ZOE
ZOONOSES ONLINE EDUCATION

HANDBOOK

OF MAIN ZOONOTIC DISEASES IDENTIFIED IN 4 EU COUNTRIES: ROMANIA, ITALY, CROATIA AND LITHUANIA

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treatment of diseases transferable from animals to humans

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Overview

The aim of this work is to create open digital educational resources in the field of veterinary medicine based on the development innovative guidelines on some zoonotic studies on diseases and veterinary, medical, pedagogical, linguistic and to raise awareness intervention related to identifying, monitoring and controlling malaria and dirofilariasis, useful for the academic, professional and general beneficiaries.

We also intend to provide didactical data and produce specific results concerning some zoonoses and vector-borne diseases in Europe, a range of relevant diseases having been selected from Romania, Croatia, Italy, Lithuania - members in ZOE project.

The selection criteria used were the preferences of members of this project to analyse some zoonotic diseases for better epidemiological knowledge or control measures in their own countries, to produce a didactical online guideline for students with news regarding details of evolution of diseases.

The handbook comprises a research and evaluation of medical literature in the veterinary field and human medicine, talking about some zoonotic diseases. This will emphasize a variety of diseases that cover the spectrum of infectious agents and will display a variety of transmission patterns, with a global geographical distribution and existence in different environments, identifying specific diseases and ways of intervention in the local context.

One of our purposes is creation of synergies between the human and veterinary medicine in elaboration of a protocol on epidemiological monitoring and disease control in public health practice, useful for academic and professionals in medicine.

SPECIFIC NOTE FROM THE AUTHORS

In order to respect the recommendations for the Standardized Nomenclature of Animal Parasitic Diseases (SNOAPAD), we decided to follow its international terminology. World Federation of Parasitologists endorsed their proposal in 1990 for the parasitic diseases, both human and animal parasitosis:

- When disease names are formed from the taxonomic name of parasite, only the suffix ‘’-osis” (-oses in the plural) should be used
- The suffix ‘’-osis” be added to the stem of parasite taxon with the last or two letters removed (e.g. *Dirofilaria*/dirofilariosis, *Leishmania*/Leishmaniosis, etc.).

The term “infection” will be applied in this book to viruses, bacteria protozoa and fungi, while the term “infestation” applies to pluricellular organisms (metazoans).

DEFINITIONS

What is zoonosis?

Zoonosis is another name for a zoonotic disease. This type of disease passes from an animal or insect to a human. Some don’t make the animal sick but will sicken a human. Zoonotic diseases range from minor short-term illness to a major life-changing illness. Certain ones can even cause death.

Types

The types of zoonosis include those caused by: a virus, bacteria, fungus, parasites. Zoonotic diseases spread by mosquitoes and ticks are some of the most serious of these diseases.

Examples of zoonotic diseases discussed in this handbook:

Malaria, Dirofilariosis, Leishmaniosis, Trichinellosis
Boreliosis, Leptospirosis, Tuberculosis, West Nile
Bartonellosis, Campylobacteriosis, Q fever, Listeriosis
Enterohaemorrhagic E.coli (EHEC), hepatitis E virus (HEV),
Salmonellosis

A specific interpretation about malaria and dirofilariosis evolution in Europe will be made in separate chapters of this handbook.

Why malaria?

Malaria is a mosquito-borne protozoan illness that is a major public health concern throughout the world. The infectious *Plasmodium* entities can have either zoonotic or human reservoirs and are endemic mainly to the tropical and subtropical regions. Patients with these infections may present in early phases of a potentially life-threatening condition, which, if not recognized by the clinician,

can lead to poor outcomes. Malaria has identified as isolated cases as well in Romania as in Italy last years due to a diversity of factors (climate change, global warming *etc.) and could make be better underline.

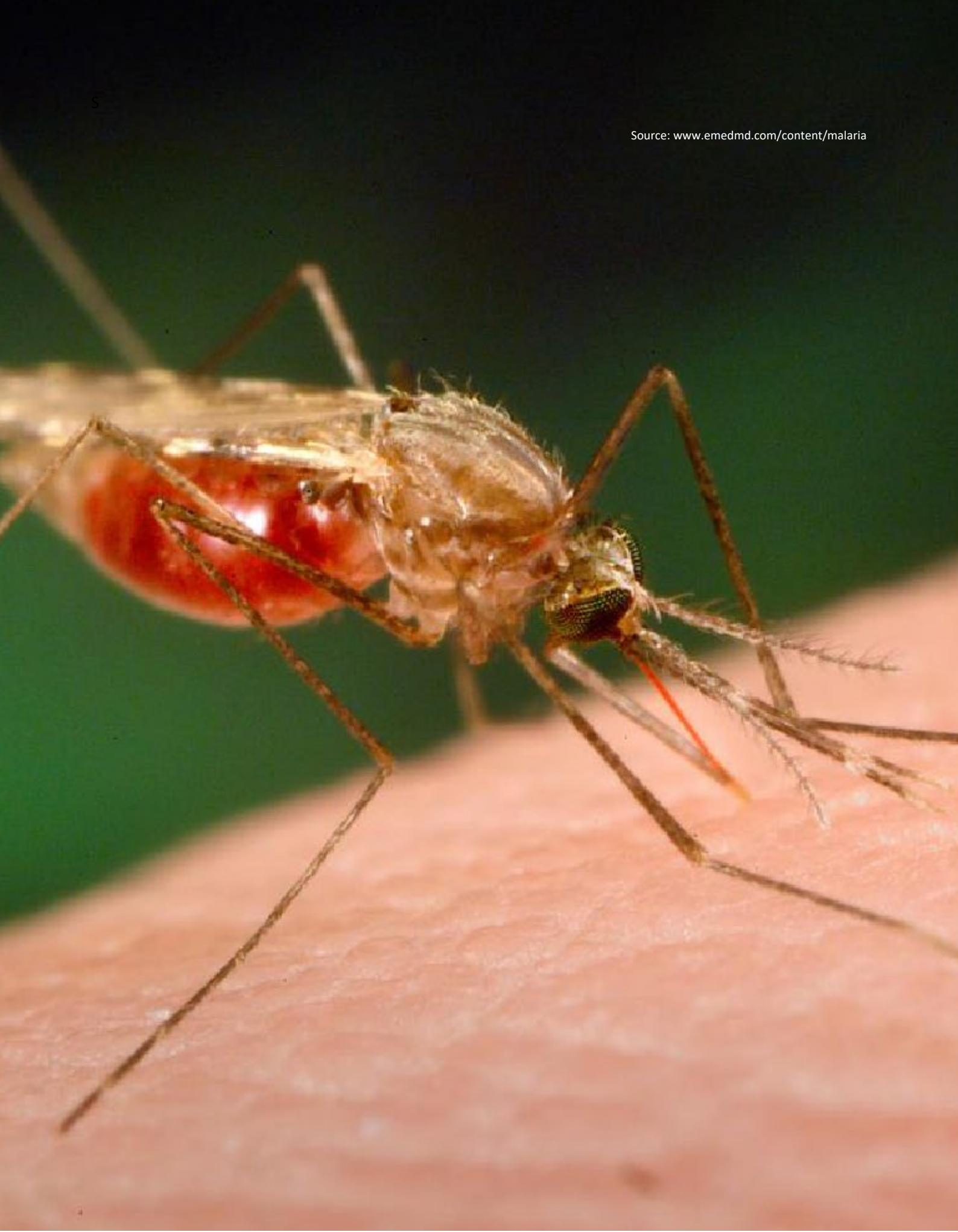
Why dirofilariosis?

Dirofilariosis is an emerging zoonotic infection caused by the filarial nematodes of dogs *Dirofilaria repens* and *Dirofilaria immitis*. The high prevalence of both *Dirofilaria* species in dogs in Romania represents a constant threat for animal and public health. However, only few cases of human infections by *D. repens* have been reported from various regions of Romania till now.

ZOONOSES ONLINE EDUCATION

STRUCTURE FOR CHARACTERISATION OF THE IDENTIFIED LOCAL ZOONOSES:

1. Name of zoonosis
2. Definition
3. Etiology
4. Epidemiology
5. Pathogenesis
6. Clinical manifestations
7. Diagnostic
8. Prophylaxis
9. Treatment
10. Scientific references



Source: www.emedmd.com/content/malaria

ONE HEALTH CONCEPT

All countries can do more to prioritize investments in education. Medical and medical-veterinary education models should shift away from narrow specializations to focus on long-term building competences for the consolidation and application of the “ONE HEALTH” concept.

The “ONE HEALTH” concept defines the idea that the health of people is connected to the health of animals and the environment. The Centre for Diseases Control and Prevention (CDC) uses a “ONE HEALTH” approach by working with physicians, veterinarians, biologists-ecologists and many other specialists to monitor and control public health threats and to learn about how diseases spread among people, animals and the environment.
[https://www.cdc.gov/onehealth]

There are many examples that show how the health of people is related to the health of animals and the environment.

For instance, some diseases can be shared between animals and people. These diseases are known as zoonotic diseases or zoonoses.

CDC is considered to be the most famous epidemiologic centre in the world. It was founded in 1946 as a centre for communicable diseases and was meant to fight malaria. There are also other specialized agencies as World Health Organization that is concerned with public health, founded 69 years ago.
[http://www.oie.int/]

The need to fight animal diseases at global level led to the creation of the Office International des Epizooties (OIE) through the international Agreement signed on January 25th 1924.O.I.E.is the World Organization for Animal Health.
[http://www.oie.int/].

GLOBAL WARMING

What is global warming?

Global warming is the current increase in temperature of the Earth's surface (both land and water) as well as its atmosphere. Average temperatures around the world have risen by 0.75°C (1.4°F) over the last 100 years about two thirds of this increase has occurred since 1975[1]. A long series of scientific research and international studies has shown, with more than 90% certainty, that this increase in overall temperatures is due to the greenhouse gases produced by humans[2].

What causes global warming?

The cause of global warming is the increasing quantity of greenhouse gases in our atmosphere produced by human activities, like the burning of fossil fuels or deforestation. These activities produce large amounts of greenhouse gas emissions, causing global warming. Greenhouse gases trap heat in the Earth's atmosphere to keep the planet warm enough to sustain life, this process is called the greenhouse effect[3]. It is a natural process and without these gases, the Earth would be too cold for humans, plants and other creatures to live. The natural greenhouse effect exists due to the balance of the major types of greenhouse gases. However, when abnormally high levels of these gases accumulate in the air, more heat starts getting trapped and lead to the enhancement of the greenhouse effect. Human-caused emissions have been increasing greenhouse levels which is raising worldwide temperatures and driving global warming.

What are the effects of global warming?

Global warming is damaging the Earth's climate as well as the physical environment. One of the most visible effects of global warming can be seen in the Arctic as glaciers, permafrost and sea ice are melting rapidly. Global warming is harming the environment in several ways including:

- Desertification
- Increased melting of snow and ice
- Sea level rise
- Stronger hurricanes and cyclones

[1] "Global temperatures." U.K. Met Office. http://www.metoffice.gov.uk/climate-change/guide/science/monitoring/global (accessed August 13, 2014).

[2] IPCC. Summary for Policymakers. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007.

[3] Le Treut, H., R. Somerville, U. Cubasch, Y. Ding, C. Mauritzen, A. Mokssit, T. Peterson and M. Prather. Historical Overview of Climate Change. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007.

Source: www.pexels.com

Source: www.pexels.com

CLIMATE CHANGE

What is climate change?

Climate change is when the average long-term weather patterns of a region are altered for an extended period of time, typically decades or longer. Examples include shifts in wind patterns, the average temperature or the amount of precipitation. These changes can affect one region, many regions or the whole planet[4]. The impact of these shifts has an impact on all life-forms on our planet including their sources of food and water.

Extreme weather has affected human society since the beginning of recorded history and certainly long before then. Humans, along with every other living thing on the Earth, have adapted to a certain range of variability in the weather. Although extreme weather can cause loss of life and significant damage to property, people and virtually every other creature have, at least to some degree, adapted to the infrequent extremes they experience within their normal climatic zone. More and more event attribution studies are being published every year and study results are increasingly requested very quickly after events occur. Some of the study methods are still relatively novel, however, and there are a range of views about how to conduct and interpret the analyses.

[4] Allison, Ian. *The science of climate change: questions and answers*. Canberra: Australian Academy of Science, 2010.

For example, warming is expected to increase the likelihood of extremely hot days and nights (Figure 1). Warming also is expected to lead to more evaporation that may exacerbate droughts and increased atmospheric moisture that can increase the frequency of heavy rainfall and snowfall events.

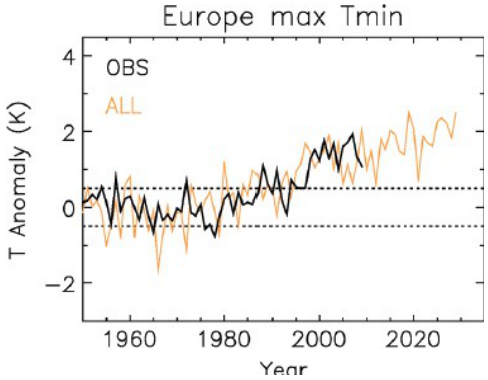


FIGURE 1 This figure shows a time series of the annual maximum night-time temperature averaged over the European Region. Temperatures are plotted as anomalies, or deviations from normal (in this case, 1961-1990), in degree Kelvin (K). Observed temperatures are represented by the black lines and are based on Caesar et al. (2006; updated). The orange lines come from model simulation (Martin et al., 2006)[5]. Both observations and model output show an increasing trend in night-time temperature anomalies over time. The horizontal dotted lines denote the uncertainty range (5-95%) due to natural climate variability. SOURCE: Stott et al., 2011[6].

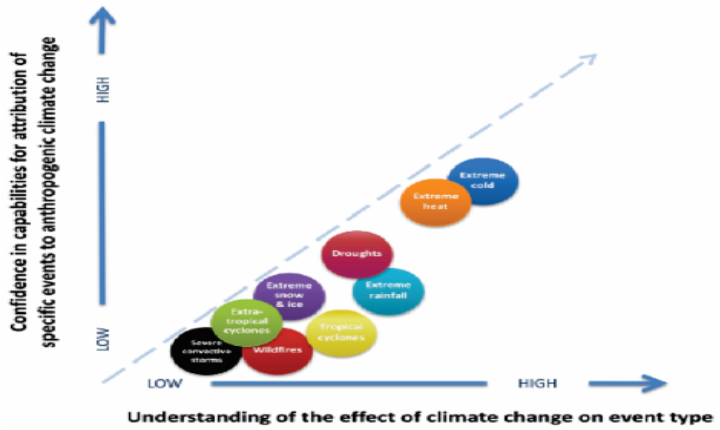


FIGURE 2 Schematic depiction of this report's assessment of the state of attribution science for different event types. The horizontal position of each event type reflects an assessment of the level of understanding of the effect of climate change on the event type. The vertical position of each event type indicates an assessment of scientific confidence in current capabilities for attribution of specific events to anthropogenic climate change for that event type.

[5] Martin, G. M., M. A. Ringer, V. D. Pope, A. Jones, C. Dearden, and T. J. Hinton. 2006. The physical properties of the atmosphere in the new Hadley Centre Global Environmental Model (HadGEM1). Part I: Model description and global climatology. Journal of Climate 19(7):1274-1301. DOI: Doi 10.1175/Jcli3636.1.

[6] Stott, P. A., N. Christidis, and R. A. Betts. 2011. Changing return periods of weather-related impacts: The attribution challenge. Climatic Change109(3-4):263-268.
DOI: http://dx.doi.org/10.1007/s10584-011-0265-8.

1. MALARIA

Source: www.yourgenome.org/facts/how-is-malaria-treated-and-prevented

1. Name of zoonosis:

MALARIA

2. Definition

Malaria is an infectious devastating disease, being widely grown in tropical and subtropical regions. It is the illness with the widest distribution in the world, millions of people being annually infected in Africa, India, Southeast Asia, Middle East, Central and South America, which makes nearly 50% of the global population, to be under the malaria risk of infestation (fig. 1.1.).

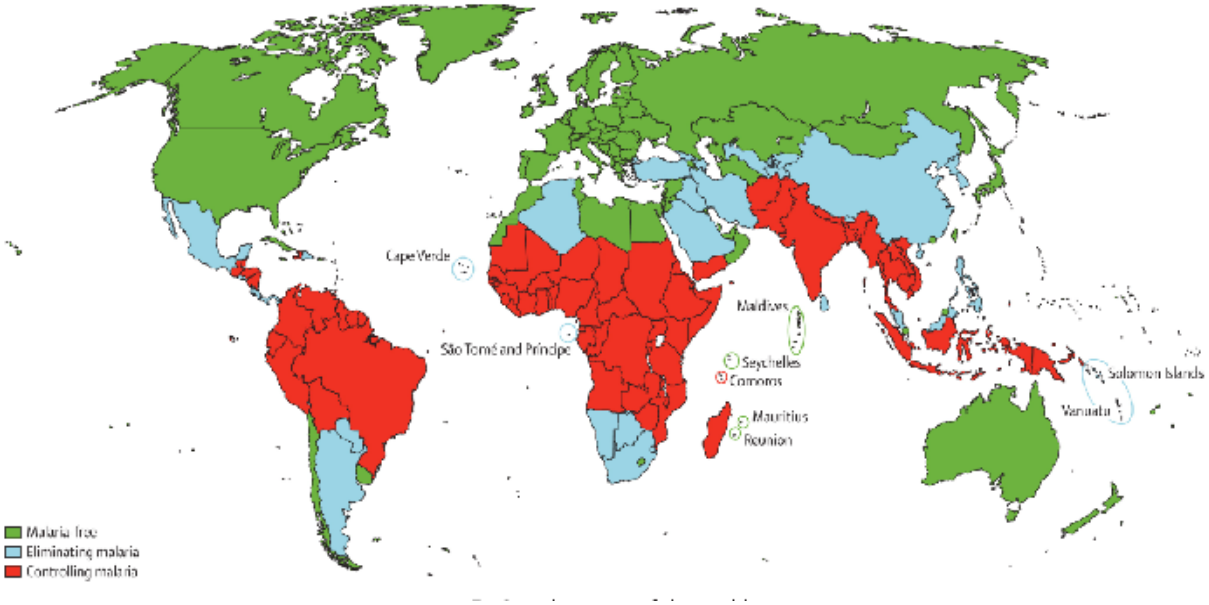


Fig.1.1. Malaria map of the world
(Source: www.thelancet.com/cms/attachment/2001010053/2003786757/gr1_lrg.jpg)

3. Etiology

Malaria is caused by infection of red blood cells with protozoan parasites of the genus Plasmodium inoculated into the human host by a feeding female *Anopheles* mosquito. The five human Plasmodium species transmitted from person to person are *P. falciparum, P. vivax, P. ovale* (two species) and *P. malariae*. Increasingly, human infections with the monkey malaria *parasite P. knowlesi* are being reported from the forested regions of the South-East Asia and particularly the island of Borneo.

3.1. Taxonomy

Plasmodium is a protozoan belonging to the family *Plasmodiidae*, order *Haemosporidia* and phylum *Apicomplexa*, which, together with dinoflagellates and ciliates, form the superphylum *Alveolata*, belonging to the Eukaryotic kingdom.

3.2. Morphological description

Malaria parasite undergoes four development stages in humans (hepatic schizonts, then intra-erythrocytic trophozoites, schizonts, and gamonts), and three development stages inside the mosquito vector (ookinete, oocyst, and sporozoite).

The schizonts are activated under the action of certain factors, transforming into merozoites, which penetrate the erythrocytes, with the following stages:

- The signet ring stage: the parasite has the shape of a ring, and takes 1/3 of the erythrocyte total volume. The protoplasm at the other end of the nucleus is thicker, coloured in blue, and the nucleus coloured in red. In the middle, there is a colourless vacuole full of nuclear liquid. The duration of this phase is approximately 12 hours at *Plasmodium falciparum*, varying according to species.
- The deformed ring stage: the ring is deformed at the side opposite the nucleus. There are dark brown granulations, representing melanin pigment resulted from the deposition of iron from haemoglobin. The duration is 12 hours.
- The amoeboid stage I: there are protoplasmic pseudopods in all directions, with various forms and sizes. The nucleus grows, and the vacuole decreases or disappears. The melanin pigment is abundant in the whole mass of the parasite.
- The adult amoeboid stage II: the parasite grows, extending in the whole erythrocyte, getting a more regular shape. The nucleus is big. The melanin pigment is abundant and uniformly spread. The duration of both amoeba stages is 16 hours.
- The pre-rosette stage: the nucleus starts to divide, 2, 3, 4 and 6 nuclei.
- The rosette stage: the nuclei are divided at maximum 16 to 24.

Sometimes we can also see the division of the protoplasm surrounding each nucleus, resulting in merozoites with 40-80 µm diameter. They place themselves at the periphery of the parasite, on several planes, giving the rosette a blackberries aspect. The melanin pigment remains in the middle of the parasite. The duration of pre-rosette and rosette stage is 8 hours, and of the whole schizogonic cycle takes 48 hours (12).

The sporogonic forms are represented by gametocytes. A hypertrophied un-deformed erythrocyte with regular contour contains the round or oval-shaped hematozoon, intense blue protoplasm, a small, dense, crimson-red nucleus, no vacuole, and very fine granulations of melanin pigment, uniformly spread. This is the female gametocyte or macrogametocyte with 7-14 µm diameter. The male gametocyte or microgametocyte has reddish-blue protoplasm, a big nucleus with diffuse chromatin, and coarse melanin pigment, placed in heaps around the nucleus. The microgametocytes of the mosquito are long (15-25 µm length), produced by exflagellation of fertilized round macrogametocyte to form ookinetes (15-20 x 2-5 µm), which migrate through the intestinal wall to form ovoid oocytes (up to 50 µm diameter) on the exterior surface. The oocyst produces thousands of thin long sporozoites

(~ 15 µm length), which eventually infect the salivary glands.

3.3. Biological cycle

Malaria biological cycle is one of the most fascinating and complicated of all the organisms, constituting a special interest, especially in molecular biology, cellular biology, and immunology. Malaria vector transmission by mosquitoes of the genus Anopheles was established by Grassi at the end of the last century. The mosquitoes involved in the transmission of malaria are: *A. hyrcanus, A. labranchiae, A. maculipennis maculipennis, A. maculipennis atroparvus, A. maculipennis messae, A. plumbeus, A. claviger, A. sacharovi*. In our country, the following species were signalled*: A. maculipennis, A. messae, A. atroparvus, A. sacharovi,* and *Anopheles labranchiae.*

Sporogonic cycle of malaria parasite

By changing the habitat, there are significant losses for the malaria parasite, creating an unbalance in population density. This unbalance is compensated by the growth and reproduction in cellular niches protected by the host's immune responses (10). In the stage of ookinete and sporozoite, the parasite has the greatest losses, these stages taking place in mosquito vector (fig. 1.2.)(23). The ookinetes develops from the zygote in the lumen of the host's middle intestine, being the result of fertilization between a female macrogametocyte and a male microgametocyte. The ookinetes crosses the layer of epithelial cells of the intestine on the apical side, reaching eventually the basal lamina. As a response to the invasion, the body activates its protection mechanisms, leading to a decrease in the parasite population density. The ookinetes are transformed into oocysts, which constitute an extracellular development stage, then the sporozoites are formed and then released in the cavity of the mosquito body, invading after wards the salivary glands (1, 26).

During this migration, there is a decrease in parasite population density, the sporozoites inoculated to the mammal host reaching the liver in a very short time. In 1948, Shortt & Garnham discovered that the sporozoites infected the hepatocytes, developing a stage there before infecting the erythrocytes. The hepatic stage is asymptomatic, interesting from a scientific point of view, with the purpose of vaccines production (27). Here is the amplification up to 10,000 times the number of parasites, culminating with the release of exoerythrocytic merozoites. The merozoites will invade the erythrocytes, consequently initiating the erythrocytic cycle, which is pathogenic (fig. 1.3).

Today, the expression of genes and proteins involved in the evolution of the mosquito's stages and the pre-erythrocytic stages are well-known (11, 13, 14). The discovery of the genes responsible for the hepatic stage led to the formation of attenuated sporozoites, obtaining a live attenuated vaccine. The parasite has sexual reproduction only once, in the mosquito body.

The erythrocytic schizogonic cycle of malaria parasite

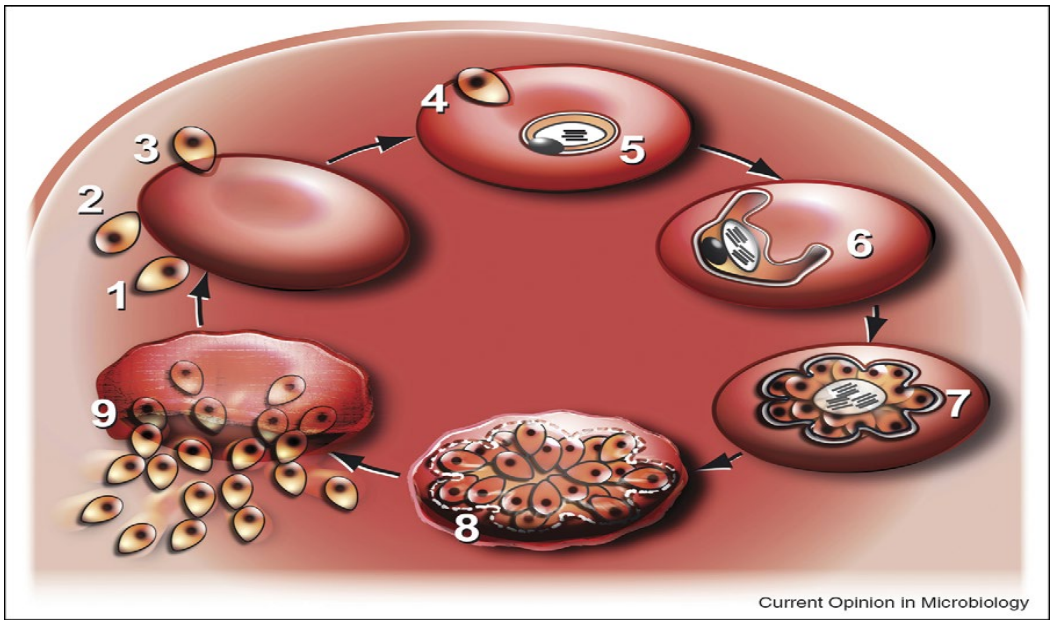


Fig.1.2. Asexual replication cycle of the parasite inside the red blood cells (Olivier Silvie, 2008):

(1) The extracellular merozoites are randomly attached to the surface of the erythrocytes. (2) Then the apical end of merozoites is directed towards the surface of the erythrocytes. (3) Formation of Tight junction and the active merozoites enter the erythrocytes. (4) Invasion of merozoites appears by simultaneous formation of a parasite porous vacuole. After the invasion, the merozoites are transformed in a ring. (5) This stage is characterised by a big digestive vacuole, where the digestion of haemoglobin results in the formation of the malaria pigment, hemozoin. (6) The parasite grows intra erythrocytic, and it is called trophozoite. The hemozoin pigment accumulates in the digestive vacuole. (7) DNA replication precedes the future cell, process called schizogony (8). The merozoites secrete exoneme, which initiates the exit from the parasite porous vacuole and red blood cells (RBC) of the host. (9) The merozoites which exited adhere to the adjacent erythrocytes in a few seconds. (1) Initiation of a new erythrocytic cycle.

The factors initiating the formation of gametocytes are not very well known. The ingestion of gametocytes during the blood meal activates the formation of gametes (gametogenesis) in the mosquito intestinal lumen. One molecule, derived from xanthurenic acid, along with the modification of the temperature and pH, may start the male gametogenesis is under the form of exflagellation (6). Protein P48\45 is essential in enabling the male gamete to fertilize female gametes (which are expressed on the surface of protein P47). The fertilization takes place to form the zygote. After the meiosis, the zygote is transformed into an-ookinete, which crosses several epithelial cells of the intestine (16). Ookinetes cross the epithelial cells to the basal lamina, and are transformed into oocysts, which have an extensive growth, and in 10-14 days, thousands of sporozoites are produced. After a migration through the hemolymph, the sporozoites resulted from oocysts attach themselves to the basal side of the acinar cells of the salivary gland. The sporozoites cross the acinar cells, and enter the ducts of the salivary glands, and then they are sent to the mammal host during the blood meal (20, 22).

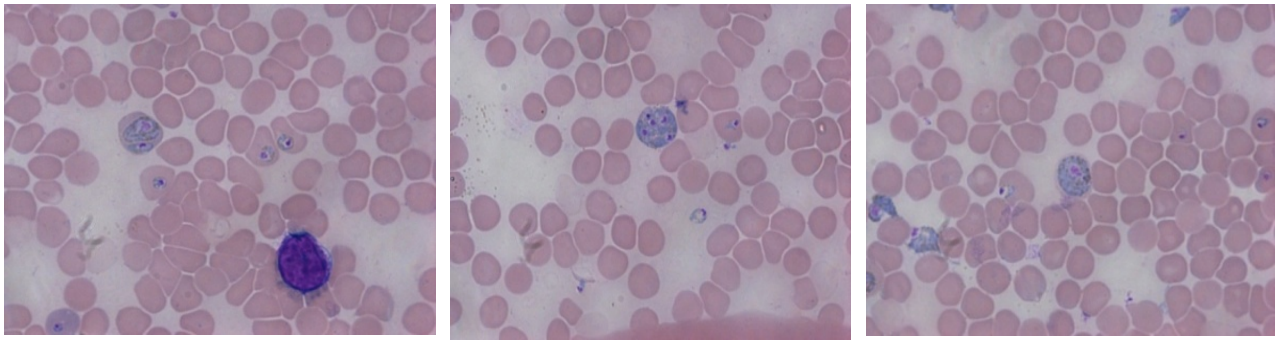


Fig.1.3. Various erythrocyte stages (1-ring form, 2-the mature schizont, 3-gametocytes) of *Plasmodium berghei*, Giemsa stained smear x 1000.

The mammal host is infected with sporozoites from the salivary glands of mosquitoes. Approximately 100 sporozoites are injected by a single mosquito (17). They remain in the derma for a period of time, they have an apparently random movement until they find a blood vessel, then they migrate to the liver (2, 18). After injection, the sporozoites remain in the derma for 1-3 hours, then they migrate to the liver (28). Not all the sporozoites get into the circulation and into the liver; some of them are stuck at the injection place, from where they will be probably eliminated by phagocytosis. Some of them reach the lymphatic system, in the lymphatic ganglions, where the response of T and CD8 cells is activated. These cells are capable to eliminate the developing parasites from the liver (2, 8). In the skin, the sporozoites actively migrate through the cells, leading to the perturbation of the plasmatic membrane of the host cell. For the cellular crossing, at least three proteins, SPECT-1, SPECT-2, and phospholipase, are involved. The sporozoites with no SPECT-1, SPECT-2 are immobilized in the derma as a result of the impairment of the cell crossing capacity (3, 24, 4). After the sporozoites inoculation by Anopheles vector, the exoerythrocytic cycle starts, with a duration according to the species, as follows: at *Plasmodium falciparum-6 days; Plasmodium vivax– 8 days; Plasmodium ovale*– 9 days; *Plasmodium malariae*– 15 days. Some sporozoites of *Plasmodium vivax* and *Plasmodium ovale* which penetrated the hepatocytes remain in a latent stage for a long period of time (a few years), and they are called hypnosoits (7). They are activated by the action of certain factors, and become merozoites, penetrating the erythrocytes, with the following stages: the signet ring stage, the deformed ring stage, the amoeboid stage I, the adult amoeboid stage II, the pre-rosette stage, the rosette stage, sporogonic forms: gametocytes, macro-gametocyte or micro-gametocyte. Inoculated in humans, the parasite under goes a series of transformations, first in the liver (this constitutes the exoerythrocytic or tissue cycle), then penetrates the RBC, where it develops until it leads to the destruction of RBC (the erythrocytic or asexual cycle). The parasite released from the destroyed cells parasitizes again other cells (24, 25). The mosquito inoculates the parasite under the form of sporozoites (infecting forms for humans), which form, when reaching the liver, a big plasmodial mass from which

merozoites come off, reaching the blood and penetrating the red blood cells. Inside the red blood cells, the parasite goes through the amoeboid stage, then the rosette stage, then it destroys the red blood cells releasing the merozoites, which will parasitize other RBC, where the cycle will start again (21). The gametocytes (males, females), also result from the erythrocytic cycle, joining in the body of the mosquito and forming zygotes. In the intestinal lumen, the zygotes transform into ookinetes, from which the sporozoites are produced after a series of transformations, located in the salivary glands, from where they are inoculated in humans (26).

4. Epidemiology

Malaria is transmitted by a female mosquito of the genus *Anopheles*; consequently, the ecological modifications favouring the prevalence of these insects facilitate the spread of infection every time there is a malaria case.

4.1. Source of infection

There are three possible modalities of malaria transmission from one diseased per on or plasmodia carrier to healthy patients: in natural conditions - by *Anopheles* mosquito bites, intrauterine, from sick mother to foetus – through placenta or during birth, by transfusion - by blood containing the pathogen during medical manipulation or injections in aseptic conditions with contaminated needles.

Vectorial - the vector transmitted to humans, and at the same time the compulsory host for the sexual stage of the parasite are the mosquitoes of the genus *Anopheles* (of the approximately 400 species on the globe, only 30-40 can be malaria vectors in natural conditions). The period of formation of sporozoites in the salivary glands varies between 7 and 30 days, according to the parasite species and environmental temperature. Blood transfusion - malaria post-transfusion known from 1911 appears from latent asymptomatic malaria carriers. In most cases, post-transfusion transmission was made with *P. malariae* (which gives persistent forms with long time blood carriers), and more rarely with *P. vivax* and *P. falciparum* (15).

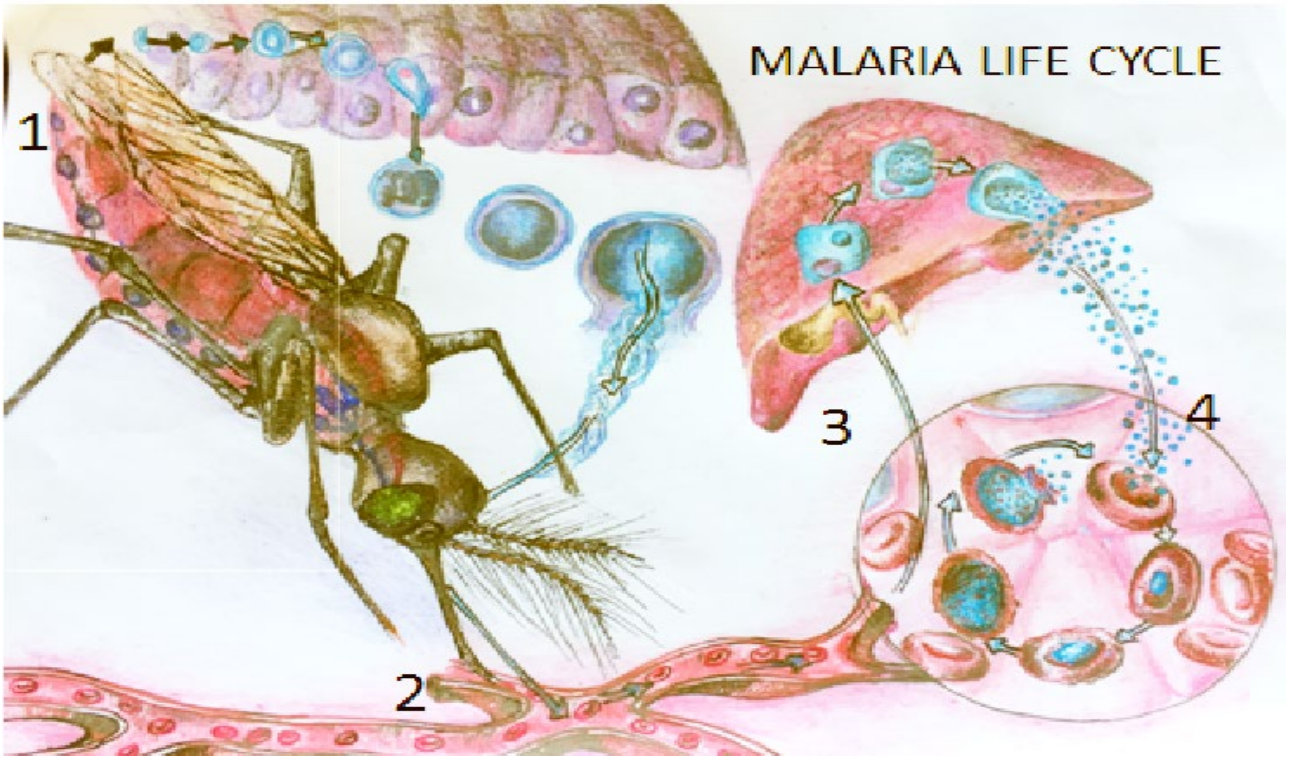


Fig. 1.4. Malaria – life cycle: 1. The sporogonic cycle of *Plasmodium*; 2. Mosquito injects sporozoites in blood meal; 3. Sporozoites infect liver cell; 4. The erythrocytic schizogonic cycle of malaria parasite (original)

4.2. Path of infection

Anopheles females biting a sick person whose blood contains gametocytes and schizonts, aspirates at the same time with the blood a variable number of parasites under these two forms. The schizonts are digested in the stomach of the mosquitoes, and the gametocytes ensure the continuation of the sexual or sporogonic cycle, from which the sporozoites result. It was demonstrated that some mosquitoes contain two genes encoding carboxypeptidase from the middle intestine, involved in parasite development at *Anopheles gambiae* (4).

4.3. Responsiveness

The receptivity is general, with some peculiarities related to resistance to malaria produced by some types of *Plasmodium*.

The immune response developed after the natural infection is of humoral and cellular type, the humoral immunity (antibodies against the asexual red blood cells) being the most important element of protection. The antibodies appear early during the parasitemia and reach a maximum level with the decrease in the number of circulating parasites. In endemic areas of malaria, passive natural immunity (IgG of maternal origin) provides protection for the newborn until around the age of 3 months, after which the level of these antibodies gradually decreases until disappearance. In the first year of life of these children, the immune system is

continuously exposed to *Plasmodium* and the body begins its own production of antibodies (IgM and IgG) in the presence of parasitemia. Epidemiological studies show that this immune response reduces the risk of severe complications and premature death in children who have lived their first year of living in endemic areas compared to those who have lived their first year of life outside endemic areas.

Among the adult population of these endemic areas, it has been observed the appearance of some easier clinical forms due to occult immunization through repeated contact with the mosquito (source of pathogen) over the years. A special category of receptive is HIV-positive people in endemic areas who develop more frequent the disease, but often have a low response to anti-malarial therapy.

4.4. Resistance to the environment

The period of human contagiousness is the time that the infesting gametocytes of the malaria parasite persist in the blood of the diseased, which varies with *Plasmodium* species. Consequently, the gametocytes of *P. malarie* may persist in the blood even up to 53 years; *P. vivax* may persist for 1-3 years, and *P. falciparum* up to 1 year. The *Anopheles* female remains an infesting agent its entire life.

4.5. Geographical distribution

The proportion of the population at risk in sub-Saharan Africa who are infected with malaria parasites is estimated to have declined from 17% in 2010 to 13% in 2015 (UI: 11–15%). In 2015, an estimated 212 million cases of malaria occurred worldwide (UI: 148–304 million). Most of the cases in 2015 were in the WHO African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). About 4% of estimated cases globally are due to *P. vivax,* but outside the African continent the proportion of *P. vivax* infections is 41%. The number of people infected with malaria parasites in sub-Saharan Africa is estimated to have decreased from 131 million in 2010 (UI: 126–136 million) to 114 million in 2015 (UI: 99–130 million). The vast majority of deaths (99%) are due to *P. falciparum* malaria. *Plasmodium vivax* is estimated to have been responsible for 3100 deaths in 2015 (range: 1800–4900), with 86% occurring outside Africa. Infection rates are higher in children aged 2–10 years, but most infected people are in other age groups. International travellers could be at risk of malaria infection in 91 countries around the world, mainly in Africa, Asia and the Americas. People infected with malaria often experience fever, chills and flu-like illness at first. Left untreated, the disease can lead to severe complications and, in some cases, death. Malaria symptoms appear after a period of seven days or longer. Fever occurring in a traveller within three months of possible exposure is a medical emergency that should be investigated immediately.

In Romania, malaria was eradicated in 1965, being considered in 1967 by the WHO, a malaria free country. The risk of re-emergence of malaria in Romania is the presence of the *Anopheles* vector, which is part of the *Anopheles maculipennis* complex, of the pathogen agent, in the nature and in the modification of climate factors. The cases of malaria diagnosed in Romania were all imported, being constantly increasing, due to the development of tourism and labour markets in malaria endemic areas.

5. Pathogenesis

All of the pathology of malaria is due to parasites multiplying in erythrocytes. The fever spike may reach up to 41^0C and corresponds to the rupture of the red cell as merozoites are released from the schizonts infected cell. If the infection is not synchronous and there are several broods of parasites the periodicity may occur at 24 hrs. intervals. Anaemia appears when red blood cells are unable to transport enough oxygen for the muscles of the body and organs, producing a sensation of drowsiness, weakness, and faintness. Anaemia during the first few weeks of infection the spleen is palpable because it is swollen due to the accumulation of parasitized red cells as well as proliferation of white cells. If the infection is treated the spleen returns to normal size, however, in chronic infections the spleen continues to enlarge, becoming hard and blackened in colour due to the accumulation of malaria pigment, hemozoin (5).

Cerebral malaria is determined by brain swallowing, which may lead to permanent cerebral lesions, producing also attacks (convulsions) or coma. Other complications which may appear are: hepatic insufficiency and icterus, shocks due to a sudden drop in blood pressure, pulmonary edema, acute respiratory distress syndrome (ARDS), hypoglycemia, kidney failure, swelling and rupturing of the spline and severe dehydration.

6. Clinical manifestations

The first symptoms of malaria are nonspecific -a minor systemic viral illness. The symptoms of malaria typically develop within 10 days to four weeks following the infection. In some people, symptoms may not develop for several months. Some malarial parasites can enter the body but will be dormant for long periods of time.

Common symptoms of malaria include: shaking chills that can range from moderate to severe, high fever, profuse sweating, headache, nausea, vomiting, diarrhoea, anaemia, muscle pain, convulsions, coma, and bloody stools. Anaemia is a condition where the red blood cells are unable to carry enough oxygen to the body's muscles and organs, leaving you feeling drowsy, weak and faint. Uncomplicated *Falciparum* malaria can progress rapidly to severe forms of the disease, especially in people with no or low immunity, and severe falciparum malaria is almost always fatal without treatment. Severe malaria usually manifests with one or the following: coma (cerebral malaria), metabolic acidosis, severe anaemia, hypoglycemia, acute renal failure or acute pulmonary edema.

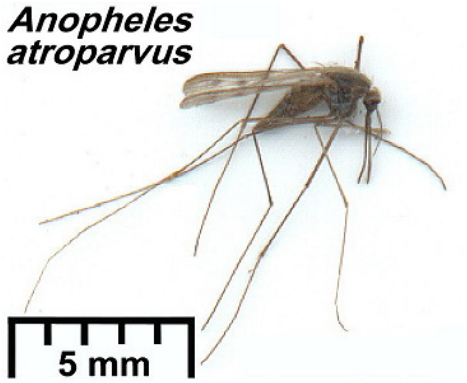


Fig.1.5. *Anopheles atroparvus*
(source: http://antsofafrica.org/ant_species_2012/personal/crhtml/anatro.htm)

Cerebral malaria

In rare cases, malaria can affect the brain. This is known as cerebral malaria, which can cause your brain to swell, sometimes leading to permanent brain damage. It can also cause fits (seizures) or coma. Other complications that can arise as a result of severe malaria include: liver failure and jaundice – yellowing of the skin and whites of the eyes, shock – a sudden drop in blood pressure, pulmonary edema- a build-up of fluid in the lungs, acute respiratory distress syndrome (ARDS), abnormally low blood sugar – hypoglycemia, kidney failure, swelling and rupturing of the spleen, dehydration.

Malaria in pregnancy

The World Health Organization (WHO) recommends that pregnant women should avoid travelling to areas where there's a risk of malaria.

If you get malaria while pregnant, you and your baby have an increased risk of developing serious complications, such as: premature birth – birth before 37 weeks of pregnancy, low birth weight, restricted growth of the baby in the womb, stillbirth, miscarriage, death of the mother.

7. Diagnostic

All patients with suspected malaria should be treated on the basis of a confirmed diagnosis by microscopy examination or Rapid diagnostic test (immune-chromatographic) testing of a blood sample. Malaria is suspected clinically primarily on the basis of fever or a history of fever. Antimalarial treatment should be limited to cases with positive tests, and patients with negative results should be reassessed for other common causes of fever and treated appropriately.

In patients with suspected severe malaria and in other high-risk groups, such as patients living with HIV/AIDS, absence or delay of parasitological diagnosis should not delay an immediate start of antimalarial treatment.

8. Prophylaxis

Prevention of mosquitoes bites between dusk and dawn is the first line of defence against malaria. Measures to prevent mosquito bites include sleeping under long-lasting insecticidal nets, and using protective clothing and insect repellents. Depending on the malaria risk in the area to be visited, international travellers may also need to take preventive medication (chemoprophylaxis)

prior to, during, and upon return from their travel.

Some groups of travellers, especially young children, pregnant women and individuals with a weakened immune system, are at particular risk of developing serious illness if they become infected with malaria. In pregnant women, malaria increases the risk of maternal death, miscarriage, stillbirth and low birth weight, as well as the associated risk of neonatal death.

Pregnant women should avoid travelling to areas where malaria transmission occurs, and parents are advised not to take infants or young children to areas where there is risk of *P. falciparum* malaria. When travel cannot be avoided, it is very important to take effective preventive measures against malaria, even when travelling to areas with *P. vivax* malaria transmission.
Prior to their travel to malaria-endemic countries or regions, individuals should consult their national disease control centres, or other institutions offering travel advice, for information regarding the preventive measures that should be taken.

Preventing or delaying resistance is essential for the success of both national and global strategies for control and eventual elimination of malaria. To help protect current and future antimalarial medicines, all episodes of malaria should be treated with combination therapy, and antimalarial drugs must be given at optimal dosages. All cases of suspected malaria should have a rapid diagnostic test (RDT) or parasitological test.

Case incidence. In 2015, an estimated 212 million cases of malaria occurred worldwide (UI: 148–304 million). Most of the cases in 2015 were in the WHO African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). About 4% of estimated cases globally are due to *P. vivax*, but outside the African continent the proportion of *4* infections is 41%. The incidence rate of malaria is estimated to have decreased by 41% globally between 2000 and 2015, and by 21% between 2010 and 2015. Mortality. In 2015, it was estimated that there were 429 000 deaths from malaria globally (UI: 235 000–639 000). Most deaths in 2015 are estimated to have occurred in the WHO African Region (92%), followed by the WHO South-East Asia Region (6%) and the WHO Eastern Mediterranean Region (2%). The vast majority of deaths (99%) are due to P. falciparum malaria. Plasmodium vivax is estimated to have been responsible for 3100 deaths in 2015 (range: 1800–4900), with 86% occurring outside Africa.

Preventing relapse in *P. vivax* or *P. ovale* malaria

To prevent relapse, treat *P. vivax* or *P. ovale* malaria in children and adults (except pregnant women, infants aged <6 months, known not to be G6PD deficient, and people with G6PD deficient) with a 14-day course (0.25-0.5mg/kg body weight) of primaquine in all transmission settings. When G6PD deficiency, consider preventing relapse by giving primaquine base at 0.75mg/kg body weight once a week for 8 weeks, with close medical supervision for potential primaquine-induced hemolysis. Children weighing <20kg should receive a higher dose of artesunate

(3mg/kg body weight per dose) than larger children and adults (2.4mg/kg bw per dose) to ensure equivalent exposure to the drug.
Antimalarial medicines should be selected for procurement based on the "WHO guidelines for the treatment of malaria", or the "WHO model list of essential medicines". Fixed-dose combination formulations are strongly preferred and recommended over co-blistered, co-packaged or loose tablet combinations, since they facilitate adherence to treatment and reduce the risk of taking the medicines as monotherapy, which can contribute to the development of drug resistance.

9. Treatment

Malaria is an entirely preventable and treatable disease. The primary objective of treatment is to ensure the rapid and complete elimination of the *Plasmodium* parasite from the patient's blood in order to prevent progression of uncomplicated malaria to severe disease or death, and to prevent chronic infection that leads to malaria-related anaemia.

From a public health perspective, the goal of treatment is to reduce transmission of the infection to others, by reducing the infectious reservoir, and to prevent the emergence and spread of resistance to antimalarial medicines. Patients with suspected malaria should have parasitological confirmation of diagnosis with either microscopy or rapid diagnostic test (RDT) before antimalarial treatment is started. Treatment based on clinical grounds should only be given if diagnostic testing is not immediately accessible within 2 hours of patients presenting for treatment. Prompt treatment – within 24 hours of fever onset – with an effective and safe antimalarial is necessary to prevent life-threatening complications.

Artemisinin-based combination therapies (are the mainstay of recommended treatment for P. falciparum malaria and, as no alternative to artemisinin derivatives is expected to enter the market for at least several years, their efficacy must be preserved.

WHO recommends that national malaria control programs regularly monitor the efficiency of antimalarial medicines in use to ensure that the chosen treatments remain efficient

Artemisinin-based combination therapies (ACT):

- Artemether + lumefantrine
- Artesunate + amodiaquine
- Artesunate + mefloquine
- Dihydroartemisin + piperaquine
- Artesunate + sulfadoxine-pyrimethamine (SP)

In low transmission areas, a single low dose of primaquine should be added to the antimalarial treatment in order to reduce transmission of the infection. Testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency is not required, as a single low dose of primaquine is both effective in blocking transmission and unlikely to cause serious toxicity in individuals with any of the G6PD-deficiency variants. Artemisinin and its derivate must not be used as oral mono-therapy, as this promotes the development of artemisinin resistance. *Plasmodium falciparum* resistance to artemisinin has been detected in five countries in the Greater Mekong sub-region. In Cambodia, high failure rates after treatment with artemisinin-based

combination therapies have been detected for four different ACTs.

In areas with highly seasonal malaria transmission in the sub-Sahel region of Africa, provide seasonal malaria chemoprevention with monthly amodiaquine +sulfadoxine-pyrimethamine for all children aged <6 years during each transmission seasons. Duration of ACT treatment- should provide 3 days with an artemisinin derivate. The dose it is very important and for young children, <25 kg treated with dihydroartemisin + piperaquine should receive a minimum of 2.5 mg/kg body weight per day of dihydroartemisin and 20 mg/kg body weight per day of piperaquine daily for 3 days.

In low-transmission areas, give a single dose of 0.25 mg/kg body weight primaquine with ACT, to patients with *P. falciparum* malaria (except pregnant women and infants aged < 6 months) to reduce transmission.

P. vivax infections should be treated with chloroquine in areas where this medicine remains effective. In areas where chloroquine-resistant P. vivax has been identified, infections should be treated with an artemisinin-based combination therapy, preferably one in which the partner medicine has a long half-life.
In order to prevent relapses, primaquine should be added to the treatment; dose and frequency of the administration should be guided by the patient's glucose-6-phosphate dehydrogenase (G6PD) enzyme activity.

Severe malaria should be treated with artesunate injection (intramuscular or intravenous) for at least 24 hours and followed by a complete 3-day course of artemisinin-based combination therapies once the patient can tolerate oral medicines. When injection treatment cannot be given, children under 6 years of age with severe malaria should receive a pre-referral treatment with rectal artesunate before being referred immediately to a health care facility where the full level of care can be provided.

In view of the latest development of resistance, it is essential that neither artemisinin-based injections nor artesunate suppositories be used as mono-therapies – the initial treatment of severe malaria with these medicines needs to be completed with a 3-day course of an artemisinin-based combination therapies. Uncomplicated *Falciparum* malaria can progress rapidly to severe forms of the disease, especially in people with no or low immunity, and severe falciparum malaria is almost always fatal without treatment. To reduce the spread of drug resistance, antimalarial medicines should be administrated only to patients who truly have malaria.

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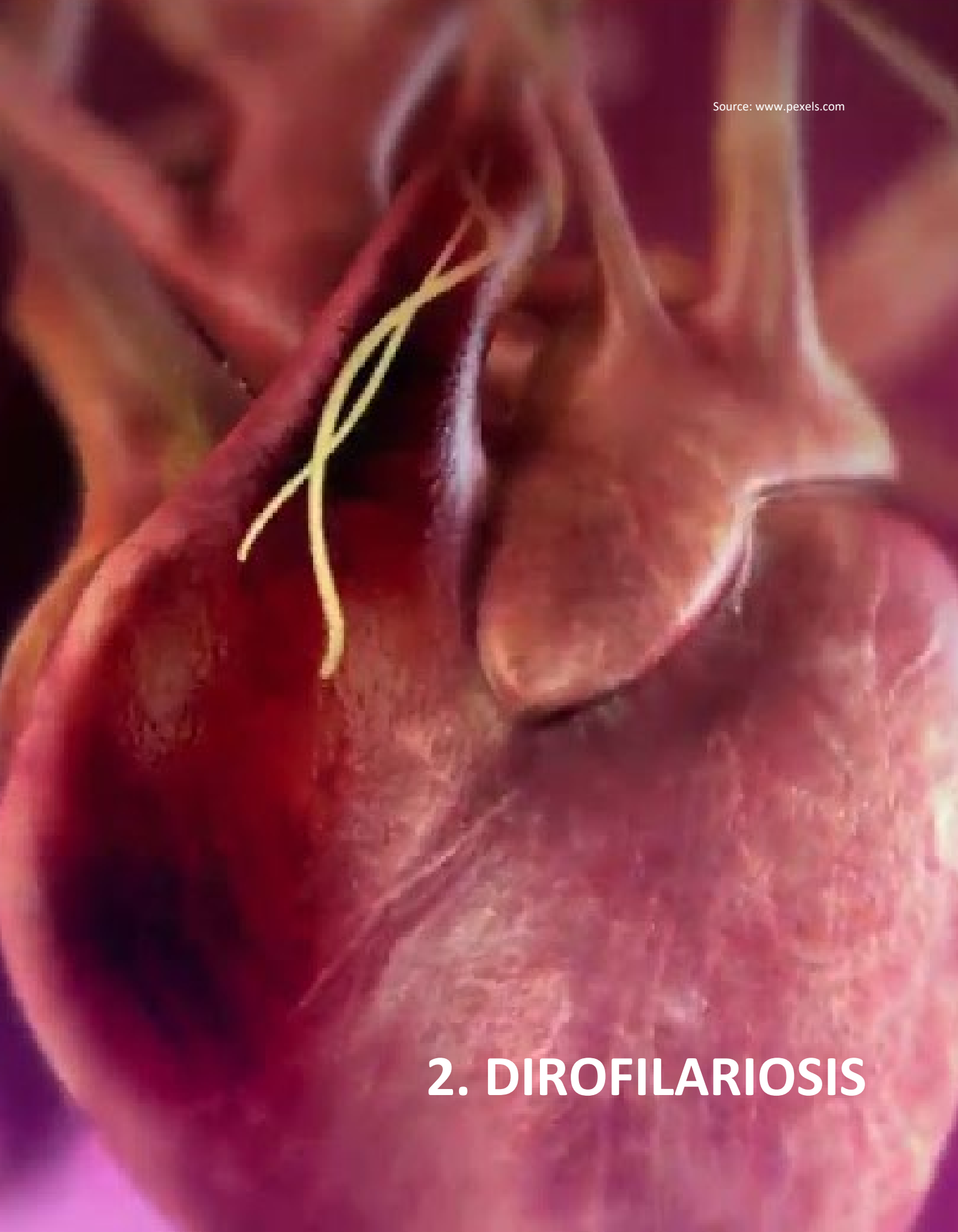

Source: www.pexels.com

2. DIROFILARIOSIS

1. Name of zoonosis:

DIROFILARIOSIS

2. Definition

Dirofilariosis is one of the anthropozoonoses that affect people randomly, through mosquito bites and it is caused by roundworms belonging to the *Dirofilaria* species, which naturally infect domestic and wild mammals, especially dogs, in which case they are called "heartworms" (1).

3. Etiology

Dirofilariosis accidentally affects humans, the most important definitive host being the dog. *Dirofilaria immitis, Dirofilaria repens, Dirofilaria ursi, Dirofilaria tenuis, Dirofilaria striata, Dirofilaria spectrans*) affect the human being as an accidental host.

4. Epidemiology

4.1 Source of infection

The main natural hosts for the three *Dirofilaria* species that most frequently cause diseases in humans are dogs and wild canids (such as wolves and foxes), as well as raccoons. People are infected with *Dirofilaria* larvae through mosquito bites. The mosquitos belonging to the *Aedes*, *Armigeres*, *Culex*, *Anopheles* genera and the *Mansonia* species are the ones reported being involved in transmitting dirofilariasis. Some species of fleas, lice and ticks are also considered to be vectors. The type of species and the vector involved in spreading the infection seem to vary according to different geographical regions (1, 2).

4.2 Path of infection. Modes and ways of transmission

The mode of transmission is direct, through the bite of the aforementioned insects as sources of pathogenic agent. The way of transmission is subcutaneous, and subsequently by blood. Humans and other mammals are accidental hosts that do not play any role in the transmission of dirofilariasis. In these hosts, the *Dirofilaria* larvae may develop into adult worms, but these remain sexually immature, so the microfilariae are not produced (3).

Dirofilariosis is not transmitted from human to human and neither from human – mosquito – other human. The transmission of the disease requires mosquitos to be intermediate host, but at the same time it requires the production of microfilariae, which does not happen in the human body (3).

4.3. Resistance to the environment

Besides the increase in the frequency of travels with dogs and cats, the climate changes are considered to play an important role in spreading dirofilariosis in Europe. The pathogenic agents transmitted through bites by hematophagous insects (vector-borne pathogens) are sensitive to the external environment; therefore the climate change may influence the incidence of dirofilariosis or the outbreaks of epidemics or epizootics. The climate changes (temperature, rainfall, humidity) determine the increase in the number of insects, mosquitos. The *Aedes albopictus* was transported to Italy in 1990 and spread through all Europe, even to the

North of Netherlands, contributing to the spread of dirofilariosis on the continent (4).

4.4. Geographical distribution

Dirofilariosis is spread in the entire world, and the *Dirofilaria* species are frequently detected (5).

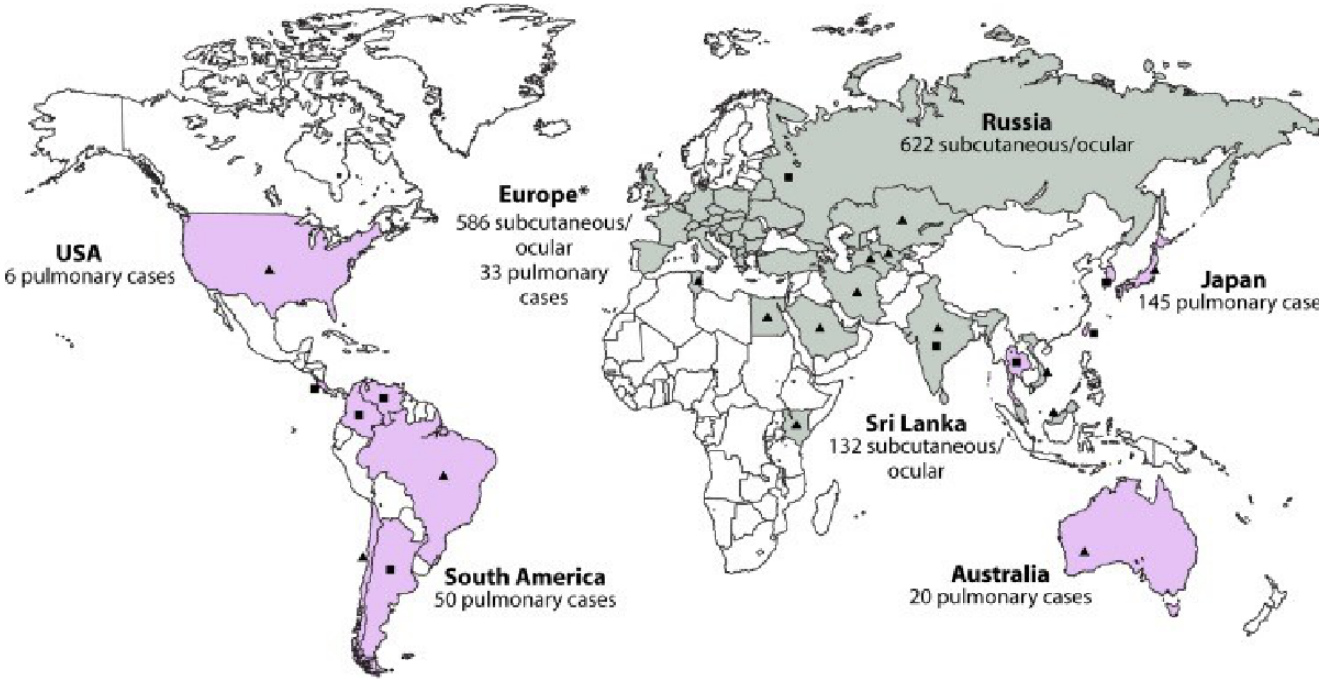

Fig. 2.1. Geographical distribution of human dirofilariasis at global level (2012) (5).
Legend: Purple, countries in which *D. immitis* cases predominate; gray, countries in which *D. repens* cases predominate; ■, sporadic pulmonary cases; ▲, sporadic subcutaneous/ocular cases. The asterisk indicates that data from European countries except for the former Soviet Union were included (5).

In the USA, canine dirofilariosis was reported in all states, and *D. tenuis* in raccoons is frequently detected in many territories where raccoons may be found. Human and canine dirofilariasis are the most predominant in the eastern and southern states, even though incidences are also rapidly increasing in a series of western states. *D. immitis* is the species most frequently reported to cause dirofilariasis in humans, in the USA (3). Endemic outbreaks of infection with *D. repens* exist in the Middle East, Central Asia, Sri Lanka (7). In Europe, *D. repens* is the species most frequently reported to cause dirofilariosis in humans, being reported especially in Spain, the South of France, Italy, Greece, Croatia, Bosnia, the Czech Republic, Turkey and Hungary (3, 4). Dirofilariosis by *D. immitis* is endemic in many countries from Southern and Eastern Europe (Portugal, Spain, the South of France, Italy, Greece, Croatia, Bosnia, the Czech Republic and Turkey) (Fig. 2.1.), but the infection was reported in Hungary as well. Italy is the European country with the highest prevalence of human dirofilariasis (66%), followed by France (22%), Greece (8%), Turkey (4,3%) and Spain (4%). In Northern Europe sporadic cases of human and canine dirofilariasis have been described, but only following exposure during a visit in Southern Europe (7). Climate changes, favourable to the development of the parasite, and the

increase of the number of pets that are taken during travels have increased the risk of infection for dogs and cats. Also, some sporadic cases of dirofilariasis by *D. repens* have been reported in Germany, the Netherlands, Poland and Austria (6).

Table. I. Epidemiological data on human dirofilariasis, with special reference to Europe[7]

Country	No. of cases					
	Pulmonary dirofilariasis			Subcutaneous dirofilariasis		
	Until 1999	2000–2011	Total	Until 1999	2000–2011	Total
Spain	5	3	8	6	2	8
France	2	2	4	24	63	87
Italy	3	10	13	135	188	323
Greece		3	3	10	25	35
Hungary			0		31	31
Croatia			0		10	10
Serbia			0	3	19	22
Germany	2	1	3	6	3	9
Turkey			0	1	21	22
Russia		2	2	61	561	622
Austria			0		>16	>16
Ukraine			0	23	1	24
Others[a]						170

[7] Countries with subcutaneous/ocular sporadic or imported cases due to D. repens include Bulgaria, Dubai, Georgia, Kazakhstan, Kenya, Iran, Israel, India, Japan, Malaysia, Poland, Romania, Slovakia, Slovenia, Sri Lanka (>132 cases), Tunisia (10 cases), Turkmenistan, Vietnam, Uzbekistan, Egypt, Saudi Arabia, Kuwait, Norway, Belgium, Australia, Brazil, Chile, and the United States. Countries with pulmonary sporadic dirofilariasis cases attributed to D. immitis include India, South Korea, Thailand, Taiwan, Costa Rica, Argentina, Venezuela, and Colombia.

Fig.2.2. Distribution of *Dirofilaria immitis* and *Dirofilaria repens* in Europe (6)

In Romania, the first cases of human dirofilariosis have been reported by Victor Babes. Only some cases of human dirofilariosis have been described in Romania, most frequently with *D. repens* (a case of subcutaneous dirofilariasis and another case of ocular dirofilariasis with immature dirofilariae) (7, 9).

4. Pathogenesis

The cardiovascular dirofilariasis in dogs and cats is characterized by acute and chronic inflammatory lesions in the lungs and other organs due to the presence of adults and microfilaria. *Dirofilaria immitis*, like most filarial worms, has metabolism conditioned by the presence of an intracellular rickettsian symbiont that has been found in abundance in Malpighi tubes in mosquitoes. *Wolbachia* would appear to have a major role in filarial physiology because the literature reports a massive decrease in the number of larvae in peripheral blood when the definitive host is treated with tetracycline, especially doxycycline, which is most active against these bacteria. The pathophysiological response in cardiovascular dirofilariasis is mainly due to the presence of *D. immitis* parasites in the pulmonary arteries. The first lesion occurs in the pulmonary artery (fig. 2.3) and in the pulmonary parenchyma due to intravascular adult localization; pulmonary hypertension occurs, which then leads to congestive heart failure. Another syndrome is the blood circulation disorder, due to the location of the *Dirofilaria* in the right cord (fig. 2.4.), at the level of the tricuspid valve. These disorders lead to massive hemolysis and hemoglobinuria, being responsible for cave vein syndrome.

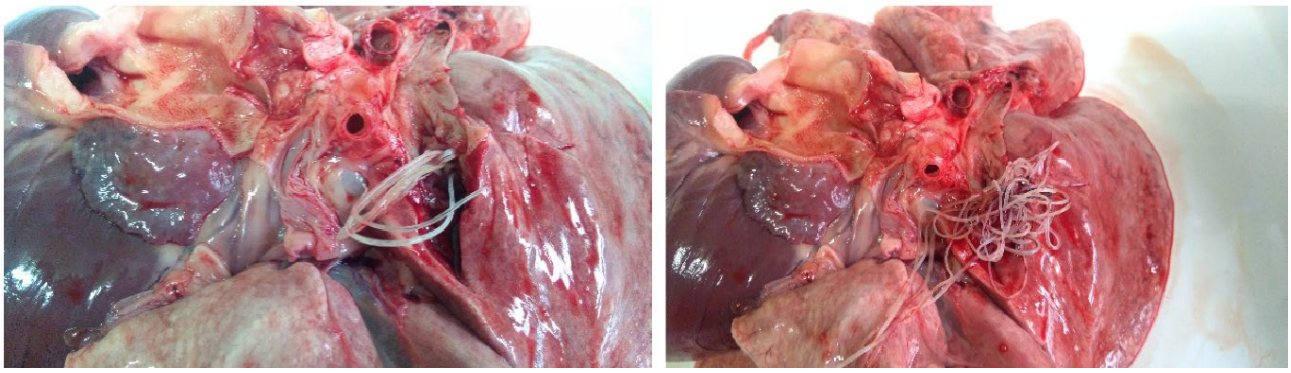

Fig. 2.3. A bunch of adults of *Dirofilaria immitis* before (a) and after mechanical extraction
(b) from a nodule from the traject of right diaphragmatic lobar branch of pulmonary artery in a male stray dog aged 12 years

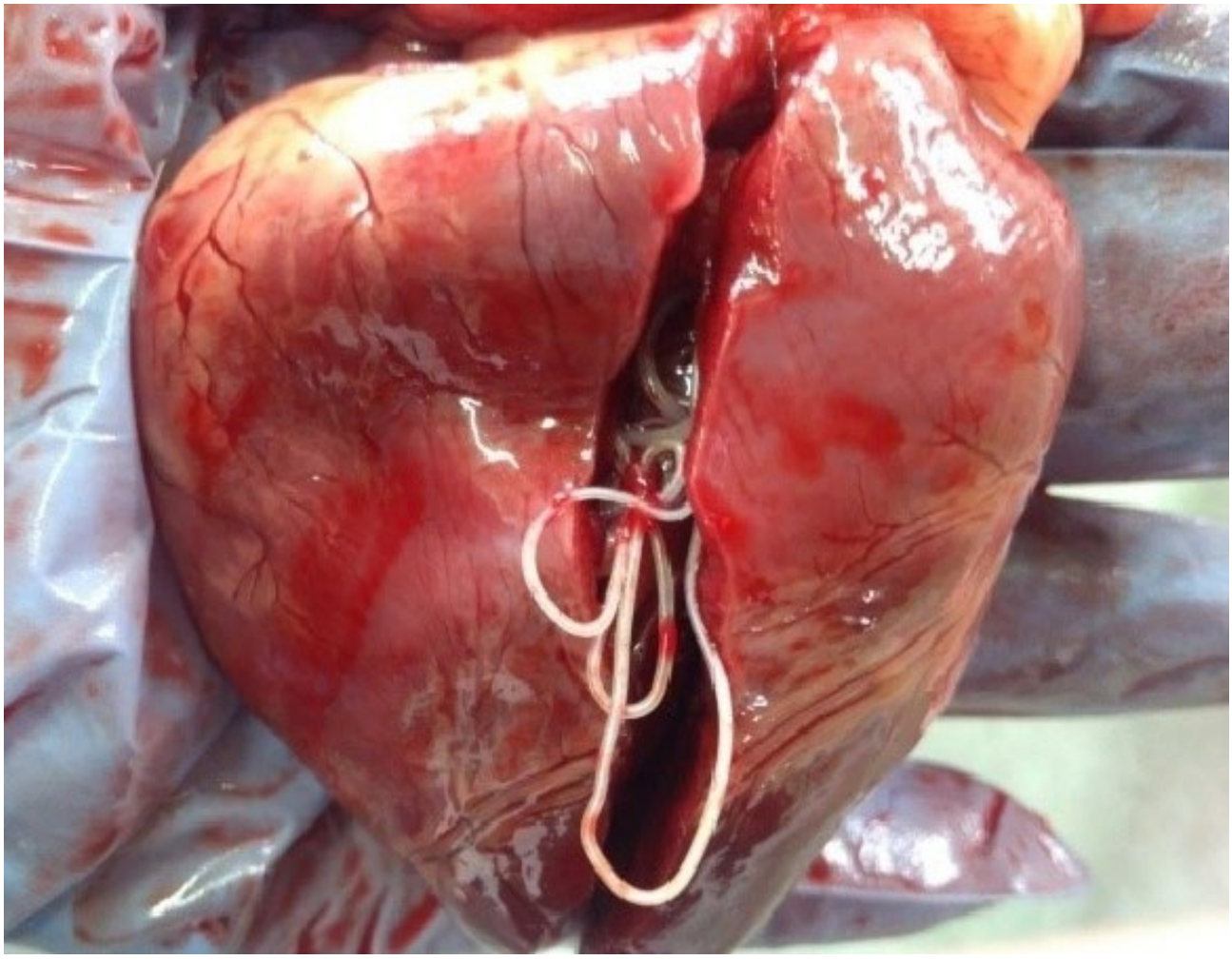

Fig. 2.4. Adults of *D. immitis* in right chambers of canine heart.

4. Clinical manifestations

Cardiopulmonary dirofilariasis in animals and humans

Normally, the expression of the cardiovascular dirofilariasis symptoms appears in the chronic form. The disease may develop asymptomatically over a period of several months or even years, the appearance of clinical signs being dependent on the number of adults in heart or pulmonary artery, individual reactivity and physical activity of the dog (injury the artery walls is directly proportional to the physical activity of the animal). Ideally, infection with *D. immitis* should be identified by serological testing prior to the appearance of clinical signs. However, at the earliest, antigenemia and microfilaemia do not occur up to 5 and 6.5 months, respectively, after the infection. Subcutaneous dirofilariosis in animals and humans. Subcutaneous dirofilariosis in dogs is usually asymptomatic. Clinical manifestations were classified into two clinical syndromes: multifocal nodular dermatitis, which is generally located on the face and prurigo papularis dermatitis.

5. Diagnostic

Diagnosis of *D. repens* infection is based on the presence of circulating microfilaria or on parasite observation in the subcutaneous nodules, as there are currently no screening tests available for antigens for serological diagnosis. Generally, most cases of cat dirofilariosis are undiagnosed. Mostly immature stages of the nematode *D. immitis* do not get mature and die as they reach the pulmonary arteries. Thus, the absence of adults makes it impossible to diagnose the infection without the cuticular antigen. The death of the larvae in the pulmonary arteries induces severe changes in the respiratory system, which is why the disease is now recognized as a pulmonary syndrome called HARD-Heartworm Associated Respiratory Disease. Clinically diagnostic is based on the evaluation by thoracic radiography, echocardiography and electrocardiography, of each patient with cardiopulmonary dirofilariosis. Chest radiographs identify pulmonary artery enlargement, lung parenchymal changes, and right cardiomegaly in the advanced stages of the disease. This technique cannot be used to evaluate parasitic burden.

6. Prophylaxis

Dirofilariosis may be prevented by avoiding mosquito bites in areas where mosquitos may be infected with dirofilariosis larvae. The risk of such mosquito bites may be reduced through as little exposure of the skin as possible, using ointments and sprays with insecticide (repellent) during the evening and night, in the rooms and the area of the bed, as well as through the correct use of mosquito nets (mosquitoes become active during the night) (3).

7. Treatment

Treatment in cardiopulmonary dirofilariosis is complex and difficult to establish in conditions where adulticides can cause thromboembolism and death of the patient. In conclusion, the therapy schedule should be used depending on the animal's health status

and burden with adults of *D. immitis*, and the association with other competing diseases.

In principle, the treatment is aimed at eliminating microfilaria in the blood and disrupting the development of larval stages in adults and the elimination of preexisting adults. For the adulticide therapy the only substance approved and recommended by AHA (American Heartworm Society) is melarsomina that is used at the 2.5 mg / kg dose, two doses at 24-hour intervals. Numerous studies suggest that macrocyclic lactone therapy (ivermectin), which has been shown to be partially adulticide when used at doses of 6-12 mcg / kg every month for 16 months or even 30 months have an efficacy of 100 %.

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3. LEISHMANIOSIS

1. Name of zoonosis

Leishmaniosis

2. Definition

Leishmaniosis is an important disease, present in over 88 countries, 350 million people are at risk. Every year there are 500,000 new cases of visceral leishmaniosis and 1–1.5 million new cases of cutaneous leishmaniosis and this causes 2.4 million disability-adjusted life years (DALY). Visceral leishmaniosis is responsible for over 59,000 deaths every year, a death rate second only to malaria among parasitic diseases (6).

There are risk factors that can exacerbate the impact of this disease on public health such as migrations, urbanization and deforestation. Conditions that lower the immune system also such as HIV and malnutrition are considered individual risk factors.
The disease caused by the species of the genus *Leishmania* are considered to be the third most important vector-borne diseases after malaria and lymphatic filariosis and the world's poorest population, living mainly in rural and suburban areas are particularly affected.

3. Etiology

Leishmaniosis is caused by diphasic protozoan parasites belonging to the genus *Leishmania*. The genus *Leishmania* is part of class *Kinetoplasta*, order *Trypanosomatida* and the family *Trypanosomatidae*. The genus *Leishmania* is itself divided in the subgenera *Leishmania* and *Viannia*.

These protozoans are transmitted by sandflies of the genus *Phlebotomus* in the Old World and genus *Lutzomya* in the New World. Sandflies (fig.3.1.) are crepuscular and nocturnal insects that are active during warm months in subtropical regions and all the year in tropical regions. There are many species of sandflies but only some can act as vector for leishmaniosis (4).

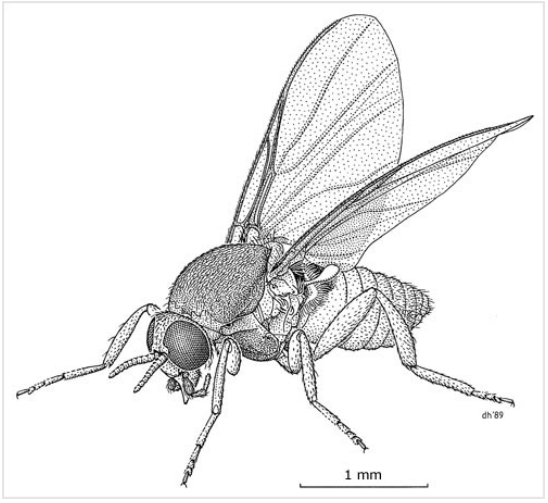

Fig.3.1. Sandfly

(source: https://teara.govt.nz/en/artwork/15352/female-sandfly)

Some sandflies species can only act as vector for only one species of *Leishmania* while some can transmit multiple species.

This protozoan has two life stages: It takes a nonflagelated form called amastigotes while it is in the vertebrate host, where it multiplies by fission in the macrophages of the host. Sandflies get infected by biting a vertebrate that has this parasite. While in the sand fly the parasite takes a flagellated form called promastigotes and replicate in the midgut or the hindgut of the sand fly.

4. Epidemiology

Depending on the species of the parasite, there can be three forms of the disease: cutaneous, mucocutaneous and visceral leishmaniosis. Cutaneous leishmaniosis is the most common form and causes ulcers on the most exposed part of the skin that leave life-long scars and disability after they heal. Most of the cases are found in Central and South America. There are few cases in the Mediterranean basin as well (1).

Mucocutaneous leishmaniosis cause the destruction of mucous membranes of the mouth, nose and throat. Most of the cases occur in Bolivia, Brazil, Ethiopia and Peru. Visceral leishmaniosis, also known as kala-azar is fatal if left untreated in most cases and is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. Visceral leishmaniosis is the most common type of leishmaniosis found in Europe. Visceral leishmaniosis can be anthroponotic if the etiological agent is *Leishmania donovani*. This species of *Leishmania* is found in the north-east of India, Bangladesh, Nepal and east Africa. Zoonotic visceral leishmaniosis is caused by *Leishmania infantum* and is present in China, Central Asia, Middle East, Central America and the Mediterranean area (6).

Although there are many species of *Leishmania* and the dog can be infected by at least 12 species, the most important etiological agent of canine leishmaniosis is *Leishmania infantum*. In Europe, canine leishmaniosis can also be caused by *L. tropica* and *L. major* but in the majority of cases *L. infantum* is still the cause and, as the following data regards canine leishmaniosis in Europe, we will be talking about *Leishmania infantum* from now on (fig. 3.2).

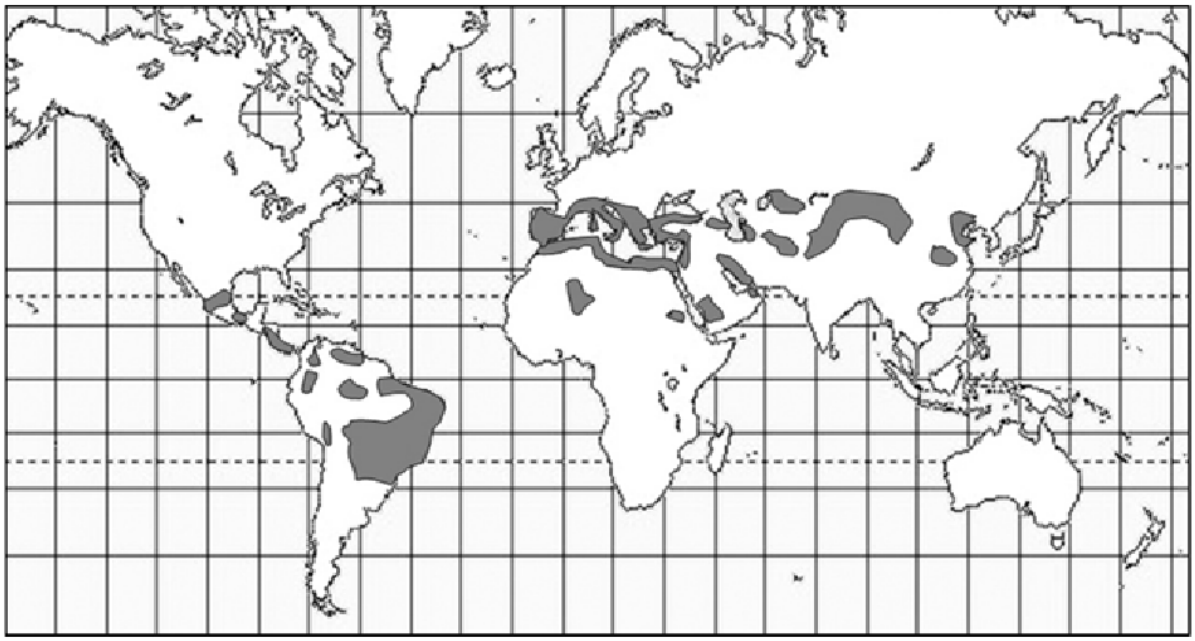

Fig. 3.2. Map distribution of zoonotic visceral leishmaniosis caused by *L. infantum* (1)

Even though humans, cats, rats and many other mammals can be hosts to *Leishmania infantum*, the dog is considered the most important reservoir (fig.3.3.).

Leishmania spp.[a]	Vectors[b]	Geographical distribution[c]
L. amazonensis	*L. flaviscutellata, L. nociva, L. whitmani*	Brazil
L. arabica	*P. papatasi*	Saudi Arabia
L. braziliensis	*L. intermedia, L. migonei, L. wellcomei, L. whitmani, and others*	South America
L. colombiensis	*L. hartmanni*	Venezuela
L. guyanensis	*L. anduzei, L. umbratilis, L. whitmani*	Colombia
L. infantum	*L. longipalpis, L. evansi, L. neglectus, P. perniciosus, and others*	Africa, America, Asia, Europe,
L. major	*P. papatasi*	Egypt, Saudi Arabia
L. mexicana	*L. ayacuchensis, L. olmeca*	Ecuador, USA
L. panamensis	*L. hartmanni, L gomezi, L. panamensis, L. trapidoi, L. sanguinaria*	Colombia, Ecuador, Panama
L. peruviana	*L. peruensis, L. verrucarum*	Peru
L. pifanoi	*L. flaviscutellata, L. youngi*	Ecuador
L. tropica	*P. sergenti*	India, Iran, Morocco, Syria

Fig. 3.3. Leishmania species reported in dogs in the Old and New Worlds (2)

Changes in the epidemiology of canine leishmaniosis caused by *Leishmania infantum. Leishmania infantum* has long been considered a disease typical of tropical and subtropical regions but reports from all around the world have shown that the epidemiology of this disease is changing. On the American continent autochthonous cases have been found in the south-east of Canada and in the north of Argentina. In Europe there are many epidemiological studies that show that this disease is spreading more to the north of the continent. Autochthonous cases

have been found in the centre of France, north of Italy, Germany and Romania (2).

5. Pathogenesis

Leishmania promastigotes are transmitted to the vertebrate host, during the bite of a sandflies vector, with the saliva. In the vertebrate host, the promastigotes are phagocytized by macrophages. In the phagolysosome, the promastigotes transform in amastigotes and multiply until the macrophage breaks and the amastigotes are released, get phagocytized again by other macrophages and repeat the cycle. By this mechanism the amastigotes spread to the hemolymphatic organs. The absence or presence of clinical manifestations in case of an infection depends on the immune response of the host (4).

If the host develops mostly a T-helper type 2 immune response, with the production of interleukin 4 and interleukin 10, there will be an important antibody response that does not eliminate the parasite. The chronic and excessive production of antibodies can cause immune-mediated thrombocytopenia, glomerulonephritis and vasculitis that can cause dermal, visceral or ocular necrosis (4).

An immune response that is mostly dependent on Th type 2 cells causes the production of IFN-γ, IL-2 and TNF-α. These cytokines cause the elimination of *Leishmania* amastigotes from macrophages by producing nitric oxide that produces apoptotic cell death (4).

6. Clinical manifestations

Infection with *Leishmania* does not mean there will be clinical manifestations. Depending on the type of immune response of the host, the infection can be eliminated, have a subclinical evolution or have clinical manifestations. A large number of dogs have subclinical infections that can evolve to have clinical manifestations if the dog becomes immunosuppressed (10).

Canine leishmaniosis is a visceral disease but often also has dermatological manifestations. The clinical manifestations that are most often encountered are: dermatitis, lymphadenomegaly, weight loss, anorexia, decreased appetite, lethargy, splenomegaly, ocular lesions, epistaxis, hepatomegaly, onychogryphosis (5).

7. Diagnostic

Diagnostic tests for leishmaniosis are done in order to confirm the disease in patients with matching clinical signs. Routine diagnostic tests such as complete blood count, biochemical profile, urine and serum electrophoresis can help to raise the suspicion. One of the oldest diagnostic methods used to confirm leishmaniosis is the detection of amastigotes in stained cytological smears and aspirates from cutaneous lesions, spleen, bone marrow and lymph nodes. This method has the advantage of being fast and cheaper than other methods but it also has the disadvantage of having a low sensibility because a low number of parasites can be present even in dogs with clinical manifestations. (fig. 3.4.) (8). A histological analysis of a biopsy from affected tissues can also be used to detect amastigotes but it has the same problem as the cytological examination and it also takes more time. The isolation of parasites from infected tissue in culture is only used for research purposes and not diagnosis

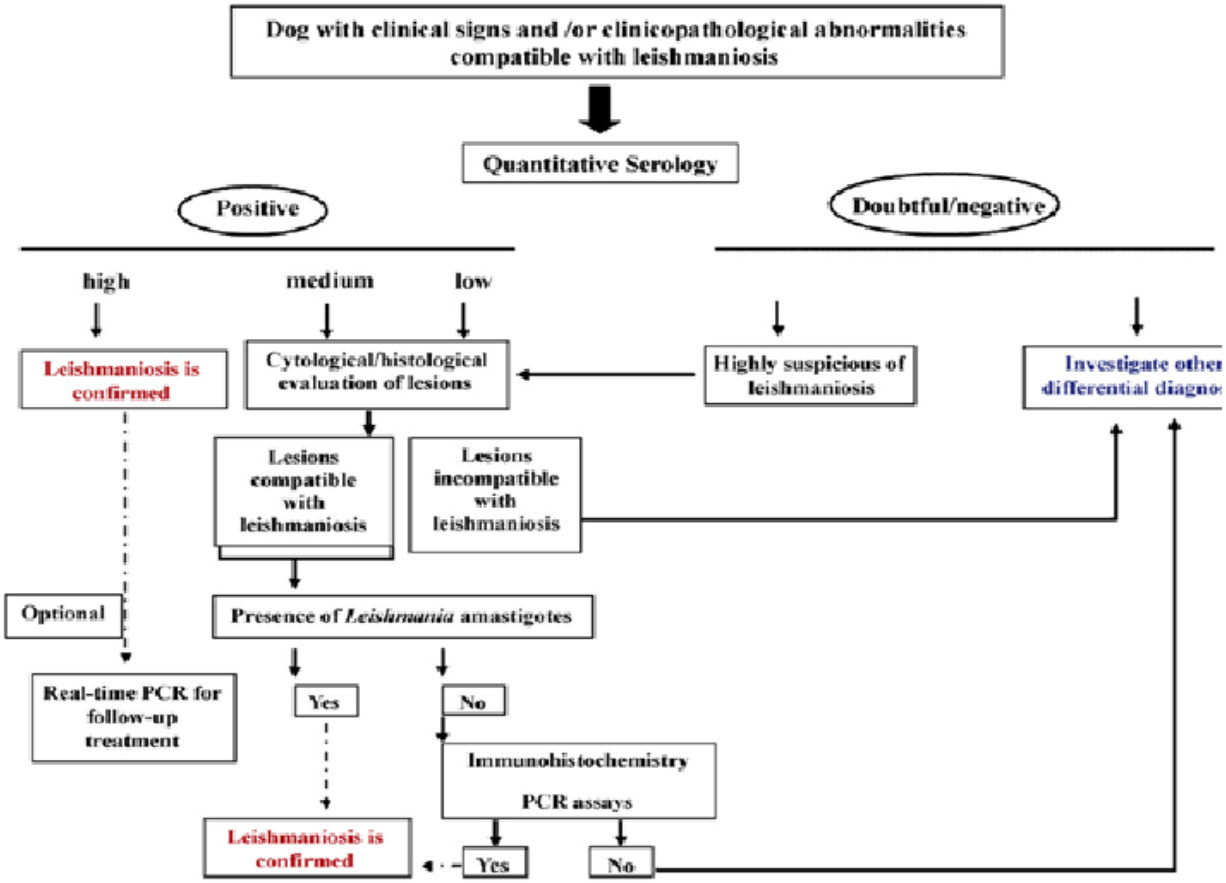

Fig. 3.4. Algorithm of diagnostic approach in dogs with clinical signs and/or clinic pathological abnormalities compatible with leishmaniosis. (3)

because it's expensive, it takes time and it is less sensible than PCR and serology. Serological techniques and PCR are the most useful diagnostic methods used for the diagnosis of leishmaniosis. Quantitative serological techniques such as enzyme-linked immunosorbent assay (ELISA) and immune-fluorescence antibody test (IFAT) can be used to detect anti-*Leishmania* serum antibodies. The seroconversion time can vary widely. Clinicians should take into account that all serological tests can have false positive results due to cross-reactivity with other pathogens like *Trypanosoma cruzi* (6).

High levels of antibodies confirm the diagnosis of leishmaniosis but in the case of low levels, the diagnosis has to be confirmed with other methods like cytology, histopathology or PCR (13).

PCR is the most sensible and specific method for the diagnosis of leishmaniosis. Assays that target the DNA of the kinetoplast seem to be the most sensible. PCR done on DNA extracted from bone marrow, lymph node, conjunctive swabs, spleen or skin is more sensitive than PCR done on whole blood, buffy coat and urine. There are three different PCR techniques available for diagnosis:

conventional PCR, nested PCR and real-time PCR. Real-time PCR is the most sensible and specific method available and it can be used to quantify the load of *Leishmania* in in tissues. The clinician should take into account data from the clinical evaluation as well as the diagnostic tests (3).

8. Prophylaxis

Culling of seropositive dogs and restrictions on the transport of dogs from between regions has been the hallmark of the measures to control the spreading of zoonotic leishmaniosis in Brazil. Even though these measures have been in place since the 1950s and approximately 3500 dogs are culled every year, data from the Brazilian Health Ministry shows an ascending trend of human visceral leishmaniosis and most scientist argue that these measures are not efficiently preventing the transmission of leishmaniosis in a hiperendemic region like Brazil. These measures have also been met with resistance by some societal groups and by dog owners. The use of topical insecticides or insecticide impregnated collars is one of the most affordable and effective measure used to control the transmission of leishmaniosis (7). Insecticides such as permethrin, deltamethrin, imida-cloprid or piriproxyfen have been shown to reduce transmission of leishmaniosis from between dogs and from dogs to humans (12). These substances are usually used alone or in combinations of two and can be applied either as spot on or as an impregnated collar. The use of an impregnated collar can be effective for up to 6 months while spot on products lose their efficiency after one month. Although the use of insecticides is an effective and cheap way to prevent the transmission of leishmaniosis, the use of only this method is insufficient to control this disease and there has been much effort put in producing effective vaccines (1).

The production of new vaccines efficient against leishmaniosis is slow and difficult because this parasite can induce an inefficient humoral immune response. The antigen used in the production of an efficient vaccine has to induce a cell mediated immune response that causes the T cells to produce cytokines that causes the production of nitric oxide by the macrophages which is the principal substance responsible for the intracellular killing of the parasite. The complex interactions between this parasite and the immune system of its host is the reason why after decades of attempts only two vaccines are registered for commercial use as a canine vaccine and have showed to provide protection in field conditions. The first vaccine registered in Brazil is Leishimune®, a vaccine that uses a glycoprotein purified from *Leishmania donovani* called fructose mannose ligand (FML) as an antigen mixed with QuilA saponin as adjuvant (11). This vaccine has been found to have an 80% clinical efficacy. CaniLeish® is the first licensed vaccine for canine leishmaniosis in Europe and it's based on an excreted secreted protein (ESP) antigen purified from the supernatant of a culture of *Leishmania infantum*. It uses QA-21 saponin as adjuvant. This vaccine has a 68% clinical efficacy (3).

9. Treatment

The drugs most commonly used for the treatment of canine leishmaniosis are: meglumine antimoniate, aminoside, miltefosine, amphotericin B, liposomal amphotericin B, metronidazole, marbofloxacin. The

combination of meglumine antimoniate with allopurinol is considered the most effective and constitutes the first line of treatment. There are many different therapeutic protocols suggested but the pharmacokinetics of antimonials may vary from case to case in patients with canine leishmaniosis because the half-life is increased in patients with renal failure. The risk of renal failure is increased as meglumine antimoniate is nephrotoxic. Because of this side effect miltefosine in combination with allopurinol has been suggested as an alternative to the meglumine antimoniate and allopurinol treatment. Amphotericin B is the 1st line drug for human visceral leishmaniosis in Europe but it's not recommended for veterinary use to avoid drug parasite resistance (9). Amphotericin B also has the disadvantage of being nephrotoxic. The response to treatment can vary a lot as some patients might show clinical improvement in a month while others require a much longer period of time. The clinical evolution of the case has to be monitored during the treatment but this can be difficult because the clinician has to distinguish the deterioration of the patient caused by the disease from the side effects of the drugs. Besides monitoring the clinical status of the patient, it is recommended to perform complete blood count, biochemical profile and urinalysis. These analyses should be repeated often during the duration of the treatment. The treatment should be ceased after a complete clinical recovery is declared after physical examination and after complete blood count, biochemical profile and urinalysis. Clinically healthy patients that have tested positive for leishmaniosis should not be treated.

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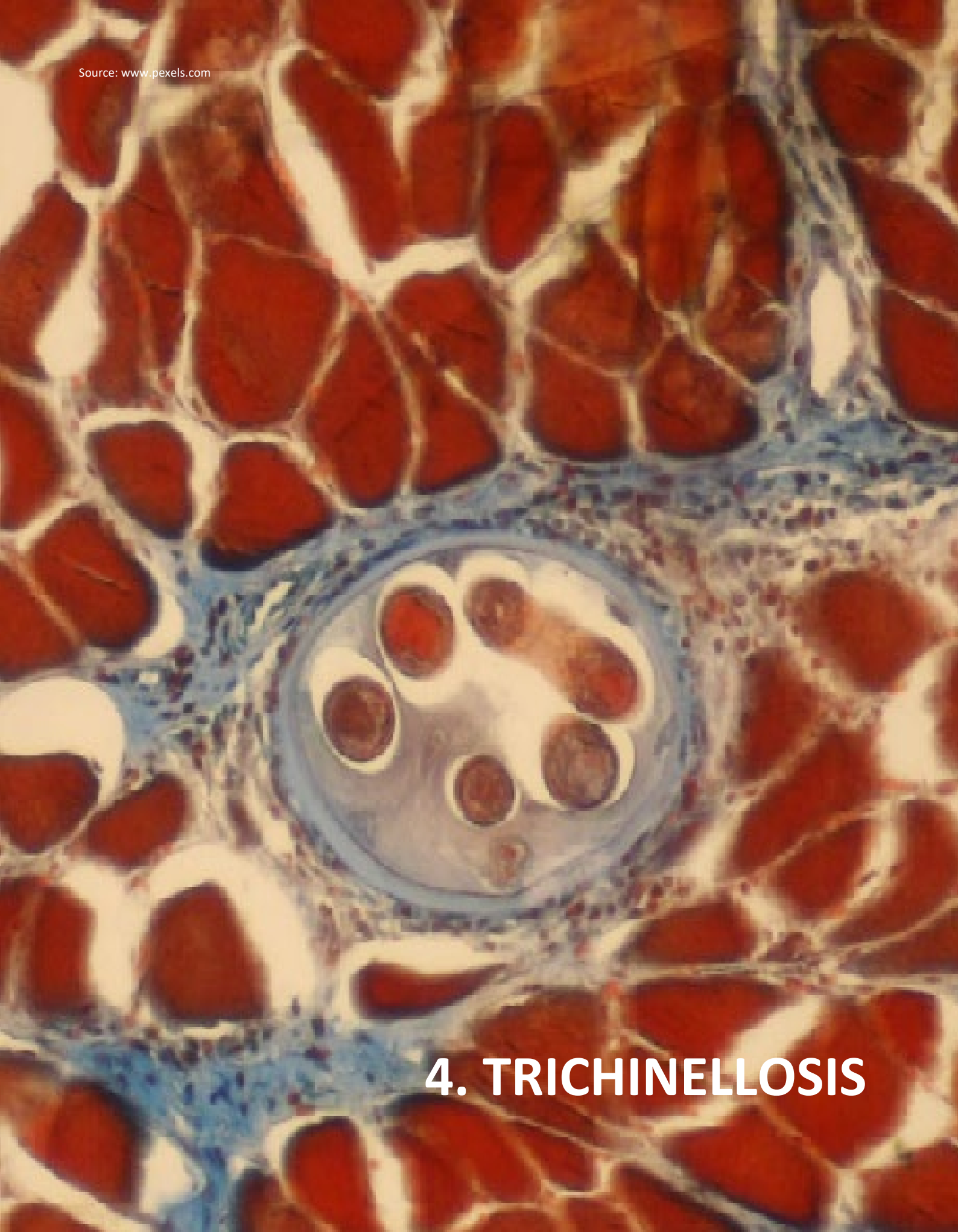

4. TRICHINELLOSIS

1. Trichinellosis

2. Definition

Trichinellosis is a serious parasitic zoonosis caused by the nematodes from the *Trichinella* genus, which parasitize in the small intestine and in the striated muscle tissue, on the same host. The disease affects the majority of domestic and wild animals (swine, rodents, carnivores, equines, aquatic animals, birds, reptiles), in which it evolves clinically asymptomatic and anatomic-pathological, with catarrhal enteritis lesions and myositis (18). In humans, trichinellosis evolves seriously, with fever, digestive disorders, facial edemas, allergic conditions and myalgia's (10, 32).

3. Etiology: *Trichinella* genus

3.1. Etiologic agent

Trichinella spp. The name *Trichinella spiralis*, comes from the Greek: *thrix, trichos*: hair; *ella*: small, and Latin, *spiralis*: spiraled, rolled.

3.2. Taxonomy

Trichinella spp. Belongs to the phylum *Nemathelminthes*, class *Nematoda*, subclass *Adenophorea*, order *Enoplida*, superfamily *Trichinelloidea*, genus *Trichinella* (3). Molecular phylogenetic studies carried out by Blaxter (2011) (2), divides the phylum *Nematoda* in three branches: *Enoplida*, *Dorylaimia* and *Chromadoria*. According to these studies, *Trichinella spp*. belongs to the branch *Dorylaimia*, order *Trichinellida*.

The *Trichinella* genus includes nine species *(T. spiralis, T. nativa, T. britovi, T. nelsoni, T. murrelli, T. papuae, T. pseudospiralis, T. zimbabwensis, T. patagoniensis*) and three unidentified genomes (*T6, T8, T9*) (14). Within the *Trichinella* genus, the species are divided into two groups: one group contains the species*: T. spiralis, T. nativa, T. britovi, T. nelsoni, T. murrelli, T. patagoniensis* and genomes T6, T8, T9, which form larvae incapsulated in the striated muscle tissue and the second group, containing the species*: T. pseudospiralis, T. papuae. T. zimbabwensis,* which don't form encapsulated larvae, these developing freely in the muscle tissue (25, 27).

3.3. Morphological description

Trichinella spiralis has a small body, unequally calibrated, thinned towards the front and thickened towards the rear, covered with a smooth cuticle. The upper part of the body displays a round of discoidal cells, called stichocytes, which form the stichosome. In the hypodermis there are four rows of glandular cells spread along the body, which open towards the exterior, through a pore. The orifice of the mouth presents a small stylet. The esophagus is long occupying one third or half of the entire length of the body (3).

The male measures 1,4-1,6 mm. The caudal bursa is replaced with a copulating piece formed out of two conical appendages, ventrally curved and two pairs of papillae, between which the cloacal orifice is opening; it has a single testicle and has no spicule (32).

The female measures 2,2-4,0 mm x 40-60 µm. The genital orifice opens in the middle third of the body, on the ventral side. It has a single

ovary and it is viviparous. In the uterus, early embryonic stages and larvae of stage I (L1) may be observed, prepared to be laid (3) (fig. 4.1.).

Fig. 4.1. *Trichinella spiralis*, adult nematodes: female (right) and male (left), taken from the small intestine of a rat that was experimentally infected. A pre-larva is coming out through the genital orifice of the female (Bowman, 2014).

Adult nematodes are localized in the small intestine, in mammals and birds (in the spotted mouse, *Trichinella nativa* extends up to the large intestine), and the larvae stages parasitize in the striated muscles, in the same host.

3.4. Biological cycle

The biological cycle is auto heteroxenous (fig. 4.2.), in which the parasitized organism is definitive host and intermediate host both at the same time. The infection of the host subject is done through the intake of the meat or meat products parasitized with *Trichinella* cysts, which contain infected larvae (17). Under the action of gastric juices, the larvae are freed from the cysts and due to the transit, migrate to the intestine (ten minutes) where they enter into the cells of the intestinal epithelium. After 10-12 hours, the larvae go through their first molting (6). More intestinal cells (around 100) go through membrane fusion, forming a syncytium or a multicellular niche in which larvae are localized. These localized larvae go through four successive molting, at small intervals (30-60 hours), transforming into adult nematodes, sexually differentiated. The sexual maturity is reached early (5).

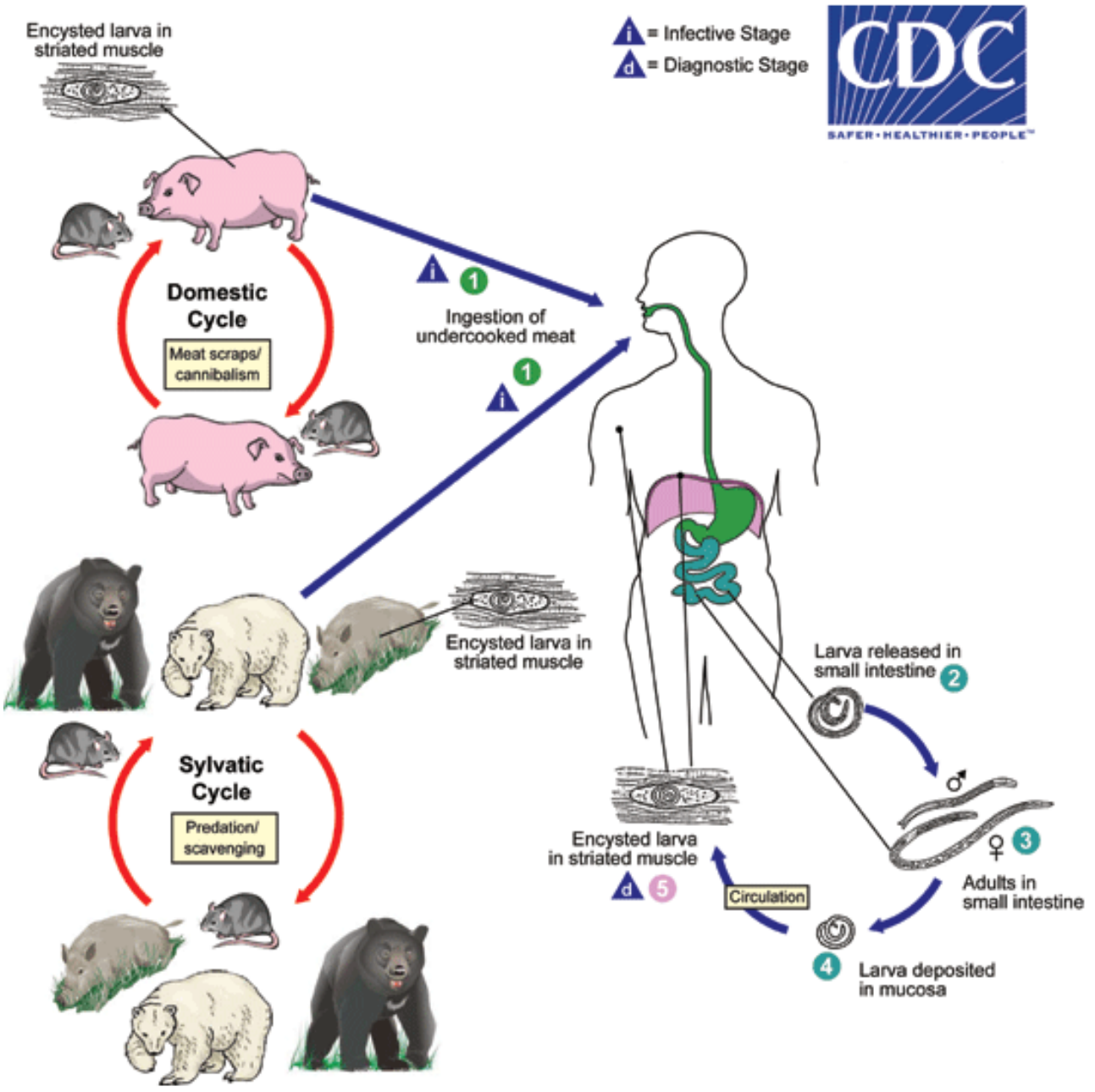

Fig. 4.2. Biological cycle of the species *Trichinella spiralis* (source: CDC)

The adult female occupies numerous cells (415-425) forming a multicellular niche in which, through pheromone release, she attracts the male for copulation. The females' fecundity varies, according to the species of the host: in mice there have been found 1600 larvae laid by a single female. The intestinal stage is influenced by the immune system status of the host species: in mice and rats it takes up to 10-12 days; in guinea pigs, 30-40 days and in humans, 42 days. The formation of embryos takes on average 90 days but the larvae (L1) are laid by the females in five days after the infection (34).

In the first hour from the moment the larvae were laid, L1 crosses the lamina propria of the epithelium and reaches into a lymphatic or capillary blood vessel (a part of the larvae pass through the interstitial tissue and the liquids from the peritoneal cavity, choosing another migration path) (10).

Through the circulatory system, the larvae get into the right side of the heart and lungs (where they stay for around ten hours, while a part of the larvae are destroyed) and through the pulmonary arteries, they get into the left side of the heart, from where they are dispersed, through the arterial system, into the whole body (3) (fig. 4.3.).

The larvae that got into the striated muscles continue their development while the larvae that have migrated to the organs are degenerating. In the case of pregnant animals, the larvae pass through the placental barrier and under some conditions, they colonize the foetus. The trans placental path has been demonstrated in the sow, rabbit doe, rat doe, guinea pig sow (5, 34). The localization of the larvae in the striated muscles is conditioned by the intensity of the vascularization and innervation of the tissue, the larvae preferring the muscles that are better irrigated, with intense activity. The larva penetrates the muscle cell with the help of some proteolytic enzymes. After it has penetrated into the muscle cell, the larva transforms it into a "feeding" cell, or a "nanny" cell, isolated from the adjacent tissue, functioning as a yolk sac, for the life and development of the larva (17).

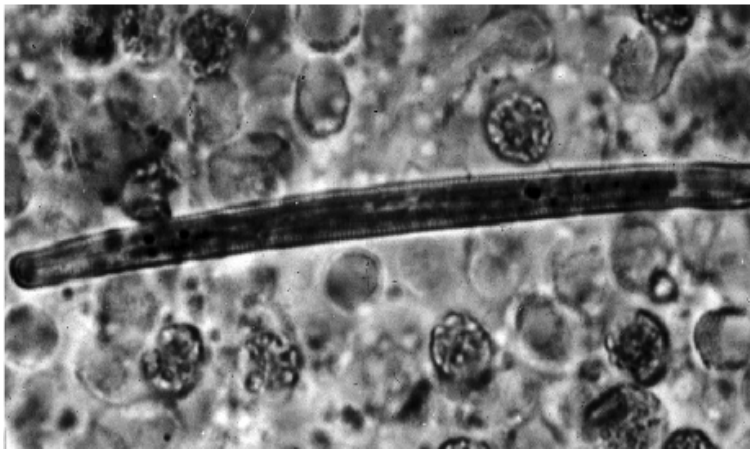

Fig. 4.3. *Trichinella spiralis* prelarva in migration identified in the blood of a cat through the Knott method (Bowman, 2014).

The changes in the muscle cell start from the fourth day and take up to the 20th day from the moment the larva penetrated into the cell. These changes are initiated by an antigen (TSL-1) created by the stichocytes and of the larva, starting from the 11th day from the infection. Under these conditions, the muscle fibre loses its contraction capacity and becomes a source of proteins for the growth and survival of the larva (3).

The host organism produces anti-stichocytes antibodies, through which it protects itself against a new infection. In the feeding cell, the larva grows in volume, the cuticle thickens, the esophagus develops (the number of stichocytes increases, which differentiate in cells with α granules and cells with β granules) and the body becomes spiralled (10) (fig. 4.4.). The host organism develops (starting from the 15^{th} day, up to five weeks post infection), an inflammatory reaction in the form of a capsule around the feeding cell, representing the *Trichinella* cyst. Inside the cyst there are one or more larvae (up to seven). The wall of the cyst represents an adventitial reaction made from collagen produced by the fibroblasts of the host organism (18).

In the encapsulated species *(Trichinella spiralis, T. britovi, T. nativa,* etc), the larva does not survive if the protective capsule or the cyst is formed. In some cases, in wild boars, the larvae of the species *T. spiralis* do not form cysts. In the case of some species (*Trichinella pseudospiralis, T. papuae, T. zimbabwensis*), the larvae develop freely, non-encapsulated. The species *T. pseudospiralis*, following the very acute immunosuppressive action, induces a much reduced inflammatory reaction from the part of the parasitized host, preventing the formation of the capsule (31).

The capsule limits the movements and shift, and protects it against the immunity reaction of the host. Inside the capsule, the larva feeds on amino acids (tryptophan, alanine, tyrosine). After a variable period (from a few months up to two years), the process of calcification of the capsule begins. The period varies according to the species of the host and the parasitized organism (3).

Fig. 4.4. *Trichinella spiralis*. Larva (L1) at the beginning of its spiralling stage (rounded extremities may be observed) Mehlhorn, 2008 LM of an unfixed coiled larva 1 (note the rounded ends).

After the larvae are localized in the striated muscles, the cycle of development may be interrupted temporarily and may restart under the conditions in which another host ingests the parasitized muscle tissue or the same host, in the case of cannibalism. In this case, the larvae from the muscles reach the infecting stage in a very short time, before the formation if the capsule, in 17-20 days from the infection (5).

4. Epidemiology

Trichinellosis is a widely distributed zoonosis, being reported around the globe in humans and in domestic and wild animals, in which it evolves with an outbreak characteristic.

4.1 Source of infection

The trichinellosis outbreaks are sustained within the synanthropic cycle, by swine, rats, dogs, cats, coypus and equines, as important sources of parasites, and by wild boars, foxes, bears, badgers, which constitute reservoirs and sources of parasites within the sylvatic cycle (26). Other sources of infection are represented by omnivorous and carnivorous birds, as well as some heterothermic animals (crocodile, varanids). Between the two cycles of development: synanthropic and sylvatic, there is a flow of communication, an inter-outbreak possibility, to which rats have an important contribution, circulating on vast areas (8).

Within the domestic cycle, the trophic relationships between pig-rat-human are the ones that interfere, in interrelations with wild animals (29).
In some cases, the sylvatic cycle exists without any human intervention, representing a direct source for the infection of humans. In the

development cycle there are untargeted hosts which contribute to spreading the disease. *Trichinella* larvae have been discovered in muscle tissues from ovine and bovine animals, deer and reindeer, in different geographical areas.

Marine animals like the seal, the walrus, have been found to be naturally infected with *T. nativa*; through parasitized walrus meat consumption, the disease is transmitted to humans. Also, the import of live animals parasitized with *Trichinella*, represent a sure way of spreading the parasite in disease-free areas (30). The dissemination of the disease is realized through rats, which carry the disease in a specific area; cannibalism; game meat; necrophagous and dipterous insects (Calliphora, *Sarcophaga* or *Musca domestica* and *Lucilia sericata*), in the bodies of which (in the nymphs) the larvae survive eight days, or through migratory birds, which carry and eliminate the larvae, on great distances.

Homo sapiens are the only species (among the Primates) that gets naturally infected with all the species of the *Trichinella* genus, except for *T. zimbawensis*. When humans will prepare accordingly the products of animal origin, they will manage to control the transmission of *Trichinella* species (*T. spiralis, T. britovi, T. pseudospiralis*) between domestic and wild animals, and when they manage to breed swine under appropriate conditions, they will stop being hosts for these parasites (25, 29).

4.2 Path of infection

The transmission of trichinellosis to humans is done through the consumption of raw meat or meat that was insufficiently thermally treated meat originating from the infected animals: pig, game (bear, wild boar), horse, dog (3). The transmission is also done through the consumption of meat preparations (salami, sausages) or meat preserved in lard, in oil, smoked products, pastrami etc., if the preparation time and/or the concentration of the brine used have not been sufficient for the destruction of the larvae (33). The method of food preparation is decisive in the transmission of the disease, within the groups of people that have consumed infected meat originating from the same source: some get seriously ill, others don't. The risk of transmission of the disease to human's increases under the conditions of lack of examination or incorrect examination of the meat destined for consumption (standardized methods of trichineloscopy, artificial digestion, serology) (12). Providing early sanitary education, expanding the medical and biological knowledge, as well as raising the awareness regarding the risks of infection, all contribute to limiting the ways of infection and the development of trichinellosis in humans.

4.3 Responsiveness

The emergence of trichinellosis in some countries is explained through a more documented knowledge of the disease (often confused with influenza), of the consumers' habits, or as a result of afforested areas in Europe, and therefore of the development of game, of meat imports from other countries where trichinellosis is endemic, of the mistakes made in the veterinary-sanitary control and the errors of diagnosis, of social events which make use of the same source of infected food (19).

Trichinellosis is conditioned by the meat consumption, in which case infection is, theoretically, easy to prevent through appropriate preparation, freezing, and veterinary-sanitary control, measures that should be taken seriously by the entire personnel involved in the public health sector, and finally, the disease could be eradicated from the swine farms as well (7, 23).

The occurrence of new cases of the disease is due to the passive spread of the parasite by rats or by accidental contamination of food with larvae of other origins. In this case, the storage of fodder contaminated at least a month before they are given to pigs, reduces the risk of infection. In Romania, trichinellosis is induced by the species *T. spiralis, T. britovi* and *T. pseudospiralis* which affect domestic and wild animals (1, 21). During 2007-2009, Romania reported to the World Health Organization a high incidence of trichinellosis in humans, where the annual average incidence was of 2,7 cases in 100.000 people (20).

4.4 Resistance to the environment

The encapsulated larvae of the species *T. spiralis* are resistant to the putrefaction phenomena of meat or cadavers, where they remain viable for up to four months. The collagen wall around the larva and the feeding cells ensures the survival of the larva and maintains the capacity of infection in the processes of complete putrefaction of the muscle tissue, due to the anaerobic metabolism. In this case, the necrophagous animals become larger reservoirs of *Trichinella* (4).

The encapsulated larvae are resistant to the climate factors for a long time, presenting a surprising viability in relative humidity and temperature oscillations (15). In negative temperatures of -18ºC, the T. spiralis larvae have resisted for more than 268 days, keeping their viability verified through experimental reproduction of the infection in lab mice (12). In the case of natural infection in pigs and wild boars, the larvae have remained viable after three successive freezing and thawing treatments, at intervals of three months each. In the case of traditional preservation methods through smoking, salting, drying, the encapsulated larvae resist both in the pork meat and in the game meat (4).

4.5 Geographical distribution

Trichinellosis is encountered in over 55 countries (16). The *Trichinella* genus has a wide geographic spread, without any climatic restrictions (tab. 4.1) (14, 27).

Table 4.1.

Geographic spread of the species from the *Trichinella* genus and description of their biological and clinical characteristics

Species	Geographic spread	Environment	Hosts	Biological characteristics	Clinical characteristics
Trichinella spiralis	Cosmopolitan	Domestic and sylvatic	Wild boar, horse, bear, fox	Is not resistant to freezing: big number of larvae/female: forms cysts	High pathogenicity, short incubation period, lethality<0,2%
Trichinella nativa	Arctic and subarctic regions from Asia, Europe and North America	sylvatic	Bear, fox, dog, wallrus, raccoon, puma	Is highly resistant to freezing, small number of larvae/female: forms cysts	Moderate pathogenicity, long incubation period, severe enteric symptoms, mortality cases have been reported
Trichinella britovi	Temperate zone from Europe and Asia	Sylvatic, rarely domestic	Fox, wolf, raccoon, wild boar, pig, horse, dog	Is moderately resistant to freezing, small number of larvae/female: forms cysts	Moderate pathogenicity, long incubation period, moderate or absent enteric symptoms, there have not been any mortality cases reported
Trichinella pseudospiralis	Cosmopolitan in Asia, Europe, North America and Tasmania	Sylvatic, rarely domestic	Pig, wild boar, fox, marsupials, carnivorous and omnivorous birds	Is not resistant to freezing, does not forms cysts	High pathogenicity, short incubation period, mortality cases have been reported
Trichinella nelsoni	Sub-Saharan Africa	sylvatic	Hyena, lion, common warthog	Is not resistant to freezing, forms cysts	High and moderate pathogenicity, long incubation period, moderate or absent enteric symptoms, mortality cases have been reported
Trichinella murrelli	USA	sylvatic	Fox, bear, raccoon, lynx, horse	Is not resistant to freezing, forms cysts	Moderate pathogenicity, long incubation period, moderate or absent enteric symptoms, mortality cases have been reported
Trichinella papuae	Papua New Guinea	sylvatic	Wild boar, crocodile	Is not resistant to freezing, does not forms cysts	Low or moderate pathogenicity
Trichinella zimbabwensis	Zimbabwe	sylvatic	Nile crocodile	Is not resistant to freezing, does not forms cysts	Unknown
Trichinella potogoniensis	Patagonia Argentina	sylvatic	Wild carnivorous animals	Forms cysts	Unknown

The etiological agent of trichinellosis in humans is practically spread all around the globe, being found in animals from both the synanthropic and sylvatic cycles, except for the Antarctica, where there have been no cases of infection reported. The global distribution of species from the *Trichinella* genus is consistent with the food habits, culture and education of the human population, favouring the infection. *T. spiralis* has a dominant distribution, being more spread in the countries from the Central and Eastern areas of Europe (24,25).

The prevalence of trichinellosis in humans is difficult to approximate, but it is considered that over 11 million people might be infected (7, 13). The geographic spread of the species from the *Trichinella* genus is illustrated in fig. 4.5.

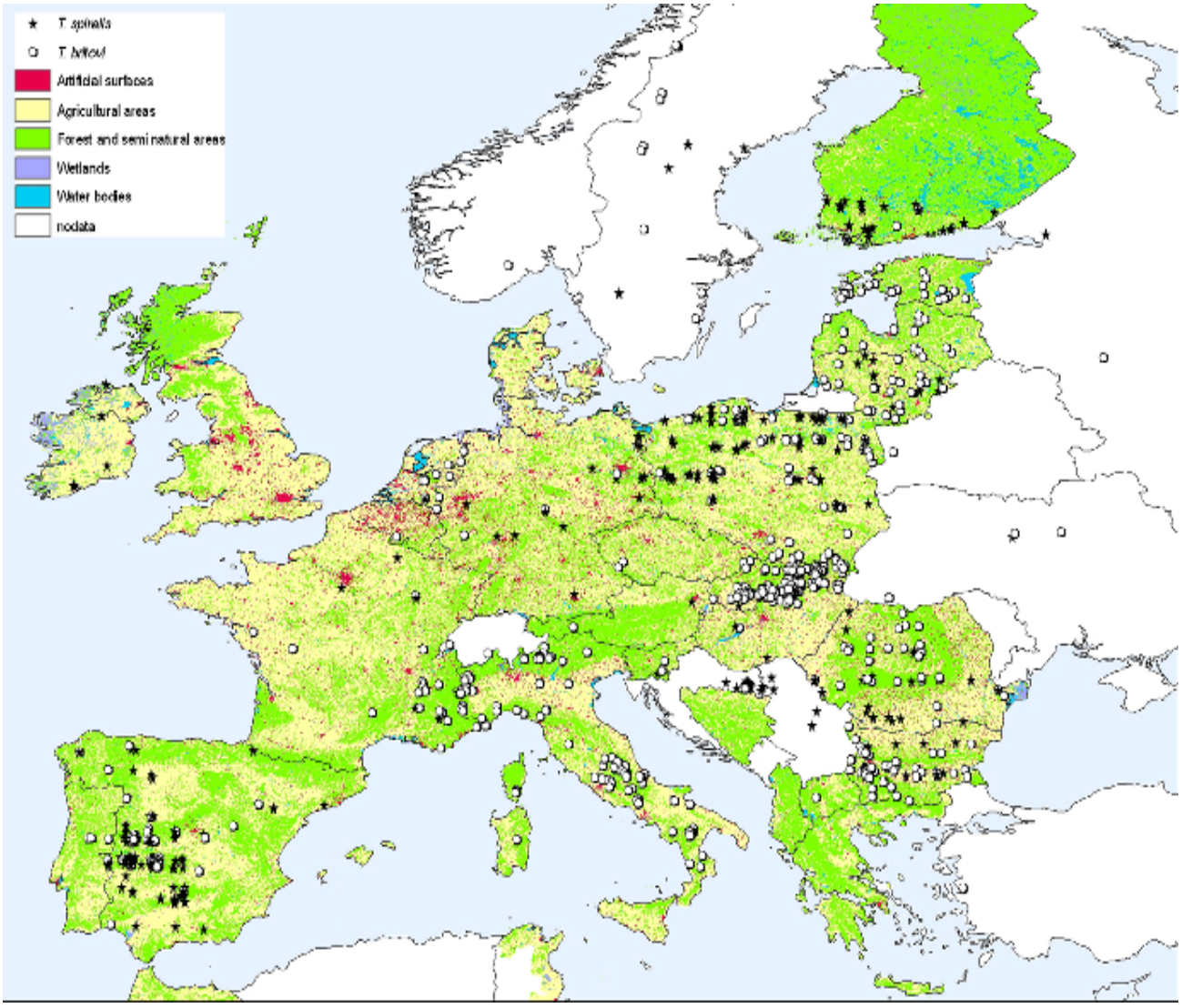

Fig.4. 5. The distribution of *Trichinella spiralis* (black stars) and *Trichinella britovi* (white circles) isolates in Europe. (Pozio et al., 2009).

5. Pathogenesis

The aggression of nematodes from the *Trichinella* genus is exerted by the larval stages, in the period of migration, after the localization in the muscle fibre, and by the adult nematodes, which parasitize deep into the glandular crypts of the mucous membrane of the small intestine (10, 17). The pathogenesis mechanisms are known in humans and lab animals that were infected experimentally and less known in animals infected naturally. During the time of migration, the larvae affect the mesenteric lymph nodes, causing, depending on the infecting dose, inflammations with an inflow of eosinophils and macrophages (16).

In the muscular localization stage, the larvae produce an irritating-inflammatory reaction and, through the metabolism products, they have significant allergic and toxic effects, inducing irreversible changes (3). The myocyte expands in volume, actin and myosin discs disappear, the nucleus suffers changes and the myofibrils are disorganized. Inside, the larva grows and develops on the basis of the nutrient sources offered by the parasitized myocyte (17) (fig. 4.6.).

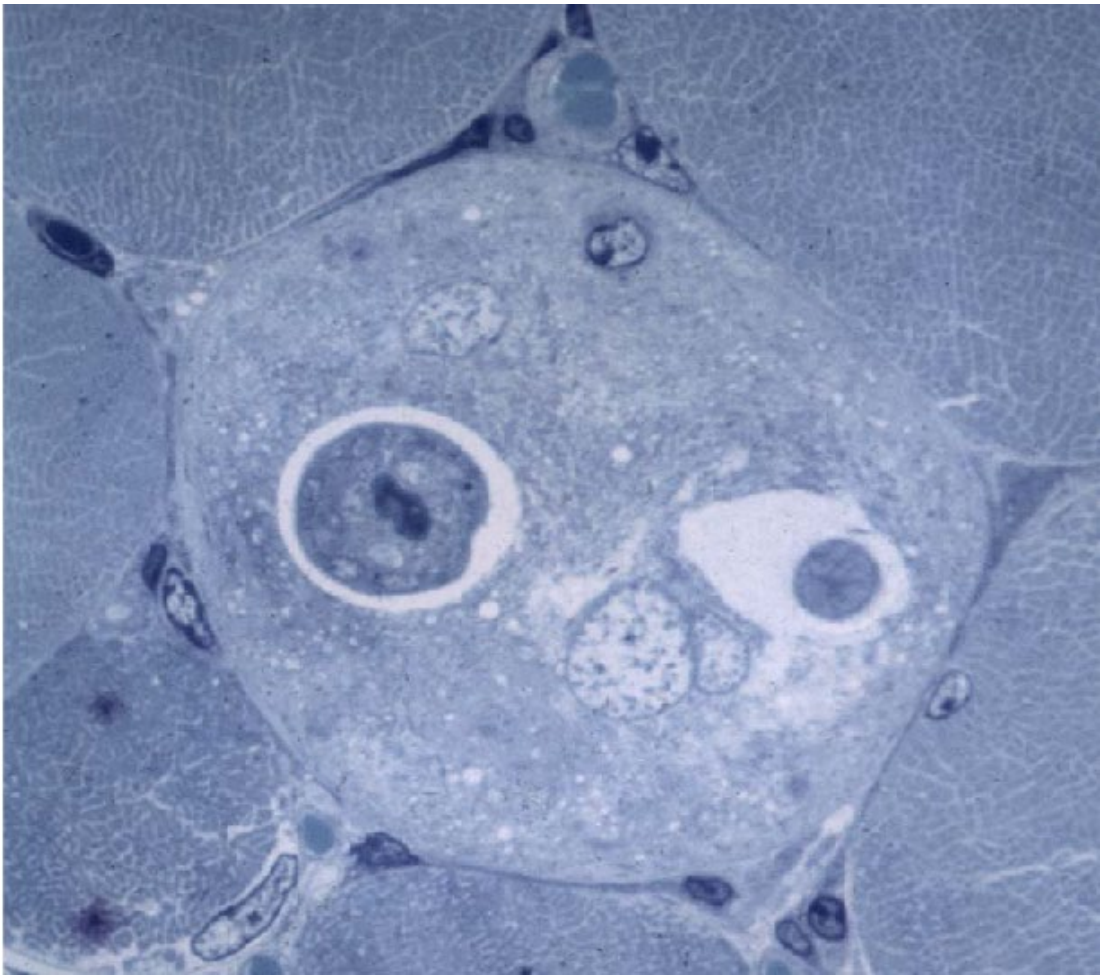

Fig. 4.6. *Trichinella spiralis*. Cross-section in muscle cell containing two larvae (Mehlhorn, 2008).
LM of a semi thin section with a twice cross-sectioned larva in muscle cell (Mehlhorn, 2008).

The myocyte has transformed into a protective connective tissue capsule, permissive to glycogen, some amino acids and lipids, ensuring prolonged survival for the larva. The larvae are usually localized in the diaphragm muscle, the lingual muscles, intercostal muscles with variable density (200-5.000 larvae/gram muscle tissue). In the muscles, the larvae induce phenomena of necrobiosis, necrosis, inflammation (myositis) (5). The toxins released induce general disturbances, reflected biochemically through: hypoalbuminemia, leukocytosis, eosinophilia (over 20%), moderate anaemia, increase in phospholipid level, change of concentration of the fatty acids from the serum etc (16).

The allergic effect, more pronounced in some *Trichinella* species, is characterized by the presence of edema, asthma, dyspnoea, urticarial form eruptions, increase in capillary permeability, serious circulatory disorders in organs and tissues, increased concentration of IgE (10).

The inoculator action of larvae is accentuated during the passage through the intestinal mucous membrane or the pulmonary tissue, by engaging some microbial agents, causing myocarditis, bronchopneumonia, meningitis, nephritis etc. Adult nematodes, by getting deeper into the intestinal mucous membrane, has a mechanical, traumatic, irritating, inflammatory effect, causing catarrhal enteritis, intestinal haemorrhage, ulcerous lesions, malabsorption. Immunity. The immunological activity is conditioned by the host species and the species or genotype of *Trichinella*. The development stages (newly laid larvae, larvae during migration, larvae localized in the muscles) and adult nematodes determine different reaction of the organism, gradually requesting the intervention of all defensive humoral and cellular elements, which limit or annuls the parasitic offense (antigens) (18). The parasites release surface or somatic antigens and secretion-excretion or functional antigens. The body responds humoral, by releasing IgA, IgG, IgM and IgE immunoglobulins, and cellular, by the intervention of lymphocytes (T, B), plasmocytes, mastocytes, macrophages, eosinophils and mucus secreting calceiform cells, initiating the antitoxic and anthelmintic defence.

The immunity appears starting from the first infection, without a premunition state and becomes stronger after reinfections (17). Vaccination with moderate doses of irradiated larvae has induced a strong immunity to the vaccinated subjects. Passive immunity was proved through the transfer of maternal antibodies to newborns by colostrum ingestion. The reactivity of the body is influenced by a multitude of intrinsic and extrinsic factors that contribute to the duration and intensity of the immune response (5).

6. Clinical manifestations

In animals, trichinellosis evolves sub clinically or with faint, uncharacteristic symptoms (16). In the case of experimental infections, eight days after infection, signs of catarrhal or haemorrhagic enteritis have been observed, with different duration and intensity, dehydration and colitis. This is the intestinal stage, which lasts, on average, three weeks. In this stage, some animals die, due to the dense population of adult nematodes that are

present in the duodenum and jejunum (3). The muscular stage starts 15 days after infection and it is characterized by symptoms corresponding to the localization of the larvae in the muscles, causing dysphagia, mastication difficulties, muscle pain and pruritus. The intestinal and muscular stages overlap for a short period, amplifying the clinical picture (32).

7. Diagnostic

Diagnosing trichinellosis is done by cross-referencing epidemiologic, clinical, paraclinically and anatomic-pathological data.

7.1. Clinically

Asymptomatic or non-specific evolution (6).

7.2. Paraclinically

The immune diagnosis is recommended, through the ELISA and PCR multiplex tests with broad applicability in human medicine (9). In animals, the examination of the muscle tissue is done after slaughter or post mortem, according to the standardized veterinary sanitary norms (22), or through direct trichineloscopic examination, through which the *Trichinella* cysts are examined (fig. 4.7.), or through artificial digestion of the muscle tissue, through which the larvae released from the cysts are examined (13) (fig. 4.8.)

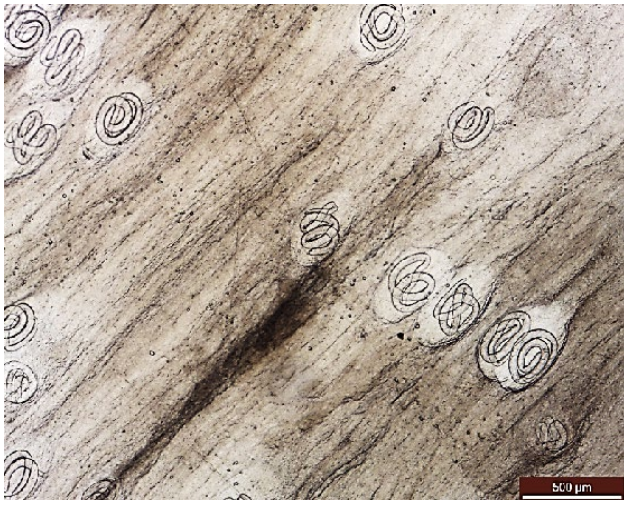

Fig. 4.7. Trichinellosis. Pig striated muscle tissue, parasitized with *Trichinella* cysts x 45 (Iacob, 2016).

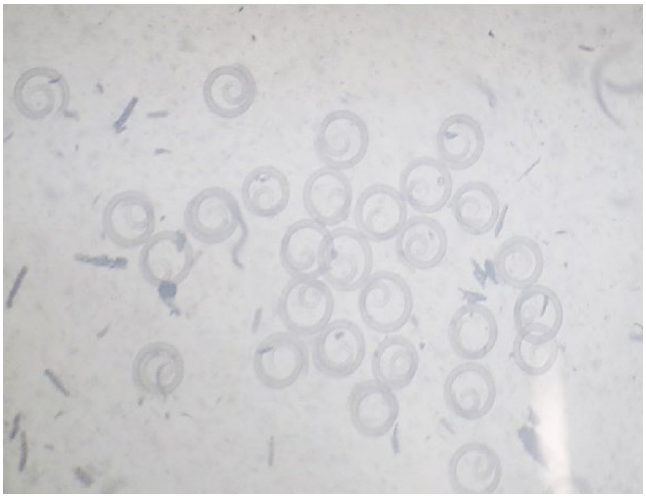

Fig. 4.8. Trichinellosis in pigs. Artificial digestion of the muscle tissue parasitized with *Trichinella spiralis*: free larvae (trichineloscope x 80) (Iacob, 2016).

The differential diagnosis of *Trichinella* cysts is done compared with *Sarcocystis* cysts; in the case of calcified cysts, acidic solutions are applied, for the dissolution of the calcium deposits, after which, either the *Trichinella* larvae or the *Sarcocystis* bradyzoites are noticed; these are differentiated against *Toxocara*, *Stefanurus*, *Strongyloides* larvae who are in the migration stage, cazeified micro-abcesses and against muscular hydatidosis (17).

7.3. Epidemiologically

The transmission of the disease is conditioned by the biological characteristics of the parasites, the attitude and behaviours of the humans, the presence of synanthropic animals and the geographic area in which it evolves. *Trichinella spiralis* and *T. pseudospir*alis have a cosmopolitan distribution, while other species or genotypes are limited to some specific geographic zones (31). Carnivorous, omnivorous, predatory, cannibalistic and necrophagous animals are receptive to infection. Humans have an important role in maintaining the infection outbreaks and the transmission of the parasite, since through food habits, instruction, biological and medical knowledge, compliance with the veterinary sanitary regulations regarding the control of products of animal origin, they contribute to either limiting or spreading the disease (28).

7.4. Pathologically

In the intestine, catarrhal enteritis lesions, oedematous infiltrations of the intestinal wall, petechial haemorrhages and adult nematodes in the mucous membrane are discovered (3) (fig. 9).

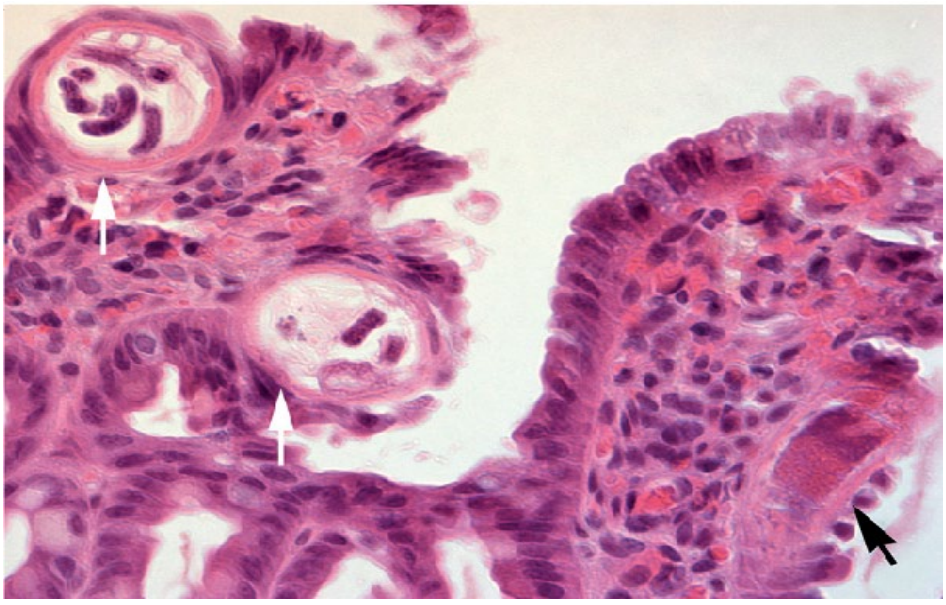

Fig. 9. *Trichinella spiralis* adult. Rat intestine histological section, 7 days after infection. Section through a female with prelarvae (Bowmann, 2014). *Trichinella spiralis* adult in the mucosa of the small intestine of a rat (×480). Two cross-sections through a female (white arrows) contain prelarvae and a longitudinal section through the stichosome esophagus (black arrow) (Bowmann, 2014).

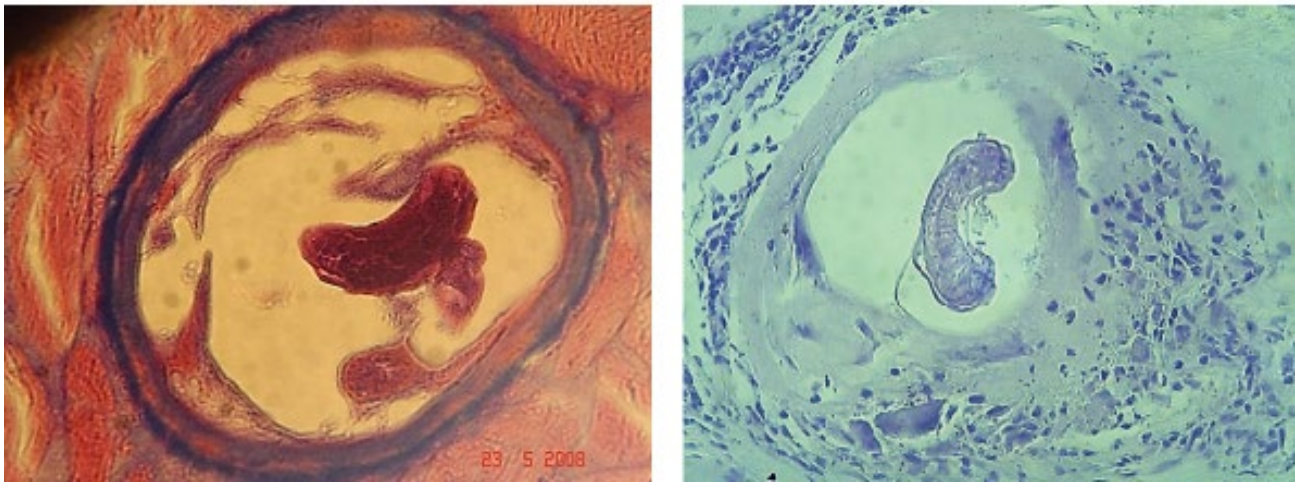

Fig. 10 a, b. Trichinellosis in pigs. Section through muscles with *Trichinella* cysts. (Col. MGG (left), HEA (right)x 400 (Iacob, 2016).

In the muscle tissue, the myositis lesions are detectable only through a microscope. In this case, there are processes of necrobiosis, necrosis and destruction of myocytes, that may be observed in the muscle tissue; cystic formations that contain a coiled larva protected by the sarcolemma, connective tissue invaded by a population of histiocytes, macrophages, eosinophils, lymphocytes, with inflammatory character, connective tissue fibres, that delimits the cyst and adipose cells located at the cystic poles (12) (fig.10 a, b)

In time, the *Trichinella* cysts regress through processes of caseification and calcification that finish after approximately two years from the time of the infection of the host or through bacterial complications; in this stage, the cysts appear as small white formations, spread in the muscle tissue (5).

8. Treatment

The treatment is effective especially when anthelmintics have a broad spectrum and they are administered during the vulnerable stages of the biological cycle. The impediment appears when it comes to the (impossible) identification of the clinical stages of development of parasites, in the case of which only the immunodiagnostic is efficient. The treatment has maximum efficacy over the adult nematodes in the intestine, a period that is not identified *intra-vitam*, as well as over the larvae that have been migrating for 8-18 days, migration that is detectable serologically through circulating antibodies. The efficacy decreases over the encapsulated forms with chronical evolution detected by muscle biopsies. The efficient medicinal substances (3, 18) are as follows:

- Mebendazole, in a dose of 20 mg/kg bw/day, orally, daily, for ten days; or in a dose of 600 mg/animal, for 6 or 10 days;
- Flubendazole, in a dose of 20 mg/kg bw/day, orally, repeated ten times at an interval of 48 hours, associated with hyaluronidase, with great efficacy;
- Fenbendazole, in a dose of 250 mg/ animal 2 times per day, for 14 days;

- Albendazole, in a dose of 20 mg/kg bw/day, orally, repeated 15 times, at an interval of 24 hours;
- Albendazole, in a dose of 50 mg/kg bw/2 times per day, for 7 days, for dogs and cats;
- Albendazole, in a dose of 20 mg/kg bw/day, orally, associated with Levamisole, in a dose of 3-4 mg/day, repeated at an interval of seven days, for several times;
- Albendazole, in a dose of 20 mg/kg bw/day, orally, associated with Ibuprofen, in a dose of 10 mg/kg bw/day, for 6 consecutive days;
- Oxfendazole, in a dose of 10 mg/kg bw/day, for 15 days, in the food, with good efficacy over incapsulated larvae;
- Thiabendazole in a dose of 25-50 mg/kg bw, for 5-10 days;
- Avermectin, in a dose of 0,3 mg/kg bw, subcutaneously, at 14 and 21 from the time of infection, with good efficacy (84%).

In animals, the use of drug preparations in great doses and for a long period of time imposes restrictions on the consumption of meat.

9. Prophylaxis

The trichinellosis prophylaxis imposes the compliance with the sanitary and veterinary norms in force, regarding swine breeding and processing of products and sub-products, the following measures being mandatory (35, 22, 30, 33):

- direct trichinelloscopic exam or by artificial ingestion of pork, game (bear, wild boar), horse, coypu meat, for both public and private consumption;
- confiscation and appropriate thermal treatment of carcasses and products originating from infected animals;
- sterilization of products and sub-products originating from slaughterhouses used for preparing animal food; rendering the cadavers of animals suspected of infection;
- periodic disinfection, collection and destruction of rat cadavers from the animal breeding enterprises, slaughterhouses, meat and meat products processing enterprises;
- minimizing the number of stray dogs and cats, the sources of connectivity between the sylvatic and synanthropic cycles;
- education of the population regarding the processing of the meat through boiling or thermal treatment (oven), for a longer period of time (4-5 hours), being known that preparing the meat by salting, smoking and freezing it at -15ºC, for 20 days, does not destroy the larvae from meat and meat preparations;
- informing people and raising the awareness on the risk of infection through consumption of pork, bear, wild boar, horse, coypus meat and meat preparations without specialized veterinary sanitary examination (trichineloscopy, artificial digestion etc.).

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Source: www.pexels.com
5. LYME DISEASE

1. Name of zoonosis:

LYME DISEASE

2. Definition

Lyme borreliosis is an infectious disease of humans and animals caused by the spiral bacteria *Borrelia burgdorferi*. In humans the disease may initially present clinically on the skin (Erythema chronicum migrans) at the site of a tick bite, and in the later course of the disease through inflamed joints, changes to the central nervous system, skin, changes to the heart muscle and the eyes (4).

3. Etiology

3.1 Etiologic agent

Lyme borreliosis is caused by the bacteria B. burgdorferi.

3.2 Taxonomy

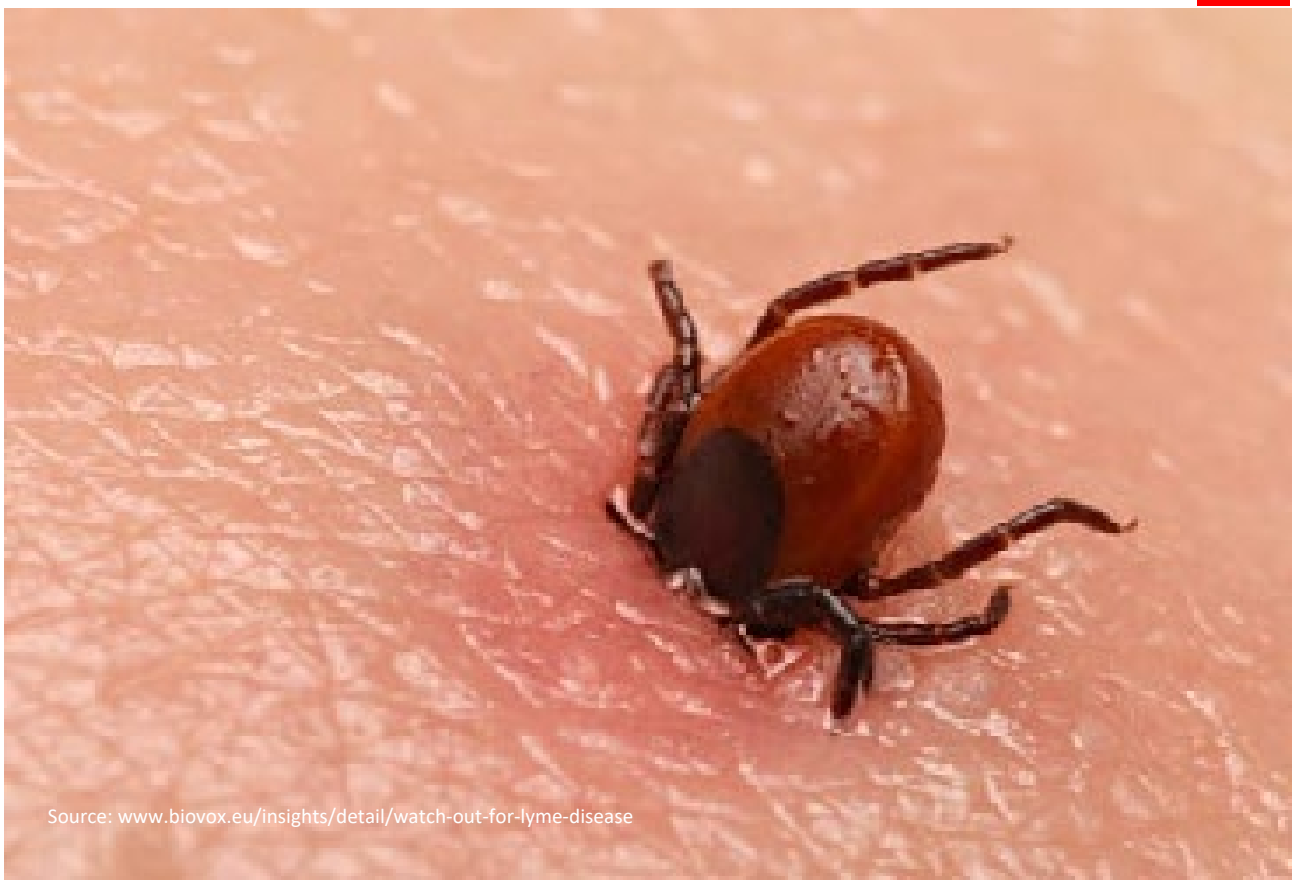
The bacteria *B. burgdorferi* is classified in the order *Spirochaetales*, the family *Spirohaetaceae*, and the genus *Borrelia*. On the basis of genetic differences, the strains of *Borrelia* belong to the genome complex *B. burgdorferi sensu lato*, within which to date about ten genomic species have been identified, of which several are pathogenic (4).

3.3 Morphological description

B. burgdorferi is a spiral, elongated slim microaerophilic bacteria, 0.2-0.5 x 3-30 µm in size, which grows in a liquid Barbour-Stoener-Kelly (BSK-II) culture medium at a temperature of 32-340C. Its ultrastructure consists of a helical protoplasmic cylinder, wrapped in an external and internal membrane, and subterminal and bipolar periplasmic flagella.

3.4 Biological cycle

In spring the female tick lays eggs (possible transovarial transmission of the pathogen) from which larvae hatch and then attach themselves as parasites onto infected mice-like rodents which are reservoirs of disease. By feeding from these disease reservoirs the larvae become infected, are then shed and remain on the ground for the entire year. The following spring, the infected larvae molt to become nymphs, which during the spring and summer feed off a new host (rodents, other animals and humans). Feeding off the new host continues over four days so that the infected nymphs can infect the new host, or uninfected nymphs can even become infected from infected hosts. In the autumn the nymphs molt again to become adult ticks. The percentage of infected larvae, nymphs and adults may increase over two years to up to 50%. The adults then mate, the males die and the infected females look for a new host, feed, drop to the ground, survive through the winter, and in spring lay infected eggs to complete the two-year life cycle (2).


Source: www.biovox.eu/insights/detail/watch-out-for-lyme-disease

4. Epidemiology

4.1 Source of infection

The reservoirs and sources of infection are small free-living rodents. In Croatia and Europe, these are mainly wood mice *Apodemus sylvaticus*, yellow-necked mice *Apodemus flavicollis*, bank voles *Clethrionomys glareolus* and dormice *Glis glis*. In the endemic areas of *Lyme borreliosis*, infected wild ruminants also play a significant role in the maintenance of the disease. Wild ruminants are important for maintaining the life cycle because adult ticks from the genus *Ixodes* feed on them. However, the level of infection in large mammals is lower than in rodents, and as a result they are an unsuitable reservoir for *B. burgdorferi*. Birds also play an important role as disease reservoirs, because as they migrate they can carry the pathogens over a large distance and into new areas (8).

4.2 Path of infection

The main vectors of *B. burgdorferi* are various species of slow-feeding ticks of the genus *Ixodes*. Infection of humans and animals mainly follows from an infected adult tick or nymph feeding on a host. When a tick attaches itself to a human or animal and begins to suck blood, the *Borrelia* in the central intestines of the tick are transferred into the salivary glands and through the saliva into the host's vascular system. It is known that the migration of the pathogen from the intestines to the salivary glands takes 12-36

hours, so the danger of infection grows if the tick remains on the animal or human for a long time. Given our knowledge of the natural cycle of ticks of the genus *Ixodes*, it must be emphasized that in the months of May, June or July, humans are infected by infected nymphs, and by adult ticks in the autumn. It has been established that nymphs, apart from being extremely small, carry a larger quantity of pathogens than adult ticks, and therefore they are a more significant vector in the spread of the disease to humans (7).

4.3 Responsiveness

The antigen structure of *B. burgdorferi* varies greatly. By analysis of the antigen structure and genotype analysis it has been established that *Borrelia* isolates comprise a complex known as *Borrelia burgdorferi sensu lato*, which includes 18 genomic species. For veterinary and human medicine, in terms of importance, potentially pathogenic species have been described such as: *B. afzelii, B. bavariensis, B. bissettii, B. burgdorferi sensu stricto, B. garinii, B. kurtenbachii, B. lusitaniae, B. spielmanii and B. valaisiana*. The most common pathogens for humans are *B. burgdorferi sensu stricto, B. afzelii* and *B. garinii* (7).

People that are most often exposed to the disease are those who spend time professionally or for leisure in endemic areas. Apart from infections in wild animals, especially small rodents, mice, voles, wild rabbits, hedgehogs, deer and birds, the disease also occurs in horses, dogs, cows and sheep (1).

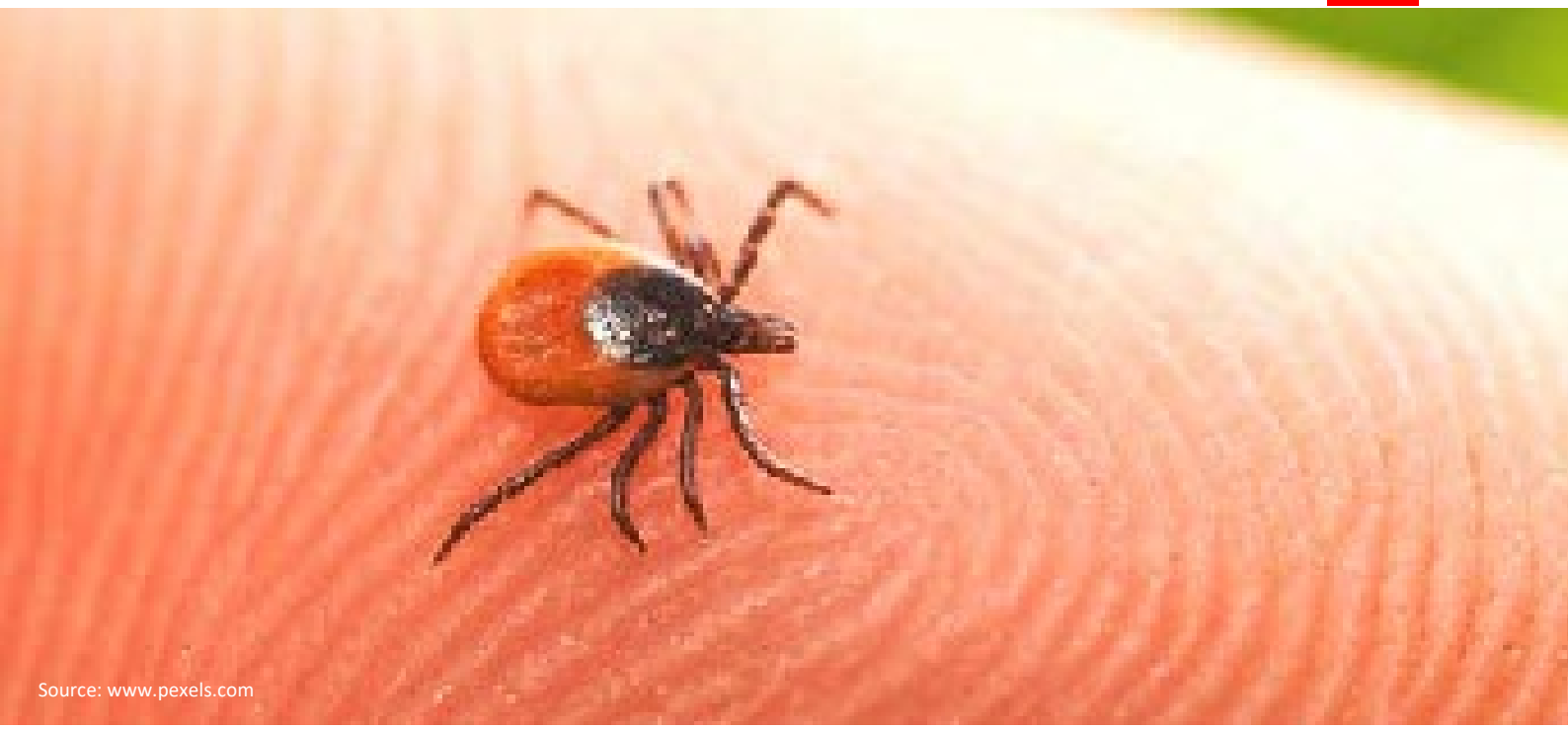
4.4 Resistance to the environment

In contrast to some other spiral bacteria, *Borrelia* cannot survive independently in the environment.

4.5 Geographical distribution

The disease has been known since 1975 and was first diagnosed in children in the small town of Old Lyme. Lyme borreliosis occurs mainly in areas with cold climates, with deciduous, dry to moderately damp forests, and sandy and humus-loamy soil. *Ixodes* ricinus and *Ixodes persulcatus* are the main vectors in Europe and Eurasia. In Europe, most cases of the disease have been recorded in Scandinavian countries and central European states, Germany, Austria, Switzerland and Slovenia, where *I. ricinus* is widespread, and it spreads *B. burgdorferi, B. garinii* and *B. afzelii* (3).

The spread of the infection stretches to the east through Eurasia, according to the presence of the tick *I. persulcatus*, which only carries *B. garinii* and *B. afzelii*. In Europe and Asia *I. ricinus* and *I. persulcatus* feed on more than 200 species of vertebrates. In the USA pathogens are carried by *I. scapularis* - black legged tick or deer tick, *I. dammini* - white-tailed deer tick and *I. pacificus*. In Europe, 85,000 cases and in the USA up to 20,000 cases of the disease are registered in humans per year. It is estimated that about 255,000 people are infected every year in the world. The annual prevalence of the disease varies depending on the size of the tick population and exposure of animals and humans, and most infections occur seasonally during the warmer months (3).



Source: www.pexels.com

5. Pathogenesis

The transfer of spirochetes from the intestines of an infected tick to the host takes at least 50 hours. Despite the many people and animals that are bitten, only a few develop clinical signs of the disease, which probably depends on the host's immunoreactivity (3).

In endemic areas, where there are more than 50% infected ticks, only 2% of people who are bitten become ill. It is presumed that *Borrelia spp.* proliferate for a certain length of time locally on the skin at the location where the tick is attached, or the infection. From that location they multiply and actively migrate through the tissue, at first close to the site of the bite, and later they infect other tissue, including joints. After infection, *B. burgdorferi* persist in the body. Trial infections suggest that spirochetes persist extra-cellular over a long period of time in the skin, connecting tissues, joints and the nervous system, and thereby avoid the action of the immune system. Research shows that *Borrelia*, upon infecting the host, change the expression of their surface proteins significantly, that is, they are capable of completely changing their surface structure. If they find themselves in an unfavorable environment their shape changes in a few minutes and so they survive over a long period of time without food, but upon returning to more favourable conditions they very quickly return to their spiral form. The clinical form of the disease is the result of the inflammatory response of the host (5).

6. Clinical manifestation

Infection in animals mainly passes in a subclinical form. It has been described in dogs, horses, cattle and sheep. The symptoms of the disease in dogs are acute fever, with raised body temperature of 39.5^0 C to 40.5^0 C, occasionally a limp, swollen joints, lymphadenomegaly, anorexia and general weakness. Later the disease may manifest in

chronic joint infections, and kidney, heart and nerve disorders. The clinical manifestation of the disease in horses is seen in stiffness and limping in one or more legs, muscle sensitivity, hyperesthesia, lethargy and behavioural changes. In contrast to humans and dogs, in horses there are fewer occurrences of joint infections. Lyme borreliosis is a multisystem disease in humans, accompanied by many and varied clinical symptoms. It occurs as an early and a late infection, that is, in three stages: early localized, early disseminated and late persistent. In the first stage, early infection - the localized form, there is erythema migrans and general weakness. In the second stage, early, infection – the disseminated form, there is weakness and exhaustion, multiple erythema migrans, pain in the muscles, tendons and bones, meningitis, neuritis, local lymphadenopathy, myopericarditis, con-junctivitis and proteinuria. In the third state, late infection - the persistent form, there is acrodermatitis chronica atrophicans, chronic arthritis, chronic encephalomyelitis mental disorders, spastic paresis, generalized lymphadenopathy and splenomegaly, and keratitis (5).

7. Diagnostics

7.1 Clinically

The disease would be suspected in cases with clinical symptoms of the disease which correspond to the initial or advanced forms of the disease (1).

7.2 Paraclinically

In terms of microbiology, *B. burgdorferi* can be proven directly and by separating out the pathogen. Direct proof of the pathogen is possible by testing samples of body fluids, such as synovia, by dark field microscopy and using the process of dying a fixed sample of tissue silver. Since the concentration of pathogen in the samples from sick animals and humans is usually small, direct microscopy is used most often only for testing samples of tissue from ticks. Much more often, pathogens are separated on a culture medium, where Barbour–Stoenner–Kelly II liquid medium (BSK-II) has been shown to be the best, as well as modified Kelly–Pettenkofer medium (MKP). The most appropriate tissue samples from dogs, suitable for isolation of *Borrelia*, both living and after death, are skin and collagen-rich connecting tissues (fascia, pericardia, peritoneum and synovia) close to where the tick is attached to the body. Use of polymerase chain reaction (PCR) in diagnosis of Lyme borreliosis is somewhat limited. The results obtained from PCR may vary for many reasons. One of these is the choice of primers for DNA sequencing of pathogens, and another is the small quantity of extracted DNA used for PCR analysis. When we also consider the small quantity of pathogen in the tested tissues, these facts significantly reduce the sensitivity of the PCR test in diagnosing Lyme borreliosis. The most suitable samples for use of the PCR test in diagnostics of humans are the skin and the synovial sac. For diagnosis of Lyme borreliosis, immunological or serological methods are most often used. The most commonly used serological tests are various versions of ELISA (enzyme-linked immune-sorbent assay) tests and indirect

immunofluorescence, when an entire or part of a microorganism can be used as the antigen (6).

Due to the serious weaknesses of serological diagnostic methods using whole microorganisms as antigens, it is necessary to use additional tests to confirm positive results obtained from these tests. To that end the Western blot technique is used, which is able to differentiate false positive results as the consequence of cross reactions, and it can also differentiate vaccination and infective immune responses in the serum of the tested animals (6).

7.3 Epidemiologic

In terms of epizootology, the disease should be suspected in cases of large scale exposure to tick findings in animals and humans. Suspicion of the disease will also be prompted by a history of previously noted symptoms, such as skin redness, inflammation of the joints, neurological disorders etc (7).

7.4 Pathologically

In the corpses of naturally and seriously infected dogs there are extremely swollen joints, with accumulation of synovia. The infection of the synovial sac is mild, and the synovia consists, in acute forms of neutrophils, whilst in chronic cases in dogs that have created specific antibodies, a non-purulent infection of the synovial sac and the joint capsule occurs. There is also a local infection of lymph nodes, especially in an infected leg. In experimentally infected dogs, there are histological changes in the lymph nodes, joints, pericardia, the skin, in the blood vessels, in the peripheral nerves and the meninges. In the lymph nodes there is follicular hyperplasia, and in biopsy specimens of the skin there are superficial perivascular lymphatic infiltrations with accumulations of mastocytes. In the corpses of naturally infected dogs, changes are found in the kidneys in the form of glomerulitis, diffuse necrosis of tubules and interstitial infection (2).

8. Prophylaxis

General prophylactic measures consist of reducing the infestation of animals and people by ticks, and control of the tick population in the wild. Reduction of exposure of animals to ticks is achieved by avoiding movement and spending time in areas rich in ticks, and individual use of acaricides. Daily checks of the skin of dogs and humans are very useful, and removal of attached and unattached ticks. Control of the tick population in the wild is difficult and expensive. Due to the two-year life cycle and the four stages of development of ticks, it is difficult to undertake disinsection using a single application. On the other hand, such mass and non-selective use is ecologically unacceptable. The main method of immuno-prophylaxis is vaccination of animals. In view of the variety of pathogens of Lyme borreliosis, the use of vaccinations in some areas of the world is difficult. Vaccination of dogs should be undertaken selectively, with respect for the epizootic situation in the area where the dog lives, or where it will be spending time, as well as the purpose of the

dog, that is, its exposure to possible infection (8).

9. Treatment

The main choice for etiological therapy for Lyme borreliosis are antibiotics. The treatment of choice is doxycycline, because it is soluble in fat, and therefore can be used in smaller doses, it becomes well-distributed in the tissue, and more easily penetrates to the cells than classical tetracycline, but it is not used for treating younger animals. In general, the best antibio-therapy is achieved in the initial phase of the disease. Already after 24 to 48 hours an improvement is observed in health, but that improvement should be taken with reserve because the symptoms of arthritis and limping may occur already after a few days or weeks. Early etiological therapy produces a weaker immune response, but also a smaller number of microorganisms in the body and is mostly administered for 30 days. However, even then the body is not free of pathogens so the symptoms may occur even after the end of treatment.

The use of non-steroid preparations removes the symptom of arthritis, but their use requires care due to possible irritation of the digestive system. The use of glucocorticoids in minimal doses is also limited because long-term use can cause immuno-suppression, or may reactivate the disease. Therefore, they should be avoided in chronically infected dogs, or may be used only in combination with antibiotics. Etiological treatment in humans depends on the stage of the disease. For the early stages or rheumatic forms of the disease, azithromycin, doxycycline and amoxicillin are used, and for treatment of neurological forms ceftriaxone, cefotaxime or doxycycline (6).

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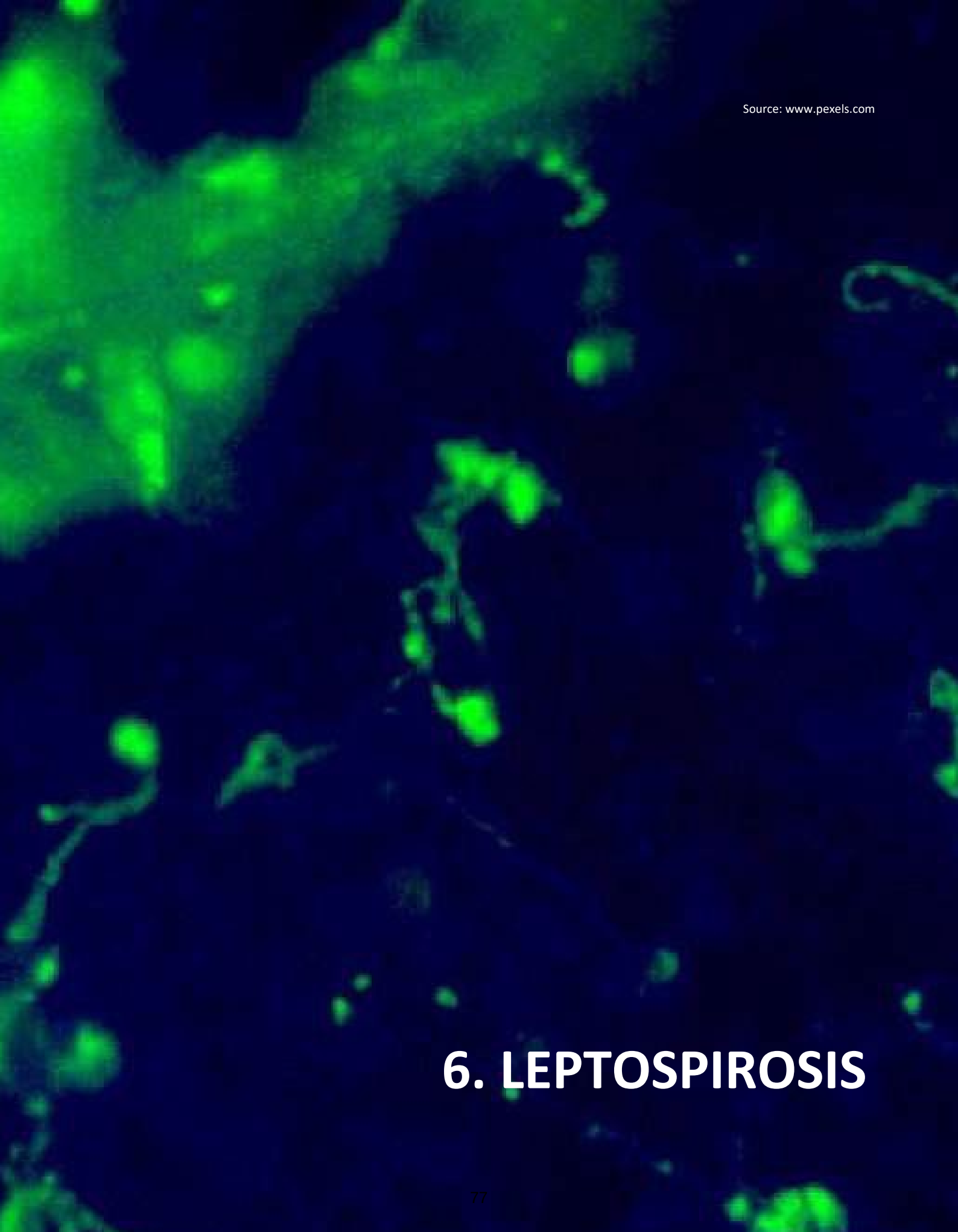

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6. LEPTOSPIROSIS

1. Name of zoonosis:

LEPTOSPIROSIS

2. Definition

Leptospirosis is an acute, septicemic, infectious disease of domestic and wild animals and humans, caused by bacteria from the genus *Leptospira*. It presents in various clinical forms which vary from unapparent and mild to severe clinical symptoms, accompanied by kidney failure or pulmonary haemorrhagic syndrome and a high mortality rate. It is spread throughout almost the entire world, and due to its increased occurrence and many epidemics recorded over the past few years, leptospirosis is classified as a (re)emerging zoonosis.

3. Etiology

3.1 Etiologic agent

The causes of leptospirosis are bacteria from the genus Leptospira, the family *Leptospiraceae* and the order *Spirochaetales* (8).

3.2 Taxonomy

Leptospira are very heterogenic spiral microorganisms, currently categorized with almost 320 “serovars”, divided between 29 serological groups, or 21 genomic species (7). In the everyday clinical work of veterinarians and physicians, when presenting the etiology of leptospirosis for the sake of practicality, the basic taxonomic unit "serovar" is used (1).

3.3 Morphological description

Leptospira spp. are thin (about 0.1 µm wide and 6-20 long), very mobile, slow growing, always aerobic, spiral bacteria, which are usually bent at the ends in the shape of a hook. They proliferate at an optimal temperature of 28 to 30 °C and may be raised artificially in liquid, semi-liquid and solid mediums enriched with rabbit serum (6).

4. Epidemiology

4.1 Source of infection

Sources of infection are sick animals and animal carriers. After recovering from the disease, the animal remains a carrier for a certain length of time, and through its urine (leptospiruria) contamination occurs of the external environment, surface water, food and objects. Reservoirs of infection are wild and domestic rodents which, having been infected, remain carriers throughout their lives. The spread of the disease in domestic animals is particularly enhanced by keeping animals out at pasture or freely in large groups, where there is a large number of rats and mice present (13).

4.2 Path of infection

The pathogen enters the host through its mouth, through visible mucosa, or macerated or damaged skin. Humans are usually infected by direct or indirect contact with contaminated urine, or through contaminated soil, food and water. Inter-human transfer of the pathogen is rarely recorded (3).

4.3 Responsiveness

The responsiveness of individual species of domestic and wild animals has been found to one or more serovars, but they may be responsive to others as well. Regarding their

relationship with *Leptospira*, animals are categorized as reservoirs, evolutionary hosts or accidental hosts. The primary reservoirs of leptospirosis are rodents, in which no disease occurs after infection but only permanent habitation of the renal tract and, as a consequence, the life-long excretion of *Leptospira* in the urine. In the other group of animals, which we term the evolutionary hosts, the process of adaptation to a specific serovar has not yet been completed. In them infection by these serovars still causes what are most often very mild symptoms, after which they remain carriers for an extended period, which may last for several months. Accidental hosts are those which are not part of the usual life cycle of a specific *Leptospira* serovar in the wild, but they have become included in the epizootological/ epidemiological cycle of leptospirosis by chance. These are animals and humans who, after infection, most often present various clinical signs of the disease and after recovery excrete pathogens in their urine for a certain length of time, as convalescent carriers. There is a higher incidence of leptospirosis in humans who are professionally exposed to sources of infection, such as farm workers, holders of livestock, butchers and veterinarians, but also professional and recreational sportsmen and others exposed to contaminated surface water (12).

4.4 Resistance to the environment

The pathogen survives for weeks and months in surface water, mud and damp alkaline soil. The ability to accumulate cells and create a biofilm additionally contributes to their survival outside any host. They are sensitive to common disinfectants, the sun and drying (12).

4.5 Geographical distribution

Leptospirosis occurs throughout the entire world, especially in warm and damp climates. Leptospirosis is seasonal in character and is more common in the summer and autumn, especially where there is abundant rain and after flooding. In any specific geographical area, only individual serovars circulate between reservoirs and evolutionary hosts, which they infect and, depending on the virulence of the type, cause mild or more severe illness in other animals and humans. The reason for this is that some *Leptospira* have adapted to certain hosts, making leptospirosis a zoonosis of the natural-foci type, whose basic patho-bio-cenoses were formed long before humans disturbed their balance by their activities. Due to the influence of new biotic factors, resulting from the activities of humans in the environment, and the introduction of new animal species (domestic animals), *Leptospira* have undergone genetic adaptation. So, from the old "archaic" foci of leptospirosis, which were related exclusively to rodents living wild, under the influence of humans, synanthropic and anthropogenic foci have developed. Synanthropic foci have appeared because individual animal species, due to more easily available food, have broken away from the wild and moved closer to or into human habitats, e.g. black and brown rats - *Rattus rattus, R. norvegicus* and house mice *Mus musculus*, whilst the anthropogenic foci have appeared through the introduction and keeping of domestic animals. The natural foci are those that shape the form of preservation and frequency of individual infective serovars which cause the disease. The best example of synanthropic foci is large, densely inhabited

settlements with poor sanitation and hygienic conditions and a large number of rats, which are the main reservoirs for the serovar *Icterohaemorrhagiae*. Along with an appropriate climate, these areas are ideal for preservation and spread of leptospirosis, and it is estimated that the number of diagnosed cases of leptospirosis in countries in the Tropics and developing countries is growing, or remains at the same level (11).

In those countries, the illness very often appears in humans in the form of an epidemic, which is the result of the lack of a proper sewerage system in suburban settlements - slums, or even the result of natural disasters, primarily flooding. In contrast, a good example of archaic natural foci are the river basins of the Sava and the Danube, which are still covered by old, untouched forests, inhabited by various species of rodents, reservoirs of individual serovars of *Leptospira*. These areas, with their favourable climate, edaphic and hydrological conditions, greatly contribute to the occurrence of leptospirosis in the Republic of Croatia (2).

So the high incidence of probably infected serovars from the serological group *Australis* in humans in Croatia differs from the situation established in other European countries (France, Italy and Germany), where *L. Icterohaemorrhagiae* is without exception the most common serovar which causes infections in humans. Apart from the difference in prevalence of the probably infective serovar, in the Republic of Croatia we also find an extremely high percentage of infections in humans, with an incidence of 1.83 to 100,000 of the population, which in terms of leptospirosis in human's places Croatia in first place in Europe and 13th in the world (2).

5. Pathogenesis

The pathogenesis of leptospirosis has not yet been completely explained. The outcome of infection depends on many characteristics of the pathogen and the macroorganism. The movement of the pathogen from its entry into the macro-organism has been well-researched, and this knowledge is used in diagnosing the disease. Following the penetration of the pathogen, after the entrance of the infection into the lymph and blood circulation systems, septicemia occurs with generalization of the disease. When the first specific antibodies appear, five to ten days after infection, the *Leptospira* withdraw from the circulation into the proximal convoluted tubule of the kidney, where they may remain for months, and are occasionally excreted in urine. As well as in the kidneys, *Leptospira* may also hide from antibodies in the brain, the anterior chamber of the eye and the genital organs (6).

The primary lesion from *Leptospira* is damage to the endothelium of the small blood vessels, which causes localized ischemia in the targeted organs, and as a result necrosis of the renal tubule, damage to liver and lung cells, and inflammation of the meninges, muscles and placenta. The autoimmune reaction is also deemed to be the cause of certain ophthalmic symptoms recorded in humans and horses (3).

6. Clinical manifestation

Infection by *Leptospira* may lead to a variety of clinical forms, from unapparent infection to pulmonary haemorrhage with fatal outcome

(1). The course and severity of the disease differ depending on the infective serovar and the general resistance capacity of the macroorganism (4). Incubation of the disease is short, from 7 to 10 days. The clinical picture is dominated by systemic inflammatory response syndrome, icterus, hemoglobinuria, kidney failure, and other clinical symptoms occur depending on the species of animal. Two to eight months after the infection, especially in the case of the serovar Pomona, periodic ophthalmia may occur in some horses (and up to 45% of animals), or recurrent iridocyclitis or uveitis, also known as moon blindness. Disturbances in reproduction in horses is manifest in foetal death and resorption, abortion, and also in cases of intrauterine infection, the occurrence of septicemic disease and the death of neonatal foals. In cattle, acute leptospirosis usually occurs in the severe clinical form in calves and heifers, caused by many serovars, of which the most common is the serovar Pomona. In older cattle the clinical manifestation is varied. The most frequent clinical symptom of leptospirosis in dairy cattle is a transient fever with a sudden drop in milk yield and mastitis, which lasts for two to ten days. It is clinically different from other forms of mastitis because the entire udder remains equally soft and flabby throughout. It is usually caused by infection by the serovar *Hardjo prajitno*, but also the serovar *Hardjo bovis* and others. The chronic forms of leptospirosis in cattle manifest in reproduction disorders, which are most often caused by serovars *Hardjo* and *Pomona*. In pregnant cows, infection of the foetus leads to abortion (most often in the second or final third of pregnancy), a still born calf, birth of immature and non-vital calves, and retained placenta is also common. The disease in pigs is characterized by systemic inflammatory response syndrome, anaemia, jaundice, anorexia, myalgia, and in pregnant animals, abortions two to four weeks before the due date, or birth of non-vital icteric piglets. The disease in dogs may occur in an acute or sub-acute form. The disease begins with severe systemic inflammatory response syndrome, generalized jaundice, with severe episcleral injection, discharge from the eyes and nose, anorexia, lack of appetite and gastro-enteritis with blood stained stools. The disease progresses with severe inflammation of the liver and kidneys, and myalgia in the back legs. Polyuria occurs, which progresses into oliguria and anuria. If the animal does not die, from the 7th day of the disease recovery begins, which continues with several months of leptospiruria, or chronic renal insufficiency. Recently a severe form of leptospirosis with pulmonary hemorrhage has appeared in dogs, with mortality higher than 50%. The clinical manifestation of leptospirosis in humans is very varied. Leptospirosis may pass with no symptoms, or may manifest in fever, muscle and stomach pain, headache and conjunctival hematoma. These clinical forms are mostly short-lived and pass without treatment after about a week. About five to ten percent of patients with leptospirosis develop the severe icteric form, which is also known as Weil's Disease, and is accompanied by fever, bleeding, kidney failure, liver damage, jaundice and fatality in 5 to 15 % of cases. In recent years a rise has been recorded in the incidence of the severe pulmonary haemorrhage form of leptospirosis, with mortality of more than 50% (10).

7. Diagnostic

7.1 Clinically

The sudden onset of acute septicemic disease with severe icterus, hemoglobinuria, kidney failure or the occurrence of abortion indicate a possible *Leptospira* infection.

7.2 Paraclinically

The disease in live animals may be objectively diagnosed by hemoculture and proof of the pathogen in the blood by PCR, up to the seventh day of infection. Serologically the disease is diagnosed using the serum reaction technique, the microscopic agglutination test. In a live animal the pathogen may be isolated or proven in the urine by PCR, and after its death by isolating it from the kidney or by demonstrating *Leptospira* in the kidney by PCR and histologically. Samples of blood, urine and, following death, the kidneys are sent for diagnostic tests, and should be sent to the laboratory as quickly as possible at a temperature of +40 ^{o}C. Differential diagnosis should consider other septicemic and parasitic diseases, which are manifest by systemic inflammatory response syndrome, icterus, hemoglobinuria and abortion (10).

7.3 Epidemiologic

A history of epizootology should be suspected in cases of occurrence of the acute disease in animals on farms where there are many rodents, in animals fed food contaminated by rodent excretion or in animals kept on pasture (9).

7.4 Pathologically

If an animal dies, upon anatomic pathological examination we find changes corresponding to the clinical condition characteristic for acute septicemia. However, the findings will be dominated by a picture of intoxication and degeneration of the parenchymal organs, especially the liver and kidneys. Beneath the serosa and in the mucous membranes we may find spotted bleeding, exudation in the serosa cavities and usually universal icterus. In dogs, haemorrhagic enteritis is possible and substantial bleeding in the lungs (8).

8. Prophylaxis

General prophylaxis presumes systematic suppression of rodents, or prevention of contamination of the environment, water and food by urine of rodents, sick animals and carriers. On large farms the use of these measures is quite effective, whilst in the wild it is almost impossible. Specific prophylaxis is conducted using inactive vaccines prepared from one or more serovars which are specific for a certain animal species. Unfortunately, commercial vaccines do not achieve long-term immunity or cross-protection between serovars (5).

9. Treatment

Etiological treatment of infected animals comes down to the use of antibiotics, of which the most effective is penicillin in the septicemic phase of the disease, and dihydrostreptomycin in the carrier phase. Sensitivity to antibiotics varies between individual serovars, which must be taken into account, particularly when treating farm animals. Since the use of streptomycin is restricted in some states, chronic carriers may be treated using oxytetracycline with extended release (20 mg/kg once or twice with a gap of 10 days) or amoxicillin with extended release (15 mg/kg twice with a gap of 48 hours). Symptomatic therapy is aimed at maintaining circulation and kidney and liver function (6).

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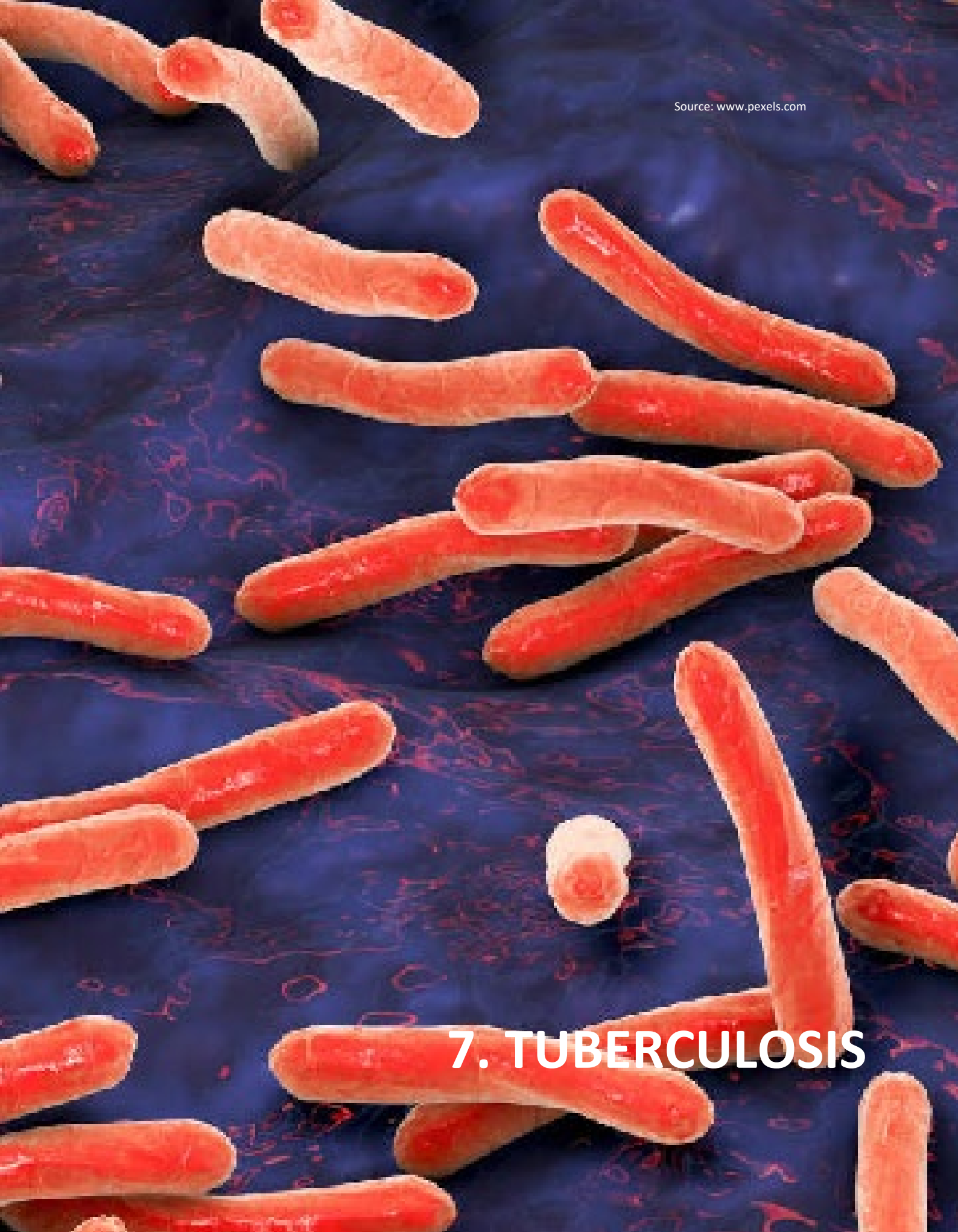


Source: www.pexels.com

7. TUBERCULOSIS

1. Name of zoonosis:

TUBERCULOSIS

2. Definition

Tuberculosis is a chronic, contagious and infectious disease of various species of domestic and wild animals, and humans. It presents in the formation of nodules (tubercles), exudative infection of the serous membrane and, in chronic forms, necrosis of the infected tissue, most frequently the lungs.

3. Etiology

3.1 Etiologic agent

The causes of tuberculosis in animals and humans are bacteria from the genus Mycobacterium (4).

3.2 Taxonomy

The number of species within the genus *Mycobacterium* is subject to frequent changes, and today they number more than 135. There are several ways to classify mycobacteria, but the most common method divides them into the bacteria of the *Mycobacterium tuberculosis* complex and the *M. avium* complex. Within the *M. tuberculosis* complex, the most common species which are pathogens for humans and animals are: *M. tuberculosis, M. bovis, M. africanum, M. canetti, M. microti, M. caprae* and *M. pinnipedii*; and within the *M. avium* complex, the species: *M. avium, M. intracellulare, M. marinum, M. ulcerans, M. leprae, M. lepraemurinum, M. avium subsp. paratuberculosis, M. senegalense, M. farcinogenes*, and unidentified fast-growing bacteria (4).

3.3 Morphological description

Mycobacteria usually have the shape of straight or slightly curved sticks, sized 0.2-0.7 x 1.0-10 µm, and in the Gram stain procedure they stain mildly gram-positive. They are acid-alcohol resistant, so they stain red in the Ziehl-Neelsen procedure (8).

4. Epidemiology

4.1 Source of infection

The sources of infection are diseased animals and animal disease carriers, whose excretions contaminate the environment, containing perpetual or occasional pathogens of tuberculosis. The primary sources of infection are also products and raw materials from infected animals, animal corpses and infected wild animals. An infected human may represent a source of infection for household pets with which he or she spends a great deal of time in close social contact. Due to the good sustainability of the pathogen in the environment, sources of infection are also contaminated soil, pasture land, water, animal feed, items and equipment, facilities and means of transport (7).

4.2 Path of infection

Infection most often occurs through airborne pathogens or by enteral infection. Animals with pulmonary tuberculosis in cohabitation with other animals, infect other animals when they cough, by means of aerosol transmission. The same pathogen falls to the ground in droplets, where it contaminates feed or is

inhaled again with dust, and so it comes into contact with the animals. For the intestinal infection, the sources are faeces and other excretions containing mycobacteria, and contaminated food and water. High density occupancy of stalls, with poor lighting and poor hygiene, favours the long-term survival of mycobacteria in the animals' environment (2).

Shared pasture also favours the intestinal infection. Animals may be infected by unpasteurized milk and whey from animals with tuberculosis, especially cattle. Fodder raw materials and mixtures contaminated with mycobacteria from the environment also play an important role in the spread of the disease. The pathogens may be brought by birds to livestock farms, but they may also be introduced in soiled litter (straw, wood shavings, saw dust) (4).

4.3 Responsiveness

The disease is not equally significant for all species of domestic animals. The greatest economic significance and public health problem is in cattle. Apart from cattle, which are the most responsive, pigs are also easily infected, as are dogs, cats and goats, but sheep and horses are naturally more resistant. The disease is very frequently found in wild animals (buffaloes, zebras, elephants, apes, lions etc.). The tuberculosis pathogen in cattle is M. bovis. Apart from in cattle, *M. bovis* may also cause tuberculosis in humans, apes, horses, sheep, goats, dogs and cats, and many wild animals. *M. tuberculosis* may be found in cattle; it rarely causes infection or causes slight non-progressive changes in the lymph nodes. *M. avium* may cause infection in cattle only in rare cases, but it causes problems in the diagnostics of tuberculosis using the tuberculin skin test. There is no species of mycobacteria specifically adapted to pigs. Apart from the three most important species of mycobacteria *M. bovis, M. tuberculosis* and *M. avium*, pigs are also responsive to other mycobacteria: *M. africanum, M. xenopi, M. microti, M. chelonae* etc.) which can cause the same or similar changes in them. Tuberculosis in dogs is a chronic disease which occurs sporadically. Tuberculosis in dogs is most often caused by *M. tuberculosis*, and in cats by *M. bovis*. Dogs are quite resistant to infection by *M. avium*. Granulomatous changes on cats' skin (feline leprosy) are most often caused by *M. lepraemurium*, and in dogs and cats granulomas may be caused by *M. chelonae*, *M. fortuitum* and *M. smegmatis* (1).

Humans are most often infected by the bacteria M. tuberculosis, but the disease may also be caused by other species from the M. tuberculosis complex. The factors that favour infection of humans are: the level of exposure to pathogens, age, the immunocompetence of the person, genetic factors, vaccination and, especially, social and economic factors (3).

4.4 Resistance to the environment

In relation to external influences, micro-bacteria are very resistant and may retain their infectiveness for a long time outside any organism, especially in bodily excretions on dirty surfaces and in corpses. In droplets from coughing on stall litter, M. tuberculosis remains infective for four weeks, in dust for three to four months, and in corpses for five months. In faeces M. bovis survives for a month, but if it is in the dark it can survive for up to two years. Pasteurization kills *M. bovis*

and M. tuberculosis in milk. Disinfectants for mycobacteria that are effective are phenol (1-3%), formalin (3%), propanol and chlorine preparations (5).

4.5 Geographical distribution

Tuberculosis is the most widespread disease in global terms. Tuberculosis in cattle used to be widespread in Europe, but systematic work to combat the disease in many countries has led to its eradication. In some developed countries it still persists (Great Britain, Italy, Ukraine, Portugal, Russia, Northern Ireland and Croatia). It is a very important disease in South and Latin America. About 85% of livestock in Africa is in areas without control, and in Asia the disease is only partially controlled. Tuberculosis in pigs is described in all countries with well-developed pig production. Tuberculosis in humans is present throughout the world. It is estimated that nine million people are infected and about three million die from it every year in the world. The vast majority of those infected (>95%) are in developing countries, especially in parts with a high incidence of HIV infection, where the disease is particularly widespread in children. The highest mortality is recorded in Africa, South-East Asia and parts of South America, with an incidence of 100-250/100,000, and in some places even more than 350/100,000 of the population (Tab.6.1). In developed countries the morbidity rate has been reduced to 9-20/100,000 of the population, and morbidity in Europe is between 4.3/100,000 in Iceland to 154/100,000 in Kazakhstan (7).
See fig.7.1

5. Pathogenesis

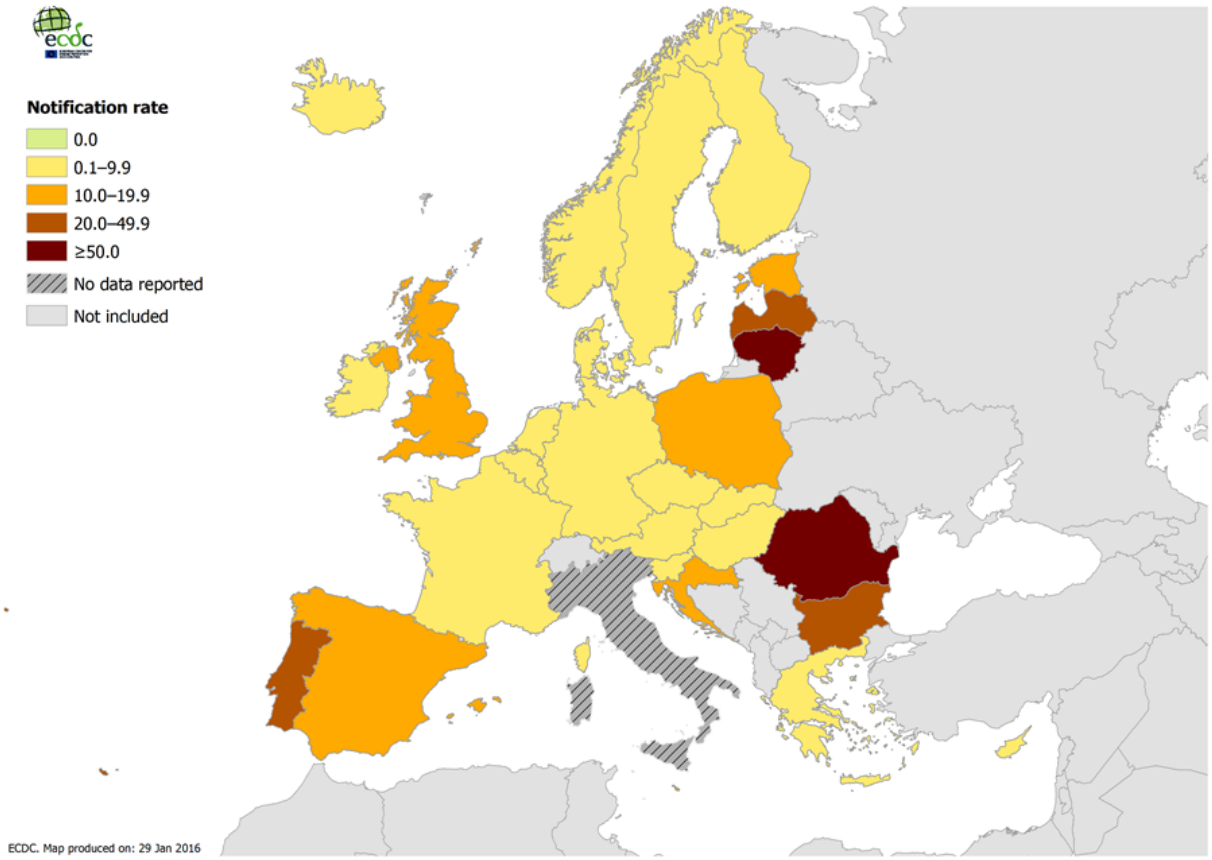
In the development of the tuberculosis process we distinguish three basic stages: the primary tuberculosis complex, the generalization stage and organic tuberculosis. The entry of mycobacteria and the initial local reaction of the organism to the invasion by mycobacteria are known as the primary site (focus). By lymphogenic transfer of the mycobacteria from that site the process moves to the lymph nodes, and this all together comprises the primary complex. In cattle and humans, the mycobacteria most often enter through the respiratory organs (airborne infection in more than 90% of cases) and in 10% of cases through the digestive organs. The primary focus in cattle is most often in the lungs. The outcome of the primary complex may vary. The animal may recover, where the organization occurs of the connecting tissues (encapsulation) of the primary focus, or there may be disintegration of the primary focus, or it may spread throughout the organism. The bacteriemia that occurs in this way, with the spread of tuberculosis lesions throughout the organism, is known as the generalization of the process. In organic, isolated tuberculosis it is characteristic that the spread of the process is usually intracanalicular, and usually affects the lungs or other organs (6).

Country	2011		2012		2013		2014		2015				
	Reported cases		Reported cases		Reported cases		Reported cases		National coverage	Reported cases			Confirmed cases
	Number	Rate	Number	Rate	Number	Rate	Number	Rate		Number	Rate	ASR	
Austria	684	8.2	646	7.7	653	7.7	586	6.9	Y	583	6.8	6.8	451
Belgium	1019	9.3	976	8.8	963	8.6	949	8.5	Y	988	8.8	9.0	775
Bulgaria	2406	32.6	2280	31.1	1932	26.5	1872	25.8	Y	1660	23.0	22.4	782
Croatia	619	14.4	575	13.4	517	12.1	499	11.7	Y	486	11.5	10.9	385
Cyprus	54	6.4	69	8.0	41	4.7	41	4.8	Y	63	7.4	7.2	42
Czech Republic	600	5.7	597	5.7	497	4.7	511	4.9	Y	518	4.9	4.8	401
Denmark	381	6.9	389	7.0	356	6.4	320	5.7	Y	357	6.3	6.5	279
Estonia	339	25.5	289	21.8	290	22.0	248	18.8	Y	217	16.5	16.0	180
Finland	324	6.0	274	5.1	273	5.0	263	4.8	Y	271	5.0	4.8	215
France	4991	7.7	4975	7.6	4934	7.5	4827	7.3	Y	4788	7.2	7.5	2492
Germany	4309	5.4	4213	5.2	4325	5.4	4533	5.6	Y	5865	7.2	7.5	4123
Greece	489	4.4	558	5.0	540	4.9	519	4.7	Y	482	4.4	4.2	305
Hungary	1445	14.5	1223	12.3	1045	10.5	851	8.6	Y	906	9.2	8.8	413
Ireland	412	9.0	359	7.8	374	8.1	311	6.8	Y	312	6.7	7.2	199
Italy	4461	7.5	4252	7.2	3973	6.7	3916	6.4	Y	3769	6.2	6.3	2609
Latvia	885	42.7	993	48.6	904	44.7	761	38.0	Y	721	36.3	35.9	592
Lithuania	1904	62.4	1781	59.3	1705	57.4	1607	54.6	Y	1507	51.6	50.9	1221
Luxembourg	26	5.1	45	8.6	38	7.1	24	4.4	Y	30	5.3	5.5	24
Malta	33	8.0	42	10.1	50	11.9	46	10.8	Y	32	7.5	7.6	24
Netherlands	1004	6.0	956	5.7	845	5.0	814	4.8	Y	867	5.1	5.3	578
Poland	8478	22.3	7542	19.8	7250	19.0	6698	17.6	Y	6430	16.9	16.7	4630
Portugal	2609	24.7	2606	24.7	2410	23.0	2278	21.8	Y	2124	20.5	19.8	1324
Romania	19202	95.1	18190	90.5	16689	83.4	15879	79.6	Y	15195	76.5	75.4	10382
Slovakia	399	7.4	345	6.4	401	7.4	336	6.2	Y	317	5.8	5.9	158
Slovenia	192	9.4	138	6.7	140	6.8	144	7.0	Y	130	6.3	6.0	119
Spain	6798	14.6	6070	13.0	5632	12.1	4917	10.6	Y	4191	9.0	8.9	2861
Sweden	580	6.2	623	6.6	639	6.7	659	6.8	Y	821	8.4	8.9	697
United Kingdom	8915	14.1	8714	13.7	7866	12.3	7025	10.9	Y	6240	9.6	10.0	3787
EU	**73558**	**14.6**	**69720**	**13.8**	**65282**	**12.9**	**61434**	**12.1**	**.**	**59870**	**11.8**	**11.8**	**40048**
Iceland	9	2.8	11	3.4	11	3.4	9	2.8	Y	7	2.1	2.1	3
Liechtenstein	.	.	.	.	.	.	.	.	.	.	.	.	.
Norway	354	7.2	374	7.5	392	7.8	324	6.3	Y	318	6.2	6.3	247
EU/EEA	**73921**	**14.5**	**70105**	**13.8**	**65685**	**12.9**	**61767**	**12.1**	**.**	**60195**	**11.7**	**11.8**	**40298**

ASR: age-standardised rate

Source: Country reports from Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Denmark, Estonia, Finland, France (provisional data for 2015) Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain (provisional data for 2015), Sweden, and the United Kingdom.

Table 7.1. Tuberculosis cases per 100.000 population: number and rate (EU/EEASource:http://ecdc.europa.eu/en/healthtopics/Tuberculosis/Pages/Annual-epidemiological-report-for-2015.aspx)



Source: Country reports from Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, the United Kingdom.

Suggested citation: European Centre for Disease Prevention and Control. Annual epidemiological report 2015. Tuberculosis. Stockholm: ECDC; 2016.

Fig. 7.1. Notification rate of TB cases per 100.000 population by country, EU/EEA 2015

Source: European Centre for Disease Prevention and Control, WHO Regional Office for Europe. ECDC/WHO EURO. Tuberculosis surveillance and monitoring in Europe, 2016. Stockholm: ECDC. 2016. http://ecdc.europa.eu/en/healthtopics/Tuberculosis/Pages/Annual-epidemiological-report-for-2015.aspx

6. Clinical manifestation

Incubation for tuberculosis in cattle is long. Several months may pass, or even years, before visible signs of the disease appear. As a rule, they appear at the stage of early generalization, organic tuberculosis or late generalization. Pulmonary tuberculosis presents as a severe, dry cough, which usually occurs upon movement or inhalation of cold or dusty air. The animal does not feed well and appears depressed. The cough becomes more frequent, dry and coarse, or damp and hoarse. During a coughing fit, thick reddish purulent muco-bronchial sputum is coughed up. Breathing is accelerated, affecting a large part of the lungs, and work and later even walking become difficult (4).

The later stage of pulmonary tuberculosis also causes almost complete loss of appetite. Signs of anaemia and pale mucous membranes appear, and the cough becomes increasingly weak and very painful. Nasal discharge is purulent and smelly, and the animal dies in great agony (8).

Tuberculosis mostly develops in a chronic form. The development of the disease is slow and it takes months or even years for us to even notice any signs of the disease, so that even with apparently healthy animals some animals may be found to have tuberculosis upon slaughter. The disease caused by micro-bacteria in pigs is difficult to recognize from clinical signs. The symptoms are mainly inconspicuous and vague. In pigs the disease most often appears in changes to the lymph nodes. First of all, the lymph nodes in the head and neck are affected; they swell up and may be palpitated as hard, painless nodular swellings of various sizes (7).

The swelling may be large and cause disturbance to movement of the head and swallowing. The clinical picture of primary tuberculosis in humans depends on the age of the patient. In infants there are always strong and severe generalized symptoms of the disease, in pre-school children the general symptoms are weaker, but symptoms of lymphadenitis are dominant (cough, wheezing, vomiting), whilst in adults the clinical symptoms are rarer and uncharacteristic. After involution of primary tuberculosis, the latent stage begins, with no signs of illness, and this may last the entire life. In cases of a drop in the person's general resistance levels, the primary focus may develop clinical manifestations of post-primary tuberculosis, with the subsequent general and localized symptoms. General symptoms are uncharacteristic: exhaustion, loss of appetite and body weight, raised temperature, increased perspiration and pain in the muscles, bones, joints and headache. In most patients with post-primary tuberculosis of the lungs, there is a dry or productive cough, with occasional blood coughed up (1).

7. Diagnostic

7.1 Clinically

Primary pulmonary tuberculosis may be suspected in cases of chronic weakness, a sub-febrile state, loss of appetite and weight loss, the occurrence of a chronic, dry cough or a productive cough with blood. Chronic processes in other organ systems, which are the result of dissemination of the process, should also be taken into account (7).

7.2 Paraclinically

Tuberculinization is an allergy skin test on which the diagnosis of tuberculosis is based in living animals, especially cattle and pigs. After positive animals have been sacrificed,

bacteriological and molecular techniques are used to confirm the mycobacteria in samples of changed organs (lymph nodes, parenchymal organs etc.) (2).

7.3 Epidemiologic

Tuberculosis should be suspected in cases when epizootology data indicate the possible intake of pathogens into a herd through purchase of animals from suspect or infected farms, or feeding with contaminated feed. Epidemiologically, the disease should be suspected in humans in cases of direct and indirect contact with persons with tubercular diseases, cases of eating animal products of uncertain quality, poor living conditions and in the presence of other illnesses (3).

7.4 Pathologically

The basic infective and pathological process is the formation of specific, histologically formed nodules - tubercles. In domestic animals, the tubercular infective process may affect all tissues and all organs at the same time (the generalized form), or only some of them individually (the localized form). Inflammation of a specific organ system most often depends on the entry route of the microorganism. In cattle, tuberculosis of the lungs is most frequent, and the primary complex affects the lungs, where we find clusters of sub-pleural nodules, and changes to the bronchial and mediastinal lymph nodes. As the process ages, fusing of the nodules may occur, with the creation of larger focuses. In those focuses there is gradual caseous necrosis, and the creation of hollows - caverns. Inflamed nodes, with the subsequent necrosis and changes to the regional lymph nodes, are also found in the digestive system, the urogenital tract, the parenchymal organs, the udders and elsewhere throughout the body (6).

8. Prophylaxis

The basic measures for prophylaxis of tuberculosis in animals are timely and reliable discovery of infected animals, their harmless removal, with the use of isolation for decontamination of facilities and equipment. This is achieved through systematic, regular diagnostic measures and procedures, of which tuberculinization has been shown to be the best. These measures are conducted in order to prevent the occurrence of the disease in animals, and combat and eradicate it, with the end goal of protection of the health of humans. Prevention and suppression of tuberculosis in humans is founded on conducting compulsory vaccination programs in children, discovering and treating newly infected persons, examinations of risk groups, and implementation and supervision of the treatment of the disease using anti-tuberculosis drugs.

9. Treatment

There is no treatment for tuberculosis in animals, but the disease must be suppressed by law so all animals with tuberculosis are harmlessly removed from the farm. The basic and compulsory treatment of infected people is the use of antituberculotics. Etiological therapy is always combined, with several preparations used together. In the initial phase treatment is daily, then in the stabilization stage every second day, over a period of from six to nine months. A significant problem in the treatment of tuberculosis in humans is the increasingly frequent appearance of strains of pathogens that are resistant to some antituberculotics.

WHO European Region

POPULATION 2016 0.92 BILLION

WHO MEMBER STATES	**53**
OTHER COUNTRIES AND TERRITORIES	**1**

Estimates of TB burden,[a] 2016

	Number (thousands)	Rate (per 100 000 population)
Mortality (excludes HIV+TB)	26 (25–27)	2.8 (2.8–2.9)
Mortality (HIV+TB only)	5 (4–6)	0.55 (0.43–0.69)
Incidence (includes HIV+TB)	290 (251–333)	32 (27–36)
Incidence (HIV+TB only)	34 (26–42)	3.7 (2.9–4.6)
Incidence (MDR/RR-TB)[b]	122 (110–134)	13 (12–15)

Estimated TB incidence by age and sex (thousands),[a] 2016

	0–14 years	> 14 years	Total
Females	15 (10–19)	87 (62–112)	102 (73–132)
Males	17 (12–22)	177 (124–230)	194 (136–251)
Total	31 (22–41)	264 (186–342)	290 (251–333)

TB case notifications, 2016

Total cases notified	260 434
Total new and relapse	219 867
— % with known HIV status	84%
— % pulmonary	85%
— % bacteriologically confirmed among pulmonary	64%

Universal health coverage and social protection

TB treatment coverage (notified/estimated incidence), 2016	76% (66–88)
TB patients facing catastrophic total costs	
TB case fatality ratio (estimated mortality/estimated incidence), 2016	0.11 (0.09–0.13)

TB/HIV care in new and relapse TB patients, 2016

	Number	(%)[f]
Patients with known HIV-status who are HIV-positive	24 871	15%
— on antiretroviral therapy	16 333	66%

Drug-resistant TB care, 2016

	New cases	Previously treated cases	Total number[c]
Estimated MDR/RR-TB cases among notified pulmonary TB cases			71 000 (71 000–72 000)
Estimated % of TB cases with MDR/RR-TB	19% (12–26)	55% (43–67)	
% notified tested for rifampicin resistance	50%	65%	145 183
MDR/RR-TB cases tested for resistance to second-line drugs			13 994
Laboratory-confirmed cases			MDR/RR-TB: 49 442, XDR-TB: 3 114
Patients started on treatment[d]			MDR/RR-TB: 47 846, XDR-TB: 4 657

Treatment success rate and cohort size

	Success	Cohort
New and relapse[e] cases registered in 2015	76%	198 754
Previously treated cases, excluding relapse, registered in 2015	58%	18 866
HIV-positive TB cases, all types, registered in 2015	62%	7 171
MDR/RR-TB cases started on second-line treatment in 2014	54%	40 698
XDR-TB cases started on second-line treatment in 2014	29%	4 404

TB preventive treatment, 2016

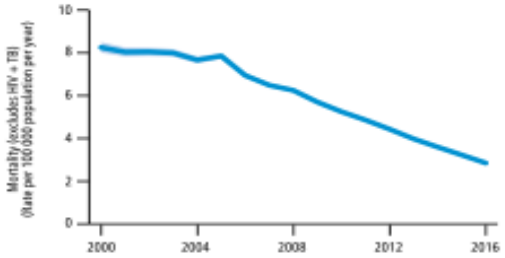
% of HIV-positive people (newly enrolled in care) on preventive treatment	54%
% of children (aged < 5) household contacts of bacteriologically-confirmed TB cases on preventive treatment	55% (52–58)

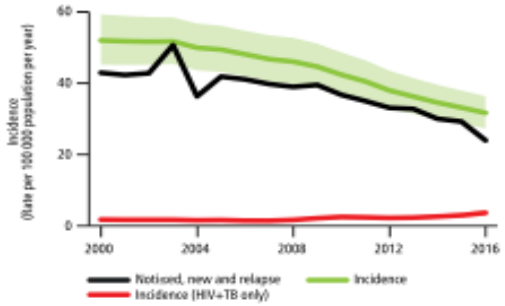
TB financing (low- and middle-income countries),[g,h] 2017

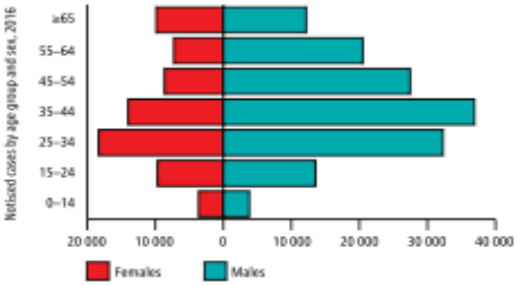
National TB budget (US$ millions)	1 572
Funding source:	93% domestic, 5.1% international, 2.1% unfunded

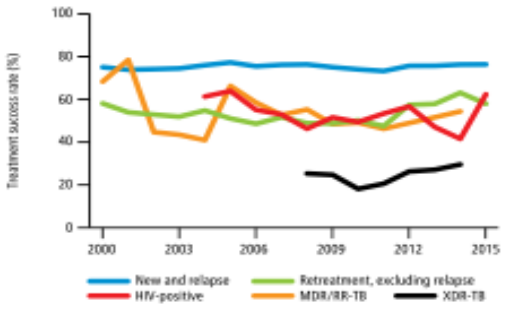
Data are as reported to WHO. Estimates of TB and MDR/RR-TB burden are produced by WHO in consultation with countries.

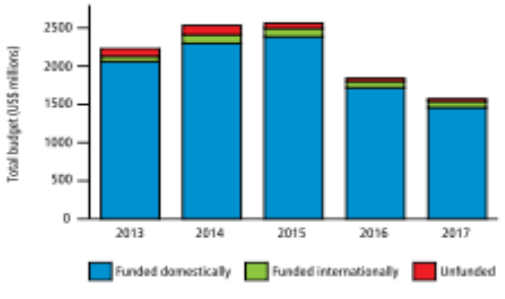
[a] Ranges represent uncertainty intervals.
[b] MDR is TB resistant to rifampicin and isoniazid; RR is TB resistant to rifampicin.
[c] Includes cases with unknown previous TB treatment history.
[d] Includes patients diagnosed before 2016 and patients who were not laboratory-confirmed.
[e] Some countries reported on new cases only.
[f] Calculations exclude countries with missing numerators or denominators.
[g] Data are not collected from all Member States.
[h] Financing indicators exclude funding for general healthcare services provided outside NTPs.











Data for all countries and years can be downloaded from www.who.int/tb/data

Source: http://www.who.int/tb/publications/global_report/en/

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Source: pixabay.com/en/photos/mosquito/

8. WEST NILE DISEASE

1. Name of zoonosis

WEST NILE DISEASE

2. Definition

West Nile Disease is an emergent infectious viral disease of birds, horses and humans, which presents clinically in non-specific and neurological clinical symptoms, with possible fatal outcome in birds and humans.

3. Etiology

3.1 Etiologic agent

West Nile Disease is caused by a virus from the family *Flaviviridae*, genus *Flavivirus*.

3.2 Taxonomy

The genus *Flavivirus* consists of several viruses, and the West Nile encephalitis virus is part of the antigen complex of the Japanese encephalitis virus in horses. The members of the Japanese encephalitis serogroup which most often cause disease in horses are the Japanese encephalitis virus, the West Nile virus and the Kunjin virus. By phylogenetic analysis of virus isolates it has been established that the original virus has developed into two separate lineages. Strains from lineage 1 have been isolated in Africa, the Middle East, Europe, North American and Australia, and from lineage 2 in South Africa and Madagascar. In the past decade, lineage 2 has spread to horses throughout central and Eastern Europe. In the literature, strains of the virus are mentioned that were isolated from arthropods, which may be ascribed to new lineages, indicating the need for frequent re-classification.

3.3 Morphological description

West Nile is an RNA virus; it is small (50 nm) and round, with a single-stranded genome with a positive strand. It has three structural (protein capsid, membrane glycoprotein, precursor membrane, membrane) and seven non-structural proteins. Of the structural proteins, the E membrane glycoprotein of the virus is important, since it enables the virus to bond to a cell, participates in viral hemagglutination, and gives rise to the creation of anti-bodies, on which immunity to this virus is based.

4. Epidemiology

4.1 Source of infection

The primary sources and reservoirs of the virus are birds in which the virus propagates, making direct transfer between them possible. During propagation in birds, viremia occurs, which enables mosquitoes of the genuses *Culex*, *Aedes* and *Ochlerotatus* to become infected whilst feeding off the birds. In the infected mosquitoes the virus is able to propagate in the salivary glands, and transovarial transfer of the virus has also been established. Therefore, mosquitoes are the biological vector which, as a result of hibernation by infected females, creates the

conditions for the endemic character of the disease.

4.2 Path of infection

Although direct transfer of the virus is possible from bird to bird, in the spread of the disease hematophagous insects are most important, when as they feed on infected birds they carry the disease to other birds and other animals, mammals and humans. The main vector in the spread of West Nile virus are mosquitoes from the genus *Culex*, and the incidence and spread of the disease depend on their activities. Due to the limited propagation of the virus in mammals, it is believed that mosquitoes cannot transfer the infection from mammal to mammal (Fig.7.1). Therefore, in an epidemiological sense, the most important form of transfer of the virus by mosquitoes is from infected birds to humans. Apart from mosquitoes, iatrogenic transfer by transfusion of infected blood, organ transplants, and intrauterine and galactogenic transfer to humans have also been proven (5).

4.3 Responsiveness

Birds, mammals and humans are responsive to infection. In the USA significant per acute mortality of birds from the crow family *Corvidae* has been recorded. Clinical cases of the disease in birds in Europe have been described mainly in birds of prey, such as the sparrow hawk Accipiter nisus, the goshawk *Accipiter gentilis* and the gyrfalcon *Falco rusticolus*. During epizootics in horses' differences have been registered in disposition in relation to the gender, age and breed of the animal, which need to be further researched. In mammals the virus propagates to a limited extent, that is, it does not cause strong viremia, which means that mammals are not a source of infection, but they are the "dead-end hosts". As risk factors for the disease in humans older age and immunosuppression are mentioned (7).

4.4 Resistance to the environment

The virus is not very stable in the environment and is inactivated easily by heat and common disinfectants (1).

4.5 Geographical distribution

The West Nile virus was isolated for the first time in 1937 from the blood of a woman from the region of the West Nile in Uganda, but the first detailed clinical description of the disease in humans' dates from the epidemic in Israel in 1951. It was proven in horses in France in the early 1960's, and later sporadic epizootics were recorded in several Mediterranean countries. Frequent sporadic occurrences of the disease, recorded in the same areas in Europe, have been linked with the flight paths of migratory birds (10). The disease reached endemic and enzootic status through frequent recurrences in a large part of Europe and North America. The disease was described in humans in the territory of Croatia in 1980, and an infection of horses in Croatia, in the area of eastern Slavonia, was proven serologically in 2007 (9). In the period from 2012 to 2013, 27 cases of neuro-invasive diseases in humans were registered in the same area. In neighbouring Hungary, the lineage 2 West Nile encephalitis virus caused fatal encephalitis in goshawk *Accipiter gentilis* in 2004. (fig.7.2.). In the following years

infections were proven in birds, sheep and horses, and 14 infections of humans with clinical symptoms of meningitis and encephalitis were proven in 2008. In the same year, the spread of the virus was also proven in Austria. In 2010, infections were reported in 42 animals in the areas of central and southern Italy, in regions which are rich in migratory birds. (Fig.7.3). In Romania three cases of subclinical infection were recorded in 2010 (2).

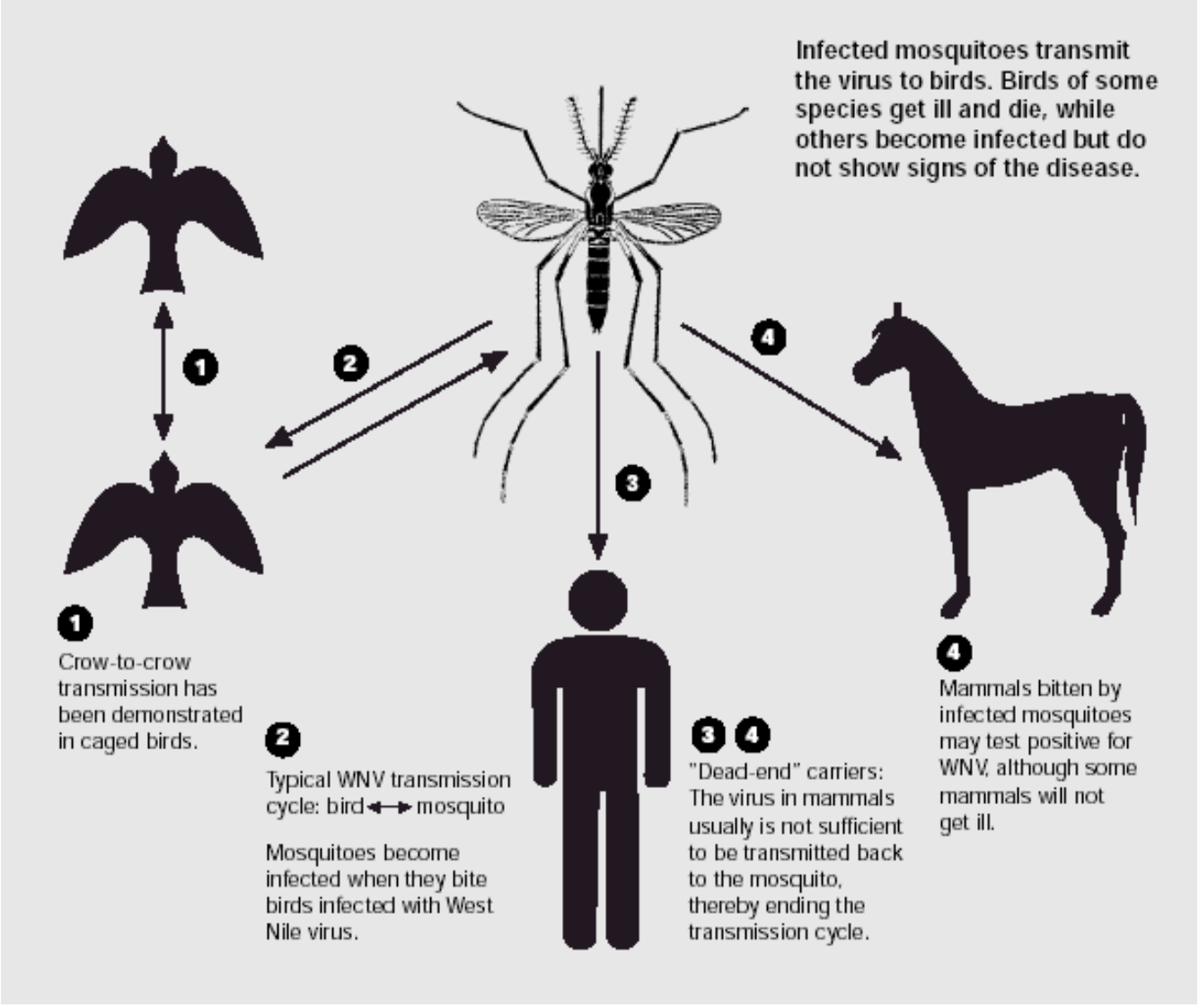

Fig. 8.1. Path of infection in West Nile disease
(Source: https://sites.google.com/a/fivestarschools.org/historyofthewestnilevirus/)

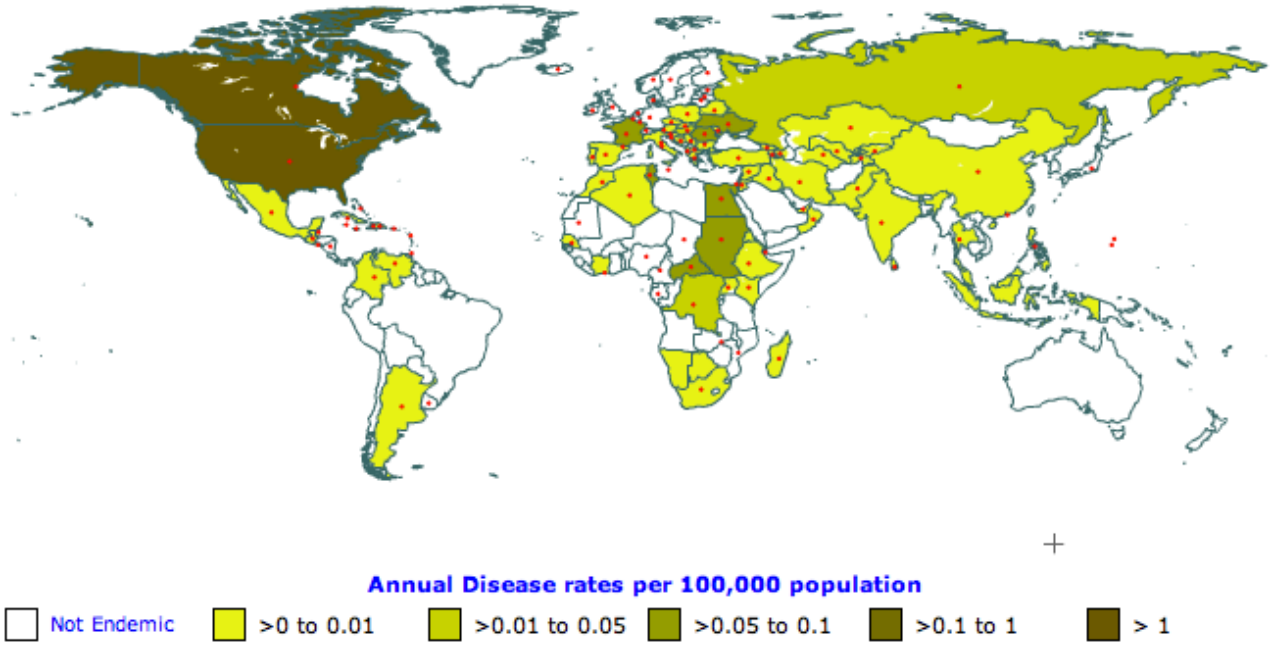

Fig.8.2. Annual W.N. disease rate

(Source: https://www.gideononline.com/cases/westnilefever/)

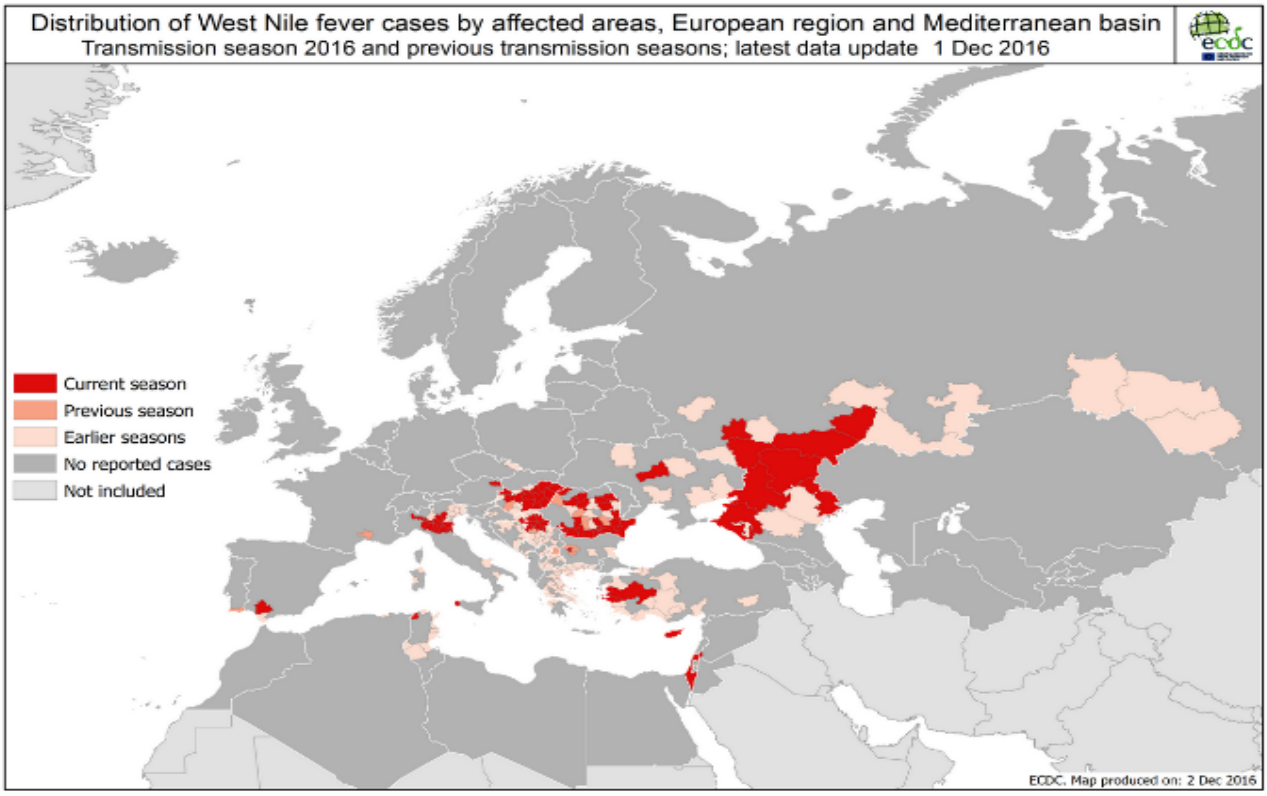

Fig.8.3. Distribution of WN disease in European and Mediterranean basin

(Source: http://ecdc.europa.eu/en/healthtopics)

5. Pathogenesis

Clinical signs in the nervous system are the result of viral infection and pathological changes to neurons. The West Nile encephalitis virus, as all the other viruses in the Japanese encephalitis serogroup, exhibits strong tropism towards nerve tissue, in which it causes lysis or apoptosis of the neurons. It is believed that the viral infection spreads through the macroorganism in two ways: first, from the entry bite, through the lymph and blood vessel systems to the parenchyma organs, and by crossing the blood-brain barrier into the central nervous system, and second, through the axon to the central nervous system. Infection by the virus presents as multifocal and asymmetric motor deficit, primarily seen as weakness and ataxia. These two clinical signs are the result of infection of the brain and spinal cord, disturbance of the motor pathways in the hindbrain, and loss of fine motor control due to infection of the thalamus and basal ganglia (5).

6. Clinical manifestation

West Nile virus infection in birds, horses and humans may be manifest or subclinical in character. In birds the clinical picture depends on the virulence of the virus type, and it varies from peracute mortality to general lethargy, ataxia, loss of feathers, hanging of the head and convulsions. The infection in horses most often passes asymptomatically. Incubation of the disease in horses lasts from three to 15 days. The clinically manifest form in horses begins with a general infection syndrome, a raised body temperature and depression. The onset of the disease is often accompanied by colicky stomach pains, difficulty in standing or moving the legs. The main symptoms of the viral infection are trembling muscles, stiffness and behavioural changes. Shivering is frequent in the area of the muscles of the head and neck, and it may also affect all four legs and the body. Changes to the cranial nerves manifest as short-term weakness of the tongue, mouth deviation and twitching of the head. In many horses a period of excitement is noticed, which may reach the level of aggression. Some horses develop symptoms similar to narcolepsy, and develop sleep disorders. In most horses there is an improvement in health after three to seven days from the appearance of the disease and in one to six months' recovery occurs in 90% of cases. In humans the viral infection is most often subclinical, but it can also cause various clinical forms (4). The manifest form is preceded by incubation of two to six, and no more than 14 days. The clinical picture varies from a mild febrile form to fatal meningoencephalitis. As a non-specific febrile disease, it begins with a raised temperature (over 39°C), weakness, anorexia, nausea, headache, myalgia, arthralgia and lymphadenitis. The illness lasts three to six days. In less than 1% of patients the infection is manifest as a neuroinvasive disease, with three forms: meningitis, encephalitis, and poliomyelitis. The mildest form is meningitis, with a good prognosis, whilst in the case of encephalitis mortality is up to 20% with neurological sequelae, such as tremor and/or ataxia, which may last up to a year. In cases

of poliomyelitis mortality may be above 50%, and recovery may leave permanent neurological sequelae (5).

7. Diagnostic

7.1 Clinically

Findings of clinical symptoms with disturbance of the central nervous system in horses or humans should indicate West Nile disease, and differential diagnosis should distinguish it from encephalitis with another etiology.

7.2 Paraclinically

The disease may be diagnosed objectively by proving the pathogen by serological methods. In living patients, proof of the pathogen by isolating the virus or PCR testing is limited by the brief viremia and the appropriate choice of primer for performing molecular tests. Serologically, the disease is diagnosed as an acute infection by showing IgM antibodies, but a finding of IgG antibodies is proof of a prior infection or possible vaccination. Serological diagnostics are also hindered by cross reactions with other viruses from the genus *Flavivirus* (12).

7.3 Epidemiologic

West Nile disease should be suspected upon the occurrence of the death of birds and clinical symptoms of a disturbance of the central nervous system in humans and horses in an area where the disease has already been established. Additional suspicion should also be aroused by the seasonal occurrence of the disease (6).

7.4 Pathologically

We find macroscopic pathological changes in horses. It is possible to find congestion of the meninges, hyperaemia in the grey matter, petechia or significant bleeding in the brain and thoracolumbar spinal cord. Histological findings in birds and horses are characterized by non-purulent lymphocyte/histiocyte polio encephalitis (3).

8. Prophylaxis

General prophylactic measures for this disease relate to avoidance or reduction of exposure of horses and humans to mosquitoes. By systematic and regular disinsectization of the immediate environment, destruction of habitats of mosquitoes and use of repellents, exposure of humans and horses is reduced. Additional measures are: keeping animals in closed, protected facilities during seasonal and daily periods of mosquito activity, and, for people, wearing appropriate clothing. In order to disturb the mosquito's life cycle, it is especially important to remove water from all places where it may remain for longer than seven days. In horses, vaccination has been used for some time using inactivated and recombined vaccines. When planning immuno-prophylaxis in horses it is necessary to consider the justification of vaccination on the basis of the prevalence of the disease, the selection of the appropriate type of virus vaccine and the possibility of precise registration of vaccinated animals (8).

9. Treatment

There is no etiological therapy. Sick horses are treated symptomatically and with support of infusion solutions, reducing the inflammatory reaction of the central nervous system. Before treatment itself, the animal should be accommodated to avoid excitement, and if it has difficulty standing it may be placed in a sling (11).

For humans there is no specific therapy for treatment of an infection by the West Nile virus. A mild form of the disease does not require therapy, apart from medication to reduce a high temperature and ease pain, and for the more severe form of the disease, the patient requires hospital treatment (3).

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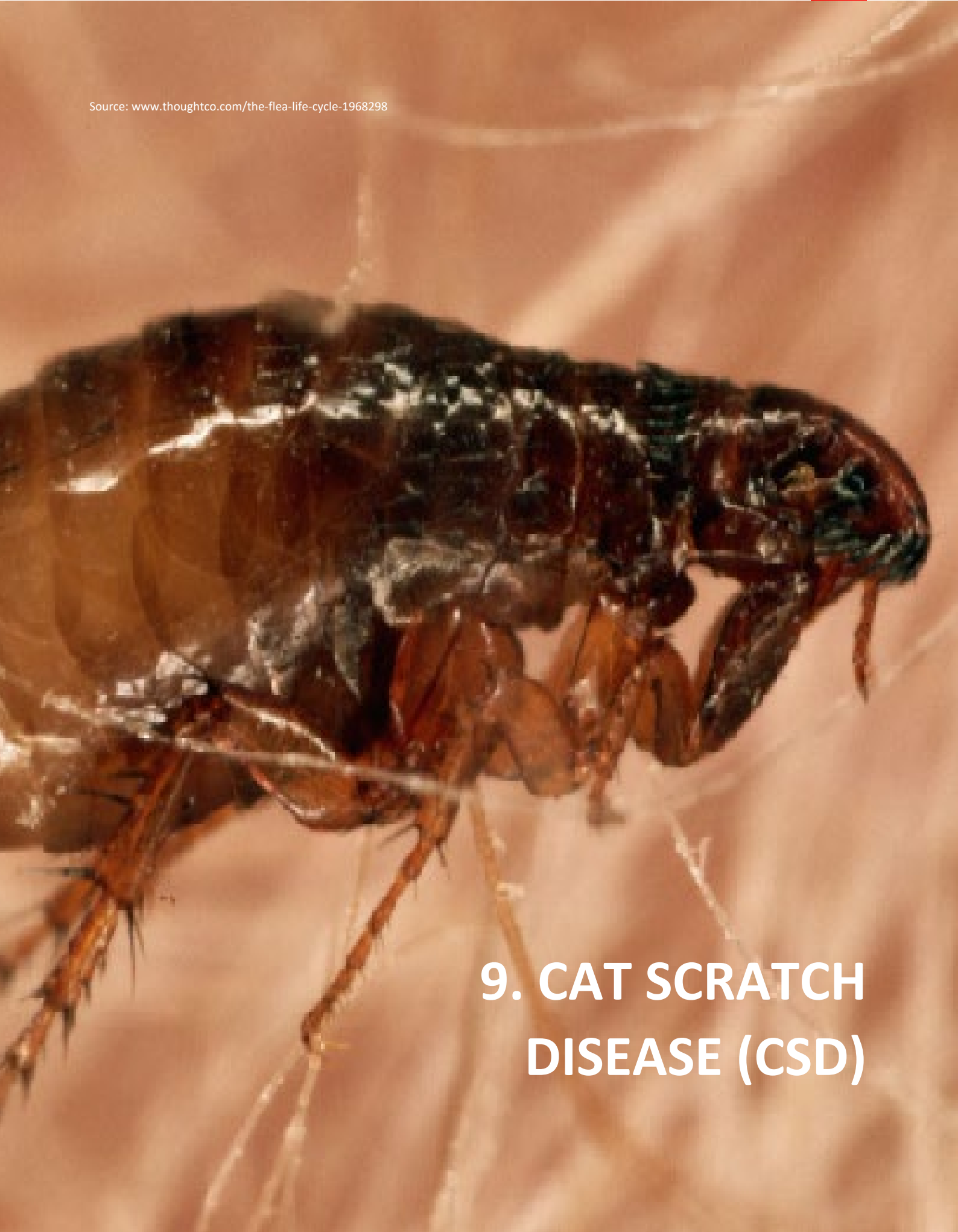


Source: www.thoughtco.com/the-flea-life-cycle-1968298

9. CAT SCRATCH DISEASE (CSD)

COUNTRY ANALYSIS ON ZOONOSES STATE OF ARTS

1. Name of zoonosis

Cat Scratch Disease (CSD)

2. Definition

Cat Scratch Disease (CSD) is a zoonotic disease naturally transmissible from animals to humans caused by bacteria belonging to the *Bartonella* genus. In the recent years, the scientific literature reported an increase of human clinical cases due to *Bartonella* infection in various parts of Europe, especially in those individuals more exposed for professional reasons: veterinarians, professionals and owner's catteries (22).

3. Etiology

3.1 Etiologic agent

The main etiologic agent of CSD is *Bartonella henselae* (fig. 9.1.) which is involved in the infection of domestic and wild cats. The main reservoir of the infection is the cat. However, other species belonging to the Bartonella genus can be pathogenic to humans and can cause CSD: *B. clarridgeiae, B. koehlerae, B. quintana, B. doshiae* (6).

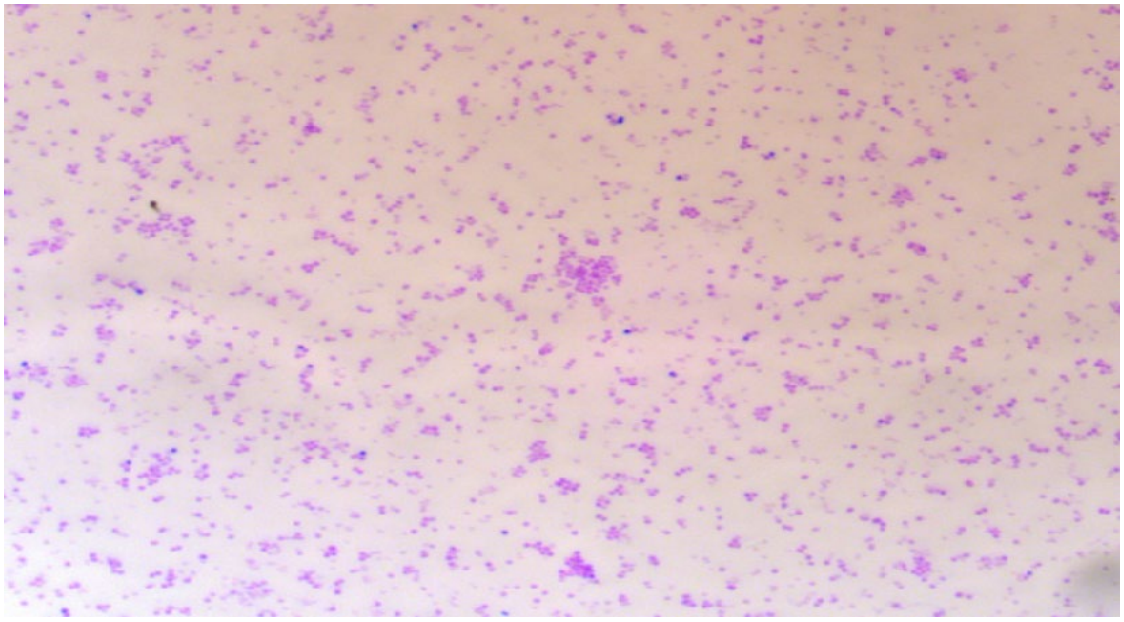


Fig.9.1. *Bartonella henselae* Gram coloration (100x)

3.2 Taxonomy

Bartonella spp. belongs to α2 sub-group of *Proteobacteria* family of *Bartonellaceae*, which actually includes some species previously classified in the genus *Rochalimaea*. In 1993 Brenner and colleagues proposed a new classification, moving these organisms from the *Rickettsiaceae* family to

Bartonellaceae, removing definitely *Bartonellaceae* from the *Rickettsiales* order (8).

Domain: *Prokaryota*
Regn: *Bacteria*
Phylum *Proteobacteria*
Class: *Alphaproteobacteria*
Order: *Rhizobiales*
Family: *Bartonellaceae*
Gender: *Bartonella*
Species: *Bartonella henselae*

3.3 Morphological description

Bartonella is a pleomorphic coconut-bacillus bacterium, gram negative, aerobic, which grows slowly on culture (3, 7).

3.4 Biological cycle

Bartonella henselae may infect various vertebrate hosts/or reservoirs (felines, canines, man, rodents) and the flea *Ctenocephalides felis* (fig. 9.2.) has as main vector, although other arthropods (e.g. *Ixodes*) may be involved (6).

Fig.9.2. *Ctenocephalides felis*

4. Epidemiology

4.1 Source of infection

The faeces of *Ctenocephalides felis*, contaminated by *B. henselae* represent the main source of infection for cats. The disease is transmitted to humans primarily through the infected cat scratch and/or bite (5).

4.2 Path of infection

Cat's nails and oral cavity, contaminated by flea's faeces, represent the main source of infection for human beings through the scratch and /or bite. The role played by the dog in transmitting *Bartonella spp*. is still not very clear, even if some researches revealed

that this animal species is susceptible to the infection due to *B. henselae* and other *Bartonella* species (Fig.9.3.). Therefore, the dog may be used as sentinel animals for human infection (31). In recent years, with the increased number of researches on bartonellosis, the knowledge of the host range is now more complete. In fact, various species of *Bartonella* have been isolated in cattle, wild canids, horses, sheep and rodents (13).

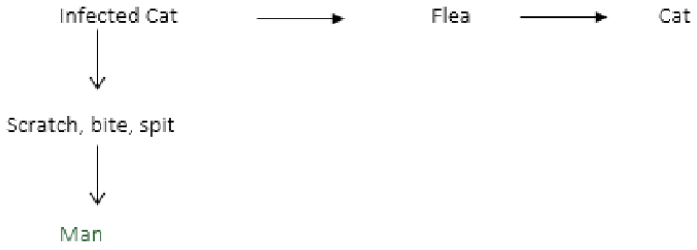


Fig.9.3. Human infection with *Bartonellla spp*.

The *Ctenocephalides felis* flea (fig.9.4.) is the competent vector of *B. henselae*. The flea becomes infected through ingestion of blood from bacteriemic cats. The bacterium replicates in the fleas and persists in their faeces. Cat nails and teeth are contaminated with the *Ctenocephalides felis* faeces. Among cats, the potential mechanism of transmission of B. henselae can be represented by contamination of skin wounds or by ingestion of flea's faeces. The flea plays a significant role in the horizontal transmission of infection among cats (5).

4.3 Resistance to the environment

Bartonella can live in blood stored more than 35 days, thus representing a potential risk associated to blood transfusions (11).

4.4 Geographical distribution

Bartonella spp. The Geographical distribution is presented in tab. 9.1. :

Tab.9.1. The Geographical distribution *of Bartonella spp*.

Species	**Disease**	**Geographical distribution**
B. bacilliformis	Carri*ón disease*	South America
B. rochalimaea	Fever, skin lesions, splenomegaly	Peru
B. quintana	Endocarditis, FQ*, MGG**, AB***, hepatic peliosis	South America, United States, Europe, Africa
B. henselae	MGG, ocular lesions, encephalopathy, meningitis, fever, abscesses, osteomyelitis, AB, pellosis, nodosum erythema, skin lesions.	South America, United States, Europe, Africa, Asia
B. elizabethae	Endocarditis	Europe, United States, Asia

B. clarridgeiae	MGG, endocarditis	Europe, United States, Asia
B. clarridgeiae-like	Fever and splenomegaly	Peru
B. koehlerae	Endocarditis, MGG	United States
B. vinsonii subsp. berkhoffii	Endocarditis, arthralgia, myalgia, headache, asthenia	Europe, United States
B. washoensis	Fever e myocarditis	United States
B. tamiae	Fever	Thailand
B. grahamii	Neuro-retinitis	Europe, Canada, Asia
B. doshiae	MGG	Europe

*Quintana fever, **Cat scratch disease ***Angiomatosibacillare

Fig. 9.4. *Ctenocephalides felis* flea – detail in SEM (Source: cosmosmagazine.com/biology/domestic-fleas-infest-scores-of-wild-mammal-species)

5. Pathogenesis

Bartonella is a bacterium not strictly intracellular. It lives adhering to red blood cells and may cause prolonged bacteriemia and vasoproliferative lesions due to invasion of endothelial cells (4).

Pathogenesis in animals:
Cat is usually asymptomatic. It can develop a bacteriemia lasting for weeks or months. It has been report cases of bacteriemic cats for more than 1 year. Young cats (< 1 year) can develop a stronger bacteremia than older cats, as well as the stray cats than domestic cats. In pregnant ruminants, Bartonella can cause abortion (2).

Pathogenesis in humans:
One to two weeks after the infection through the scratch or the bite of an infected cat, the first clinical signs may appear as fever and asthenia (15).

6. Clinical manifestations

In humans, the typical forms of bartonellosis are characterized by clinical signs in the scratch or bite site, such as cutaneous suppurative lesions, vesicles and cutaneous redness and lymphadenopathy of local lymph nodes (21). The prognosis is favourable if the disease is promptly diagnosed and treated appropriately. It exists a detectable immune response, but not very effective to eliminate the microorganism (10).

Immunosuppressed individuals may present vasoproliferative injuries in the liver and spleen (peliosis visceral) and also on the skin (bacillary angiomatosis) (20).

Cases of atypical forms of bartonellosis may occur especially in children who often do not have the typical symptoms of the disease: tonsillitis, pneumonia, involvement of the conjunctiva, osteomyelitis, granulomatous liver and/or spleen, encephalitis, thrombocytopenia). The bartonellosis is also associated with cases of endocarditis (12).

7. Diagnosis

For several years, the diagnosis of CSD in humans was based on clinical criteria, exposure to a cat, failure to isolate other bacteria, and/or histological examination of biopsies of lymph nodes. The atypical forms are more difficult to recognize. In cats, because of the usual lack of symptoms, clinical diagnosis is not easy, while in dogs the infection can be suspected for the presence of fever or endocarditis (1).

The existence of epidemiological circumstances, such as the occurrence of cat's bite and/or scratch, the possible contact with rodents, fleas, ticks may all suggest to request more specific tests (serology, blood culture or PCR) (11, 32).

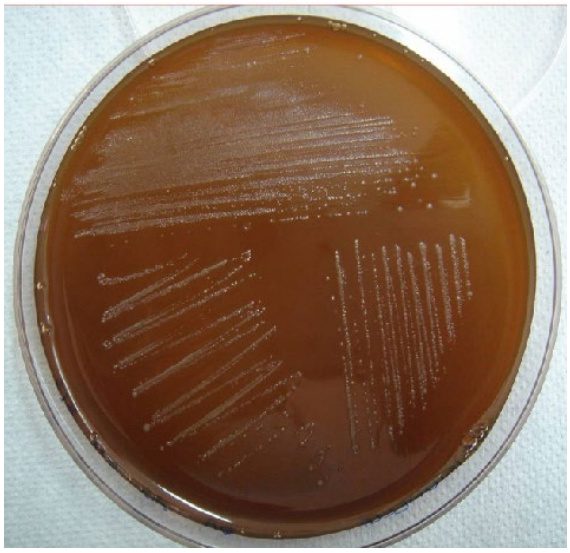
The laboratory diagnosis of infections caused by *Bartonella* can be based on various methods (23):

- indirect immunofluorescence for the detection of specific antibodies;
- immunoblotting for the identification of *B. henselae* immunodominant

antigens recognized by IgM and IgG isolation from blood samples through cultural methods;

- isolation through molecular biology techniques, such as PCR followed by restriction fragment length polymorphism -RFLP- and single-run real-time PCR.

For the diagnosis of CSD the detection of antibodies against *B. henselae* through IFA is indicated. In cats, in addition to IFA, an accurate study on the animal conditions concerning bartonellosis, recommends the application of direct techniques for bacterium isolation through blood cultures or molecular biology (1).

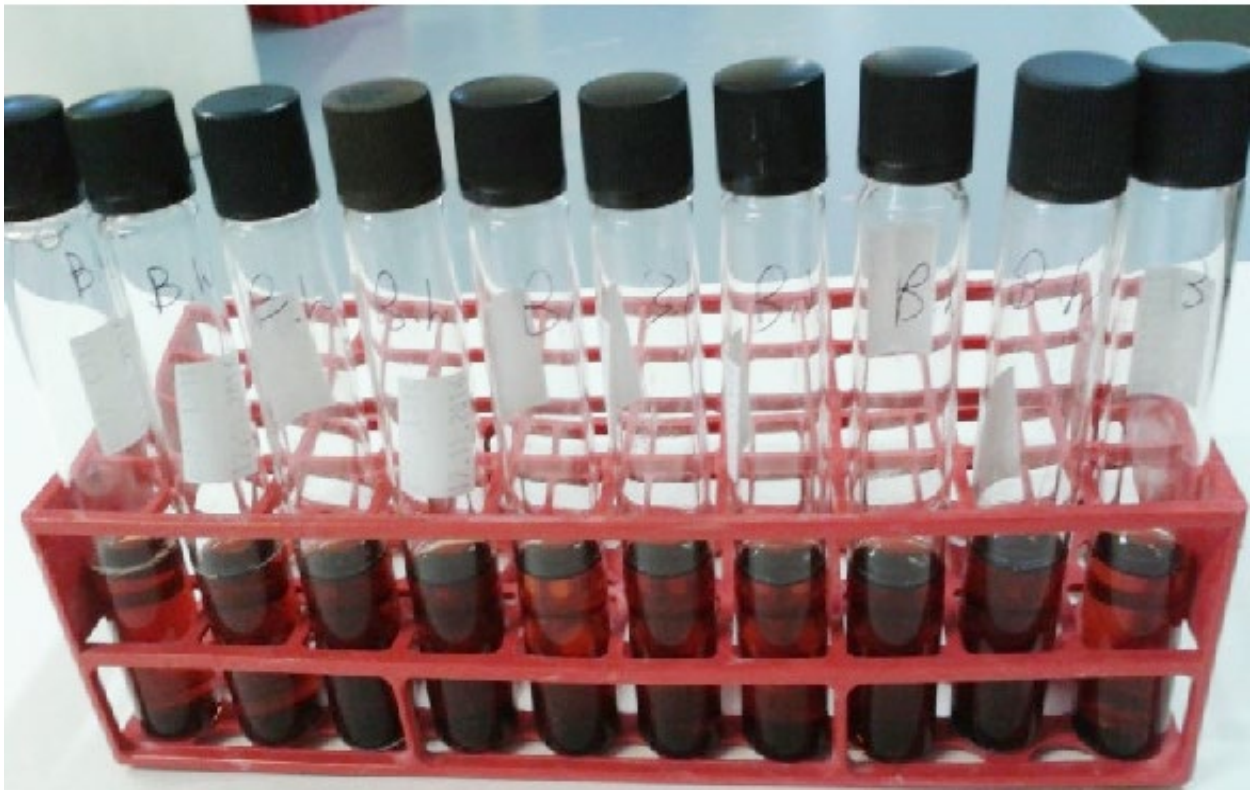


B. henselae in AGAR blood (photo G.Masala)

B.henselae in AGAR blood

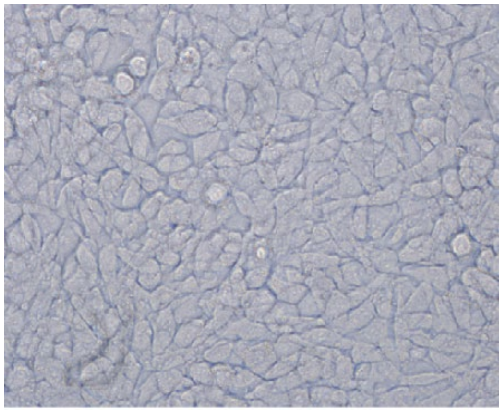
Bartonella henselae grows in cultures on solid terrains (AGAR blood) at 37°C in 5% of Co2 and in liquid terrains: *Brucella* broth with Emina, 8% Fildes solution, 37°C - 5% CO2 in agitation. It is also possible to cultivate on cell cultures, such as the L929 cell lines, mouse fibroblasts (29).

For antigen production, in order to prepare the slides for the indirect Immunofluorescence staining, you can be grown in the Bartonella Flask (25cm^2) with basis of L929 cells, mouse fibroblasts, at 37°C - 5% CO_2 (14).

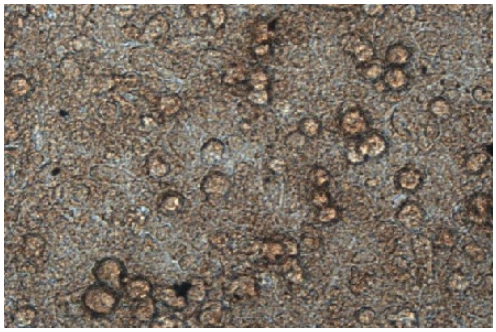


B. henselae in *Brucella* broth col. Emina, 8% Fildes solution

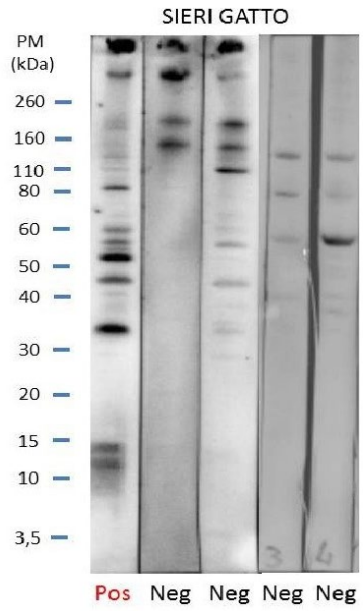
B. henseale in flask (25cm^2) with L929 cellular basis, inoculum from the culture broth.



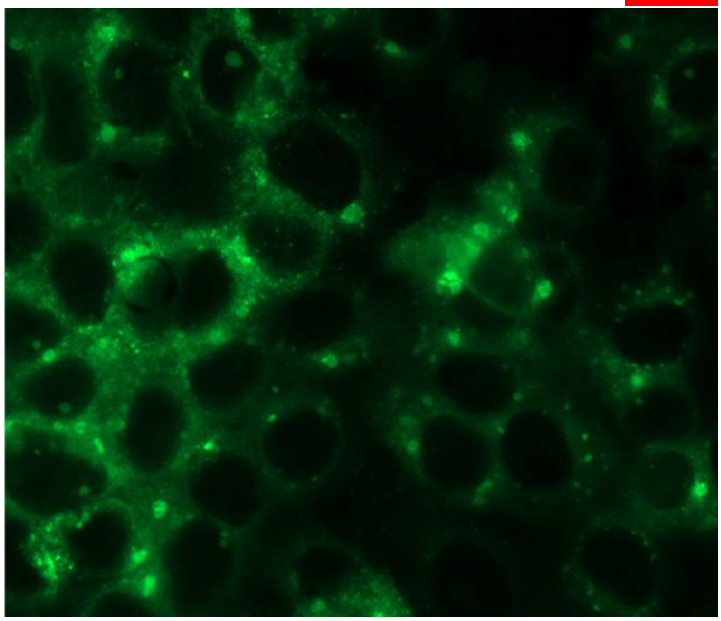
Cellular basis L 929 flask land



B. henseale (small bacterium) in L929 basis



Immunoblotting *Bartonella spp*. positive serum: strongly presence of PM 54kDa immune-reactive band.



Indirect immunofluorescence *Bartonella spp*. (positive cat sera)

7.1 Clinical

a. Paraclinically

Hematologic abnormalities are infrequent, but in infected cats have been reported thrombocytopenia, lymphocytosis, neutropenia and eosinophilia (19).

b. Epidemiological

Cat scratch disease is the most common zoonotic disease caused by *Bartonella* all over the world (18).

Assay	Type of sample
Serological diagnosis (antibodies search) IFI	Serum (200-300 µl)
Direct methods (Cultural isolation)	EDTA blood (2-3 ml)
Direct methods (PCR)	EDTA blood (300µl)

Diagnostic protocol for the laboratory diagnosis of *Bartonella* infection in cats

Human cases have been reported in many countries including North America, Europe, Australia. It has been reported with high serological prevalence in humid hot climates, which contribute to maintaining the flea in the environment. The prevalence of the disease varies considerably in the cats (stray or domestic cats) with lower values in cold climates (0% in Norway) and higher in humid hot climates (68% in the Philippines) (28).

Two genotypes have been identified: Houston -1 (type I) and Marseille (type II). The prevalence of the two genotypes varies among cat populations of different geographical areas. *B. henselae* type Marseille is dominant in the Western United States, Western Europe (France, Germany, Italy, the Netherlands, UK) and Australia; while Houston-1 is dominant in Asia (Japan and the Philippines). However, differences in the prevalence of the two genotypes can occur even within the same country (24, 26).

Few studies in Western Europe and Australia have reported that most human cases of CSD were caused by *B. henselae* type Houston -1, despite the fact that Marseille type was found dominant type among cats. Type I could be more virulent for man (17).

In Europe the highest prevalence has been reported in the Netherlands with 22% of 113 domestic cat's positives to the blood culture (isolation) (30).

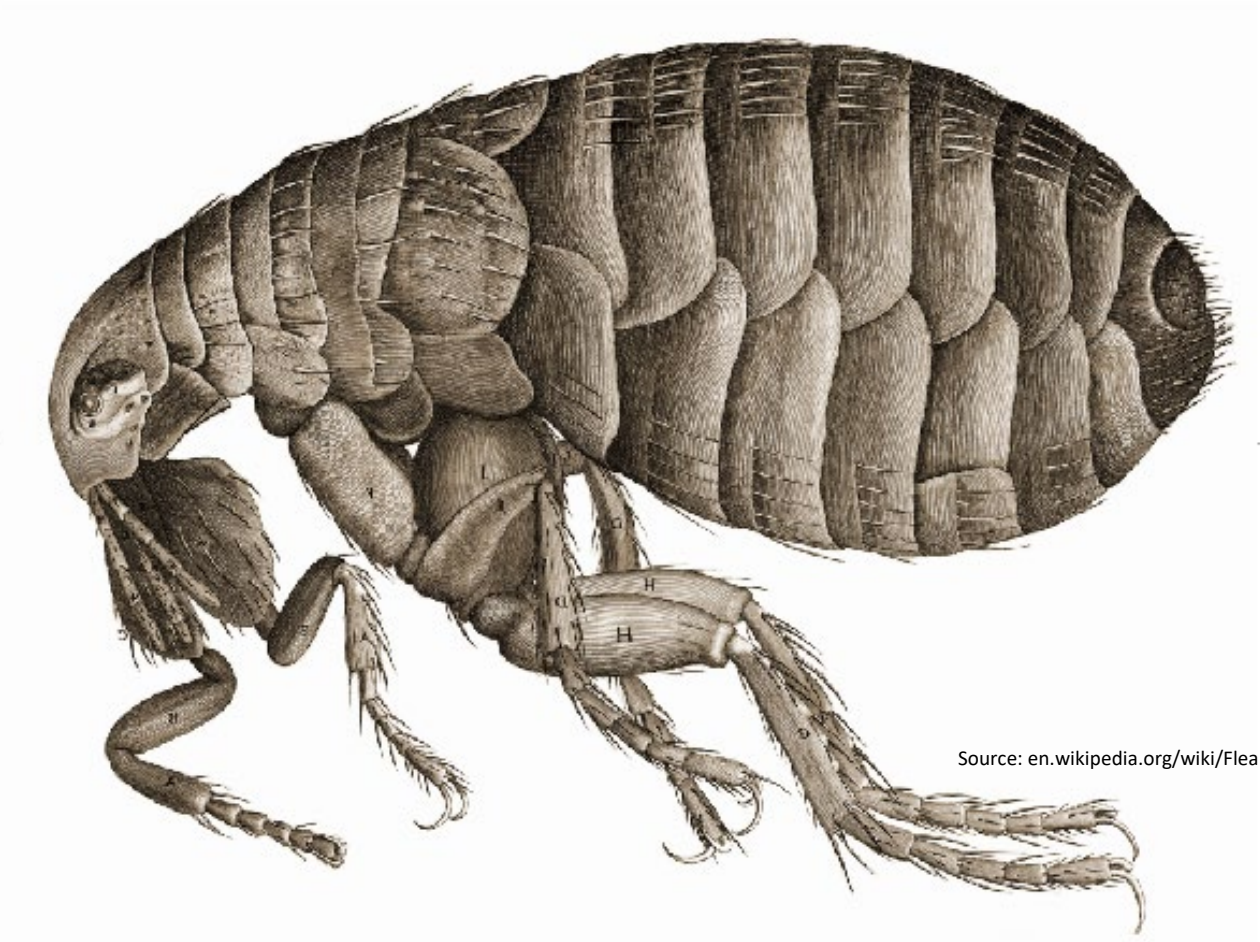
In Italy, the ISTISAN 1/16 report, analysing the data of dismissals from Italian hospital from 2009 to 2013, suggests that the mean incidence rate of hospitalizations due to human bartonellosis is 1.85 / 1 million inhabitants (9).

PROVINCE	AVERAGE INCIDENCE RECOVERIES (1.000.000 INHABITANTS)
Teramo	3.19- 9.06
Pescara	0.01- 1.90
L'Aquila	0.01- 1.90
Chieti	1.90- 3.19

Mean incidence rate of hospitalizations due to human bartonellosis (cat scratch disease) in Abruzzo (2009-2013) (30)

5. Prophylaxis

The prevention of the disease is mainly based on the control of the ectoparasites in the environment, as well as on regularly treating the cats against ectoparasites. Further studies are needed to clarify the role of dogs in disease transmission. The data in the literature show that experimentally infected cats become resistant and do not develop any bacteriemia after reinfection (16). Immuno-prophylaxis among cats could be considered to reduce a risk to public health. Immunosuppressed individuals should be particularly careful in having behaviours that increase the risk of cat's bites and scratches (2).



Source: en.wikipedia.org/wiki/Flea

6. Treatment

For the pharmacological treatment of cats, the following drugs can be used: doxycycline, amoxicillin, enrofloxacin, combinations of amoxicillin and clavulanic acid (28). Humans can use several types of antimicrobial, although some studies show a limited efficacy of some compounds in most instances, administration of antimicrobials does not appear to improve response to or shorten the duration of the infection (2).

The treatment differs from immunocompetent subjects with classic symptoms to immunocompromised individuals: in the first case, for CSD without complications, it can be used azithromycin, ciprofloxacin, rifampin and cotrimoxazole; in the second case, immunosuppressed with angiomatosis bacillary and peliosis hepatitis, it is recommended tetracycline, doxycycline, erythromycin, rifampin, azithromycin, doxycycline, also in combination each other. In the presence of endocarditis, the therapy should also include aminoglycosides (2).

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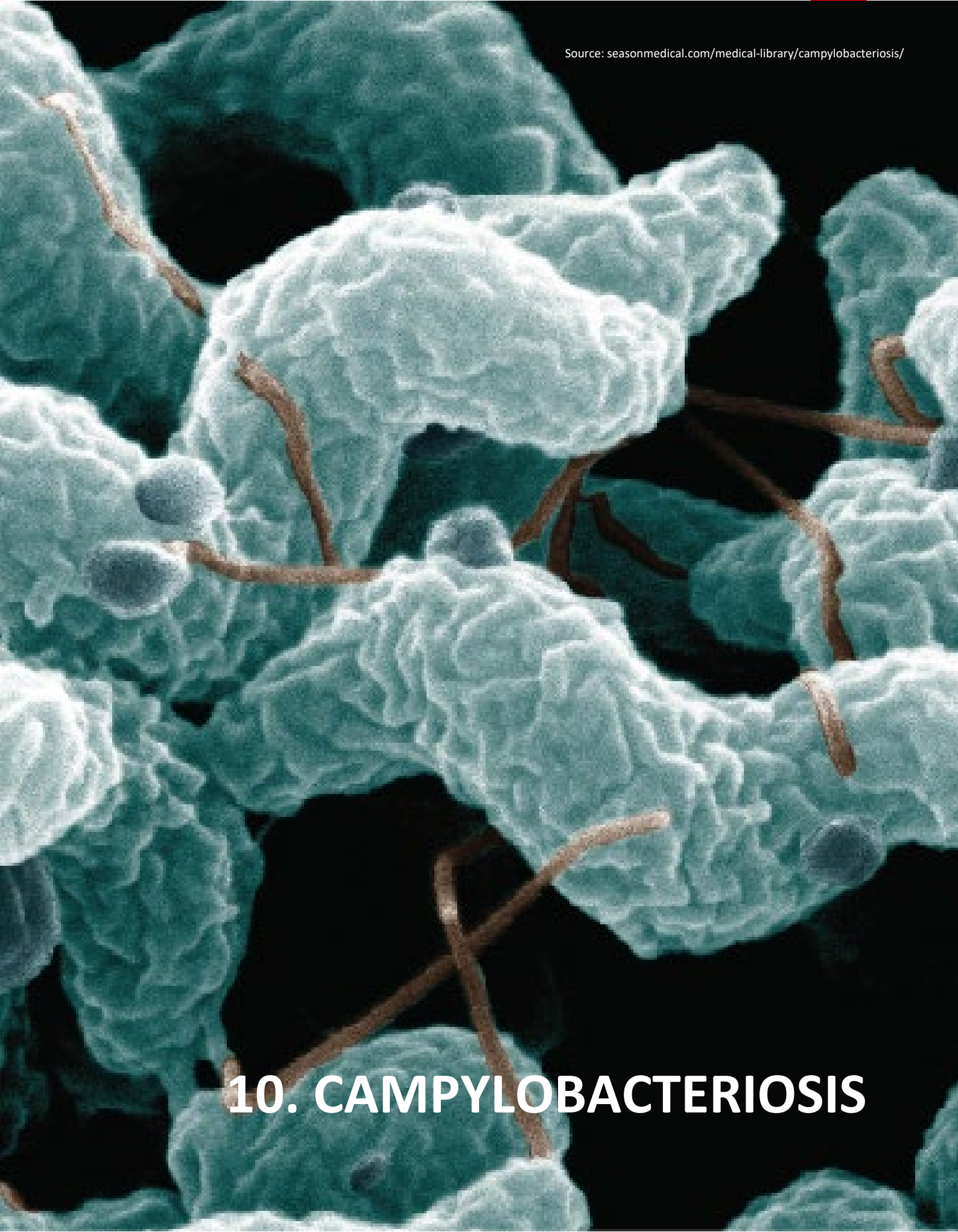

Source: seasonmedical.com/medical-library/campylobacteriosis/

10. CAMPYLOBACTERIOSIS

1. Name of zoonosis

Campylobacteriosis

2. Definition

Campylobacteriosis is a foodborne zoonotic disease caused by bacteria of the genus *Campylobacter*.

3. Etiology

3.1 Etiologic agent and Taxonomy

The genus *Campylobacter* currently includes 34 species (2015) and this number is constantly increasing due to the identification of new species. *Campylobacter jejuni* followed by C. *coli,* and *C. lari,* but other *Campylobacter* species are also known as cause of human infection and of the most important species in terms of food-borne disease. Thermophilic *Campylobacter* (*C. jejuni, C. coli, C. lari, C. upsaliensis* and *C. helveticus*) do not grow below 30°C and have an optimal growth temperature at 42°C. Many healthy animals can show *Campylobacter* spp. asymptomatically. *Campylobacter* spp. also causes enteritis, abortions and infertility in various species. Some strains of *C. jejuni, C. fetus subsp. venerealis,* and *C. fetus subsp*. *fetus* (also known as *C. fetus*) cause infertility and abortions in ruminants (2).

Campylobacter fetus subsp. fetus is transmitted by ingestion in cattle, sheep and goats. Animals can be infected after contact with faeces, vaginal discharges, aborted foetuses and foetal membranes. This organism and *C. fetus subsp. venerealis* are also transmitted venereal in cattle. *C. fetus* is occasionally isolated from humans with septicemia. Additional zoonotic species include *C. helveticus, C. hyointestinalis, C. lari*, *C. upsaliensis, C. sputorum*, and *C. ureolyticus*. Species that have been associated only with disease in humans to-date include *C. concisus* and *C. curvus* (gastroenteritis, periodontitis); *C. gracilis C. rectus*, and *C. showae (periodontitis); C. jejuni subsp. doylei (septicemia, enteritis)*; and *C. lari subsp*. *concheus* and *C. peloridis (enteritis)* (11).

3.2 Morphological description

Campylobacter, a gram-negative, non-spore–forming, S-shaped or spiral shaped bacteria (0.2–0.8 µm wide and 0.5–5 µm long) and able to move via unipolar or bipolar flagella with a characteristic corkscrew-like motility. These bacteria are microaerophilic but some strains also grow aerobically or anaerobically. Some species are thermophilic, growing optimally at 42°C (12).

3.3 Biological cycle

Campylobacter jejuni and *C. coli* are transmitted by the faecal - oral route. Although it is clear that livestock, and particularly poultry, are the most common source. The natural environment (soil and water) plays a key role in transmission, either directly to humans or indirectly via farm

animals. The principal reservoirs are the alimentary tract of wild and domesticated birds and mammals. These bacteria are prevalent (20):

- in food-producing animals such as poultry, cattle, pigs and sheep;
- in pets, including cats and dogs;
- in wild birds;
- in environmental water sources.

Furthermore, the bacteria are present in the intestinal tract of humans where it can exist without causing illness. These bacteria are transmitted through the faecal oral route, sexual contact, the ingestion of unpasteurized milk and raw poultry, ingestion of contaminated water (waterborne) and the exposure to sick puppies. Transmission cycle of Campylobacter jejuni can also be transmitted through poor sanitation practices and unsafe food handling in kitchens, food processing plants, retail establishments and farms . (20)

4. Epidemiology

4.1 Source of infection

Campylobacter can speedy contaminate various foodstuffs, including meat, raw milk and dairy products, and less frequently, fish and fishery products, mussels and fresh vegetables. Poultry meat is one of the major sources. Among sporadic human cases, contact with live poultry, consumption of poultry meat, drinking water from untreated water sources, and contact with pets and other animals have been identified as the major sources of infections (15).

Raw poultry contaminated meat, eating undercooked meat or ready-to-eat food and cross-contamination during food preparation at home, are also described as an important transmission route. Raw milk and contaminated drinking water have been implicated in both small and large outbreaks. There have been a number of reports implicating environmental water as the source of campylobacteriosis outbreak. EFSA opinion estimates that handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases, while 50% to 80% may be attributed to chicken reservoir as a whole (17).

These results suggest that strains from the chicken reservoir may reach humans by pathways other than food (e.g. via the environment or by direct contact). All types of poultry (e.g. broilers, layers, turkeys, ducks, geese, quails, ostriches) and wild birds can be colonized with *Campylobacter*. Eggs consumption does not contribute to the human campylobacteriosis problem as *Campylobacter* is rarely, if ever, transmitted vertically. The proportion of Campylobacter-positive samples (single or batch) of fresh pig or fresh bovine meat was generally low (15, 16).

4.2 Path of infection

Routes of transmission flowing through the environment, farm animals and wild animals through to humans interact in complex ways. These interactions would be driven by factors such as the defecation of wild birds or farm animals, water flow due to climatic

conditions, spread by flies and other complex ecological parameters. The infective dose of this bacteria is low (<500 cells). Zoonotic transmission of Campylobacter spp. to human occurs primarily through the consumption and handling of livestock, with poultry being the most common source. However other infection routes, including the natural environment, may also contribute (7).

Campylobacter does not multiply outside a warm-blooded host (e.g. on meat samples) because of the absence of micro aerobic conditions and no permissive temperatures. The amplification vessel, and therefore the reservoir, of *Campylobacter spp*. are warm-blooded animals, including food-producing animals (cattle, sheep, pigs and poultry), wildlife and domestic pets (3).

4.3 Responsiveness

Guillain-Barré syndrome (GBS) is a post infectious neuropathy most frequently caused by Campylobacter jejuni. Lipo-oligosaccharides (LOS), expressed by *C. jejuni* induce antibodies that cross-react with self-glycolipids in peripheral nerves, causing neuropathy (1, 14)).

4.4 Resistance to the environment

Campylobacter grows optimally at 37–42 °C but cannot tolerate drying and is unable to grow in atmospheric levels of oxygen, requiring instead conditions with reduced oxygen levels (5–10% v/v) but raised carbon dioxide levels (5–10% v/v). *Campylobacter* is sensitive to many external physical conditions like low water activity, heat, UV light and salt. Although generally considered to be poor survivors outside of their animal hosts, some *C. jejuni* appear to be able to survive and persist in environmental niches and *Campylobacter* employs a number of strategies enabling it to survive in a wide range of environment (catalase, protein as Cj1386 an ankyrin-containing protein involved in the same detoxification pathway as catalase, etc.) (13).

Natural environment (soil and water) plays a key role in transmission, either directly to humans or indirectly via farm animals. Biofilm formation, the viable but non cultivable state, and interactions with other microorganisms can all contribute to survival outside the host. It has been shown that *Campylobacter* can survive for as long as 7 months in phosphate-buffered saline at 4 °C, with cellular integrity and respiratory activity being maintained for much longer than cultivability. Survival in water was temperature dependent, with *Campylobacter* generally surviving much better at low temperatures (10–16 °C) compared with room temperature. Similarly, different *C. jejuni* strains from various origins exhibited origin-dependent ability to survive in sterilized drinking water (18).

Campylobacter jejuni strains can also survive for long periods in well water. Cold temperatures (4-10 º C), darkness and a moist atmosphere support the survival of the organism. The conditions that poultry meat is stored at for retail are ideal for its survival. *Campylobacter* species do not tolerate drying or heating but can often survive for a time in

moist environments. *Campylobacter* can survive for weeks in water at 4°C (39°F), but only a few days in water above 15°C (59°F). *C. jejuni* may remain viable for up to 9 days in faeces, 3 days in milk and 2 to 5 days in water. *C. jejuni and C. coli* can remain infective in moist poultry litter for prolonged periods. *C. fetus* can survive in liquid manure for 24 hours and soil for up to 20 days (18).

4.5 Geographical distribution

C. jejuni, C. coli and *C. fetus* infections are spread worldwide. Recent studies investigated urban–rural differences in campylobacteriosis incidence in Germany, Canada, the Netherlands, Scotland, Denmark and Sweden. Some of these studies found higher incidences in rural environments and other in urban and urbanized environments. Recent studies suggested the higher incidence of campylobacteriosis in urban and urbanized areas related to higher consumption of ready-to-eat foods.

5. Pathogenesis

Campylobacter is the most common cause of acute bacterial gastroenteritis worldwide. The infective dose of this bacteria is low (<500 cells). The typical incubation period is from 2 to 4 days. Campylobacter colonized the intestines and get entrance keen on the mucus layer of the intestinal cells (4).

The mechanism of pathogenesis of *Campylobacter* comprises four main stages: adhesion to intestinal cells, colonization of the digestive tract, invasion of targeted cells, and toxin production. To initiate infection, the organism must penetrate the gastrointestinal mucus, which it does by using its high motility and spiral shape. The bacteria must then adhere to the gut enterocytes and once adhered can then induce diarrhoea by toxin release. *C. jejuni* releases several different toxins, which vary from strain to strain, mainly enterotoxin and cytotoxins, and these correlate with the severity of the enteritis. During infection, levels of all immunoglobulin classes rise. Of these, IgA is the most important as it can cross the gut wall. *C. jejuni* can also stimulate the cellular immune system, but this seems to play only a small role in preventing infection (7).

The capsular polysaccharide plays a vital role in bacteria survival and persistence in the environment, which contributes to its pathogenesis. There are multiple factors that have been implicated in adhesion, among them CadF which binds to host fibronectin as well as the C. jejuni polysaccharide capsule (CPS) which alters adherence in vitro. The flagella also appear to be necessary for both successful adhesion and colonization. This capsular polysaccharide (CPSs) display different structural variation misleading to the host antigens and the resistance phagocytizes and the complement- mediated killing. CPSs allow the bacterial to invade host immunity through several mechanisms (10).

C. jejuni is a unique pathogen, being able to execute N-linked glycosylation of more than 30 proteins related to colonization, adherence, and invasion. Moreover, the flagellum is not only depicted to facilitate

motility but as well secretion of Campylobacter invasive antigens (Cia). The only toxin of C. jejuni, the so-called cytolethal distending toxin (CdtA,B,C), seems to be important for cell cycle control and induction of host cell apoptosis and has been recognized as a major pathogenicity-associated factor. The flagella also appear to be necessary for both successful adhesion and colonization. Additionally, the flagellar components function as a type III secretion system and secrete a number of proteins through a central filament, including Cia (Campylobacter invasion antigens) proteins. The flagella proteins FlaC and FspA have been found to be important in the modulation of the invasion of epithelial cells in different experimental systems (21).

The best characterized virulence factor *of C. jejuni* is cytolethal distending toxin (Cdt). It's a tripartite toxin that arrests intestinal epithelial cells in G2 phase of the cell cycle through the action of the active subunit CdtB which is transported to the host cell nucleus where it induces double strand breaks in DNA, a factor believed critical in increasing host pathogen contact time and is associated with IL-8 secretion from infected epithelial cells (14).

6. Clinical manifestations

In humans, *C. jejuni* and *C. coli* are associated with campylobacteriosis causing abdominal pain, sometimes bloody diarrhoea, fever, headache, nausea and vomiting. The incubation period in human's averages from two to five days. Usually infections are self-limiting and last only a few days, but some cases may require medical treatment including hospitalization. Extra-intestinal infections or post-infection complications such as reactive arthritis and neurological disorders can also occur. Occasionally, in immunocompromised patients, the pathogen can spread systemically, leading to more severe sequelae, and it is also a major predisposing cause of the peripheral nervous system disorder, Guillain-Barré Syndrome, Miller Fisher syndrome, and functional bowel diseases, such as irritable bowel syndrome. The vast majority of human cases (about 99%) are sporadic rather than outbreaks (3).

Animals rarely succumb to disease caused by these organisms. However, *C. jejuni* is considered cause of abortions in sheep. In the United States recently emerged a highly virulent clone that causes outbreaks of ovine abortions and its zoonotic nature has been recognized. *C. jejuni* and *C. coli* rarely cause disease in animals other than humans. Additional species cause reproductive disease in sheep and cattle (11).

7. Diagnostic

7.1 Clinically

Campylobacter is a common bacterial enteropathogen that can be detected in stool by culture, enzyme immunoassay (EIA), or PCR. Clinical diagnosis of enteric Campylobacter infection is established by demonstrating the organism via direct examination of faeces or by isolation of the organisms. Different techniques are used for isolation from faecal specimens. These

include growth at 42°C, use of antibiotic-containing media, and micropore filtration to keep larger bacilli from contaminating the culture. Specific types of selective media are blood-based, antibiotic-containing media such as Skirrow, Butzler, Campy-BAP, mCCDA and others. Micropore filtration is based on filters with pores small enough to prevent the passage of microbes but large enough to allow passage of organism-free fluid (12).

Filters with a pore diameter of 25 nm to 0.45 µm are usually used in this procedure, which can also be used to remove microorganisms from water and air for microbiological testing. The optimum atmosphere for *C. jejuni* growth is 85% N2, 10% CO2, and 5% O2. Antigen tests to directly detect *Campylobacter* in faecal samples are increasing for *Campylobacter* diagnosis (14).

There are no serological assays in routine use for the detection of colonization of *C. jejuni* and *C. coli* in livestock. However, antigen-capture enzyme-linked immunosorbent assays (ELISAs) have been described in the literature for all host species. A standardized assay has recently been described in humans for use in sero-epidemiological studies. A range of tests are available for testing food samples, microbiological test (ISOAFNOR, NKLM, FDA etc.) and molecular tests how PCR and RealTime PCR (4, 6).

7.2 Epidemiologic

Genotyping methods have been developed and applied to differentiate *Campylobacter* isolates and clonal spread of food poisoning pathogens between animals and humans. A number of genotyping schemes have been developed to identify the sources and route of transmission of these foodborne pathogens so that proper control measures can be developed. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed to compare their genetic relationship for correlate epidemiologically campylobacter strain isolated from different sources (5).

Some studies aimed at determining the genetic diversity of *Campylobacter* strains isolated from animal/food/environmental ruminants and study by multilocus sequence typing (MLST) their links to human isolates in same or different countries. MLST data will help to obtain a more comprehensive image of the population structure of C. jejuni and establish reliable source attribution schemes. The application of whole-genome sequencing (WGS) in *Campylobacter jejuni* epidemiology, the leading cause of bacterial gastroenteritis worldwide, is used. WGS analysis increased understanding on the evolutionary and epidemiological dynamics of this pathogen and on the WGS potential to improve surveillance and outbreak detection (3, 9).

8. Prophylaxis

Currently there is no commercially available vaccine for Campylobacter for human use. There are no effective vaccines available for the prevention of enteric *Campylobacter* colonization in birds or mammals but there are studies underway investigating approaches to develop effective vaccines.

Vaccines are not available for enteritis, but can prevent abortions in sheep. They are also useful for both prophylaxis and treatment in bovine genital campylobacteriosis; however, vaccinated cows may remain carriers. Artificial insemination can control or prevent bovine genital campylobacteriosis (2).

9. Treatment

Liquid treatment for symptoms due to campylobacteriosis and electrolyte replacement are sufficient in human infections. The use of antibiotics to treat *Campylobacter* infections is controversial. Antibiotics may be indicated if any of the following symptoms occur: high fever, bloody diarrhoea, HIV infection and other immunocompromised states, pregnancy. Macrolide antibiotics (erythromycin, clarithromycin, or azithromycin) are the most effective agents for *Campylobacter jejuni* (8,12).

Azithromycin therapy would be a primary antibiotic choice for *Campylobacter* infections, when indicated with a typical regimen of 500 mg/d for 3 days. Erythromycin is one of the classic antibiotics used, its resistance remains low, and it can be used in pregnant women and children. Fluoroquinolone antibiotics (ciprofloxacin, levofloxacin, gatifloxacin, or moxifloxacin) can also be used, but resistance to this class has been rising, at least in part due to the use of this class of antimicrobial in poultry feed. Ciprofloxacin and tetracycline are alternatives but should be avoided in young children. In addition, the use of fluoroquinolones in food animals has resulted in fluoroquinolone-resistant *Campylobacter* strains worldwide. High levels of ciprofloxacin resistance have also been reported in developing countries, with resistance ranging from 30% to greater than 70% (19).

Clindamycin is another therapeutic alternative. Patients with endovascular *C. fetus* infections require at least 4 weeks of treatment; gentamicin is believed to be the agent of choice. Treatment with ampicillin or third-generation cephalosporins is an alternative. In poultry, *Campylobacter* colonisation is asymptomatic. In chickens there is no commercial benefit for the therapeutic treatment of flocks to eliminate the colonisation (8).

Treatment of healthy animals is not recommended for several reasons: there is a high likelihood of re-exposure and there is no evidence that treatment is effective. Antibiotic treatment may not completely prevent shedding in colonized animals, though it may prevent exposed sheep from aborting during an outbreak. Bulls with bovine genital campylobacteriosis are sometimes treated (7).

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11. Q FEVER

1. Name of zoonosis

Q fever

2. Definition

Q fever is a zoonosis caused by a bacterium called *Coxiella burnetii*.

3. Etiology

3.1 Etiologic agent

Coxiella burnetii.

3.2 Taxonomy

For a long time *Coxiella burnetii* has been classified within the order of *Rickettsiales*, even if at genetic and biological level, it presents various similarities with Legionella, *Francisella* and *Rickettsiella*. Actually it has been classified within the *Coxiellaceae* family, as the unique member of the *Coxiella* genus. The use of innovative molecular methods allowed to identify numerous genotypes within the species, whose recognition was very helpful for epidemiological purposes during humans and animals' outbreak investigations (5).

3.3 Morphological description

Electronic microscope observation showed the presence of two morphologically distinct forms of Coxiella burnetii: small and big cells variants, respectively comparable to vegetative and spore-forming of *Bacillus* and *Clostridium* (5).

Thanks to the presence of a resistance form C. *burnetii* has a high stability outside the infected host. For this reason, *C. burnetii* is not exclusively transmitted by vectors, as for example for *Rickettsia* (5).

3.4 Biological cycle

Moreover, two different antigen forms were identified: one pathogenic, called phase 1, mainly presents within the infected tissues and another not virulent, called phase 2, after serial passages in cell cultures. The transition from one phase to the other is irreversible because it happens after a chromosomic deletion at LPS chains level (2).

3.5. Source of infection

The main transmission route for humans is through the exposure to aborted foetus, fluids and placenta during parturition (109 germs per gram of placenta). Transmission takes place also through tick bite. Around 40 species of ticks have been identified naturally infected by *Coxiella*. The species more frequently involved in *C. burnetii* transmission belong to *Ixodes*, *Rhipicephalus, Dermacentor* and *Amblyomma* genera of ticks. With exception of the latter, all the others are widespread in Europe (9).

Ticks are both vector and reservoir of infection due to the capacity of transovarial transmitting the infection to the progenies. The higher virulence of *C. burnetii* when transmitted by tick bite in respect to animal-to-animal direct transmission, suggests a possible amplification of the pathogen within the vector. Among all products of animal origin, raw milk represents the food at highest

risk for humans given the high concentration of *Coxiella* in this product (103 to 105 Infectious Units/ml) (6).
Coxiella burnetii persists for a long time in not-pasteurized milk products: refrigerated butter has been found infectious for 41 days after its preparation. Even soft cheese remains infectious for few weeks. The milk stored in the refrigerator is infectious for at least 3 months, whereas it is killed at 63°C for 30 minutes (6).

Animals eliminate *C. burnetii* with milk, especially in the early stage of lactation. Humans, in fact, are more at risk during animal parturition and the following days. Carcasses from serologically positive animals can contain the bacterium, albeit in a significantly lower quantity than offals and in particular uterus and udder. In any case heating at 80°C kill any *C. burnetii* in the meat. Nevertheless, butchers could be infected by aerosol and percutaneously slaughtering infected animals and manipulating highly contaminated meat and organs thereof (4).

4. Epidemiology

4.1. Responsiveness

Considering the amplification role of ticks, animals at pasture in ticks infected areas are those more exposed to the transmission of *Coxiella burnetii* from the wild cycle. In this case the infection is often completely asymptomatic apart for the abortion in pregnant animals with possible source of human contagion (1).

4.2. Resistance to the environment

Thanks to its resistance capacity, *Coxiella burnetii* has a big stability outside his vector:
in dry materials (leather, wool), in powder and food until 500 days;
in milk treated at 65°C for 30 minutes, with inactivation when the pasteurization process is applied (3).

4.3. Geographical distribution

Coxiella has a cosmopolitan distribution. To date Q fever has been notified throughout the world with the exception of New Zealand (4).

5. Pathogenesis

Researches carried out by various authors in different geographic areas identify two distinct cycles. The first one, called “domestic cycle”, mainly involves domestic ruminants. In case of abortion *Coxiella burnetii* is spread into the environment. In this cycle, man could be infected by direct exposure to abortion. Clinical symptoms are fever which can evolve in pneumonia or, in the most serious chronic forms, in endocarditis (8). (fig. 11.1.)

In the so called “wild cycle” *C. burnetii* is transmitted by ticks to susceptible animals, which further contaminate the environment through the elimination of the bacterium with faeces. In both cycles, wild or domestic carnivores (shepherd dogs) can also be infected either by ticks or by ingestion and inhalation of infected products. Once infected, they can spread *Coxiella burnetii* during parturition, or infecting other ticks (8).

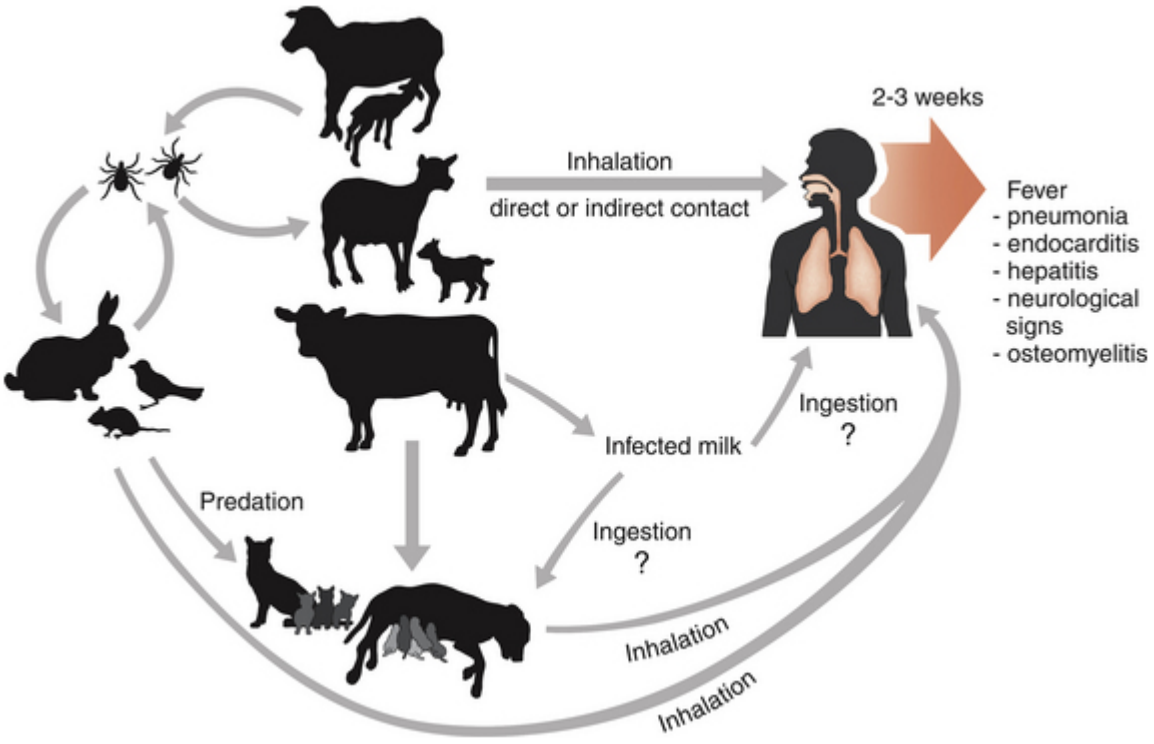

Fig. 11.1. Domestic cycle of *Coxiella burnetii* (www.microbenotes.com)

6. Clinical manifestations

Affected persons can develop a high fever with headache, muscle pains, sore throat, nausea and vomiting, chest and stomach pains. The fever can last for one or two weeks, and lead to pneumonia or affect the liver. Treatment involves long term antibiotic therapy (1).

7. Diagnostic

7.1 Clinical

In humans, Q fever is mainly an occupational disease and it occurs on higher percentage in breeders, veterinarians, laboratory technicians, slaughterers, stingers, carders and tanners. Unlike what happens in wild animals, ticks usually play a marginal role in human infections. The diagnosis takes place on a clinical basis. In Italy, two outbreaks, in 1987 and 1988 respectively, were reported in a farm employing people under drug addiction rehabilitation programmes. In this case, the data analysis showed a greater prevalence of infection in HIV-positive individuals. In all 235 cases the source of the infection was related to the contact with infected sheep.

In ruminants' serological examination is not useful for identifying the single infected animals, but it can be used for the detection of infected herds (12).

Animals, following an acute infection, are seropositive for several years. Some of them

may eliminate *C. burnetii* before showing any clinical sign, while in some others the infection occurs completely asymptomatic. Therefore, for proper diagnosis it is not enough the use of serology, but it is necessary to find the presence of the parasite or its DNA in the secretions (vaginal mucus, milk) or faeces of infected animals. For the isolation of *C. burnetii,* the richest tissues and organs are the aborted foetuses, placenta and vaginal swabs taken after the abortion or parturition. Milk samples (either mass or individual), colostrum or stool can be also used as diagnostic material. Being samples coming from farms with abortions, the sampling, packing and transportation of this material must follow strict biosecurity procedures (3).

7.2 Epidemiological

Q fever in humans is mainly associated with animal parturition, since this is the time of highest dispersion of *C. burnetii* in the environment. In addition, the persistence of dry and windy weather conditions has often been associated with the onset of human infection. Such conditions, in fact, on one hand, encourage the survival of resistant forms of the bacterium, and at the other the dissemination of the germ and therefore the contamination by air. Similar conditions were considered in the outbreak occurred in the Netherlands together with the transmission through contaminated food (4, 11).

7.3 Pathological

In the animals' Q fever causes a persistent chronic infection with localization of *C. burnetii* in the lymph nodes of the reproductive system and udder. During birth and abortion and during lactation there is a strong shed of *C. burnetii* into the environment. No specific or pathognomic lesions can be observed. Frequent lesions are: endometriosis and retention of placenta with a purulent necrotic placentite, characterised by a white-yellowish exudate around the cotyledons (5, 10).

8. Prophylaxis

Direct. Because of the complexity of the biological cycle, in order to prevent the Q-fever an effective control action is required at different levels (7, 14):

- serological screening to detect any infected herds;
- fight against ticks and tick infestations;
- elimination of animals proven to be infected by *Coxiella burnetii*;
- safe removal and destruction of foetuses and other products of abortion;
- thermal processing of milk.

Indirect

In the laboratory, strict controls are needed and *C. burnetii* has to be handled under biosafety level 3 standards, as outlined in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (7). Numerous vaccines against Q fever have been developed for animals. Currently, the available studies show up that vaccines using *C. burnetii* in Phase 1 are 100 times more effective than those using the same pathogen in Phase 2. Since 2014 it is available a cow and goat vaccine (Coxevac) containing *Coxiella burnetii* in Phase 1. Studies

on cattle and goats showed that the use of the vaccine reduces the spread of the microorganism (which is the main factor for disease spread) through vaginal secretions and milk. While in goats the vaccine has reduced the spread of the germ in the stools as well as in the placenta. Studies on goats also revealed a lower percentage of abortions in vaccinated goats than unvaccinated. The protection lasts for 280 days in bovine and 1 year in goats. The massive use of vaccine has been considered as the reduction of Q fever in Slovakia (7, 13).

9. Treatment

In case of acute form in humans, the treatment foresees (7):

- Doxiciclin (1st choice) - Highest effectiveness if started within 3 days after his manifestation
- Standard Posology: 100 mg per os, each 12 h, for 15-21 days
- Chinolonics (2nd choice) - In case of relapse, it's necessary to restart the therapy.

In chronic forms, endocarditis is hard to fight and the treatment foresees:

- long period anti biomedic treatments
- doxiciclin in association with chinolonics, for less than 4 years
- doxiciclin in association with hydroxychloroquine, for 1, 3-5 years
- Less is the percentage of relapse
- Oftamological exams are needed in order to check chloroquine accumulation
- Surgical removal of damaged valves may be required

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Source: ewn.co.za/2018/01/19/officials-urge-residents-not-to-panic-after-listeria-strain-found-in-food-outlet

12. LISTERIOSIS

1. Name of zoonosis

Listeriosis

2. Definition

Listeriosis is a foodborne zoonotic disease caused by bacteria Listeria *monocytogenes*.

3. Etiology

3.1 Etiologic agent and taxonomy

In the family *Listeriaceae*, the genus *Listeria* is constituted of 17 species, belonging to 4 different taxa. Taxa 1: *L. monocytogenes, L. marthii, L. innocua, L. welshimeri, L. ivanovii, L. seeligeri.* Taxa 2: *L. grayi. Taxa 3: L. fleischmannii, L. floridensis, L. acquatica*. Taxa 4: *L. newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia, L. booriae. L. monocytogenes* has 13 serotypes, including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7. Among them, serotypes 1/2a, 1/2b, and 4b have been associated with the vast majority of foodborne infections and animal disease. No association between serovar and animal species has been established (11).

The infectious disease caused by these bacteria is known as listeriosis. *L. monocytogenes* causes serious localised and generalised infections in humans and a variety of other vertebrates, including domesticated and wild birds and mammals. Animal listeriosis is mainly a result of infection transmitted by *Listeria monocytogenes* and *L. ivanovii*. Some possible cases of human disease caused by *L. seeligeri* have been described, including a human meningitis case. *L. ivanovii* an associated to abortions in sheep and cows or to septicemia in sheep. The etiological agents of human listeriosis are mainly *L. monocytogenes* and rarely *L. ivanovii* (11).

3.2 Morphological description

L. monocytogenes is a Gram-positive rod-shaped, motile at 20-25°C, catalase and aesculin positive, facultative intracellular pathogen, non-encapsulated, aerobic or facultative anaerobic, motile by means of flagella (10).

3.3 Biological cycle

L. monocytogenes is a resistant bacterium to many stressful environmental conditions. It is salt-tolerant and can survive in temperatures below 1°C, but can also grow in these conditions, unlike many other pathogens. It is also notable for its persistence in food-manufacturing environments. The bacterium is ubiquitous in the environment and can be found in moist environments, soil and decaying vegetation (4).

4. Epidemiology

L. monocytogenes is spread worldwide and widely distributed in the environment and usually can cause a hyperacute or acute disease in humans and animals. In 2015, 28 MS reported 2,206 confirmed human cases of listeriosis. The EU notification rate was 0.46 cases per 100,000 populations, which was similar to 2014. There was a statistically

significant increasing trend of listeriosis over 2008–2015. Nineteen Member States (MS) reported 270 deaths due to listeriosis in 2015, which was the highest annual number of deaths reported since 2008. The EU case fatality was 17.7% among the 1,524 confirmed cases with known outcome (9). Listeriosis infections are most commonly reported in the elderly population in the age group over 64 years old and particularly in the age group over 84 years. The proportion of cases in the over 64 age group steadily increased from 56% in 2008 to 64% in 2015 (3). In the period 2008–2015, a seasonal pattern was observed in the listeriosis cases reported in the EU/EEA countries, with large summer peaks followed by smaller winter peaks (2). Despite the significant increasing trend ($p < 0.01$) over this period, the number of cases stabilized in 2015. Twelve Member States (France, Germany, Greece, Hungary, Malta, the Netherlands, Poland, Romania, Slovakia, Slovenia, Spain and Sweden) had increasing trends ($p < 0.01$) since 2008. None of the MS observed any decreasing trend between 2008 and 2015 (3).

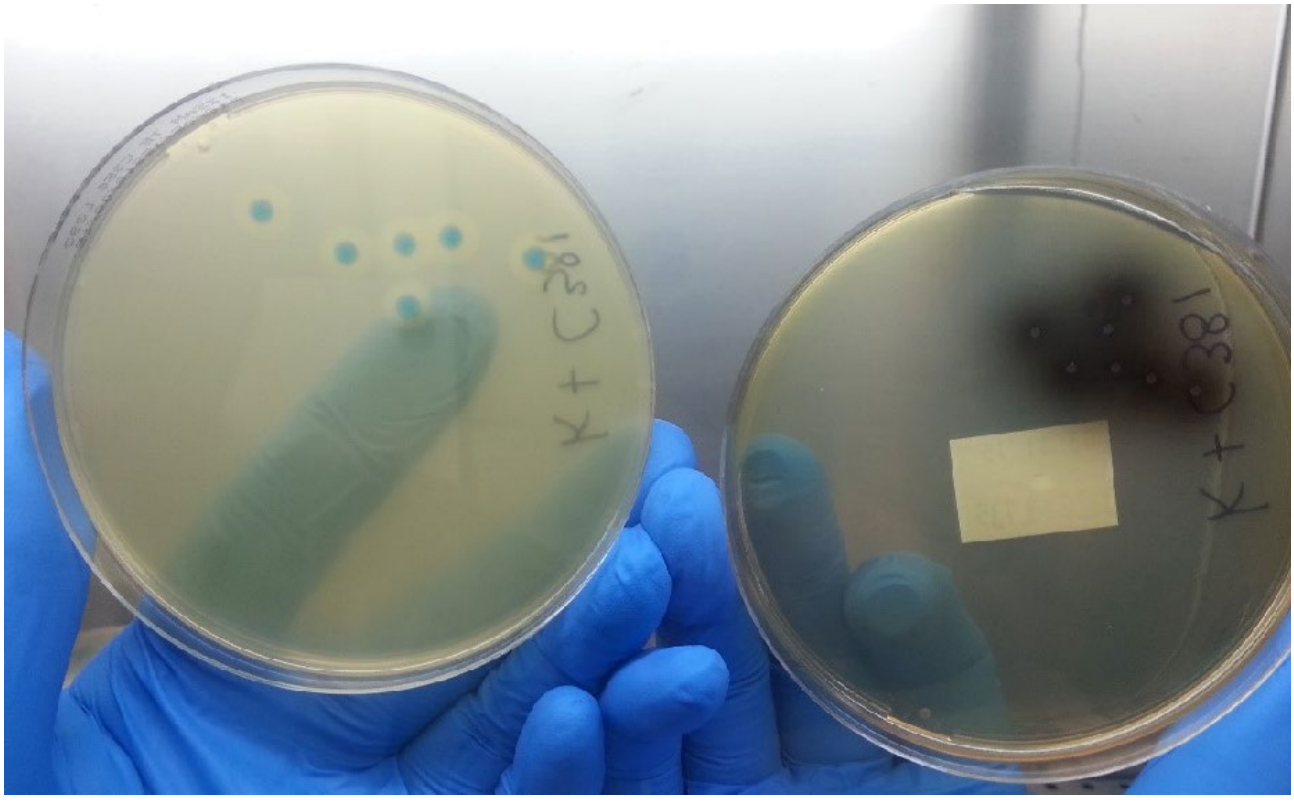

Fig. 12.1. *Listeria monocytogenes* colonies on ALOA agar (left side) and Modified Oxford agar (right side)

A wide variety of domestic and wild mammals, birds, fish, crustaceans and insects carry L. monocytogenes asymptomatically in the digestive tract. Numerous mammals are susceptible to Listeria: ruminants, rodents, lagomorphs, more rarely horses, pigs and carnivores. In wild mammals it has been found in fallow deer (*Dama dama*), roe deer

(*Capreoleus capreoleus*), red deer (*Cervus elaphus*), moose (*Alces alces*), reindeer (*Rangifer tarandus*), red fox (*Vulpes vulpes*), European brown hare (*Lepus europaeus*), mountain hare (*Lepus timidus*), red squirrel (*Sciurus vulgaris*), Japanese wild boar (*Sus scrofa leucomystax*), scimitar-horned oryx (*Oryx dammah*), Colobus monkeys (*Colobus guereza*) western capercaillie (*Tetrao urogallus*), Willow Ptarmigan (*Lagopus lagopus*) white stork (*Ciconia ciconia*), Common pheasant (*Phasianus colchicus*) and in 22 different species of birds (such as chicken, turkey, duck, canary, goose, faison, pigeon, partridge, grouse, eagle) (5).

1.1 Source of infection

The biological cycle of *Listeria* starts from the soil (pH> 5, 6), usually contaminated by infected or carrier animals. The source of infection for animals is usually represented by feed and water except in case of transplacental transmission. Infected animals can shed *L. monocytogenes* in faeces, milk and uterine discharges. It has also been found in aborted fetuses and occasionally in the nasal discharges and urine of symptomatic animals (10).

In ruminants, listeriosis (also called circling disease) typically occurs after the consumption of contaminated silage or other contaminated feed. The bacterium enters the body through an opening in the mucosa of the oral cavity, it reaches the bloodstream and disseminate to the brain where it multiplies and causes inflammation. The incubation period for encephalitis in ruminants' ranges from 10 days to 3 weeks rather than incubation period for septicemia and abortions which appears after one day or more. Mainly acquired by ingestion, *Listeria* can also spread by inhalation or direct contact. Venereal transmission might also be possible. Vertical transmission is the usual source of infection in newborn human infants and ruminants; infections are transmitted transplacentally or during the delivery (9, 10).

Human infection usually occurs, through contaminated food, including raw meat and fish, unpasteurized dairy products and uncooked vegetables. *L. monocytogenes* has also been found in ready-to-eat products contaminated after processing (soft cheeses, deli cold cuts, sliced or grated cheese and ice cream). One study estimates that 90% of cases are foodborne (Lake et al., 2000). Other reports describe foodborne transmission as the primary source of human infections. Alternative routes include infections acquired in hospital and occupational exposure (e.g. veterinarians). Low contamination frequency in game meat (Chamois, roe deer, red deer and wild boar) has been detected. Veterinarians and breeders can also be infected by direct contact with infected animals during calving, lambing or necropsies (6).

5. Pathogenesis

The pathogenesis of *L. monocytogenes* is unique, because the organism is able to spread directly from cell to cell in the host, rather than having to "travel" interstitially to reach other cells. Once the bacterium enters

the host's monocytes, macrophages, or polymorphonuclear leukocytes, it can reproduce, and it is bloodborne. Groups of proteins on the *L. monocytogenes* cell surface enable it to survive in phagocytic cells and enhance its cell-to-cell spread. The infective dose of *L. monocytogenes* is undetermined, but believed to vary in relation to strain virulence and susceptibility of the host. The food matrix involved also could affect the dose-response relationship. In human cases associated with raw or inadequately pasteurized milk, for example, it is likely that fewer than 1,000 cells may cause disease in susceptible individuals. As noted, however, the infective dose may vary widely and depends on a variety of factors (5).

1.3 Resistance to the environment

It is widespread in the environment including soil, vegetation, water and sewage. Has been isolated from dairy environments (e.g. water used to wash cheese prior to ripening, cheese ripening rooms) and domestic environments.
The environmental persistence and resistance of these bacteria, coupled with the high mortality rates in humans, makes safe food handling paramount to ensure public health (8).

1.4. Geographical distribution

Worldwide, with sporadic incidence.

6. Clinical signs

Listeriosis is usually characterised by encephalitis, abortions or septicemia. Clinical disease is reported most often in ruminants. Occasional cases occur in rabbits, guinea pigs, pigs, dogs, cats, poultry, canaries, parrots and other species. Clinical cases in wildlife have been reported in fallow deer, reindeer and roe deer. *Listeria spp*. seems absent from seawater fish but is present in freshwater species such as rainbow-trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*). In humans is associated in most cases with contaminated foodstuffs (11).

Ruminants: *Listeria* can cause encephalitis or meningoencephalitis, abortions and septicemia in sheep, cattle and goats. In the encephalitic form the symptoms are depression, anorexia, decreased milk production, and fever followed by neurologic signs (often unilateral) that progress to neuromuscular incoordination where animals circle in the same direction. Other progressive signs include facial paralysis with profuse salivation, ear droop, lack jaw, impaired swelling and death. The course of the disease is usually short in sheep and goats, with death as soon as one or two days. Septicemia occurs most often in newborns and young ruminants and quickly results in animal death. Localised infections can also be seen, including subclinical, acute or chronic mastitis in cattle and ophthalmitis in sheep. Listeriosis is more chronic in cattle (survival for 4 to 14 days). Abortions and stillbirths mainly occur late in gestation. In wild ruminants (fallow deer) septicemia, drooping ear on one side, circling, opisthotonus, nystagmus, serous epiphora, blepharospasm, myosis, anisocoria were reported (11).

Lagomorphs: Listeriosis can cause an acute meningoencephalitic form affecting young animals and killing them in a few days and a sub-acute form in adults characterized by a stiff neck, disorders of equilibrium and convulsions. Abortions are also observed. In rabbits *L. monocytogenes* usually causes abortion or sudden death, encephalitis is rare. Infected rabbits may also have nonspecific clinical signs.

Birds: Clinical listeriosis is rare in birds, with most cases occurring in young animals. Mortality is variable but could reach 40%. Septicemia is the most common syndrome and results in sudden death. Hyperacute deaths can be seen, sometimes without other clinical signs. No symptoms are generally noticed, except that the bird is generally prostrated, is easily captured and presents an important loss of weight (caused by anorexia), cyanosis of mucous membranes and sometimes diarrhea are observed. Meningoencephalitis is occasionally reported. In young geese, both encephalitis and sepsis can be seen concurrently. It is frequently associated with intercurrent conditions that weaken the immune system such as salmonellosis or coccidiosis.

The characteristic lesions in birds with *Listeria* septicemia are areas of myocardial necrosis and degeneration, and serofibrinous pericarditis. There may also be petechial hemorrhages in the proventriculus and heart, as well as splenomegaly, hepatomegaly, bile retention, and focal hepatic necrosis. There are no gross brain lesions in the encephalitic form (11).

Ruminants: gross lesions are absent or minimal in animals with *Listeria* encephalitis: to turbid CSF, areas of softening in the medulla oblongata, and congested meningeal vessels. The septicemic form is typically associated with necrotic foci in the internal organs, particularly the liver. Aborted foetuses may be slightly to significantly autolyzed. There can be clear or blood-tinged fluid in the serous cavities, and shallow erosions in the mucosa of the abomasum. Foci of necrosis may be found in the liver and sometimes the lung, spleen or other organs.

Swine: Listeriosis is uncommon in swine. The most common form is septicemia in young piglets, with death within 3 to 4 days. Encephalitis, abortions and conjunctivitis are also seen occasionally.

Cats and dogs: Rare cases of encephalitis or sepsis occur in cats. Septicemia and neurologic signs resembling rabies have been reported in dogs.

Humans: Symptoms of human listeriosis vary from mild flu-like symptoms, such as nausea, vomiting and diarrhoea, as non-invasive gastrointestinal illness, which generally resolves in otherwise healthy people to more serious infections, invasive form of the illness, which may cause septicemia and meningitis, and other potentially life-threatening complications (1).

Manifestations of *L. monocytogenes* infection tend to be host-dependent. In immune-competent humans, it may cause acute febrile gastroenteritis, the less severe form of the disease. In immune-suppressed population,

however, the more severe form of the disease may result in sepsis and spread to the nervous system, potentially causing meningitis. In elderly and immunocompromised people who develop the severe form, it usually manifests in this manner (1).

Gastroenteritis caused by *L. monocytogenes* has a relatively short incubation period, from a few hours to 2 or 3 days. The severe, invasive form of the illness can have a very long incubation period, estimated to vary from 3 days to 3 months. Pregnant women, who are disproportionately affected *with L. monocytogenes*, may experience mild, flu-like symptoms. One-third of confirmed cases of maternal-foetal *L. monocytogenes* infections lead to abortion or stillbirth. Otherwise healthy people might have mild symptoms or no symptoms if infected with *L. monocytogenes,* while others may develop fever, muscle aches, nausea and vomiting, and, sometimes, diarrhoea. When the more severe form of the infection develops and spreads to the nervous system, symptoms may include headache, stiff neck, confusion, loss of balance and convulsions. The duration of symptoms generally depends on the health status of the infected person. The symptoms can last from days to several weeks (1, 11).

Histological lesions

The histopathology is characteristic of the disease, consisting of foci of inflammatory cells with adjacent perivascular cuffing, predominantly of lymphocytes and histiocytes, plasma cells and occasional neutrophils. The microabscesses in the brain stem often more severally affect one side of the brain. More extensive malacic pathology may occur. The medulla and pons are most commonly involved. In the septicaemic form, multiple foci of necrosis in the liver and, less frequently the spleen, may be noted protracted in cattle (10).

7. Diagnostic

Listeriosis must be differentiated from abortion diseases, from pregnancy toxemia in ewes or ketosis in cattle, bovine spongiform encephalopathy, thromboembolic encephalitis, polioencephalomalacia sporadic bovine encephalomyelitis, lead poisoning, rabies, brain abscesses, coenurosis. *L. monocytogenes* can be isolated and identified from blood or cerebrospinal fluid (CSF), nasal fluids, organs and tissues of affected animals. Serology is not routinely used for diagnosis (7, 11).

Recommended diagnostic method(s) and preferred samples.

Microbiological examination by enrichment, culture on solid agar plate of samples and identification by means of appropriate morphological, physiological, biochemical and molecular typing tests including whole genome sequencing (WGS) (11).

Culture and identification of the agent: Isolation of the organism by direct plating is relatively easy, as in the case of septicemic form of the disease, but isolation is difficult when the organism is present in low numbers, as in the case of the encephalitic form or when samples are heavily contaminated. (Fig.12.2.)

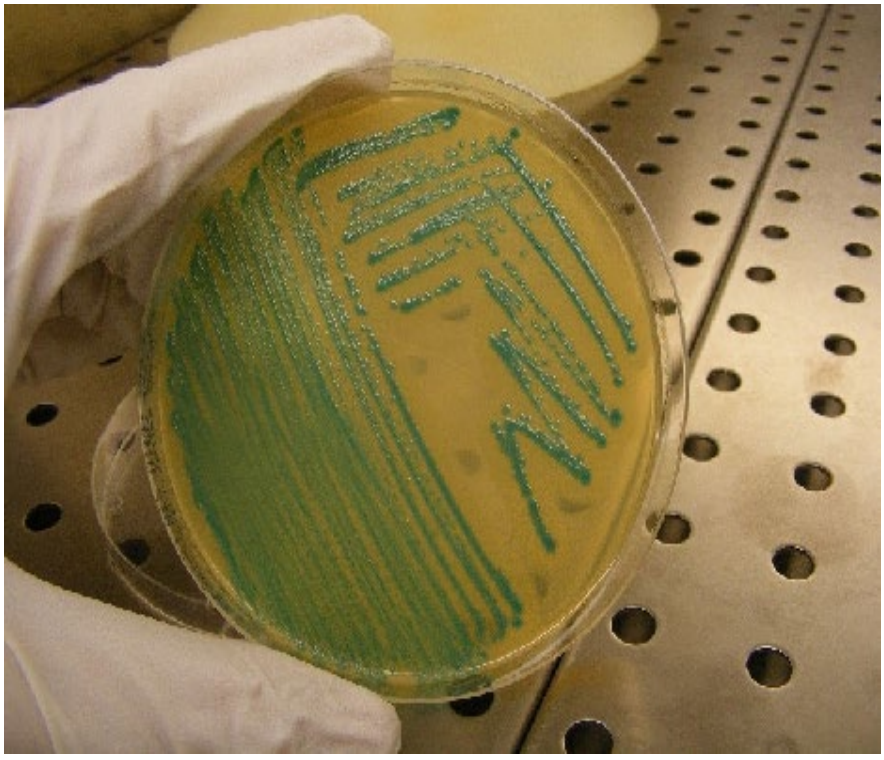

Fig.12.2. Culture of *Listeria monocytogenes*

Immunohistochemical detection of *L. monocytogenes* antigens in formalin-fixed tissue has proven to be more sensitive than direct plating for the diagnosis of the encephalitic form of the disease in ruminants. Samples: In liver, kidneys or spleen in the case of the septicemic form; spinal fluid, pons and medulla in the case of the rhombencephalitic form; and placenta (cotyledons), foetal abomasal contents or uterine discharges in the case of abortion (10).

Refrigeration temperatures (4 ± 3 °C) must be used for handling, storing and shipping specimens. If the sample is already frozen, it should be kept frozen until analysis.

The protocol recommended for isolation of *L. monocytogenes* from animal necropsy material is described in Chapter 2.9.7. of L. monocytogenes OIE Terrestrial Manual 2014, point 1.1.1 (8).

Point 1.1.2 reports the description of an alternative protocols, used for isolation from feces, silage and placental envelop. The first phase of isolation has to be finalised with the conventional identification methods, as biochemical tests, classical or molecular serotyping, DNA macro restriction (PFGE), multi-locus sequence typing (MLST) and Rapid identification methods based on polymerase chain reaction (PCR). Subtyping of the isolates including next generation sequencing (NGS) can be useful in outbreak investigations,

environmental tracking and public health surveillance (8).

Serological tests (ELISA, complement fixation and micro agglutination) have been found largely unreliable, lacking sensitivity and specificity. Many healthy animals have high levels of anti-Listeria antibodies, and cross-reactions occur with enterococci, Staphylococcus aureus and other organisms (11).

8. Prophylaxis

Control measures should be applied at farm and food-processing level, in order to prevent contamination of animals and during the processing food products.

It should be very useful to provide appropriate information to consumers through appropriate risk communication campaigns in order to communicate recommendations for people at higher risk, including pregnant women, older adults, and people with weakened immunity and Recommendations for everyone to avoid risky food categories (10).

9. Treatment

All clinical presentations are treatable with prolonged courses of antibiotics, but the prognosis of the most serious invasive infections is poor (10).

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Source: phys.org/news/2013-05-environment-patterns-emerge-tracks-coli.html

13. ENTEROHAEMORRHAGIC E COLI

1. Name of zoonosis

Enterohaemorrhagic *E. coli* (EHEC)

2. Definition

A strain of *Escherichia coli* that causes haemorrhage in the intestines. The organism produces shiga-toxin, which damages bowel tissue, causing intestinal ischemia and colonic necrosis. Symptoms are stomach cramping and bloody diarrhoea. An infectious dose may be as low as 10 organisms. Spread by contaminated beef, unpasteurized milk and juice, sprouts, lettuce, and salami, as well as contaminated water, the infection can be serious although there may be no fever. Treatment consists of antibiotics and maintenance of fluid and electrolyte balance. In advanced cases, surgical removal of portions of the bowel may be required.

3. Etiology

3.1 Etiologic agent

Verocytoxin-producing *Escherichia coli* (VTEC), also known as Shiga-toxin producing *E. coli (STEC)*, is a food-borne zoonotic agent associated with outbreaks worldwide that poses a serious public health concern (14, 19).

3.2 Taxonomy

Domain –*Bacteria*, Phylum –*Proteobacteria*, Class – *Gammaproteobacteria*, Order – *Enterobacteriales*, | Fam. *Enterobacteriaceae*, Genus – *Escherichia*, species –*E. coli*, subspecies –*O157:H7,* prototype strain – *EDL93* (1).

3.3 Morphological description

Escherichia coli serotype *O157:H7* is a mesophilic, Gram-negative rod-shaped (Bacilli) bacterium, which possesses adhesive fimbriae and a cell wall that consists of an outer membrane containing lipopoly-saccharides, a periplasmic space with a peptidoglycan layer, and an inner, cytoplasmic membrane. Some strains are piliated and capable of accepting and transferring plasmid to and from other bacteria. Though it has extremely simple cell structure, with only one chromosomal DNA and a plasmid, it can perform complicated metabolism to maintain its cell growth and cell division. *E. coli* possesses operons for transport and utilization of sucrose, urease, and sorbose, but the sorbose operon is disrupted by the insertion of a Mu-like phage. The strain also possesses a glutamate-fermentation system and two aromatic acid degradation systems that are not present in *E. coli* K-12 (2, 8).

4. Epidemiology

4.1 Source of infection

Escherichia coli can be commonly found in lower intestines of human and mammals and help with digestion processes. However, different strains of E. coli like E. coli O157:H7 is one of the most infective strains that can cause food poisoning. E. coli O157:H7 is found in the intestines of healthy cattle and are used as reservoir. The Shiga toxin released from *E. coli* requires highly specific receptors on the cells' surface in order to attach and enter the

cell; species such as cattle, pig, and deer which do not carry these receptors may harbour toxigenic bacteria without any ill effect, shedding them in their faeces. Shiga toxin is the main virulence factor of *E. coli O157:H7* infection. Implicated foods are typically those derived from cattle (e.g., beef, hamburger, raw milk); however, the infection has also been transmitted through contact with infected persons, contaminated water, and other contaminated food products. Most available information on STEC relates to serotype *O157:H7*, since it is easily differentiated biochemically from other *E. coli* strains. The reservoir of this pathogen appears to be mainly cattle. In addition, other ruminants such as sheep, goats, deer are considered significant reservoirs, while other mammals (such as pigs, horses, rabbits, dogs, and cats) and birds (such as chickens and turkeys) have been found infected. *E. coli O157:H7* is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods, as well as cross-contamination during food preparation (with beef and other meat products, contaminated surfaces and kitchen utensils), will also lead to infection. Examples of foods implicated in outbreaks of *E. coli O157:H7* include undercooked hamburgers, dried cured salami, unpasteurized fresh-pressed apple cider, yogurt, and cheese made from raw milk. An increasing number of outbreaks are associated with the consumption of fruits and vegetables (including sprouts, spinach, lettuce, coleslaw, and salad) whereby contamination may be due to contact with faeces from domestic or wild animals at some stage during cultivation or handling. STEC has also been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. Waterborne transmission has been reported, both from contaminated drinking-water and from recreational waters. Person-to-person contact is an important mode of transmission through the oral-faecal route. An asymptomatic carrier state has been reported, where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of STEC is about 1 week or less in adults, but can be longer in children. Visiting farms and other venues where the general public might come into direct contact with farm animals has also been identified as an important risk factor for STEC infection (7).

4.2 Path of infection

The EHEC “cycle” in the environment is probably driven primarily by bovine faecal contamination, which contributes to widespread faecal contamination of the agricultural environment and, hence, can affect a wide variety of raw foods. In addition, many wild animals and birds are also now known to be carriers of verotoxin-producing E. coli (VTEC) pathogenic to humans, and they perpetuate the reservoir of these organisms in the environment and add to the sources of contamination of the raw food supply (9).

4.3 Responsiveness

The attack rate of persons exposed to *E. coli 0157:H7* is 2-50%. An estimated 265,000 Shiga-toxin positive infections occur each year in the United States, of which 36% are caused by *E. coli 0157:H7*. Persons who are infected can develop a range of symptoms, including mild diarrhoea or no symptoms at all. Usually the first symptom is non-bloody diarrhoea, abdominal cramps and pain, and fever of short duration. Vomiting occurs in about one-half of patients during this first phase of infection. In the following 1-2 days the diarrhoea becomes bloody. This stage of infection lasts for 4-10 days. Normally the disease is self-limiting. However, in 5-10% of cases, primarily in children and the immuno-compromised, the patients develop a potentially life-threatening complication, HUS. The leading cause of renal failure in children is HUS and often requires dialysis. There is a 3-5% incidence of mortality with HUS. EHEC also can cause neurological complications in 25% of HUS patients, including seizure, stroke and coma. Patients with bloody diarrhoea symptoms should seek medical attention (11, 17).

4.4 Resistance to the environment

E. coli O157:H7 can survive and persist in numerous environments such as soil, water, and food as well as in animal reservoirs. *E. coli O157:H7* has been shown to survive for a year in manure-treated soil and for 21 months in raw manure that had not been composted. Composting manure is effective in destroying *E. coli O157:H7*, if the temperature is maintained above 50°C for 6 days. *E. coli O157:H7* can survive for a long time in water, especially at cold temperatures. Water trough sediments contaminated with bovine faeces can serve as a long-term (>8 months) reservoir of *E. coli O157:H7*, and the surviving bacteria in contaminated troughs is a source of infection (3). Barker et al. showed that *E. coli O157:H7* survives and replicates in *Acanthamoeba polyphaga*. *A. polyphaga* is a common environmental protozoan that is widely distributed in soil, water, and faecal slurry. Thus, it can be an efficient transmission vehicle of *E. coli O157:H7* in these environments (4, 7).

4.5 Geographical distribution

EHEC O157:H7 infections occur worldwide; infections have been reported on every continent except Antarctica. Other EHEC are probably also widely distributed. The importance of some serotypes may vary with the geographic area (4).

5. Pathogenesis

After passage through the acidic barrier, EHEC forms attaching and effacing (A/E) lesions on the mucosal epithelium at the RAJ, allowing for its colonization at the RAJ. A/E lesions are characterized by destruction of microvilli, intimate attachment of the bacteria to the cell, and accumulation of polymerized actin beneath the site of bacterial attachment to form a pedestal-like structure cupping individual bacteria (11).

6. Clinical manifestations

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7. Diagnostic

7.1 Clinically

Symptoms of the diseases caused by STEC include abdominal cramps and diarrhoea that may in some cases progress to bloody diarrhoea (haemorrhagic colitis). Fever and vomiting may also occur. The incubation period can range from 3 to 8 days, with a median of 3 to 4 days. Most patients recover within 10 days, but in a small proportion of patients (particularly young children and the elderly), the infection may lead to a life-threatening disease, such as haemolyticuraemic syndrome (HUS). HUS is characterized by acute renal failure, haemolyticanaemia and thrombocytopenia (low blood platelets). It is estimated that up to 10% of patients with STEC infection may develop HUS, with a case-fatality rate ranging from 3 to 5%. Overall, HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of HUS patients and chronic renal sequelae, usually mild, in around 50% of survivors. Persons who experience bloody diarrhoea or severe abdominal cramps should seek medical care. Antibiotics are not part of the treatment of patients with STEC disease and may possibly increase the risk of subsequent HUS (10).

7.2 Paraclinically

Diagnoses of *E. coli 0157:H7* must be reported to the Nationally Notifiable Diseases Surveillance System (NNDSS) at CDC. In all cases of acute community-acquired diarrhoea stool samples should be cultured for *E. coli* 0157:H7 on cefixime tellurite-sorbitol MacConkey agar (CT-SMAC), or CHROMagar O157. Sorbitol non-fermenting colonies should be assayed for Shiga-toxin using EIA or PCR. Sero-diagnosis of O157 and H7 antigens is necessary to confirm the isolate is *E. coli* 0157:H7. Since all *E. coli* O157:H7 strains produce Shiga toxin, identification of O157 and H7 antigens is sufficient to consider it STEC. Clinical laboratories should report and send *E. coli* 0157:H7 isolates and Shiga toxin-positive strains to state or local public health laboratories for additional characterization as

soon as possible (15). Early diagnosis of *E. coli* O157:H7 infection is important for prompt treatment since initiating parenteral volume expansion early in the course of infection is thought to improve patient outcome by decreasing the risk for renal damage (5).

7.3 Epidemiologic

Diagnoses of *E. coli* 0157:H7 must be reported to the Nationally Notifiable Diseases Surveillance System. A variety of molecular subtyping methods have been developed to improve the understanding of the epidemiology of *E. coli* O157:H7 outbreaks. These methods include pulse-field gel electrophoresis (PFGE), restriction fragment length polymorphisms (RFLP), amplified fragment-length polymorphisms (AFLP), and phage typing. Among them, the PFGE method was standardized by CDC and has been applied successfully to discriminate outbreak-associated, sporadic, or unrelated infections since 1993 (14).

7.4 Pathologically

EHEC strains, also called shiga toxin-producing *E coli* (STEC), have acquired shiga toxin, which causes cell death, edema, and haemorrhage in the lamina propria. There are two kinds of shiga-toxin (Stx-1 and Stx-2). EHEC strains can produce only one or both of the shiga toxins. The blood from the lamina propria can enter the lumen of the intestine and cause hemorrhagic colitis. Strains of EHEC that produce Stx-2 are more likely to cause kidney damage resulting in hemolytic uremic syndrome (HUS). The shiga toxin can kills glomerular endothelial cells in the kidneys this damage activates platelets and thrombin deposition. This decreased glomerular filtration and results in acute renal failure. The most common cause of hemorrhagic colitis and HUS is EHEC serotype *O157:H7*; however, *non-O157:H7* serotypes of *E coli* can produce shiga-toxin and cause hemorrhagic colitis and HUS. Shiga-toxin released by EHEC binds to endothelial cells expressing Gb3, allowing absorption into the bloodstream and dissemination of the toxin to other organs. The tissues and cell types expressing Gb3 varies among hosts, and the distribution of Gb3 targets the pathology of toxin-mediated disease to cells expressing Gb3. For example, renal glomerular endothelium expresses high levels of Gb3 in humans, and Shiga-toxin production results in acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia, all typical characteristic of HUS (6, 11).

8. Prophylaxis

E. coli 0157:H7 is transmitted by the faecal-oral route. It is most frequently associated with hamburger or other ground beef products, unpasteurized milk and fruit juices, and leafy green vegetables. Hence it is important to avoid consuming undercooked meats and unwashed vegetables. *E. coli 0157:H7* can also be transmitted via person-to-person contact. Sanitization and hand washing are important after using the bathroom or changing diapers, before preparing or eating food and after contact with animals or their environments (13).

9. Treatment

The disease usually is self-limiting and most patients recover without specific treatment within five to 10 days. Antibiotics are counter-indicated for treatment of *E. coli 0157:H7* infections as studies have shown that antibiotic treatment may increase the risk of HUS. Non-specific supportive therapy, including hydration, is essential (19).

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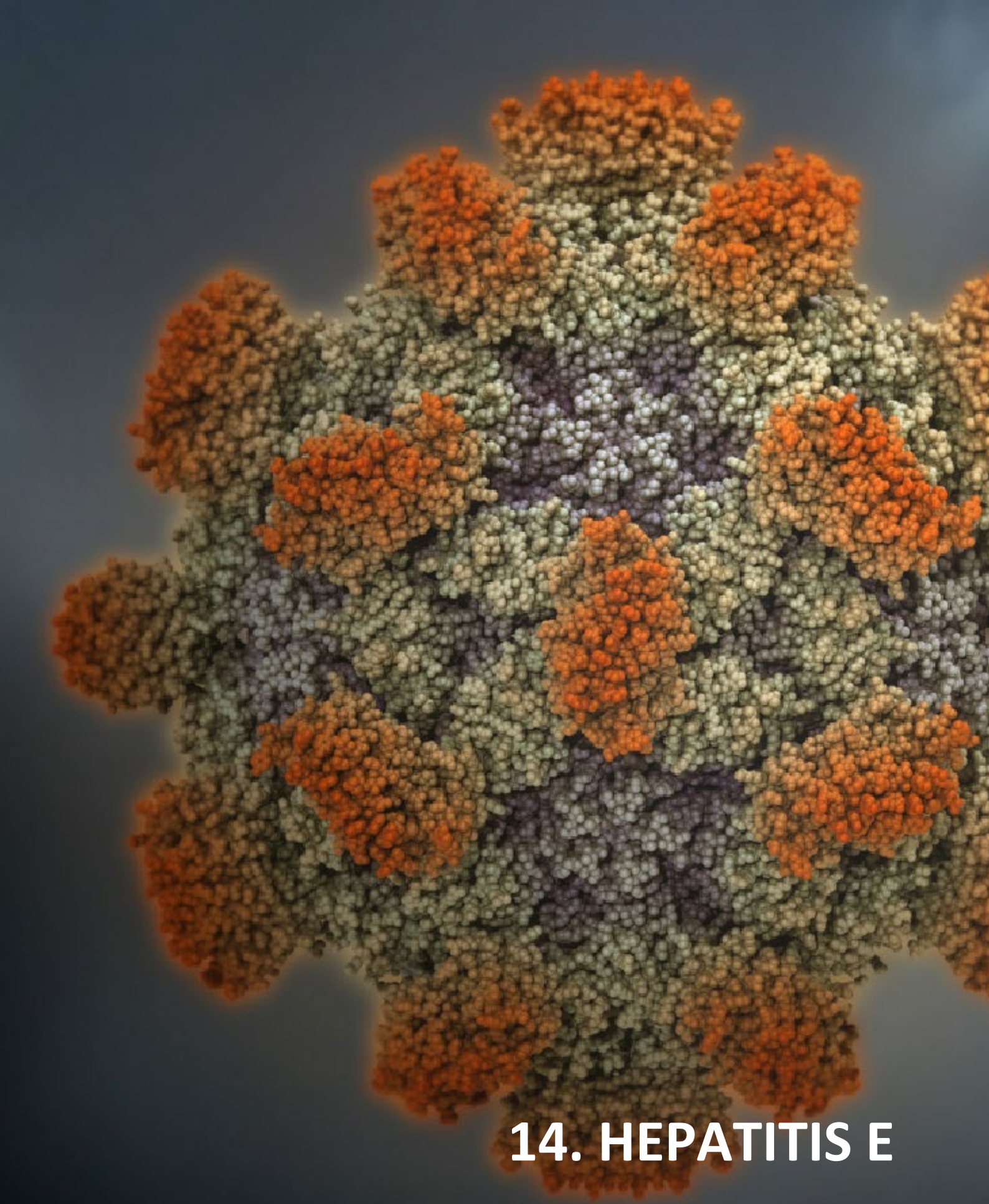

14. HEPATITIS E

Source: www.pharmamicroresources.com/2015/12/hepatitis-e-symptoms-transmission.html

1. Name of the zoonosis:

Hepatitis E

2. Definition

Liver inflammation caused by hepatitis E virus (HEV)

3. Etiology

3.1 Etiologic agent Hepatitis E virus

3.2 Taxonomy

Hepatitis E virus (HEV) is a single stranded, positive RNA virus belonging to the *Hepesviridae* family. Since the end of the 1990s, additional HEV-related agents have been identified in a large variety of animals ranging from domestic swine, wild boar, deer, rabbit, mongoose, ferret, rat and chicken to bat and cutthroat trout. Following the identification of these novel strains, a new classification has been proposed that divides the Hepeviridae family into two genera: *Orthohepesvirus* and *Piscihepevirus*. Most of the HEV strains identified so far belong to the *Orthohepevirus* genus that is divided into four species: *Orthohepevirus A, B, C* and *D.* Four main genotypes of HEV that belong to the *Orthohepevirus* A species are able to infect humans (HEV-1 to -4). Genotypes 1 and 2 (HEV-1 and HEV-2) infect only humans and are associated with large waterborne epidemics in tropical and subtropical areas. Genotypes 3 and 4 (HEV-3 and HEV-4) are present in humans and other animals, and are the main cause of autochthonous cases of hepatitis E in industrialized countries (1, 15).

3.3 Morphological description

It is a spherical, non-enveloped virus with an icosahedral symmetry. Viral particles are approximately 32 to 34 nm in diameter and composed of a capsid protein (ORF2) assembled into a highly structured multimer (60 copies) (10).

3.4 Biological cycle

The life cycle of HEV is poorly understood, largely because of the nonavailability of efficient in vitro culture methods or small animal models of infection. The viral particles are concentrated on the surface of hepatocytes, bind a specific yet uncharacterized receptor and are internalized. The virus then uncoats to release genomic RNA that is translated in the cytoplasm into nonstructural proteins. RNA-dependent RNA polymerases replicate the positive-sense genomic RNA into negative-sense transcripts; the latter then act as templates for the synthesis of a 2.2-kb subgenomic RNA as well as full-length positive-sense transcripts. The positive-sense subgenomic RNA is translated into ORF2 and ORF3 proteins. The ORF2 protein packages the genomic RNA to assemble new virions, while the ORF3 protein may optimize the host cell environment for viral replication. The ORF3 protein is also associated with endomembranes or plasma membranes and may aid in viral egress. Recent studies suggest that mature virions excreted from the liver into the circulation are enveloped by the ORF3

protein and lipids, which are subsequently removed through a process that is not understood at present, to resume a fresh infection cycle (12).

4. Epidemiology

4.1 Source of infection

The source of hepatitis E virus is considered to be water or food contaminated by human faeces. Recently, Meng et al (1997) isolated a HEV strain from the feces and sera of pigs from herds in the midwestern United States which was closely related, but not identical, to human HEV strains. Seropositivity to HEV was demonstrated in 79% (192/243) of pigs 2 months old. The implication of the findings obtained by Meng and coworkers suggest that HEV is a zoonotic virus with far-reaching ramifications in terms of hepatitis E epidemiology and infection (6).

4.2 Path of infection

The hepatitis E virus is spread in a way similar to hepatitis A, known as 'faecal-oral' transmission. This means that the virus is passed out in bowel motions (faeces) and finds its way into the mouth (orally), usually through contaminated food or water. This is one of the reasons why it is important to wash your hands after going to the toilet. The illness does not usually spread easily within families, except when all members of the family have been drinking the same infected drinking water and/or contaminated food (2).

In European countries, such as the UK, the illness can also be caused by what is known as 'zoonosis'. This means the virus can be found in animals such as pigs, wild boar, deer, rabbits and rats. It does not cause the animals any illness, however, the virus can sometimes be passed from the animal to humans. One way this can happen is by eating raw or undercooked meat. In most cases the source and route of infection is unknown. Widespread outbreaks of the virus can occur frequently or constantly in overseas countries (referred to as 'endemic areas') where water supplies are contaminated with sewage after monsoons and flooding. Unlike hepatitis B, C or D, there is no evidence of the hepatitis E virus being transmitted through sharing needles, bodily fluids or through sexual contact. However, there is a risk of transmission if there is mouth contact with the anal area. There have also been a number of cases reported where hepatitis E has been transmitted through blood transfusions and organ transplants (6).

4.3 Responsiveness

Host cell injury in a viral infection may be mediated by either a direct effect of the infectious agent or indirectly through the antiviral host immune response, or through a combination of these. The direct cytopathic effect of HEV has been difficult to study because the virus has not been cultured efficiently in vitro. Immune responses, both humoral and cellular, can play a role in the pathogenesis of viral infections (5).

The humoral response consists of antibodies that may neutralize the virus; for instance, antibodies to hepatitis B virus (HBV) can neutralize the virus and play an important role

in recovery from acute HBV infection. In HEV infection, both IgM and IgG antibodies appear concomitantly with the development of jaundice, and persist for variable periods of time. Although these antibodies appear to have neutralizing activity, their longevity and exact role in protection against HEV reinfection remain unclear (13).

4.4 Resistance to the environment

Virus is resistant to heating at 56 °C for 1 h; however, it is susceptible to boiling and frying for 5 min and to chlorination.

4.5 Geographical distribution

Hepatitis E infection is found worldwide. Two different patterns are observed, where hepatitis E is found in: resource-poor areas with frequent water contamination; and areas with safe drinking water supplies. The disease is common in resource-limited countries with limited access to essential water, sanitation, hygiene and health services. In these areas, the disease occurs both as outbreaks and as sporadic cases. The outbreaks usually follow periods of faecal contamination of drinking water supplies and may affect several hundred to several thousand persons. Some of these outbreaks have occurred in areas of conflict and humanitarian emergencies, such as war zones, and in camps for refugees or internally displaced populations (IDP), situations where sanitation and safe water supply pose special challenges. Sporadic cases are also believed to be related to contamination of water or food, albeit at a smaller scale. In these areas, an estimated 20 million infections and 3.3 million acute cases occur annually worldwide1 with an estimated 56 600 deaths2. The cases in these areas are caused mostly by infection with genotype 1 virus, and much less frequently by genotype 2 virus. In areas with better sanitation and water supply, hepatitis E disease is infrequent with only occasional sporadic cases. Most of these cases are caused by genotype 3 virus, and are caused by infection with virus originating in animals, usually through ingestion of undercooked animal meat (including animal liver) and are not related to contamination of water or other foods. Serological evidence of prior exposure to the virus has been found in most areas, with higher seroprevalence rates (proportion of people who test positive for IgG antibodies to HEV) in regions with lower standards of sanitation and thus higher risk for transmission. However, presence of these antibodies does not imply presence of or increased risk of disease. The usefulness of such data for epidemiological purposes may also be limited due to variable and possible sub-optimal performance of available serological assays, and possible disappearance of the antibody with the passage of time among those exposed to the virus (6,).

5. Pathogenesis

The pathogenesis of hepatitis E is poorly understood. Since HEV is presumably transmitted by the faecal-oral route, it is unclear how the virus reaches the liver. Perhaps there is an extra-hepatic site of virus replication. The virus could replicate in the intestinal tract before reaching the liver.

Negative strands of HEV RNA, indicating virus replication, have been detected in the small intestine, lymph nodes, colon, and liver of pigs, indicating extra-hepatic HEV replication. HEV then replicates in the cytoplasm of hepatocytes and is released into both blood and bile. The liver damage induced by HEV infection may be immune-mediated by cytotoxic T cells and natural killer (NK) cells since HEV is not cytopathic. The virus is shed in the stool (2, 4).

6. Clinical manifestations

The incubation period following exposure to the hepatitis E virus ranges from 2 to 10 weeks, with an average of 5–6 weeks. The infected persons are believed to excrete the virus beginning a few days before to around 3-4 weeks after the onset of disease. In areas with high disease endemicity, symptomatic infection is most common in young adults aged 15–40 years. In these areas, although infection does occur in children, they often have either no symptoms or only a mild illness without jaundice that goes undiagnosed. Typical signs and symptoms of hepatitis include: an initial phase of mild fever, reduced appetite (anorexia), nausea and vomiting, lasting for a few days; some persons may also have abdominal pain, itching (without skin lesions), skin rash, or joint pain. jaundice (yellow discolouration of the skin and sclera of the eyes), with dark urine and pale stools; and a slightly enlarged, tender liver (hepatomegaly). These symptoms are often indistinguishable from those experienced during other liver illnesses and typically last between 1–6 weeks. In rare cases, acute hepatitis E can be severe, and results in fulminant hepatitis (acute liver failure); these patients are at risk of death. Fulminant hepatitis occurs more frequently when hepatitis E occurs during pregnancy. Pregnant women with hepatitis E, particularly those in the second or third trimester, are at an increased risk of acute liver failure, foetal loss and mortality. Case fatality rates as high as 20–25% have been reported among pregnant women in their third trimester. Cases of chronic hepatitis E infection have been reported in immunosuppressed people, particularly organ transplant recipients on immunosuppressive drugs, with genotype 3 or 4 HEV infection (3, 9).

7. Diagnostic

7.1 Clinically

Cases of hepatitis E are not clinically distinguishable from other types of acute viral hepatitis. Diagnosis can often be strongly suspected in appropriate epidemiologic settings however, for example in the occurrence of several cases in localities in known disease-endemic areas, in settings with risk of water contamination, if the disease is more severe in pregnant women, or if hepatitis A has been excluded (8).

7.2 Paraclinically

Definitive diagnosis of hepatitis E infection is usually based on the detection of specific IgM antibodies to the virus in a person's blood; this is usually adequate in areas where disease is common. Additional tests include reverse transcriptase polymerase chain reaction (RT-PCR) to detect the hepatitis E virus RNA in

blood and/or stool; this assay requires specialised laboratory facilities. This test is particularly needed in areas where hepatitis E is infrequent, and in cases with chronic HEV infection. A test for viral antigen detection in serum has been developed; its place in the diagnosis of hepatitis E is currently being studied (4).

7.3 Epidemiologic

Recent advancements in the understanding of global epidemiology of HEV infection reveal a picture quite more complex than initially assumed. Nevertheless, distinct epidemiological patterns are clearly distinguished in endemic regions compared to nonendemic areas. Endemic countries were shown to have an overall HEV prevalence of 25% of all non-A, non-B acute hepatitis cases, and while the anti-HEV immunoglobulin (Ig)G prevalence among healthy blood donors may be as high as 45% in some hyperendemic countries, reports from industrialized countries, although highly variable from study to study, show prevalence ranging from 1% to 4%. Additional dramatic differences were observed in the size and frequency of outbreaks, overall attack rates, and duration of viremia. These issues are extensively reviewed by Kumar et al (6).

7.4 Pathologically

Pathologically the symptoms are characteristic to hepatitis. Only few reports deal with histopathology of the indigenous form of hepatitis E in Europe namely infection with genotype 3. The study of Malcolm et al describes histopathology of sporadic hepatitis E in more details and underlines the cholestatic appearance of the lesion with ductular proliferation and cholangiolitis in the portal tracts (8, 12).

8. Prophylaxis

Prevention is the most effective approach against the disease. At the population level, transmission of HEV and hepatitis E disease can be reduced by: maintaining quality standards for public water supplies; establishing proper disposal systems for human faeces. On an individual level, infection risk can be reduced by: maintaining hygienic practices such as hand-washing with safe water, particularly before handling food; avoiding consumption of water and/or ice of unknown purity; and adhering to WHO safe food practices. In 2011, a recombinant subunit vaccine to prevent hepatitis E virus infection was registered in China. It has not yet been approved in other countries (7).

9. Treatment

There is no specific treatment capable of altering the course of acute hepatitis E. As the disease is usually self-limiting, hospitalization is generally not required. Hospitalization is required for people with fulminant hepatitis, however, and should also be considered for symptomatic pregnant women. Immunosuppressed people with chronic hepatitis E benefit from specific treatment using ribavirin, an antiviral drug. In some specific situations, interferon has also been used successfully (12).

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15. SALMONELLOSIS

1. Name of zoonosis

Salmonellosis

2. Definition

Salmonella is a major cause of zoonotic infections on a worldwide scale. Salmonellosis - human and animal infectious disease caused by *Salmonella* species of gram-negative rod-shaped bacteria. The most commonly reported are *Salmonella enteritidis* and *S. typhimurium* serotypes. In Lithuania every year there are about 3,000 cases and more.

3. Etiology

3.1. Etiologic agent

Salmonellosis has been recognized in all parts of the world but is most prevalent in regions with intensive animal husbandry. Although this facultative intracellular pathogen is primarily an intestinal bacterium, it is commonly found in an environment subject to faecal contamination. Faces of infected animals can contaminate feed and water, milk, fresh and processed meats from abattoirs, plant and animal products used as fertilizers or feedstuffs, pasture and rangeland, and many inert materials. The organisms may survive for months in wet, warm areas such as in feeder pig barns and poultry houses or in water dugouts, but they survive <1 wk in composted cattle manure. Rodents and wild birds are also sources of infection for domestic animals. Pelleting of feeds reduces the level of contamination by salmonellae largely as a result of the heat treatment involved (2).

3.2 Taxonomy

Although many other *Salmonella* spp may cause enteric disease, the more common ones (to some extent varying according to geographic location) in each species are as follows: cattle—*S .typhimurium*, *S. dublin*, and *S. newport*; sheep and goats—*S. typhimurium*, *S. dublin*, *S. abortusovis*, *S. anatum*, and S. *montevideo*; pigs—*S. typhimurium* and *S. choleraesuis*; horses—*S. typhimurium*, *S. anatum, S. newport, S. enteritidis*, and Salmonella serovar IIIa 18:z4:z23; and poultry—*S. enteritidis*, *S. typhimurium*, *S. gallinarum*, and *S. pullorum*.

Salmonella can be grouped into more than 2,400 serotypes (1).

3.3 Morphological description

Salmonella, a rod-shaped gram-negative bacterium belonging to the family Enterobacteriaceae, is the causative agent of salmonellosis. Salmonellosis in warm-blooded vertebrates is in most cases associated with serovars of *Salmonella enterica*.

The type III secretion system of *Salmonella* typhimurium directs the translocation of proteins into host cells. Evolutionarily related to the flagellar assembly machinery, this system is also present in other pathogenic bacteria, but its organization is unknown. Electron microscopy revealed supramolecular structures spanning the inner and outer

membranes of flagellated and nonflagellated strains; such structures were not detected in strains carrying null mutations in components of the type III apparatus. Isolated structures were found to contain at least three proteins of this secretion system. Thus, the type III apparatus of S. typhimurium, and presumably other bacteria, exists as a supramolecular structure in the bacterial envelope (4).

3.4 Biological cycle

In order to reproduce or even survive the bacteria needs to have a host, as only certain strains can live for semi-long periods outside a host body. Once the bacteria have entered the body, either through eating contaminated foods or touching infected faeces, the bacteria begin to grow. Some animals that carry salmonella for long periods of time are either resistant to the bacteria or just long-term hosts. Animal and bird faeces have salmonella bacteria in them even after the bacteria are no longer active inside them. The bacteria invade the walls on the intestinal track, thus causing inflammation and damage to the body, causing a variety of symptoms (2).

The liver and spleen have a higher concentration of salmonella while the blood, heart, kidneys, gallbladder, and pancreas have much lower concentrations. The gallbladder, however, is the main site of carriage. If left untreated or serious the infection can spread through the bloodstream to other organs, joints, placenta or foetus, and membranes around the brain. The toxic substances released by the bacteria can affect the rest of the body, as well, damaging it even further.

4. Epidemiology

4.1 Source of infection

Salmonella are found in the intestinal tract of wild and domesticated animals and humans. Some serotypes of *Salmonella*, such as *S. typhi* and *S. paratyphi* are only found in humans. For ease of discussion, it is generally useful to group *Salmonellae* into two broad categories: typhoidal, which includes *S. typhi* and *S. paratyphi*, and non-typhoidal, which includes all other serotypes (1).

4.2 Path of infection

The usual route of infection in enteritis is faecal-oral, although infection through the upper respiratory tract and the conjunctiva have also been reported. After ingestion, the organism colonizes the digestive tract and invades and multiplies in enterocytes and tonsillar lymphoid tissue. Penetration of bacteria into the lamina propria contributes to gut damage and diarrhoea. The complex process involves attachment through fimbrial appendages and the injection by the attached Salmonella organisms into epithelial cells of proteins, which induce changes in the actin cytoskeleton that induce membrane ruffling at the cell surface. This entraps the *Salmonella* bacteria and results in fluid secretion and their ingestion by the cell (1).

The cellular infection results in activation of a host alarm process through signalling molecules as a result of the detection of bacterial surface proteins, which in turn

induces a strong inflammatory response that generally is able to restrict the bacteria to the intestine. Some serotypes also become localized in the reproductive tract. Serotypes that are able to cause typhoid can modulate the initial host response and suppress the inflammatory response. Cell destruction follows, and the bacteria are ingested by phagocytic cells such as macrophages and neutrophils. Although neutrophils are generally able to kill *Salmonella*, the bacteria can survive and multiply within macrophages, which represent the main host cell type during infection (5).

4.3 Responsiveness

The most common type of infection is the carrier state, in which infected animals carry the pathogen for a variable period of time without showing any clinical signs. Clinical disease is characterized by two major syndromes: a systemic septicemia (also termed as typhoid) and enteritis. Other less common clinical presentations include abortion, arthritis, respiratory disease, necrosis of extremities, and meningitis (3).

4.4 Resistance to the environment

Bacteria sensitive to disinfection, but it doesn't end their existence, so it is particularly important that meat processing plants would be exchanged in the requirements of hygiene standards, qualified staff so that poultry would not be infected (2).

4.5 Geographical distribution

Salmonellosis occurs worldwide but seems to be most common where intensive animal husbandry is practiced. *Salmonella* eradication programs have nearly eliminated the disease in domesticated animals and humans in some countries (e.g., Sweden), but reservoirs remain in wild animals. Serovars vary in their distribution. Some, such as *Salmonella* ser. *enteritidis* and *Salmonella* ser. *typhimurium*, are found worldwide. Others are limited to specific geographic regions (1).

5. Pathogenesis

Pathogenic salmonellae ingested in food survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of proinflammatory cytokines which induce an inflammatory reaction. The acute inflammatory response causes diarrhoea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease (6).

6. Clinical manifestations

Salmonella infections can have a broad range of illness, from no symptoms to severe illness. The most common clinical presentation is acute gastroenteritis. Symptoms include diarrhoea and abdominal cramps, often accompanied by fever. Other symptoms may include bloody diarrhoea, vomiting, headache and body aches (2, 7).

Some infectious disease texts recognize three clinical forms of salmonellosis: (1) gastroenteritis, (2) septicemia, and (3) enteric fevers. This chapter focuses on the two extremes of the clinical spectrum—

gastroenteritis and enteric fever. The septicemic form of salmonella infection can be an intermediate stage of infection in which the patient is not experiencing intestinal symptoms and the bacteria cannot be isolated from faecal specimens. The severity of the infection and whether it remains localized in the intestine or disseminates to the bloodstream may depend on the resistance of the patient and the virulence of the *Salmonella* isolate. The incubation period, or the time from ingestion of the bacteria until the symptoms start, is generally 6 to 72 hours; however, there is evidence that in some situations the incubation can be longer than 10 days (7).

7. Diagnostic

Diagnosis of salmonellosis depends on clinical signs and isolation of the pathogen from faeces, blood, or tissues of affected animals. The presence of organisms may also be sought in feed, water supplies, and faeces from wild rodents and birds that may inhabit rearing premises to determine the source of the organism. Bacteria are usually identified by a range of biochemical tests. Identification to serotype may be done, followed by further subdivision on the basis of susceptibility to selected bacteriophages (phage typing) (3).

Serologic tests are available and are increasingly used as a diagnostic tool in salmonellae surveillance and control programs. These tests are normally developed to identify a limited spectrum of S*almonella* serovars and serogroups. Serologic tests are difficult to interpret in individual animals, because a seropositive animal may no longer be infected. Furthermore, specificity issues mean that in countries with low infection prevalence, many positive results are false-positive.

The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens. Laboratory identification of the genus *Salmonella* is done by biochemical tests; the serologic type is confirmed by serologic testing. Faces, blood, or other specimens should be plated on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, Salmonella-Shigella, and brilliant green agars) as well as enrichment broth such as selenite or tetrathionate (6).

Any growth in enrichment broth is subsequently subcultured onto the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar iron agar and lysine-iron agar, and a presumptive identification is made. Biochemical identification of salmonellae has been simplified by systems that permit the rapid testing of 10–20 different biochemical parameters simultaneously. The presumptive biochemical identification of Salmonella then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Fortunately, approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. *Salmonella* isolates then should be sent to a central or reference laboratory for more

comprehensive serologic testing and confirmation(8).

8. Prophylaxis

Salmonella is difficult to eradicate from the environment. However, because the major reservoir for human infection is poultry and livestock, reducing the number of salmonellae harboured in these animals would significantly reduce human exposure. All animal feeds are treated to kill salmonellae before distribution, resulting in a marked reduction in salmonellosis (2).

Other helpful measures include changing animal slaughtering practices to reduce cross-contamination of animal carcasses; protecting processed foods from contamination; providing training in hygienic practices for all food-handling personnel in slaughterhouses, food processing plants, and restaurants; cooking and refrigerating foods adequately in food processing plants, restaurants, and homes; and expanding of governmental enteric disease surveillance programs (2).

Carrier animals and contaminated feedstuffs and environment are major problems. Drain swabs or milk filters may be cultured to monitor the salmonellae status of a herd. The principles of control include prevention of introduction and limitation of spread within a herd. In many countries and in the EU, government-backed programs have been introduced to control and reduce levels of infection in food animals, especially poultry and pigs.

Every effort must be made to prevent introduction of a carrier; ideally, animals should be purchased directly only from farms known to be free of the disease and should be isolated for ≥1 wk while their health status is monitored. Ensuring that feed supplies are free of salmonellae depends on the integrity of the source. Some countries also test for contamination of and regulate importation and home production of feedstuffs and feed components (1).

Salmonellae are facultative intracellular bacteria, and a live vaccine is therefore expected to be necessary for optimal immune protection against disease; however, there is some evidence that inactivated bacterins can induce a lower level of protection. In several studies, live attenuated Salmonella vaccines in pigs, cattle, and chickens stimulated a strong cell-mediated immune response and protected animals against both systemic disease and intestinal colonization. A live attenuated S Choleraesuis vaccine licensed for use in swine appears to effectively reduce colonization of tissues and protect pigs from disease after challenge with virulent organisms and under field conditions (2).

This vaccine also protected calves against experimental challenge with *S. dublin* and serogroup C1 salmonellae after intranasal or SC administration. A live *S. gallinarum* vaccine has been shown to be effective not only against *S. gallinarum* (fowl typhoid) but also in significantly reducing the infection of laying hens challenged with *S. enteritidis* (3).

9. Treatment

Early treatment is essential for septicemic salmonellosis, but there is controversy regarding the use of antimicrobial agents for intestinal salmonellosis. Oral antibiotics may be ineffective and may deleteriously alter the intestinal microflora, thereby interfering with competitive antagonism and prolonging shedding of the organism. There is also concern that antibiotic-resistant strains of salmonellae selected by oral antibiotics may subsequently infect people. By suppressing antibiotic-sensitive components of the normal flora, antibiotics may also promote transfer of antibiotic resistance from resistant strains of E coli to Salmonella. Use of chemotherapeutic antibiotics for growth stimulation has been banned in many countries for this reason (2).

Broad-spectrum antibiotics administered systemically are indicated for treatment of septicemia. Initial antimicrobial therapy should be based on knowledge of the drug resistance pattern of the organisms previously found in the area. Nosocomial infections may involve highly drug-resistant organisms. Trimethoprim-sulfonamide combinations may be effective (2).

Alternatives are ampicillin, fluoroquinolones, or third-generation cephalosporins. Resistance to ampicillin, trimethoprim, sulfonamide, tetracyclines, and aminoglycosides is generally plasmid mediated and transfers readily between different bacteria. Resistance to quinolones is mutational, but random mutations may be selected by antibiotic use and may be transferred by bacteriophages. Treatment should be continued daily for up to 6 days (8).

If oral medication is chosen, it should be given in drinking water and not mixed into solid food, because affected animals are thirsty due to dehydration and their appetite is generally poor. Fluid therapy to correct acid-base imbalance and dehydration may be necessary. Calves, adult cattle and horses need large quantities of fluids. Antibiotics such as ampicillin or cephalosporins lead to lysis of the bacteria with release of endotoxin, and NSAIDs or flunixin meglumine may be used to reduce the effects of endotoxemia (2,8).

The intestinal form is difficult to treat effectively in all species. Although clinical cure may be achieved, bacteriologic cure is difficult, either because the organisms become established in the biliary system and are intermittently shed into the intestinal lumen, or because the animals are reinfected from the environment at a time when their normal gut flora, which is inhibitory to colonization by pathogens, is depleted by antibiotic therapy (4).

A concern with antimicrobial therapy is that it may increase the risk of creating carrier animals; in people and other animal species, antimicrobial therapy prolongs the period after clinical recovery during which the pathogen can be retrieved from the GI tract (6).

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