGURE 5.1.1)

Based on their ubiquity, *Microsporum* spp. can be found almost everywhere and, besides host factors (e.g., ichthyosis, atopy, topical and systemic glucocorticoids, collagen vascular disease, mechanical lesions and injuries), local factors are also important in dermatophytic infection (e.g., sweating, occlusion, occupational exposure, geographic location, tropical or semitropical climates with high humidity).

Dermatophytes preferences for elevated temperatures and humidity, conditions present in sub-tropical and tropical regions and in enclosures such as shoes (that preserve these conditions), will help their proliferation. Salvo and Fabiano (2007) assessed Ligurian beaches (Italy) for the presence of dermatophytes in sediments. They found that *Microsporum* spp. represented 5% of the total filamentous fungi screened, particularly during the tourist season. Beside the risk of contracting a fungal infection on the beach it should be emphasized that contamination sources are humans themselves, animals and sewage. Sewage enrichment of soils (clay and sand) was shown to increase microfungal communities compared to untreated ones, including human pathogens (*Microsporum* spp.) (Kacprzak & Woszczyk, 2004). Next to sewage, sewage sludge was also shown to comprise a large variety of fungi including dermatophytes. If soils are amended with sewage sludge without any further disinfection treatment, present dermatophytes pose a human and animal increased risk alongside other numerous pathogens (Ulfig, 2009).

Table 5.1.1 Some characteristics of zoonotic *Microsporum* spp.

Microsporum spp.	Animal main sources	Other sources	Zoonosis	Category
Microsporum canis	Cat, dog, horse	All mammals	Frequent	Zoophilic
Microsporum gallinae	Poultry	Dog, cat	Rare	Zoophilic
Microsporum gypseum	Soil	All mammals	Possible	Geophilic
Microsporum nanum	Soil	Pig	Rare	Geophilic
Microsporum persicolor	Rodents	Dog, cat	Possible	Geophilic
Microsporum audounii	Humans	Dog	Frequent	Anthropophilic

Dermatophytes are distributed ubiquitously as shown by Yu *et al* (2004) who used advanced molecular methods and found the same strains of *M. canis* in an outbreak of tinea capitis in school children (age 3.5 to 10 years old) and their environment, pets and even fomites (pillows and carpets). In Nigeria, school age children were found to harbor *Tine capitis* (*Microsporum* spp.) at high frequency, following head lesions (Ayanbimpe *et al.*, 2008). Dermatophyte prevalence was affected by social and cultural habits (frequent interaction with soil and animals, poor health education and environmental hygiene) rather than population density. In relation to population, another aspect related to dermatophytes was reported by Monod *et al.* (2002) who screened autochthonous dermatophytes in Switzerland: new detected strains seem to be introduced by human immigration.

Sharma *et al.* (2010) studied the prevalence and distribution of keratinophilic fungi in a semi-arid region. Among the mixture of different fungi, *Microsporum canis* and *Microsporum gypseum* were also reported. These authors mentioned that industrial soil samples were most diverse in fungal flora. This observation is in agreement with industrial soil pollution with a large variety of anthropogenic chemicals that only fungi are competent to biodegrade, an obvious advantage of these microorganisms. Tampiri (2006) described a change in human dermatomycoses in Italy in the past century. The change consisted of a shift from anthropophilic to zoophilic fungi based on animals as substrata for pathogenic fungi growth. The life style in Italy for the last five decades, with overpopulation of birds, pets, wild and domestic animals together with humans in crowded urban area supports the growth of those fungi, increasing infection risk. In addition, animal debris creates optimal conditions for the growth and the multiplication of geophilic or saprophyitic fungi, such as *Microsporum gypseum* and other fungi. As a final point, the

author emphasized the importance of environments heavily populated by fungi as a ground for allergic pulmonary reactions and worse, as already described by Nakachi *et al* (1999).
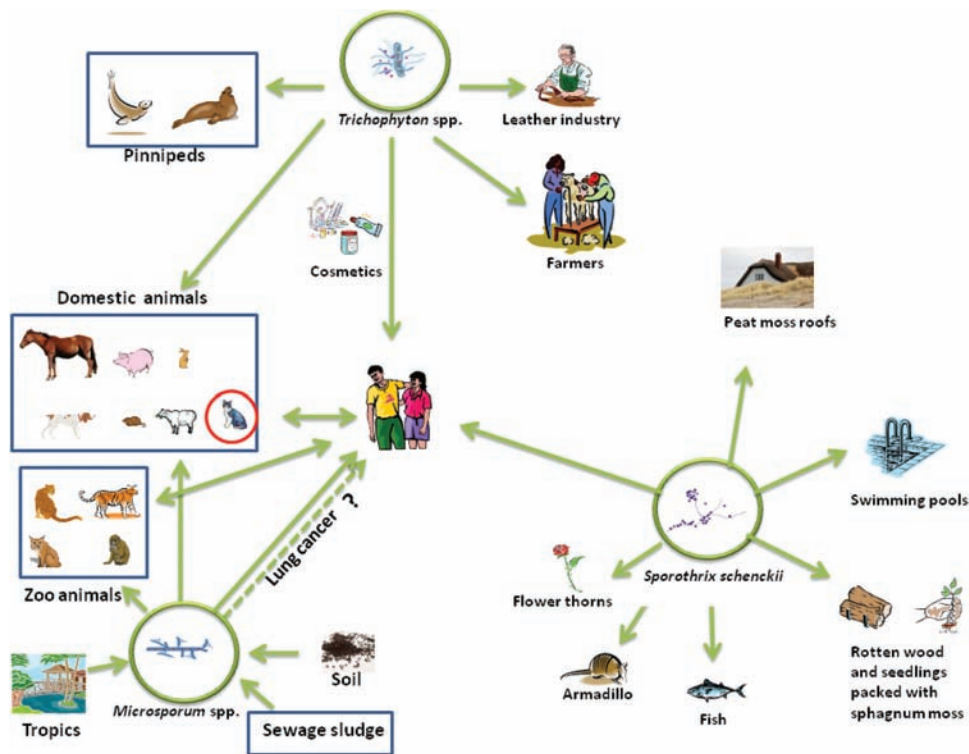
Figure 5.1.1. Zoonotic dermatophytes and environmental factors involved in human infection

Perhaps the most important report on the life threatening aspects of dermatophytes was presented by Nakachi *et al* (1999). These authors performed a comprehensive epidemiological study in northern Thailand where in women lung cancer incidence is very high (40.9 per 100,000, one of the highest in Asia) and compared it with a lower incidence rate (8.5 per 100,000), both groups being in closely related areas, geographically and culturally. Interestingly, among the analyzed parameters linked to high cancer prevalence, cigarette smoking was lower in the high lung cancer incidence area; nevertheless, *Microsporum canis*, most commonly found indoors in this area, was significantly correlated in comparison with the low incidence area (control). In addition, these authors found significant high serum concentration of a fungus constituent, concluding that *M. canis* chronic infection is likely to be involved in the etiology of female lung cancer in North Thailand. These findings are in agreement with a previous publication, in which *Candida parapsilosis* cell extract was demonstrated to cause sarcoma in mice. In the same study, increased frequency of leukemias and pulmonary tumors were observed with lipids extracts from *Microsporum*, *Trichophyton*, *Epidermophyton* and *Scopulariopsis* (Blank *et al*, 1968). This primary epidemiological link between dermatophytes and human lung cancer deserves further research in the future.

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Chapter 5.2

Pneumocystosis (*Pneumocystis* pneumonia)

[*PNEUMOCYSTIS JIROVECII* FORMERLY *CARINII*]

Pneumocystis is a yeast-like fungus (in the past erroneously classified as protozoa) that infects humans and animals (Lainson and Shaw, 1975). *Pneumocystis* pneumonia is mainly found in immunocompromised individuals, such as AIDS patients, transplant recipients, cancer patients and those exposed to ionized radiation (Hui and Kwok, 2006; Hennequin *et al.*, 1995; Lavdovskaia *et al.*, 1996). The old name, *Pneumocysitis carinii* applies now to an animal variant. Domestic and free-living animals, such as rodents, pigs, rabbits, sheep, goats and dogs, are possible sources of human infections. However, there is an increasing collection of evidence that this fungus is mostly transmitted through the airborne route (aerosols and contaminated dust) (Schmoldt *et al.*, 2008). Wakefield *et al.* (1992), applying DNA amplification and subsequent sequencing of a portion of the mitochondrial gene encoding the large subunit ribosomal RNA, found that *P. carinii* is closely related to the ustomycetous red yeast fungi, a group of fungi environmentally distributed with airborne spores characteristics. The life cycle is not completely known; nevertheless, inhaled mature cysts containing trophozoites break up and release these haploid bodies along lung alveolus surface. Then, asexual and sexual pathways take place. In the asexual path, trophozoites multiply by binary fission, while in the sexual path two trophozoites fuse to form a zygote. The zygote will form the early precyst with eight haploid nuclei following meiotic and mitotic divisions (Figure 5.2.1). The mature cyst contains eight trophozoites that will be further spread in the host or a new host after cyst wall rupture. In humans, clinical manifestations are: pneumonia (*Pneumocystis* pneumonia), dry cough, moderate fever, dyspnea, retrosternal pain, tachypnea, tachycardia and cyanosis. In AIDS patients, other organs are also infected (lymph nodes, bone morrow, liver, and spleen) where fatality can reach~20%.

5.2.1 *PNEUMOCYSTIS* AND ENVIRONMENT

Hughes (1982) clearly showed that *Pneumocystis jirovecii* (formerly *carinii*) is transmitted by air. This author exposed axenic rats to natural air, food and water versus filtered air. Experimental animals fed with contaminated lung tissue and contaminated water did not acquire infection. Those exposed to

unfiltered air but fed with sterile food and water did contract pneumocytosis. Thus, this fungus is mainly air-borne as also shown by positive nasal swab samples taken from contact health care workers and their family (Powierska-Czarny *et al.*, 2004). In this context, it will be interesting to find out if mostly children (but some adults too) that pick their nose are indirectly inoculating themselves to develop immunity against this opportunistic pathogen.
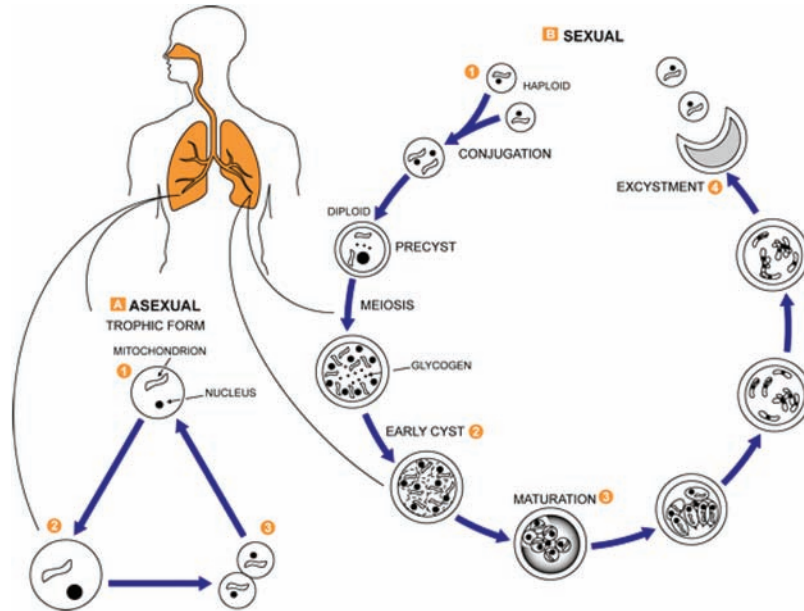
Figure 5.2.1. *Pneumocystis jurovecii* life cycle (with permission from CDC, Atlanta, USA; http://dpd.cdc. gov/dpdx/HTML/Pneumocystis.htm)

Kunakbaeva *et al.* (2006) used PCR to detect *P. jirovecii* (*carinii*) in air and rinse from medical equipment. These authors reported on intact DNA from the fungus for 12 days including 2 hours of UV irradiation. A 0.1% solution of chloramine with a subsequent 30 minutes UV irradiation eliminated *Pneumocystis* DNA. Similar results connected to hospital environments have been reported from samples taken by air-filtration from rooms of *P. carinii*-infected patients (Bartlett *et al.*, 1997; Olsson *et al.*, 1998).

Using multiple linear regression statistics, Sing *et al.* (2009) looked for a correlation between *P. jirovecii* pneumonia and climatic factors in Germany. Pneumonia prevalence caused by this fungus was found to be positively correlated to temperature (being maximal in the summer months, temperature 14.2–18.6°C) but not to rainfall and wind strength. In Spain, Varela *et al.* (2004) reported also that minimum mean temperature (7–9.7°C) was the main meteorological factor influencing infection; in other words the seasonality of *P. jiroveci* infection could result directly from increased infection, but in their case during the colder months. From these studies, it can be epidemiologically speculated that in Spain, during winter time, people will reside more indoors possibly infecting each other, while in Germany during summer time, people are more outdoor and their behavior or type of leisure activity might be relevant for *Pneumocystis* transmission. Thus, it is still unclear how this opportunistic pathogen is transmitted, though it is very possible that different animals surrounding human habitat and environment can play a role as reservoirs.

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Chapter 5.3

Trichophyton spp.

[*TRICHOPHYTON MENTAGROPHYTES, VERRUCOSUM, EQUINUM, QUINKEANUM, ERINACEI*]

Trichophyton is a fungus genus characterized by formation of smooth-wall macroconidia and microconidia. Macroconidia (clavate to fusiform shape) have a size range from 40 to 8 by 8 to 50 μm, organized directly on hyphae or on short pedicels. Macroconidia are few or absent in many species. Microconidia (clavate, pyriform, spherical or irregular shape) have a size range from 2 to 3 by 2 to 4 μm. The genus comprises approximately 26 spp., where *Trichophyton mentagrophytes*, *T. verrucosum*, *T. equinum*, *T. quinckeanum* and *T. erinacei* are zoonotic (Table 5.3.1). The distribution is worldwide in many animals including farm animals and more exotic ones such as pinnipeds (Takatori *et al.*, 1993; Pollock *et al.*, 2000; Tanaka *et al.*, 1994; Torres-Rodriguez *et al.*, 1992; Otcenasek *et al.*, 1980). Children are at increased risk by direct and continuous contact with their pets as well laboratory workers with animals (Van Rooij *et al.*, 2006; Hironaga *et al.*, 1981). Transmission occurs mainly directly between humans and animals, but indirect contamination may occur via fomites (mainly leather products). Infected humans are carriers of these dermatophytes (anthropization) even into extreme conditions such as Antarctic environments, but their survival there is still unknown (Mercantini *et al*, 1993). Presentation of clinical manifestations is via acute inflammation (deep-seated) called "kerion celsi" (pustular inflammation of hair follicles), subcutaneous abscesses, lymphoadenopathy, fever and malaise (Van Rooij *et al.*, 2006).

5.3.1 *TRICHOPHYTON* SPP. AND ENVIRONMENT

Besides clinical damage, dermatophytes (e.g. *Trichophyton* spp.) are sturdy biodegraders of hair, nails and skin (typically of keratin nature) and consequently destructive of a variety of natural products such as wool, leather and horn made tools. Shrivastava *et al.* (1996) described *T. simii* with increased keratinolytic and sulphitolysis (oxidation of cystine to S-sulphocysteine) able to biodegrade wool. A similar destructive activity of these fungi was also reported in France, where 40% of the skins to be treated by tannery are damaged with ringworm lesions (Chermette *et al.*, 2008). In environment, arthrospores of *Trichophyton*

mentagrophytes were found to germinate under certain conditions, such as high temperature (optimal 37°C), pH from 5.5 to 8.0, in the presence of certain single amino acids or oligopeptides known to be present in the human cutaneous tissues, oxygen dependent and relative tolerance to NaCl and various fungicides (clotrimazole, cycloheximide, griseofulvin, and tolnaftate) (Hashimoto and Blumenthal, 1977). Together with other pathogenic fungi, *T. mentagrophytes* was exposed *in vitro* to seawater (various salinity, 6–50%) at different temperatures (20-35°C) in a diurnal rhythm of 12 hour cycles and found to survive for up to 52 weeks, suggesting possible human infection from this environmental source (Hashimoto and Blumenthal, 1977). Related to seawater, Pollock *et al.* (2000) reported on fungal infection in captive pinnipeds and concluded in accordance with their findings that this infection was caused by two key environmental factors: pool water excessive chlorination (excessive chlorination can cause scorching skin damage that facilitates fungi infection) and warm water temperature.

Table 5.3.1 Some characteristics of zoonotic *Trichophyton* spp.

Trichophyton spp.	Animal main sources	Other sources	Zoonosis	Category
Trichophyton mentagrophytes	Rodents (hamster, mouse, chinchilla, guinea pig, rat), rabbit, dog, cat, horse, cattle, sheep, pig, zoo animals (particularly monkeys)	All mammals, pinnipeds	Frequent	Zoophilic, Anthropophilic
Trichophyton equinum	Horse	Cat, dog (rare)	Rare	Zoophilic
Trichophyton erinacei	Hedgehog	Dog	Possible	Zoophilic
Trichophyton sarkisovii	Camel	Cattle, horse, dog, cat	?	Zoophilic
Trichophyton simii	Primate, chicken	Fowl, dog, cat	Rare	Zoophilic
Trichophyton verrucosum	Cattle, other ruminants (sheep)	All mammals	Frequent	Zoophilic

A clear characteristic of these fungi is their inactivation by nitrogenous compounds (urea, ammonia, and nitrate) at high concentrations (Kosuge *et al.*, 2002). Distribution of these compounds in cattle breeding soil inhibited the growth of *T. verrucosum*, reducing soil as a potential reservoir for infection. In relation to soil, wild animals are the closest organisms to this environment, suggesting that wild animals play a significant role as carriers of dermatophytes (geophilic and zoophilic) (Mantovani *et al.*, 1982) (Figure 5.1.1).

From data on dermatophytoses' cases reported for a period of five years in the Florence area (Italy), *T. mentagrophytes* represented 6% of people infected with different dermatophytes (Sberna *et al.*, 1993). The authors pointed out that the largest part of the patients resided in rural areas with pets, and hence were infected through direct contact with soil or domestic animals. An important feature that may boost dermatophytoses is the growing use of cosmetics. In the past, cosmetics were based on mainly natural products (with less antifungal protection) while in the last century almost all cosmetics are amended with

antidermatophytic compounds. However, Mahe *et al.* (1994; 2003) reported dermatophytic infections following use of skin bleaching agents in Mali and Senegal.

Industrial utilization of keratinolytic fungi to biodegrade poultry feathers waste was also suggested (Kalyankar and Kalyankar, 2005). Among the dominant keratinolytic fungi isolated were *T. verrucosum* and *T. equinum*, both known human pathogens, therefore raising the question of this process' safety as such.

Finally, although less directly connected to zoonotic dermatophytes, socio-ethnological factors involved and favoring transmission were studied in places of worship. Raboobee *et al.* (1998) found a significantly higher prevalence of Tinea pedis and unguium among regular male worshippers in a Muslim community (Durban, South Africa) related to the spread of fungal organisms in the communal ablution areas and prayer carpets of the mosques. Consequently, other communal environments, such as gymnasia, health spas, swimming pools, changing rooms of sports clubs, public showers, and even hotels should be considered at high risk as assembling the most favorable conditions requested by fungi to infect: humidity, high temperature, crowded people and physical contact.

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Chapter 5.4

Sporotrichosis

[*SPOROTHRIX SCHENCKII*]

Sporothrix schenckii is the only active species of genus *Sporothrix* that ccurs worldwide. *S. schenckii* is a thermally dimorphic fungus (conversion from mould form to yeast form). The dimorphism is used to identify *Sporothrix schenckii*. Septate hyphae grow conidiophores at right angles, where conidia are clustered in a flower like arrangement. Conidia are hyaline, elongated ovals, 3 to 6 by 2 to 3 µm with smooth walls. Optimal growth requires 92 to 100% humidity at 26 to 29°C, conditions found mainly in moist tropical and sub-tropical zones, such as South America, Southeast Asia, Australia, south USA (Mississipi River Delta) and Europe (mainly in Italy). Environmentally, the fungus is ubiquitous in nature found on rotting organic matter (wood, peat moss, debris, etc.), surface water and wet sites (swimming pools). Beside humans, it can infect horses, mules, dogs and cats. Clinically, it can manifest as cutaneous form (nodule formation with purulent lesions), mucosal form affecting nose, mouth, pharynx, larynx and trachea (causing tonsillitis, stomatitis, glossitis, laryngitis and rhinitis like syndromes) with ulcerative nodules and lymphoadenopathy development (Rippon, 1988). In more rare cases, internal mucosal spread of sporotrichosis may affect lungs, bones and joints (osteomyelitis and synovitis), eyes (chorioretinitis), muscles, testes and epididymis, brain (meningitis) (Ewing *et al*, 1980).

5.4.1 *SPOROTHRIX SCHENCKII* AND ENVIRONMENT

As mentioned before, the potential growth and development of *S. schenckii* on peat moss was emphasized by the famous 1988 largest outbreak in the USA of cutaneous sporotrichosis (Coles *et al.*, 1992). The contamination sources seem to be a one to three years old sphagnum moss, used to pack evergreen tree seedlings, wetted with pond water and kept under favorable conditions for the development of *S. schenckii*. In South America, natural habitats/reservoirs of this particular fungus are armadillo nests, flowers, hay, trees, mosses, soil, etc. where it can grow and multiply saprobically or parasitically (Conti Diaz, 1989; Mendoza *et al*., 2007; Hajjeh *et al.*, 1997; McDonough *et al.*, 1970) (Figure 5.1.1). Infection routes can be through direct contact as pointed out earlier, but also through contaminated spines or thorns, corn-stalks, armadillo's claws, hooks, animals and also human nails (that also cause skin injuries)

(Mehta *et al*, 2007). Among animal' sources of contamination, fish was reported in Guatemala, where fish workers contracted this dermatophyte showing clinical manifestations, such as ascending lymphocutaneous sporotrichosis of the limbs with some cases of ulcerative or verrucous lesions (Mayorga *et al.*, 1978).

From the environmental point of view, soil supplemented with sewage sludge was found to contain a large variety of fungi, including human pathogenic ones such as: *Sporothrix schenckii* (Kacprzak and Woszczyk, 2004). In the same study, soil supplemented with sewage sludge was reported to increase soil fungi content independently of sludge, soil and plant types. Sludge supplementation of soils can also increase its salinity; however, Brazilian *Sporothrix schenckii* isolates were shown to be highly resistant to salt concentrations (Fernandes *et al.*, 2009). Finally, high temperatures were found to be inhibitory to experimental infections, in both animals and humans, influencing also the clinical forms (Mackinnon and Conti Diaz, 1962). Based on these characteristics, MacKinnon (1972) proposed a hypothesis that high temperature may modify the clinical course of *Sporothrix schenckii* in human infection from lymphangitis to fixed cutaneous forms of the disease.

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Index

Environmental Aspects of Zoonotic Diseases

Robert Armon and Uta Cheruti

Environmental Aspects of Zoonotic Diseases provides a definitive description, commentary and research needs of environmental aspects related to zoonotic diseases.

There are many interrelated connections between the environment and zoonotic diseases such as: water, soil, air and agriculture.

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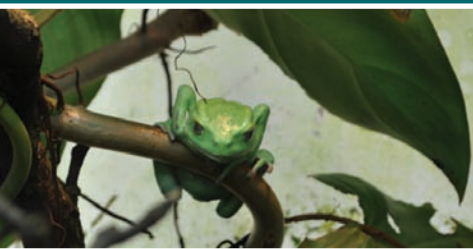
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