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PART I. ZOONOZIS – TICK-BORNE DISEASES
CZĘŚĆ I. ZOONOZY – CHOROBY PRZENOSZONE PRZEZ KLESZCZE

TICK-BORNE BACTERIAL DISEASES IN POLAND

WYSTĘPUJĄCE W POLSCE BAKTERYJNE CHOROBY PRZENOSZONE PRZEZ KLESZCZE

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Summary

Many infectious diseases are spread by a vector. Some microorganisms require both the vertebrate as well invertebrate host to complete their life cycle. In this way, many bacterial diseases are transmitted to humans.

Within *Borrelia burgdorferi* sensu lato species, 15 genospecies have been identified; 7 of which are pathogenic to humans. Lyme borreliosis, classified as a zoonosis, occurs throughout the Northern Hemisphere. The symptoms caused by these spirochetes, in addition to the characteristic erythema migrans, may affect many body systems and organs.

Infections caused by *Bartonella* spp. are classified as emerging and re-emerging diseases. Over 25 species of *Bartonella* species have been currently recognized, 14 of which are pathogenic to humans. The infections of these microorganisms are transmitted by ticks, lice and fleas, and manifest themselves as endocarditis, meningitis, pneumonia, bacillary peliosis, Parinaud's oculoglandular syndrome, the mildest of them being the cat scratch disease.

Recently, spotted fever group rickettsioses are the most commonly recognized. In Poland, the etiological agents of these diseases are various species of *Rickettsia* spp., such as *R. helvetica*, *R. monacensis, R. slovaca* and *R. raoultii*.

Human granulocytic anaplasmosis (HGA) is an acute infectious disease caused by *Anaplasma phagocytophilum.* The symptoms of anaplasmosis include: a high fever, headache, malaise, muscle pains and chills. It is characterised by thrombocytopenia, leukopenia, elevated levels of liver transaminases, increased number of neutrophils and mild anemia.

There is no specific prophylaxis to avert tick-borne infections. The best prevention method is to avoid tick bites and to remove them from the skin immediately. It is also recommended to use tick repellents.

Keywords: tick born diseases, Lyme borreliosis, bartonelosis, spotted fever group rickettsiosis, anaplasmosis

Streszczenie

Wiele chorób infekcyjnych rozprzestrzenia się z udziałem wektora. Drobnoustroje chorobotwórcze wymagają zarówno gospodarza kręgowca jak i bezkręgowca dla zamknięcia pełnego cyklu życiowego. W ten sposób wiele chorób bakteryjnych przenoszonych jest na człowieka.

W obrębie gatunku *Borrelia burgdorferi* sensu lato wyróżnia się 15 genogatunków, z których 7 jest chorobotwórczych dla człowieka. Borelioza z Lyme jest zoonozą występującą na całej półkuli północnej. Objawy wywoływane przez te krętki, oprócz charakterystycznego rumienia wędrującego, mogą dotyczyć wielu układów i narządów.

Zakażenia wywoływane przez bakterie z rodzaju *Bartonella* zaliczane są do grupy nowo pojawiających i nawracających chorób (group of emerging/re-emerging diseases). Obecnie znanych jest ponad 25 gatunków bakterii z rodzaju *Bartonella*, wśród których 14 jest chorobotwórczych dla ludzi. Wektorem zakażenia mogą być kleszcze. Zakażenia te są przyczyną zapalenia wsierdzia, zapalenia opon mózgowo-rdzeniowych, zapalenia płuc, naczyniakowatości, plamicy wątrobowej, zapalenia gałki ocznej i najłagodniejszej wśród nich choroby kociego pazura.

Najczęściej rozpoznawanymi obecnie riketsjozami są gorączki plamiste. Czynnikiem etiologicznym w Polsce są różne gatunki takie jak: *R. helvetica, R. monacensis, R. slovaca* i *R. raoultii*.

Ludzka granulocytarna anaplazmoza (human granulocytic anaplasmosis – HGA) jest ostrą chorobą zakaźną wywoływaną przez bakterie *Anaplasma phagocytophilum*. Objawy anaplazmozy to wysoka gorączka, bóle głowy, złe samopoczucie, bóle mięśniowe i dreszcze. Charakterystyczna jest trombocytopenia, leukopenia, podwyższony poziom transaminaz wątrobowych, zwiększona liczba niedojrzałych neutrofili i łagodna anemia.

Nie istnieje swoista profilaktyka pozwalająca uniknąć zakażeń przenoszonych przez kleszcze. Należy unikać ukłuć kleszczy i niezwłoczne usuwać je ze skóry. Zalecane jest stosowanie repelentów odstraszających kleszcze.

Słowa kluczowe: choroby przenoszone przez kleszcze, borelioza z Lyme, bartonelozy, gorączki plamiste, anaplazmoza

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I. Introduction - ticks as vectors

Many diseases can be spread by vectors, which are invertebrates, especially by different types of arthropods, such as insects or arachnids. Zoonotic diseases that are transmitted this way are classified into the metazoonosis group. Thus, it is invertebrates that spread infections among vertebrates. A pathogenic microorganism requires both a vertebrate and invertebrate host to complete the full life cycle.

Ticks are arthropods belonging to the Arachnida (*Arachnida*) family of mites. They are external parasites of vertebrates. The size of the hungry tick ranges from one to several millimetres, which characteristically increases after it blood feeds. The tubular hypostom, adapted to suck blood and tissue fluids, is armed with a number of teeth to hold on to the host's skin. The very moment the epidermis is punctured and feeding begins, i.e., the blood is sucked, often remains unnoticed because the tick saliva acts as a local anesthetic. It is only after 2-3 days that the irritated area begins to itch, and the blood-filled tick enlarges, which makes it more visible. Also, there may appear edema, pain, itching or redness at the site of the sting.

In their life cycle, ticks undergo transformation: from eggs are hatched larvae, which turns into a nymph after the first feeding, which in turn after another meal reaches maturity. They are characterized by a seasonal activity that depends on environmental conditions. It is assumed that the mature forms are already active at 5°C and nymphs at 8°C. Larvae attack hosts from May to September, most often however in June, July and August.

Species of the family *Ixodidae* suck blood three times in their life: at the larval, nymph, and adult stage. These meals enable the transition from one stage to the next and egg laying. At each of its development stage of the tick, i.e. larvae, nymphs, and imago (the mature form), must suck blood one time from the vertebrate to develop further. Without food, they can live up to two years.

On average, the development cycle of one tick generation lasts 2 years. The increase in temperature results in an increase in tick activity, which usually begins in March / April, and lasts until October / November. The maximum activity depends on the climatic factors and in Central Europe it takes place in two phases, i.e. in May / June and in September / October. In Poland, it starts in mid April (sometimes earlier, even in March) and lasts until early November with two summits – the first one from May to mid June and the other one in September. After hot summers, it happens that the peak of autumn activity does not appear.

The most common tick in Poland is *Ixodes ricinus* (a common tick, grazing tick). Immature forms of the tick, i.e. larva and nymph, can be encountered on grass and in low bushes. Adult forms dwell primarily in bushes, even at a height of 3 m. They recognize their potential host by responding to the infrared radiation emitted by warm-blooded vertebrates. They parasitize on many animal species, most often small mammals (insectivorous mammals, rodents), which are hosts for larvae and nymphs. Nymphs also feed on larger mammals, such as deer, rabbits, rabbits and hare, as well as birds. Adult forms parasitize on cattle, sheep, goats, elk, bison, deer, wild boar, wild boar, fox, hare, dog, rabbit and hedgehog. Man is an accidental host for each developmental form. In our country, the most important tick species for medical and veterinary sciences are: the pigeon tick, the common tick and the meadow tick.

Meadow ticks (*Dermacentor reticulates*) can transmit and be a reservoir of tick-borne encephalitis (TBE), rickettsiae causing spotted fevers (e.g. *Ricketsia conori*, *R. slovaca*), tularemia (*Francisella tularensis*), salmonelloses (*Salmonella spp*.) and others as well as protozoa (*Babesia spp*.).

The common tick (*Ixodes ricinus*) belongs to the three-host ticks, which at each developmental stage (larva, nymph and female) sucks blood from another host, once – before transforming into the next form (larvae and nymphs) or, in the case of females, before laying eggs. Males do not feed with blood. Almost all species of terrestrial vertebrates, including man, may become hosts. The tick cycle lasts several years (about 3 or longer) in our climate zone, depending on the environmental conditions.

At the greatest risk of being infected are forest rangers, urban greenfield workers, those living in rural areas and collecting forest berries and mushrooms. Another group that is at an increased risk of tick-borne diseases are hikers as well as those doing extreme sports and participating in survival camps [1,2].

II. Lyme borreliosis

The etiological factor of Lyme borreliosis, the spirochetes of *Borrelia burgdorferi* sensu lato, are, according to many researchers, the most fascinating and, at the same time, enigmatic bacterial pathogens. These microorganisms have developed unique mechanisms of interaction with their host, which makes them distinctive forms among other bacteria [3,4,5].

The spirochetes of *Borrelia burgdorferi* sensu lato belong to the order *Spirochaetales*, family *Spirochaetaeceae.* The species is genetically diverse (Figure 1, 2).

- *Phylum: Spirochaetes*
- *Class: Spirochaetes*
- *Order: Spirochaetales*
- *Family: Spirochaetaceae*
- *Species: Borrelia: grupa recurentis – Borrelia recurentis*
  B. miyamotoi
  Borrelia burgdorferi sensu lato
  Treponema

Figure 1. Systematics of spirochetes

Borrelia burgdorferi sensu stricto
B. garinii
B. afzelii
B. bisetti
B. spielmanii
B. valaesiana
B. lusitaniae
B. andersoni
B. californiensis
B. japonica
B. sinica
B. turdi

Figure 2. Geno-species of *Borrelia burgdorferi* sensu lato

Within the species, 15 genospecies were distinguished, 7 of which are pathogenic to humans. In the 1980s *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* were described and characterised as pathogenic genospecies for man. In Europe, all these three genospecies occur, while in North America only *B. burgdorferi* sensu stricto is encountered. Lyme borreliosis is a zoonosis found all around the Northern Hemisphere. Transmission of infection from one individual to another is done by the tick vector of the genus *Ixodes* [4,5]

In *in vitro* conditions, the *B. burgdorferi* sensu lato spirochetes grow very slowly and only in exceptionally rich media. Several weeks, or even months are needed, to obtain a visible bacterial growth, since the period between successive cell divisions of the spirochete lasts 14-16 hours; in contrast, the *E. coli* cell can divide on average every 20 minutes. For this reason, the isolation of the etiological agent is not used in routine diagnostic procedures to identify the infection.

They are long, mobile Gram-negative bacteria. Their cell is elongated, strongly twisted, with the underlying axial filament. The cell's shape and its twists are variable features that depend, to a large extent, on the environment in which the bacteria are located. The cell's shape and its mobility allow it to penetrate environments of different structures and density, including dense and viscous ones such as a connective tissue [5].

Besides, these are microaerophilic bacteria, living in different periods of their life cycle as external or intra-cell parasites.

They are the predominant cause of Lyme borreliosis classified as meta-zoonosis, which is transmitted in vertebrate animals and humans by the tick vector of the genus *Ixodes*. Depending on the geographic region, these are different species of ticks – in Europe, it is primarily *I. ricinus*, in Asia *I. persulcatus*, while in America mainly *I. scapularis* [4,5].

The symptoms of the disease, in addition to erythema migrans, may affect different systems and organs. Lyme strains exhibit tissue-specific tropisms that may be species- or strains-specific. The *Borrelia burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* spirochetes, the three main pathogenic spirochetes responsible for Lyme borreliosis, share only some clinical symptoms. The species *Borrelia burgdorferi s.s*. is closely related to the Lyme arthritis (*Lyme arthritis*), *B. garinii* causes *neuroboreliosis*, whereas *B. afzelii* is isolated primarily from late skin lesions (ACA) [4].

The cause for different tissue affinities (tropisms) and the severity of the disease is not fully understood. The occurrence of specific pathogenic factors in *B. burgdorferi* s.s. may be different, indicating the strain-dependent specificity [5].

The spirochetes of *Borrelia burgdorferi* s.l. produce numerous adhesion proteins on their surface, i.e. DbpA, DbpB, Bgp, BBK32, P66, which bind to their respective receptors, enabling them to recognize various matrix components and adhesion to various mammalian cells.

The spirochetes of *Borelli burgdorferi* s.l. are encoded so as to produce many adhesives, which have been found to recognize various elements of the matrix and, in this way, bind to different types of mammalian cells. The two related A and B dextrin-binding proteins (DbpA and DbpB) combine with decorin and dermatan sulphate. DbpA decorin-binding protein is an adhesion whose structure can vary from one strain to another. It determines the strain-specific affinity for different tissues.

By studying the tropism of *B. burgdorferi* strains, which contain various *dbpA* alleles, the properties of *dbpA* alleles of strain *B. afzelii* VS461, strain N40-D10 / E9 *B. burgdorferi* s.s. and strain PBr *B. garinii* were compared. It has been observed that DpbA of strain N40-D10 / E9 *B. burgdorferi* s.s., which exhibits the weakest decorin binding activity, causes at the same time severe colonization of articular cartilage and consequently arthritis. It seems that the *dpbA* allele present in strain N40-D10 / E9, determines the occurrence of arthralgia. On the other hand, there is a noticeable correlation, i.e. the greater activity of DbpA binding with decorin , the stronger the heart muscle colonization and the most severe *carditis*. The studies indicate that *dpbA* allele influence the colonization and occurrence of specific, allelic, Lyme borreliosis - related symptoms, and that are involved in the etiology of various clinical symptoms associated with infection with various strains of *B. burgdorferi* s.l.

The *B. burgdorferi* spirochetes have a characteristic Gram-negative bacterial cell wall structure. There are numerous proteins that are potent antigens on the outer membrane, involved in the colonization and tissue penetration as well as in host immune response to infection [3].

There is also a group of lipoproteins - designated as OspA (outer surface protein A), OspB, up to OspF. However, their functions are poorly understood. So far, the role of only OspA, OspB, OspC, and decorin binding proteins DbpA and DbpB, which also occur on the outer membrane, has been described. All these proteins are encoded on plasmids, and their expression depends on the conditions in which the bacterium is located [4,5,6].

Life cycle of *B. burgdorferi* sensu lato. The life cycle, circulation of *B. burgdorferi* spirochetes in the environment, can be divided into several stages, each of which is essential for the survival of the microorganism. The first, the initial phase, is living in a hungry tick, the second one is their development in the blood or body fluids of warm-blooded animals, the third stage consists in the residence time in the host cell – a mammal, and the last one, the fourth phase, is the death of the infected host cell and the release of the bacteria into the blood, from which they again get to the tick and start the cycle once again.

The ticks' share in the transmission of infection determines the seasonality of the disease, as well as its geographic range, which coincides with the range of *Ixodes* spp. In different regions of the Northern Hemisphere, different tick species are responsible for the transmission of infection. In North America, it is primarily *Ixodes scapularis*, in Europe *Ixodes ricinus*, and in Asia *Ixodes persulcatus*.

The spirochetes of *B. burgdorferi* are inserted in ticks transovarially and stransstadially, and infect the animals on which they feed. The infection is maintained not only in subsequent ticks development stages, but also in later generations.

The animal reservoir of spirochetes is very diverse. One host species is sufficient to keep the circulation of the microorganism in the environment. Under natural conditions, spirochetes can live cyclically in a variety of animal species, such as small rodents, lizards, deer, other large mammals or birds. The change of host species is primarily related to their availability. At different life stages, ticks are present on different levels of the ecosystem. Thus, there are always some hosts available, either small rodents, birds, deer or other large animals [4,5].

Diagnostic procedures

EIA methods. A proper diagnosis of the disease is conditioned by an appropriate selection of serological methods and diagnostic antigens as well as correct interpretation of the obtained results. In 2000, an international group of experts published recommendations for proper laboratory diagnostics of Lyme borreliosis (www.dghm.org/red/index.html?cname=MIQ), which are regularly reviewed and updated [4,5,6].

Because of the numerous non-specific cross-reactions and false-positive results that could not be eliminated in commonly used ELISA tests and, despite the introduction of different diagnostic antigens (whole-cell sonicate, isolated proteins, recombinant antigens), it is the two-stage serological diagnostics that is currently recommended. In the first stage, it consists of determining the level of antibodies with semi-quantitative serological tests with high sensitivity. Subsequently, serum samples in which positive or equivocal results were obtained are tested with immunoblot to verify specificity of positive results of the first stage [6].

ELISA tests, used as screening tools, should be included in at least the second generation of tests because they have improved their specificity and the cross-reactivity has been significantly reduced. In the latest, third generation tests, recombinant proteins are used as diagnostic antigens [7,8].

Immuno-blot. An elaboration on the criteria of immuno-blot evaluation in patients from Europe accounts for the occurrence of several pathogenic genomes in this continent.

In order to correctly interpret the findings of the study, it is necessary to know the duration of the disease, which is closely related to the appearance of antibodies to specific antigens.

By using recombinant antigens, one might be certain that the resulting reactions concern only specific proteins. In addition, it is possible to use specific proteins derived from different genospecies as well as specific peptides, being protein fragments, which as a whole cause cross-reactions, such as, for example, the p41 protein and the p41 peptide.

Thus, antibodies of both classes should always be determined. Obtaining a negative serological result at an early stage of infection is not decisive in the ultimate diagnosis. It is recommended to repeat the test with a new serum sample 4 weeks after the onset of the first symptoms. The interpretation of results is done according to strictly defined criteria (Table 1) [6,9]

Table 1. Interpretation of Western blot test results

Diagnostic antigen	Specific IgM antibodies[1]	Specific IgG antibodies[1]	Number of IgM and / or IgG fractions[2]
Borrelia afzelii	p39, OspC, p17 or strong reaction with p41 antigen	p83/100, p58, p43, p39, p30, OspC, p21, p17, p14	1 /2 fractions
Genetic recombinants	p39, OspC, p41 int., p17, or strong reaction with OspC	p83/100, p58, p39, OspC, p41 int., p17	2 /2 fractions

[1]Specific IgM or IgG antibodies for *B. burgdorferi* sensu lato protein fractions, relevant for interpreting Western blot test result

[2]The smallest number of protein fractions of *Borrelia burgdorferi* sensu lato with which the patient's antibody reacts to meet the positive result

Other tests. The basis for laboratory diagnosis of Lyme borreliosis is serological testing. However, there have been described some serologically negative cases of the disease. This may be due to the decrease of immune response due to use of immunosuppressants or various devastating diseases. Consequently, if an advanced stage of Lyme borreliosis is still suspected clinically despite the negative serological result, other tests such as PCR, bacterial cultures, etc. [5,6] may be used.

III. Bartonelosis

Bartonella infections are a serious public health problem as they belong to a group of emerging / re-emerging diseases.

Recurrent threats are, for example, diseases caused by *Bartonella quintana* (formerly *Rochalimea quintana*), an etiological agent of trench fever carried by a clothes louse (Wolhynia fever, five-day fever) in the first half of the 20th century. It was a disease spreading epidemically, with a characteristic course of a rapid rise in body temperature every five days with a simultaneous loss of consciousness resulting from the infection of the central nervous system. In the early eighties of the last century, the disease was thought to be a "historic one", presently non-existing. However, at the end of the eighties, infectious diseases recognised as: endocarditis, fever and lymph nodes of unknown etiology were found in some people first in North America, then in France, and finally all over Europe. After isolating the etiological factor and describing its characteristics, it turned out to be the same microorganism that causes trench fever several decades earlier.

Further research led to the discovery and description of several other new species of the genus *Bartonella*. Initially, they were diagnosed in AIDS patients as opportunistic infections. In the subsequent years, they were also found in people free of HIV infection. In 1989, a new species was described – *Bartonella henselae,* producing very similar symptoms. The reservoir for *Bartonella henselae* are cats. Taxonomic studies showed differences in the sequence of 16 S rRNAs, G + C pairs, and different phenotypic characteristics between the newly formed family *Bartonellaceae* and other species belonging to the order *Rickettsiales*, which resulted in the transfer of this family from the order *Rickettsiales* to the order *Rhizobiales* [10,11].

At present, there are over 25 known species of *Bartonella*, 14 of which are pathogenic to humans. The infection with these microbes is classified as a meta-zoonsis, which is transmitted by vectors that can include, among others, lice, fleas and ticks. The widespread prevalence of *Bartonella spp.* among various animal species and frequent human infections indicates the existence of multiple vectors that carry these infections. Recent studies have shown a significant share of ticks in this process [12,13,14].

Bartonella infections manifest themselves with various symptoms. These infections can cause endocarditis, meningitis, pneumonia, bacillary angiomatosis peliosis hepatitis, ocular inflammation, cat scratch disease, and

others. The mildest form of bartonelosis is the cat scratch disease. According to a study conducted in Germany and France, *B. henselae* bacteria are responsible for most of the lymphadenopathy in children and adults in these countries.

B. henselae and *B. quintana* are also common in Poland. At the highest risk are alcoholics, cats owners, and veterinarians. Cat owners and homeless people are the most vulnerable to *B. henselae* infection [10,12,13].

B. quintana, an etiological agent of the currently absent trench fever, is found in some European countries, including Poland, in homeless alcoholics, and in a new form – in patients with chronic bacteremia and no fever symptoms.

The basis for the diagnosis of bartonelosis is serological testing. However, in some cases it is insufficient. Limphadenopathy, which occurs due to the cat scratch disease, caused by the *B. henselae* infection, needs to be distinguished from the proliferative disease. In such cases, the detection of *B. henselae* DNA in the sections of the altered lymph nodes is decisive [15].

IV. Ricketsioses transmitted by ticks

Rickettsioses are diseases caused by obligatory intracellular bacteria. The species is divided into a group of spotted fever group (SFG), most commonly transmitted by ticks, and a typhus group (TG), transmitted by lice, fleas and mites. By improving hygiene in the 21st century, the risk of developing outbreaks of typhus infections in Europe is assessed as very low. The most commonly recognized rickettsioses are spotted fever group rickettsioses. The oldest and most common is the Mediterranean Spotted Fever (MSF) caused by *Rickettsia conorii*. The extent of its occurrence overlaps with the range of ticks of the species *Rhipicephalus sanguineus*, its vector and the reservoir of the infection. It is found in the Mediterranean and Caspian seas. The number of the reported cases of this infection reaches several hundred a year. In Poland, there are also reported some imported cases [16,17].

An etiologic agent of spotted fever are Gram-negative, pleomorphic bacteria, *Rickettsia spp*., which can present as cocci (0.1 μm in diameter) or rods (1-4 μm in length). They have a number of characteristic features such as intracellular obligatory parasitism, inability to grow on bacteriological media and the role of vector in the transmission of infection. The bacteria's inability to grow at laboratory conditions makes it difficult to isolate and culture from the clinical material. The detection of these bacteria has been made possible by the introduction of molecular biology techniques, which also resulted in an identification of a number of new species occurring in Europe: *R. raoulti*, *R. helvetica*, *R. aeschlimannii*, *R. massilliae*, *R. sibirica mongolotimonae*, *R. slovaca* and subspecies of *R. conorii* [16].

The main vector and reservoir of spotted fever group rickettsiae in Poland are *Ixodes ricinus* ticks (transmitting *R. helvetica*, *R. monacensis* and *R. slovaca*) and *Dermacentor reticulatus* (transmitting *R. raoultii*). Ticks collected from vegetation showed that the infection rate with *I. ricinus* was 9.5% in West Pomerania, 8.5% in Świętokrzyskie Province and 2.8-27.5% in Mazovia. In addition, individual cases of *R. monacensis* were reported in West Pomerania as well as in Warsaw urban parks) and of *R. slovaca* in Świętokrzyskie Province [18,19,20]. The presence *of R. raoultii* in *D. reticulatus* ticks was found in 57% of these arthropods in Podlasie Province, in 53% in Lublin Province, and in 42.7% in Masovian Province [19,20,21]. In ticks, rickettsiae are transmitted transstadially and transovarially. Small mammals and birds also participate in the circulation of this microorganism in the nature [16].

In the areas where infected ticks were found, almost 15% of the foresters, have shown presence of antibodies to the spotted fever group rickettsiae with the highest detected titres - 128 [22].

Only some species of *Rickettsia spp*. are present in the ticks, in Poland. Until now, cases of endemic spotted fevers in the form of TIBOLA / DEBONEL (*R. slovaca*, *R. raoultii*) have been recognized and a possibility of uneruptive fever (*R. helvetica*) and the Mediterranean fever (*R. monacensis*), which have not yet been diagnosed. Due to the increasing tourists travels several cases of African tick bite fever (ATBF) are reported in Poland every year [17].

Tick-Borne Lymphadenopathy / *Dermacentor*-Borne-Necrosis,-Erythema-Lymphadenopathy / Scalp Eschar Neck Lymphadenopathy: the syndrome caused by *R. slovaca*, *Candidatus Rickettsia rioja* and *R. raoultii* is known under three names. The name TIBOLA is used in Central and Western Europe except for the Iberian Peninsula, where the DEBONEL name is used. SENLAT consists of a similar set of symptoms, whose etiological factors, in addition to rickettsiae, include other tick-borne organisms such as *Francisella tularensis*, *Bartonella henselae* and *Coxiella burnetii* [16,23]

The characteristic feature of these infections is a single painful a *tache noire* in the tick bite spot, most often found on the scalp, and is accompanied by enlarged lymph nodes around the neck. Rash and swelling on the face area are less common. Fever and redness occur in about half of the patients. Infection may result in a local

hair loss and chronic fatigue syndrome. Most cases occur between October and April (with a peak in November) [16,17,23].

***R. helvetica* infection**

R. helvetica infections are reported sporadically. Single cases have been found in Sweden, France, Switzerland and Italy. Their clinical picture, however, is very diverse. The most common symptom is a tick-bite fever, with no rash and a *tache noire* (hence the name: *uneruptive fever*). In Sweden, 2 cases of sudden death of patients with *perimyocarditis* have been reported. This infection can also lead to meningitis, or just resemble it, which is manifested by stiffness of the neck, severe headache associated with photophobia, weakened muscular strength, and neck muscle pain [16].

***R. monacensis* infections**

So far, only four cases of this infection have been described in southern Europe (Spain, Croatia and Italy). Two of them resembled the Mediterranean fever – with a *tache noire* and papular rash, and two were diagnosed with rash only.

***R. africae* infections**

Its symptoms usually develop after 5-7 days. Most people have flu-like symptoms: fever, nausea, fatigue, headache, and muscle aches (mainly in the neck). Approximately, 80% of the patients experience neck stiffness, which indicates that the central nervous system has been affected. A characteristic *tache noire* with localized lymphadenitis occur in 50-100% of the patients, and maculopapular rash in less than half [24].

The basis for the diagnosis of all rickettsioses and the differentiation of spotted fever is the study of the presence of specific serum IgM and / or IgG antibodies. Usually, they are detected 7-15 days after the onset of symptoms. The exception is African fever, where antibodies are identified after 25-28 days. For serological diagnosis, the reference method is indirect immunofluorescence assay. The most commonly used pairs of antigens are *R. typhi* or *R. mooseri,* characteristic of typhus, and *R. rickettsii* or *R. conorii* antigens, characteristic of spotted fever. The higher, detected antibody titre for a particular antigen is the basis for the diagnosis of typhus or spotted fever group rickettsiosis. The identification of the *Rickettsia* sp. causing spotted fever requires the use of an indirect microimmunofluorescence (MIF) test with rickettsia antigens that are most prevalent in the area. It is recommended to take two blood samples, the first after the onset of symptoms and the other 2 weeks later to determine the dynamics of the antibodies. In rickettsioses diagnostics, the isolation of the microorganism is not routinely used because they are grown only in cell lines. The PCR method is used to detect an early infection. The test material may include peripheral blood leukocytes (a buffy coat), biopsy material from skin lesions (*taches noires*) and / or lymph nodes collected before treatment.

The drug of choice in the treatment of all diseases caused *by Rickettsia spp*. in patients of all ages, including children <8 years of age, is doxycycline. The recommended dose of doxycycline is 100 mg twice daily (orally or intravenously) for adults and 2.2 mg / kg twice daily (orally or intravenously) for children up to 45 kg. The treatment should be implemented immediately, as delay can lead to serious consequences or death [24].

V. Human granulocytic anaplasmosis (HGA)

Human granulocytic anaplasmosis (HGA) is a severe infectious disease caused by bacteria *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophilum*). They were considered to be the etiological agent of infections occurring only in animals. The first cases of this disease found in humans were identified in the 1990s [25,26]. In Poland, human granulocytic anaplasmosis was first reported in 2001.

A. phagocytophilum belongs to obligatory intracellular parasites of white blood cells (neutrophils). HGA cases occur both in Europe and in North America. The vector transmitting the infection of *A. phagocytophilum* to humans are the ticks of the genus *Ixodes* (*I. ricinus* in Europe, and *I. scapularis* and *I. pacificus* in North America) [25,26].

The ticks infected with *A. phagocytophilum* are encountered all over Poland. Their percentage ranges from 4% in western Poland to 15% in the vicinity of Białystok [27,28,29].

In the occupational groups belonging to the group of high risk of infection (foresters), antibodies to *A. phagocytophilum* are detected in about 20% of the population in Białowieża Forest and in the vicinity of Lublin [30], 5% in Świętokrzyskie Province (unpublished data) and 4% in the vicinity of Białystok [28]. Every year, several dozen persons are diagnosed with the full symptoms of this infection, although this figure appears to be underestimated (unpublished data from the Laboratory of Rickettsiae, Chlamydiae and Spirochetes in National Institute of Public Health-NIH)

The symptoms of anaplasmosis usually appear 5-14 days after being bitten by the infected tick. They mostly include: fever (92% -100% of cases), headache (82%), malaise (97%), muscle pain (77%) and chills. Skin lesions are present in less than 10% of the patients. Rarely are there any symptoms in the gastrointestinal tract and nervous system.

In most cases, anaplasmosis is a self-limiting disease. No chronic cases have been reported. Severe, life-threatening illness affects patients in an advanced age, with chronic diseases (diabetes) or those undergoing immunosuppression. These patients require hospitalization and sometimes admission to the intensive care unit. The case fatality rate among patients is <1%. The clinical symptoms of this disease in untreated people can persist for up to 60 days.

In the first week of the disease, direct detection methods of a blood etiological agent are used in the diagnosis of HGA, such as PCR (sensitivity 67-90%), a microscopic examination of peripheral blood smear stained with Giemsa method in order to detect characteristic inclusions, or the so called morulae (microcolonies) in granulocytes cytoplasm (sensitivity 25-75%). *A. phagocytophilum* isolation is rarely used because it requires security in the form of a BSL-3 lab and the cultivation of the HL-60 cell line derived from promyelocytes. Antibodies usually appear 7-10 days after the onset of the disease. A routine serological test (a reference method) in the diagnosis of anaplasmosis is an indirect immunofluorescence assay. Two serum samples are recommended to show a fourfold increase in antibody titre, i.e. taken at the beginning of the disease (in the first week of symptoms) and after 2-4 weeks.

In additional examination, which are significant at the onset of the disease before the antibodies appear, the following characteristic symptoms are commonly recognised: thrombocytopenia, leukopenia, elevated levels of hepatic transaminases, increased neutrophil numbers and mild anemia. In cases of CNS involvement, there are usually no abnormalities in the cerebrospinal fluid.

In the treatment of HGA, the drugs of choice are tetracyclines, mainly doxycycline. People over 8 years of age with HGA symptoms are advised to take doxycycline (2 x 100 mg) for 7-14 days. The length of antibiotic therapy is relevant for the complete cure of both anaplasmosis and a possible mixed infection with *Borrelia burgdorferi*, for which the common vector are ticks. Persons suffering from intolerance for doxycycline or those in whom the drug is contraindicated may be treated with rifampicin for 5-10 days. Its effectiveness has been confirmed, among others, in the treatment of HGA in children under 8 years of age as well as in women in the third trimester of pregnancy [24,25,26].

VI. Prevention of tick-borne diseases

The best prevention against arthropods is to avoid tick bites and to remove them immediately from the skin, and the best way to reduce exposure is to avoid ticks habitats – forests and grasslands. Such a behaviour is particularly important during the periods of tick activity (from early spring to late autumn). It is also vital to wear appropriate protective clothing, including headgear, long sleeved shirts, trousers, socks and covered shoes, which makes it difficult for the tick to reach the skin. Besides, it is a good idea to wear clothes in bright colours to better see creeping ticks. In addition, one should move along the well-marked trails avoiding, if possible, direct contact with vegetation.

It is also important to scare ticks away, by using repellents containing DEET (N, N-diethyl-toluamide). They can be applied on exposed skin and clothing, also in children. On the other hand, preparations containing permethrin are intended for use only on top clothing (e.g. shirts and trousers).

Finally, it is essential to quickly remove the arthropods from the skin of both humans and domestic animals before they bite or to remove them right after they do so in order that pathogens are not transmitted. In animal studies, it was estimated that the time needed to infect with, for example, bacteria *A. phagocytophilum*, is less than 24 hours.

To remove the tick, it is best to use the pointed tweezers to grab it near the skin and gently pull with a constant force (not with a single firm move). Ticks should never be covered with such substances as petrol, kerosene, petroleum jelly or nail varnish, as the suffocating tick introduces pathogens into the skin along with its vomit. The spot where the tick was removed should be next disinfected. Further, one should not crush the removed tick with bare toes or fingernails to avoid contact with secretions (containing infectious microorganisms) or a minor damage to the skin on the hands or conjunctiva, which could cause accidental infection.

A prophylactic use of one-two-dose antibiotics is advisable only for the persons with multiple tick bites. It is more important for the physician to inform the person about the possible symptoms, the time of their appearance as well as observe the spot [24].

References:

1. Siuda K. Kleszcze (*Acari: Ixodida*) Polski. Wydawnictwo Naukowe PWN, Warszawa 1991 (in Polish).
2. Swanson SJ, Neitzel D, Reed KD, Belonga EA. Coinfections aquired from Ixodes ticks. Clin Microbiol Rev. 2006; 19: 708-729.
3. Chmielewski T, Tylewska-Wierzbanowska S. Factors responsible for the development of Lyme carditis. Post Mikrobiol. 2017; 1: 100-105 (in Polish).
4. Stanek G, Strle F. Lyme borreliosis. Lancet. 2003; 362(9396): 1639-47.
5. Wilske B. Epidemiology and diagnosis of Lyme borreliosis. Ann Med. 2005; 37(8): 568-79.
6. Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol. 2007; 49(1): 13-21.
7. Goettner G, Schulte-Spechtel U, Hillermann R, Liegl G, Wilske B, Fingerle V. Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DbpA homologues. J Clin Microbiol. 2005; 43(8): 3602-9.
8. Nohlmans MKE, Blaauw AAM, van den Bogaard AEJ, van Boven CPA. Evaluation of nine serological tests for diagnosis of Lyme borreliosis. Eur J Clin Microbiol Infect Dis. 1994; 13: 394-400.
9. Robertson J, Guy E, Andrews N, Wilske B, Anda P, Grandstrom M, et al. A European multicenter study of immunobloting in serodiagnosis of Lyme borreliosis. J. Clin. Microbiol. 2000; 38: 2097-2102.
10. Broqui P, Lascola B, Roux VR, Raoult D. Chronic Bartonella guintana bacteremia in homeless patients. N Engl J Med. 1999; 340: 184-189.
11. Chomel BB, Boulouis HJ, Breitschwerdt EB. Cat scratch disease and other zoonotic Bartonella infections. J Am Vet Med Assoc. 2004; 224: 1270-1279.
12. Breitschwerdt EB, Kordick DL. Bartonella infection in animals: carrieership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13: 428-438.
13. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB. Factors associated with the rapid emergence of zoonotic Bartonella infections. Vet Res. 2005; 36: 383-410.
14. Brenner DJ, O'Connor S, Winkler HH, Steigerwalt AG. Proposals to unify the genera Bartonella and Rochalimaea, with descriptions of Bartonella quintana com. nov., Bartonella henselae comb. nov. , and Bartonella elizabethae comb. nov., and to remove the family Bartonellaceae from order Rickettsiales. Intern J Syst Bacteriol. 1993; 43: 777-786.
15. Welc-Falęciak R. Współczesna wiedza o zakażeniach wywołanych przez pałeczki Bartonella (Current state of the knowledge of Bartonella infections). Przegl. Epidemiol. 2009, 63: 11-17.
16. Oteo JA, Portillo A. Tick-borne rickettsioses in Europe. Ticks Tick Borne Dis. 2012; 3(5-6): 271-8.
17. Chmielewski T. Riketsjozy przenoszone przez kleszcze - grupa gorączek plamistych w Polsce. Klinika Pediatryczna. 2016; 23(2): 224-227 (in Polish)
18. Rymaszewska A, Piotrowski M. Use of DNA sequences for Rickettsia identification in Ixodes ricinus ticks: the first detection of Rickettsia monacensis in Poland. Microbes Infect. 2013; 15(2): 140-6.
19. Chmielewski T, Podsiadly E, Karbowiak G, Tylewska-Wierzbanowska S. Rickettsia spp. in ticks, Poland. Emerg Infect Dis. 2009; 15(3): 486-8.
20. Stańczak J, Biernat B, Matyjasek A, Racewicz M, Zalewska M, Lewandowska D. Kampinos National Park: a risk area for spotted fever group rickettsioses, central Poland? Exp Appl Acarol. 2016; 70(3): 395-410.
21. Wójcik-Fatla A, Cisak E, Zając V, Sroka J, Sawczyn A, Dutkiewicz J. Study on tick-borne rickettsiae in eastern Poland. I. Prevalence in Dermacentor reticulatus (Acari: Amblyommidae). Ann Agric Environ Med. 2013; 20(2): 276-9.
22. Podsiadły E, Chmielewski T, Karbowiak G, Kędra E, Tylewska-Wierzbanowska S. The occurrence of spotted fever rickettsioses and other tick-borne infections in forest workers in Poland. Vector-borne and Zoonotic Dis. 2011; 11(7): 985-9.
23. Parola P, Rovery C, Rolain JM, Brouqui P, Davoust B, Raoult D. Rickettsia slovaca and R. raoultii in tick-borne Rickettsioses. Emerg Infect Dis. 2009; 15(7): 1105-8.
24. Biggs HM, Behravesh CB, Bradley KK, Dahlgren SF, Drexler NA, Dumler JS, et al. Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever and Other Spotted Fever Group Rickettsioses, Ehrlichioses, and Anaplasmosis — United States. MMWR Recomm Rep 2016; 65(No. RR-2): 1–44.
25. Bakken JS, Dumler JS. Human granulocytic anaplasmosis. Infect Dis Clin North Am. 2015; 29(2): 341-55.
26. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin Infect Dis. 2007; 45(Suppl 1): S45-51.

27. Skotarczak B, Rymaszewska A, Wodecka B, Sawczuk M, Adamska M, Maciejewska A. PCR detection of granulocytic Anaplasma and Babesia in Ixodes ricinus ticks and birds in west-central Poland. Ann Agric Environ Med. 2006; 13(1): 21-3.
28. Grzeszczuk A. Anaplasma phagocytophilum in Ixodes ricinus ticks and human granulocytic anaplasmosis seroprevalence among forestry rangers in Białystok region. Adv Med Sci. 2006; 51: 283-6.
29. Welc-Falęciak R, Kowalec M, Karbowiak G, Bajer A, Behnke JM, Siński E. Rickettsiaceae and Anaplasmataceae infections in Ixodes ricinus ticks from urban and natural forested areas of Poland. Parasit Vectors. 2014; 7: 121.
30. Zwoliński J, Chmielewska-Badora J, Cisak E, Buczek A, Dutkiewicz J. Prevalence of antibodies to Anaplasma phagocytophilum and Borrelia burgdorferi in forestry workers from the Lublin region. Wiad Parazytol. 2004; 50(2): 221-7.