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Cats as a reservoir of *Bartonella henselae* for dogs

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Abstract
Bartonellosis is a disease affecting a variety of animals. Many *Bartonella* infections are zoonotic, including cat scratch disease. Within the genus *Bartonella* are 45 species, of which more than 10 can infect cats and dogs. Companion animals serve as reservoirs for several zoonotic species of *Bartonella*, and may also serve as sentinels for zoonotic *Bartonella* species harbored by wildlife. The aim of this study was to determine the frequency of the occurrence of *Bartonella* spp**.** DNA in dogs from households where cats with clinical bartonellosis were kept. The presence of DNA with 99–100% compliance of the nucleotide sequence with the sequence of the *Bartonella* DNA isolated from cats was demonstrated in the body of 10% of tested dogs. The results indicate that cats serve as a *Bartonella* reservoir for dogs, and the dogs can play the same role with regard to humans.

Key words
Bartonella spp., dogs, PCR, vector-borne disease

INTRODUCTION

Bartonellosis is a disease caused by *Bartonella* spp., which belong to the order *Rickettsiales*. Within the genus *Bartonella* there are 45 species, of which more than 10 can infect cats and dogs. *Bartonella* are pleomorphic, Gram-negative, intracellular bacilli with a size of 0.3–0.6 × 0.3–1.0 µm [1]. The disease is a zoonosis, *B. henselae*, which in humans causes a cat-scratch disease. Although cats are considered to be the primary reservoir host for *B. henselae*, DNA of this bacterial species has been found in dogs, cows, horses, feral swine, marine mammals and sea turtles [1]. Dogs infected with *B. henselae* display a wide clinical spectrum similar to that observed in humans [2, 3].

Data concerning bartonellosis in dogs in Poland are scarce. The presence of the DNA of these *Rickettsiales* has been demonstrated in the blood of 1% of dogs with suspected borreliosis [4]. In turn, in one study, Podsiadły et al. [5] demonstrated the presence of low titers of antibodies against *Bartonella* in the blood of 49% dogs in Poland.

Cat fleas (*Ctenocephalides felis*) are considered to be the primary vector for transmission to reservoir and potentially non-reservoir hosts, such as dogs and humans; however, *B. henselae* DNA has also been amplified from *Ixodes* ticks [6] and woodlouse hunter spiders [5, 7]. It is not entirely clear what serves as a *Bartonella* reservoir for dogs. It is assumed that in households where dogs are kept together with cats, the latter may serve as such reservoirs [8].

The aim of the study was to determine the frequency of occurrence of *Bartonella* spp. DNA in dogs from households where cats with clinical bartonellosis were kept.

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MATERIALS AND METHOD

The study involved 40 dogs: 32 males and 8 females (Tab. 1) from households located in the east of Poland, where cats with clinical bartonellosis caused by *B. henselae* were identified. None of the dogs showed any disease symptoms: body temperature, heart rate and respiratory rate were within the reference ranges, palpable lymph nodes were not enlarged, none of the dogs showed signs of systemic illness (Tab. 1). All 40 dogs had contact with sick cats. Twenty-six dogs were kept inside houses, the other 14 in dog pens. All dogs were vaccined against parvovirosis, distemper, adenovirosis, parainfluenza and rabies, and also received prophylaxis against ectoparasites (depending on the form of the used product: tablets, spot-on, collar, every 1–3 months). During the anamnesis, it was established that the owners did not record the presence of ticks or fleas on the dogs' bodies. Also during the clinical examination of the animals (in most cases the wet paper test was performed) no ectoparasites were observed on the body surface of the animals. Blood samples were taken from all dogs during a visit in the clinic, and tested for bartonellosis using molecular methods. Blood examination was part of regular control clinical examination which is performed on patients of the Clinic of Infectious Diseases in Lublin at least once a year. All owners agreed to the collection and examination of blood.

DNA extractions for molecular tests were performed using the DNA Blood kit (A&A Biotechnology Gdańsk, Poland). The extracted DNA was subjected to PCR, performed with the primers targeting fragments of the citrate synthase gene: one generic forward primer (BART- LC-GEN-F: 5' – ATGGGTTTTGGTCATCGAGT – 3'); one specie-specific reverse *B. henselae*, primer (BART-LC-HEN-R: 5'–AA ATCGACATTAGGGTAAAGTTTTT – 3'); and one species specific reverse *B. clarridgeiae* primer (BART-LC-CLA-R: 5'- CAAGAAGTGGATCATCTTGG – 3'), according to the method described by Staggemeter et al. [9], with small

Table 1. Description of the dogs using in the study

No	Gender	Age	Breed	Body weight (kg)	Way of keeping	Body temp. (º)	HR (min)	RR (min)	Prophylaxis against ectoparasites
1.	Female	2 years	German Shepherd	30	Pen	37,8	83	12	Collar
2.	Female	4 years	Mix	7	House	38,0	106	26	Spot –on
3.	Female	1,5 year	Boxer	28	House	37,7	96	21	Spot-on
4.	Female	5 years	Alaskan malamulte	35	Hose		88	18	Tablets
5.	Female	2 years	Mix	14	Pen	38,2	110	30	Tablets
6.	Female	7 years	Mix	12	House	38,6	104	27	
7.	Female	6 months	Irish setter	25	House	38,0	88	22	Tablets
8.	Female	3 years	Mix	6	Pen	38,9	112	29	Spot-on
9.	Male	5 years	Mix	10	Pen	38,1	99	21	Spot-on
10.	Male	1,5 year	Dachshund	9	House	37,7	92	26	Tablets
11.	Male	6 years	Mix	17	House	38,2	85	20	Spot-on
12.	Male	6 years	Doberman	40	Hose	38,7	74	19	Tablets
13.	Male	10 years	Schnauzer	15	House	37,6	80	20	Tablets
14.	Male	1,5 year	Mix	18	House	38,4	91	22	Spot-on
15.	Male	7 years	Mix	10	Hose	38,9	111	28	Spot-on
16.	Male	1 year	Dog	46	House	37,9	77	16	Collar
17.	Male	4 year	Bernese mountain dog	52	Pen	37,6	75	20	Collar
18.	Male	5 year	Mix	10	House	38,0	115	30	Spot-on
19.	Male	11 years	Irish setter	33	House	38,1	78	11	Tablets
20.	Male	5 years	Mix	16	Hose	38,6	118	32	Spot-on
21.	Male	2 years	German Shepherd	32	House	38,0	86	21	Tablets
22.	Male	3 years	Doberman	37	House	37,8	97	27	Tablets
23.	Male	10 years	Mix	19	Pen	38,2	102	28	Spot-on
24.	Male	8 years	Mix	15	Pen	38,7	113	25	Collar
25.	Male	8 years	Mix	10	Pen	38,5	99	18	Spot-on
26.	Male	4 years	Cane corso	44	House	38,0	72	15	Tablets
27.	Male	7 years	Mix	9	House	38,8	93	16	Spot-on
28.	Male	8 years	German Shepherd	37	Pen	37,9	82	19	Tablets
29.	Male	2 years	Mix	12	Pen	38,1	90	13	Spot-on
30.	Male	3 years	Jack Russel Terrier	10	House	37,9	88	22	Spot-on
31.	Male	6 years	Mix	8	Pen	37,6	107	26	Spot-on
32.	Male	2 years	Cocker spaniel	14	House	37,7	95	23	Tablets
33.	Male	4 years	Mix	23	House	38,1	82	20	Spot-on
34.	Male	10 years	German Shepherd	35	Hose	37,8	80	20	Collar
35.	Male	6 years	Jack Russel Terrier	11	House	38,8	103	26	Tablets
36.	Male	2 years	Mix	8	House	37,9	109	24	Spot-on
37.	Male	8 years	Mix	17	Hose	38,3	100	25	Spot-on
38.	Male	5 years	Mix	20	Pen	38,6	88	27	Spot-on
39.	Male	7 years	German Shepherd	41	Pen	38,1	81	19	Spot-on
40.	Male	2 years	Mix	11	Pen	38,5	92	22	Tablets

modifications: an initial denaturation of 2 min at 95 °C, followed by 37 cycles consisting of denaturation at 96 °C for 60 s, annealing at 55 °C for 60 s and elongation at 72 °C for 90 s. Reaction mixture (50 µL) contained 100 µM of each dNTP, 1.6 mM of MgCl2, 0.25 µM of each primer, 2.5 U of Taq DNA polymerase, and 5 µL of DNA template. A negative control, consisting of distilled water, and positive control, consisting of extracted DNA from a blood sample known to contain *B. henselae* (obtained from Institute of Parasitology, Academy of Science, Kosice, Slovakia), were used in each PCR run.

The size of each PCR product was analysed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. The PCR products were purified using QIAquick spin columns (Qiagen) and eluted in 50 µl of Tris 10 mM, pH 7.6. The DNA sequence was determined on both strands using the same primers employed for PCR at a DNA sequencing core facility (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland). The DNA sequences were assembled and edited using SeqMan (DNAstar, Lasergene, Madison, USA), and ClustalV alignments.

RESULTS AND DISCUSSION

The presence of *B. henselae* DNA was detected in the blood of four out of 40 tested individuals (10%). The size of the amplified citrate synthase gene fragment was 250 bp. Sequencing the PCR product obtained from the dogs and comparing its nucleotide sequence with the sequence of the gene fragment obtained from the cats from the same hosehold, as well as with the sequence deposited in GenBank under Accession No. L38987.1, showed a high level of homology: 99–100%. The dogs infected with bacteria came from urban (two individuals) and from rural areas (two individuals).

These results indicate that the problem of bartonellosis in dogs in Poland may be more significant than is assumed. In own studies the presence of the DNA of these pathogens were detected in 10% of the tested animals which manifested no clinical symptoms of the disease. However, the results concerned a small group of dogs, which additionally were more likely to become infected due to their contact with cats which were infected with *Bartonella*.

In 2006–2008, Welc-Falęciak et al. [10] examined 82 sled dogs for tick-borne diseases but did not detect the *Bartonella spp* DNA in the blood of any of the tested animals. Rymaszewska and Adamska [4] found such DNA in 1% of dogs with suspected Lyme disease, but none in 100 healthy subjects.

A comparison between those results and the results of the presented study leads to the cooonclusion that the problem of dog bartonellosis in Poland may be underestimated. In neighbouring countries, a molecular monitoring of dog bartonellosis has been conducted only in the Czech Republic, where Konvalinova et al. [11] demonstrated the presence of the DNA of these pathogens only in 0.7% of 286 dogs, both healthy and with various health problems. Although the mode of infection was not determined, the bacteria could have been transmitted by scratching during a fight between a dog and a cat from the same household, or by flea transmission between these animals, as identical citrate synthase gene fragments of *B. henselae* were amplified and sequenced from the dog and cat blood.

Flea-infested cats may be a source of infection for domestic dogs. Flea transmission from *B. henselae* bacteremic cats to specific pathogen-free dogs has been documented experimentally [8]. On the other hand, dogs might serve as a source for human *Bartonella* spp. infections. In China, the seropositivity rate among dog bite victims was significantly higher than the seroprevalence among blood donors [12]. Despite an increasing number of canine bartonellosis reports being published, dogs are still not considered to be a major reservoir for *B. henselae*.

B. henselae detected in the blood of four dogs in the current study is a serious zoonotic agent, and the obtained results show the necessity of considering dogs as a potential source of human infection. On the other hand, it is necessary to point to the fact that cats are also a potential source of direct (by scratching or by bite) or indirect (by fleas) infection for dogs.

The prevention of bartonellosis generally lies in the protection of animals against flea and tick infestation. It is therefore advisable to treat animals with anti-parasitics on a regular basis, either in the form of spray, spot on, oral drugs or collars. These measures do not give full protection against infection, but they reduce significantly the possibility of its development.

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