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Review articles

Feline bartonellosis key issues and possible vectors

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ABSTRACT. Bartonellosis is a disease caused by *Bartonella* spp. microorganisms which belong to the Rickettsiales order. This disease is a zoonosis, *B. henselae*, whose primary reservoir is the cat, which in humans causes a cat-scratch disease. In infected cats, symptoms such as fever, lymphedema, reproduction disorders, myocarditis, rhinotracheitis, gingivitis, and arthritis may be observed. *Bartonella* appears to be transmitted among cats and dogs in vivo exclusively by arthropod vectors (excepting perinatal transmission), not by biting or scratching. In the absence of these vectors, the disease does not spread. On the other hand, the disease can be spread to humans by bites and scratches, and it is highly likely that it is spread by arthropod vectors as well. This review presents a potential role of ticks and fleas in the transmission of bartonellosis. Clinicians should be aware that a common illness, such as infection with *Bartonella*, can be transmitted by arthropod vectors, and that a history of animal scratches or bites is not necessary for disease transmission.

Key words: *Bartonella* spp., cats, vector-borne disease, ticks, fleas

Introduction

Bartonellosis is a disease caused by *Bartonella* spp., which belong to the order Rickettsiales. Within the genus *Bartonella* are 24 species, of which more than 10 can infect cats and dogs (Table 1). *Bartonella* are small, pleomorphic, Gram-negative, intracellular bacilli [1]. The disease is a zoonosis, *B. henselae*, whose primary reservoir is the cat, which in humans causes a cat-scratch disease. This pathogen is widespread in the global population of cats (8–56% of clinically healthy animals have bacteraemia) [2].

In cats, mostly *B. henselae* are detected, rarely *B. clarridgeiae* or *B. koehlerae*. The microorganisms are more frequently isolated in young cats below one year old and in wild cats or those living in larger groups (e.g. in shelters). Bartonellosis is endemic, it can be found in warm and moist climates. In countries where the average annual temperature is low (e.g. Norway) it is practically non-existent in cats. In the *B. henselae* species, there are at least two genotypes of bacteria (Houston-1 and Marseille). The Marseille genotype is prevalent in western USA, in Europe and Australia, while the Houston-1 genotype is prevalent in Asia [3].

Pathogenesis and clinical symptoms

Bartonellosis is considered to be transferred on cats by ectoparasites (fleas, ticks). Other infection routes include blood transfusion and intrauterine. After penetrating into the body, the bacteria adhere to epithelial cells, including vascular endothelium, and proliferate inside them in phagosomes. They attack bone marrow progenitor cells as well [4]. *B. henselae* forms large aggregates which bind to the cell surface and are absorbed, creating a vacuole called an „invasome". Inside, the bacteria proliferate while being inaccessible to the immune system of the host and lysosomal enzymes. During division, the bacteria release proinflammatory factors, growth factors and apoptosis inhibitors. This activity may result in the proliferation of the host's cells and the formation of tumorous structures in the vascular endothelium. Then the bacteria are released to the blood, where they attack erythrocytes. Bacteraemia may persist for weeks, months or even years. After penetrating into the erythrocytes, the bacteria divide rapidly and remain inside until the cell disintegrates [1]. There are many factors which decide whether the disease

Table 1. Species of *Bartonella* spp. isolated from cats and dogs

Species	Vector
B. henselae	Cat flea (*Ctenocephalides felis*)
B. clarridgeiae	Cat flea (*Ctenocephalides felis*)
B. vinsonii subsp. *berkhoffi*	Unknown (fleas, ticks?)
B. koehlerae	Cat flea (*Ctenocephalides felis*)
B. bovis	Unknown
B. quintana	Body louse (*Pediculus humanus*)

develops in an infected cat or not. Apart from the virulence of the strain, these factors include: living and feeding conditions, concurrent diseases, congenital defects, use of immunosuppressive drugs, etc.

In many cases, an infection with *Bartonella* spp. is asymptomatic or has non-specific symptoms, such as gingivitis, arthritis, chronic rhinotracheitis, reproduction disorders, neurological symptoms, uveitis etc.

In cats, the first symptoms of bartonellosis are fever and enlarged lymph nodes [5,6]. Also, fertility disorders may occur, and endocarditis and myocarditis may develop. The bacterial DNA was isolated from the blood, from inflammatory lesions of the wrist and metacarpal bones and from the heart [7,8]. It is presumed that infections with these microorganisms may be responsible for the development of various feline pathological conditions that were earlier considered as idiopathic, such as lower respiratory tract infections [9,10], pancreatitis [11], choroiditis [12,13], gingivitis [10,14,15] and rhinosinusitis [16].

Diagnosis

Diagnosing bartonellosis is difficult. The disease should be suspected in animals from places where there is an endemic occurrence of *Bartonella* spp., with clinical symptoms which may be a consequence of endocarditis or myocarditis, and with the confirmed presence of fleas.

Haematological examinations of infected animals reveal minor non-regenerative anaemia, leucocytosis, neutrophilia and thrombocytopenia. Biochemical tests of the blood serum may show slight azotaemia, hypoalbuminemia, less frequently hyperglobulinaemia. The elevated activity of AST, ALT and AP in the blood serum is observed in specimens which developed hepatic impairment in the course of the disease [17,18].

Lesions typical of heart failure, such as pulmonary oedema, are visible in a chest X-ray, while an abdominal ultrasound reveals enlarged lymph nodes, spleen and liver [17].

A definitive diagnosis of bartonellosis may be achieved on the basis of positive results of a blood culture. However, routine bacteriological tests are not very sensitive, which is clearly related to the low bacteraemia in *Bartonella* infections. The blood is collected in test tubes with EDTA, and cultures are prepared on special beds, such as chocolate agar, and then incubated at 35–37°C in an atmosphere enriched with 5% CO_2 [19]. The culturing process may take up to 6–8 weeks.

Currently, the diagnosis of bartonellosis is based on serological tests and PCR assays. Commercial serological tests are available only for some bacterial species, such as: *B. henselae, B. quintana* and *B. vinsonii* subsp. *berkhoffii*. Moreover, these tests are not 100% specific, and their results depend on the time from infection [3]. The tests used most frequently in the diagnosis of the disease include immunofluorescence, ELISA and Western blotting. As the course of bartonellosis is usually chronic, testing pairs of serum samples collected from animals with a suspicion of this disease is not useful as the determination of the increasing titre of anti-*Bartonella* antibodies between the tests is impossible. Serological tests are used most often in the cases when the PCR assay and culture give negative results, while the clinical course of the disease indicates bartonellosis, or as supplementary tests, apart from molecular analyses [20].

Due to the imperfections of the traditional diagnostic methods of *Bartonella* infections, a search was initiated for molecular methods helpful in the detection of these bacteria. The most frequent

molecular method used for this purpose is PCR, and ftsZ gene coding one of the proteins crucial for bacterial cell division is the molecular marker useful for the detection and identification of *Bartonella* [20,21].

Treatment and prevention

The prognosis for patients with bartonellosis complicated with endocarditis is unfavourable. None of the antibiotics used currently for this disease in cats and dogs is capable of total elimination of bacteraemia. In cats, the most frequently used chemotherapeutics include: ampicillin, gentamicin, doxycycline, enrofloxacin and amoxicillin with clavulonic acid. It must be remembered that resistance of *B. henselae* to azithromycin and fluoroquinolones has been reported [22]. In cats, the treatment should be initiated with high doses of doxycycline or amoxicillin with clavulonic acid. If there is no improvement after seven days of therapy, a change in the antibiotic is recommended, e.g. to enrofloxacin or azithromycin [23].

Patients with circulatory failure usually require supportive treatment, including the administration of furosemide, for example.

Currently, there are no vaccines for feline bartonellosis available on the market. Attempts to immunize using one bacteria species resulted in the development of immunity to the strain in the vaccine, and did not achieve cross-immunity [24,25].

The best method of prevention for the disease is the regular application of anti-ectoparasitic products and keeping animals at home.

Fleas as vectors of bartonellosis

It seems that cat fleas (*Ctenocephalides felis*) are the most important vector of bartonellosis. This stems from the fact that they are the most common parasitic arthropods living on cats, and the transfer of bartonellosis is associated with parasitic arthropods [26]. *B. henselae* is spread by the transfer of fleas feeding on infected cats to specific-pathogen free (SPF) cats and by the intradermal administration of flea feces. However, the percentage of vectors infected with these rickettsia is highly variable: from 0% in Spain [27] to over 18% in New Zealand [26] and 57.1% in Italy [28].

In 2017, an extensive molecular study was conducted in Tunisia in order to detect the DNA of *Bartonella* spp. in fleas collected from various pets and farming animals: cats, dogs, sheep and goats. In total, the analysis included 2,178 (67% female, 33% male) arthropods belonging to the following species *C. felis* (83%, n=1803) *C. canis* (12%, n=266) and *Pulex irritans* (5%, n=109). Using the amplification of citrate synthase gene (gltA), the DNA of *Bartonella* was detected in 14% (121/866) of fleas. This rate was lower than in the studies conducted in the UK (17% of infected fleas) and France (26.2% of infected fleas), but higher than in the studies conducted in the USA (11.3%), Israel (7.8%), Hungary (4%) and the neighbouring countries of Tunisia, such as Morocco (4%) and Algeria (9.33%). Of all the studied flea species, the highest *Bartonella* infection intensity was observed in *C. canis* (55% of studied parasites), followed by *P. irritans* (23.5%) and *C. felis* (6.7%). The arthropods were the carriers of three species of rickettsia, classified as *B. elizabethae, B. henselae* and *B. clarridgeiae.* There was one more genotype, but it was impossible to classify it on the level of species. The study showed differences in the frequency of infection with *Bartonella* between populations of fleas and confirmed that the species of the vector plays an important role in the transfer of the disease [29].

Interesting observations were made by Ying Bai et al. [30], who demonstrated a correlation between the occurrence of the rickettsia in the fleas and the places from which the fleas were collected. The authors studied 632 fleas collected from household animals and the environment. The infection rate was significantly higher in fleas collected from their hosts (97%) than in those collected from the environment (3%). The majority of the fleas infected with *Bartonella* (14% in total) collected from the hosts belonged to *D. lypusus* (61%), *X. cheopis* (20%) and *Ctenophthalmus calceatus* (14%), while the majority of the infected fleas collected from the external environment were *C. felis.* The molecular analysis of the pathogens found in the arthropods enabled the identification of 12 genetic variants of *Bartonella*, 9 of which belonged to *B. elizabethae.*

The genetic material of the rickettsia was found in fleas living not only on household animals, but also on wild mammals. Osikowicz et al. [31] conducted PCR-based epidemiological studies aimed at detecting the DNA of *Bartonella* spp. in *Oropsylla montana*, *Hoplopsyllus anomalus* and

Echidnophaga gallinacea feeding on *Otospermophilus beecheyi*. The authors analysed 194 fleas. The results showed that out of the 3 studied flea species, only *Oropsylla montana* was a vector of *Bartonella*, and the infection rate for this species was 30.9%. *Bartonella* was not found in any of the analysed specimens of *Hoplopsyllus anomalus* and *Echidnophaga gallinacea.* These results show that fleas as a group of arthropods do not constitute a universal vector of bartonellosis, and that the pathogen exhibits preferences for certain species of these arthropods. On the other hand, they show that wild animals such as squirrels, in which the *Bartonella* infection rate ranged from 25% to 100%, depending on the location, may be a significant reservoir of the bacteria for the arthropods, which then transmit the bacteria to other hosts.

This thesis is confirmed by the results published by Silaghi et al. [32]. They studied the occurrence of *Bartonella* in wild rodents and in the fleas living on them in Germany. The fleas collected from the forest mammals included: *Ctenophthalmus nobilis*, *Megabothris turbidus*, *Nosopsyllus fasciatus*, *Doratopsylla dasycnema* and *Megabothris walkeri.* The total occurrence of *Bartonella* in the fleas was 54.1%. The majority of the 135 studied ectoparasites (n=45) carried an unclassified genotype of *Bartonella* sp., followed by *B. grahamii* (n=14), *B. taylorii* (n=8), *B.* sp. N40 (n=5) and *Bartonella elizabethae* (n=2).

Without doubt, the above literature review shows the large epizootic potential of fleas in spreading bartonellosis. However, it should be emphasized that how these arthropods transfer the disease is unclear, although it seems that the main transfer route is via the saliva. However, the most recent studies [25] show that cats exposed to fleas infected with *Bartonella* closed in bags that enabled feeding but prevented the contamination of the cats' skin and hair with flea faeces were not infected with *B. henselae.* This suggests that the transmission does not occur via the saliva, and encourages the search for other, additional vectors of the disease, possibly ticks.

Ticks as vectors of *Bartonella*

The presence of *Bartonella* in ticks has been confirmed in Europe, Asia, South and North America and Africa. In European countries, rickettsia are most frequently found in arachnoids belonging to *Ixodes ricinus* [6]. It has been confirmed that these ticks are able to transfer a pathogen of mice, *Bartonella birtlesii,* between rodents in *in vivo* conditions. This has not been observed for cats and *B. henselae,* but it has been confirmed that in *in vitro* conditions *Ixodes* may be infected with the pathogen and excrete it with the saliva [33]. In Europe, the occurrence of the DNA of *B. henselae* in *Ixodes ricinus* ranges from 0% in Finland to 60% in the Netherlands.

Muller et al. [34] conducted a study aimed at identifying the species of *Bartonella* transferred by *I. ricinus* in Austria. The study population involved 515 ticks, including 8 larvae, 348 nymphs and 159 adult forms. The infection rate in the study group of arthropods was 2.1% (11/515), and the main species identified were *B. quintana* and *B. henselae* [34].

The results presented by Rogovskyya [35] confirm that *I. ricinus* may be a vector of bartonellosis in the Ukraine. Out of 378 ticks collected in the Kiev area, the DNA of the rickettsia was detected in 2.7% of the arachnoids.

In the Italian province of Belluno, this rate was slightly lower, at 1.48% [36]. Still lower occurrences of *Bartonella* in these arachnoids were observed in Luxembourg (0.3%) [37] and Belarus (0.7%) [38]. Data from Germany, France and Portugal indicate that *Bartonella* infection rates in tick populations are 0.5–11.8%, 9.8–38.2% and 0–32.3%, respectively [39–42]. The results of these studies showed that the occurrence of *Bartonella* was significantly higher in adult ticks than in nymphs. From the observations of Dietrich et al. [39], this rate was fifteen times higher in the adult forms.

In the years 2007–2009, the potential role of *I. ricinus* as a vector of *B. henselae* was studied in eastern Poland. The results of the studies on ticks were compared with the occurrence of anti-*B. henselae* antibodies in people professionally exposed to arachnoids (94 farmers and 238 foresters). PCR-assays were performed for 1,603 *I. ricinus* (403 females, 450 males, 750 nymphs). The occurrence of *B. henselae* in ticks was 1.7%; the infection rates in adult males (3.1%) and adult females (2.7%) were almost 10 times higher than in nymphs (0.3%).

The presence of *B. henselae*-specific antibodies was detected in 30.4% of the study subjects (27.7% of farmers, 31.5% of foresters, and 8.9% of the control group). The results show a weak positive correlation between the *Bartonella* infection rate in ticks and the presence of the antibodies specific for

these bacteria in the serum of humans living in the area from which the studied arachnoids were collected. The lack of a direct relationship between these two factors indicates that human-tick contact is only one of the factors contributing to *Bartonella* infections in people. Other factors, such as contact with cats, fleas and other arthropods, must be taken into account in the epidemiology of the disease.

The results of monitoring conducted in Slovakia [43] causes some doubts as to the role of ticks as a vector of *Bartonella.* Tests for *Bartonella* were carried out on 5,767 *I. ricinus,* including 4795 nymphs and 972 adult forms. The genetic material of the rickettsia was not found in any of the studied arachnoids. Interestingly, the presence of the genetic material of *Bartonella* in wild rodents (n=641) from the territory of Slovakia, which were the source of some of the analysed ticks, was detected in as much as 64.8% of the study population.

This means that the role of ticks in the transmission of *Bartonella* requires further study. Although fleas and ticks are considered to be the main sources of infection in household animals, especially cats, the analysis of the transmission capability of these arthropods is in some doubt as to whether vector-based transmission is the only transmission route of the disease.

There are now increasing numbers of cases of bartonellosis seen in cats from territories previously considered as non-endemic to *Bartonella.* This proves that the pathogens are extending their range, and more understanding about their transmission routes is needed to protect animals against the disease.

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