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Epizootic situation of feline *Bartonella* infection in eastern Poland

Łukasz Mazurek[1], Alfonso Carbonero[2], Maciej Skrzypczak[3], Stanisław Winiarczyk[1], Łukasz Adaszek[1]

[1]Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, 20-612 Lublin, Poland
[2]Departamento de Sanidad Animal, Facultad de Veterinaria, UCO, Campus Universitarios de Rabanales, 14071, Córdoba, Spain
[3]Second Department of Gynaecology, Prof. F. Skubiszewski University School of Medicine, 20-090 Lublin, Poland
ukaszek0@wp.pl

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Abstract

Introduction: The aim of the study was to establish the prevalence of *Bartonella* spp. in cats in eastern Poland, and to determine the factors associated with the infection. **Material and Methods:** PCRs were performed to detect *Bartonella* DNA in the whole blood of 672 cats from four regions in eastern Poland (the Lublin, Podlasie, Masovian, and Subcarpathian provinces). The association between the previously selected variables and the dependent variable (presence of *Bartonella* DNA) was investigated using a logistic regression model. **Results:** The overall prevalence of infection was 40.48%. All PCR positive cats were infected with *B. henselae*. The living conditions of the animals (free outdoor roaming), mixed breed cats, Subcarpathian region, and absence of tick control were significant risk factors associated with *Bartonella* infection at a 95% confidence level. **Conclusion:** Cats in eastern Poland appear to be at risk of a bartonellosis epizootic. Factors which seem to impact the likelihood of infection in cats and factors which seem not to impact it have been suggested. We advocate additional research into the ways bartonellosis spreads, its geographical scope, and the factors that favour its development.

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

Introduction

Bartonella are small, pleomorphic, Gram-negative, intracellular bacilli belonging to the order *Rickettsiales*. Currently the genus *Bartonella* includes 24 species (9). *Bartonella* microorganisms can infect dogs, cats, and humans, causing cat-scratch disease. These pathogens are very common in the population of cats (bacteraemia is present in 8%–56% of clinically healthy cats) (4, 22).

The main aetiological factors of feline bartonellosis are *B. henselae*, *B. clarridgeiae*, and *B. koehlerae*. At least two genotypes can be distinguished in *B. henselae*: Houston-1 and Marseille. The Houston-1 genotype is prevalent in Asia, while the Marseille genotype has a larger presence in the USA, Europe, and Australia (3).

The infections they cause are mainly observed in cats which are less than one year old and live in large groups (*e.g.* in shelters). The disease is observed in areas where the climate is hot and humid.

The main vectors of feline bartonellosis are ectoparasites (fleas and ticks). In an infected organism the bacteria colonise the vascular endothelium cells, erythrocytes, and bone marrow progenitor cells (19). In the course of the disease, the bacteraemia may persist for weeks or even years (9). The factors that determine the development of the disease include the virulence of the *Bartonella* strain, living and feeding conditions, concurrent diseases, congenital defects, use of immunosuppressive drugs, *etc*. The course of bartonellosis may be asymptomatic or the infection may involve non-specific clinical signs, such as gingivitis, arthritis, reproduction disorders, neurological symptoms, uveitis *etc*.

To date, no epidemiological studies focused on feline bartonellosis have been conducted in Poland. The presence of *Bartonella* has only been demonstrated in Poland in ticks (25, 29). The aim of this study is to estimate the epizootic situation of feline bartonellosis in eastern Poland.

Material and Methods

Animals and sampling. In the period from January 2017 to July 2018, a total of 672 cats were randomly selected from the nine largest veterinary clinics in eastern Poland for blood sampling for molecular tests concerning *Bartonella* spp. None of the cats had ever left Polish territory. Of these, 288 were referred to the clinics for prophylactic reasons (vaccination as prophylaxis against ecto- and endoparasites). The remaining 384 cats were referred to veterinary practices with various clinical problems (infectious disease, kidney disease, diarrhoea, diabetes, and allergy). The samples were collected by veterinary practitioners upon request. The owners of all the cats agreed that the cats could participate in the study.

Epidemiological data from all the cats were recorded by means of a questionnaire, completed for each animal and containing the following information:

- Region: cats were examined from four provinces (the highest administrative subdivision level of Poland). The study comprised cats from eastern Poland (the Lublin, Podlasie, Masovian, and Subcarpathian provinces). The characteristics of the regions are described by Gorzelak *et al*. (13).
- Breed and sex: the sample included cats from many breeds (504 European Shorthaired cats, 28 Persian, 28 Cornish Rex, 28 Maine Coon, 20 Sphinx, 20 Siamese, 16 Norwegian, 12 Siberian, 12 British, and 4 Japanese), including both males (208) and females (464). As a consequence, it was decided to classify as purebred or mixed breed.
- Living conditions: the cats were classified by whether they were kept indoors (336) or could roam freely outdoors (336).
- Use of tick- or vector-borne disease control measures (prophylaxis against ectoparasites in the form of a spray, collar, or spot) (272).
- Age: the cats were classified as young (under one year old) (112) or adult (one year old or older) (560).

The questionnaires for the cats were completed by the authors.

DNA extraction and PCR amplification. DNA extractions for molecular tests were performed using the DNA Blood kit (A&A Biotechnology, Gdynia, Poland). The extracted DNA was subjected to PCR.

The reaction was performed according to the method described by Staggemeier *et al*. (28) with minor modifications of the primer targeting fragments of the citrate synthase gene: one generic forward primer (BART-LC-GEN-F: 5′ – ATGGGTTTTGGTCATCGA GT – 3′), one species-specific reverse *B. henselae* primer (BART-LC-HEN-R: 5′–AA ATCGACATTAGGGTAA AGTTTTT – 3′), and one species-specific reverse *B. clarridgeiae* primer (BART-LC-CLA-R: 5′- CAAGAAGTGGATCA TCTTGG – 3′).

The products of the PCR were analysed using the electrophoresis method in 1.5% agarose gel, in TBE buffer, at a voltage of 10 V/cm. The gel was stained with ethidium bromide (1 μg/mL) for 15 min. The size of the products was determined according to the weight standard DNA ladder 100 bp (Fermentas, Vilnius, Lithuania).

Statistical analysis. The chi-squared test was used to determine the risk factors associated with the presence of *Bartonella* DNA in the blood. Explanatory variables associated with *Bartonella* spp. infection with $P < 0.15$ were initially selected. Spearman's Rho (Rho) was used in the second step to determine the collinearity among the selected explanatory variables. If Rho was > 0.4, only the variable more logically associated with *Bartonella* infection was retained (P associated with Rho was < 0.001 in all cases).

The association between the explanatory variables previously selected and the dependent variable (presence of *Bartonella* DNA) was investigated using a logistic regression model. Changes in the odds ratio (OR) value greater than 25% were considered indicative of a confounding factor. A forward introduction of the variables was used. The model was re-run until all remaining variables presented statistically significant values (likelihood ratio, Wald's test, $P < 0.05$). The statistical analysis was performed using SPSS v22.0 software (IBM SPSS Inc., Armonk, NY, USA).

Results

Among the four regions located in eastern Poland, the Subcarpathian province revealed the highest PCR-determined prevalence of *Bartonella* infection in cats (53.3%), and this was nearly double the lowest prevalence from the Masovian province (28.5%). The other two regions returned prevalence rates of 47.8% for Podlasie province and 38.0% for Lublin province (Fig. 1).

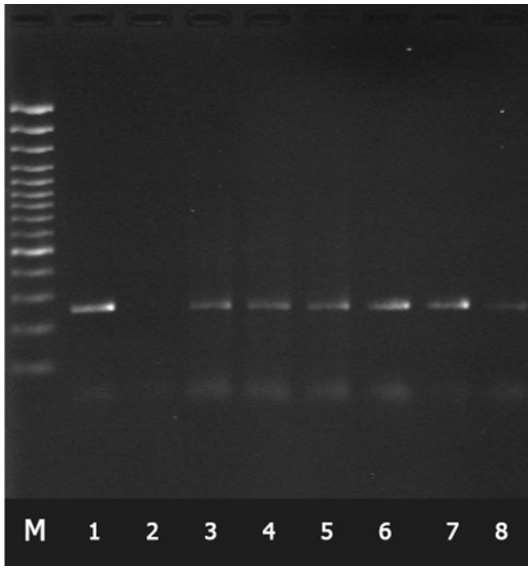

Fig. 1. PCR amplification of a partial sequence of *B. henselae* citrate synthase gene (product size: 250 bp). M – molecular weight marker (100 bp); 1 – positive control; 2 – negative control; 3–8 – positive samples

Table 1. Variables associated (P < 0.15) with *Bartonella* infection in cats from eastern Poland

Variable	Category	Total (percentage of PCR positive cats)	Total (percentage of PCR negative cats)	P
Sex	Male	79 (38.0)	129 (62.0)	0.378
	Female	193 (41.6)	271 (58.4)	
Age	Young (<1 year old, (≥1 year old)	42 (37.5)	70 (62.5)	0.482
		230 (41.1)	330 (58.9)	
Living conditions	Indoors only	84 (25.0)	252 (75.0)	<0.001
	Outdoors and indoors	188 (56.0)	148 (44.0)	
Breed	Mixed breed	241 (47.8)	263 (52.2)	<0.001
	Purebred	31 (18.5)	137 (81.5)	
Region	Lublin	84 (38.0)	137 (62.0)	<0.001
	Masovian	53 (28.5)	133 (71.5)	
	Subcarpathian	81 (53.3)	71 (46.7)	
	Podlasie	54 (47.8)	59 (52.2)	
Disease	Yes	169 (43.2)	222 (56.8)	0.087
	No	103 (36.7)	178 (63.3)	
Tick control	Yes	76 (27.9)	196 (72.1)	<0.001
	No	196 (49.0)	204 (51.0)	

Table 2. Logistic regression model showing variables significantly associated with *Bartonella* spp. in eastern Poland (at a 95% confidence level)

Variable	Category	P (Wald's Statistic)	OR	95% CI
Living conditions	Outdoors and indoors	<0.001	3.36	2.46–4.95
	Indoors only*			
Breed	Mixed breed	0.001	2.81	1.75–4.51
	Pure*			
Region	Lublin	0.684	0.9	0.55–1.48
	Masovian	0.471	0.82	0.48–1.4
	Subcarpathian	0.013	2.01	1.16–3.47
	Podlasie*			
Tick control	No	<0.001	2.02	1.41–2.92
	Yes*			
Constant		0.001	0.33	

* Reference category. Its OR value was 1 in all cases

The chi-squared test results are presented in Table 1. Five variables were selected for collinearity analysis, at an 85% confidence level (living conditions, breed, region, disease and tick control).

Collinearity, evaluated by Spearman's Rho, was not detected among the selected variables (all values were under 0.4). The variables included in the logistic regression model, at a 95% confidence level, are shown in Table 2. It can be observed that the living conditions of the animals (freely roaming outdoors), mixed breed, Subcarpathian region, and absence of tick control were significant risk factors associated with *Bartonella* infection.

Bartonella DNA was demonstrated in the blood of 56% of the free-roaming cats and only in 25% of the cats kept exclusively indoors. It was observed in 47.8% of the mixed-breed cats and only in 18% of the purebred cats, in 49% of the cats that did not receive ectoparasite prevention treatment, and in 27.9% of the cats that received such treatment.

Overall, the presence of *B. henselae* DNA was detected in the blood of 272 out of the 672 tested animals (40.48%). The size of the amplified citrate synthase gene fragment was 250 bp (Fig. 1). When the PCR product obtained from the cats was sequenced and the nucleotide sequence compared with the one deposited in GenBank under accession number L38987.1, the two showed a level of similarity of 99%–100%. The DNA of *B. clarridgeiae* was not found in the blood of any animal.

Discussion

In this article we studied a population of domestic cats from eastern Poland to determine the prevalence and associated risk factors for *Bartonella* infection and to identify endemic *Bartonella* species.

In our study, the only *Bartonella* species found in cats was *B. henselae.* This is the most common *Bartonella* species type found in cats in Europe (2, 7, 16, 18, 26). We found no presence of *B. clarridgeiae* in any of the study cats, unlike in studied cat populations in France and the Netherlands (2, 16)

Bartonella henselae has worldwide distribution; high seropositivity to *B. henselae* in cat populations (18%, 50%, and 58.8%) was recorded in Italy, Spain, and Greece, respectively (8, 14, 24)

The results of our study indicated that eastern Poland is a territory at risk of a feline bartonellosis epizootic. The DNA of this bacterium was found in the blood of 40.48% of animals, and this proportion appears to be high compared to other countries in Europe. For example, in France the bacteraemia caused by *Bartonella* was found in 11% of pet cats (6), in Germany in 13% of pet cats (26), and in the Netherlands in 22% of shelter cats (2).

Despite some reports suggesting that the sex of a cat may be a factor that predisposes the animal to the development of the disease (22, 25), most studies on the

subject, including ours, do not confirm such a relationship (11, 12, 15, 16). Similarly, in our studies we did not observe a relationship between frequency of infection and age of the cat; however, previous studies have indicated that young cats are more often infected with *Bartonella* (5, 18, 26).

Our study also failed to reveal any statistically significant differences between the frequency of *Bartonella* DNA detection in the blood of healthy cats and this frequency in those with various health issues. However, it is possible that the immunosuppression associated with some diseases like FeLV or FIV increases the pathogenicity of *B. henselae* infection in cats, and may be a predisposing factor for disease development (30). It is interesting that the *Bartonella* infection itself is often asymptomatic in cats, and in the haematological results no statistically significant differences were found between *Bartonella* spp.-positive and -negative cats (27).

The statistical analysis of the results from our study enabled us to demonstrate that such factors as breed (mixed-breed cats), area of origin (the Subcarpathian province), living conditions (free-roaming), and lack of ectoparasite-prevention treatments are factors conducive to *Bartonella*-related bacteraemia in cats.

Warm, humid environments, density of the cat population, and unbeneficial aspects of the habitat including state of hygiene and flea infestation, may correlate with a higher prevalence of *B. henselae* infection (20). A positive correlation between the origin of the cats used in this study (the Subcarpathian province) and the presence of *Bartonella* infections confirm these observations. Among the four provinces of Poland covered by the study, the Subcarpathian province had the highest annual mean air temperature and mean air humidity.

The fact that bacteraemia is more frequently observed in mixed-breed cats than purebreds is more likely a result of the greater popularity of these animals in Poland than of any breed predisposition. On the other hand, the higher prevalence of *Bartonella* infection in mixed-breed cats may be connected with the cats' living conditions, as they are more likely to be kept outdoors. Outdoor or free-ranging animals are more prone to being infected by ectoparasites and transmitted pathogens (24).

The positive association of bacteraemia with the lack of anti-ectoparasite prophylaxis and the living conditions of the cats (free-roaming) is confirmatory of previous studies demonstrating that cat fleas (*C. felis*) and ticks (*Ixodes* spp.) may serve as the vectors of *B. henselae* between cats (5, 10, 17, 21, 30). This association may be connected with a greater exposure to *Bartonella* vectors in this group of animals and confirms the previous observation of Chomel *et al* (5), who identified an association between bacteraemia and flea infestation.

Similar investigations were performed by Barradas *et al.* (1), who did not find significant differences regarding *B. henselae* infection between age groups, sexes, breeds, living conditions, extents of contact with other animals including rodents, or seasons of sample collection. A statistically significant association was observed only between *B. henselae* infection and the lack of access to prophylaxis against ectoparasites.

The increased frequency of bartonellosis in cats may be the result of climate change. However, the use of new diagnostic techniques, including molecular methods, in the disease recognition process and better understanding of bartonellosis among veterinarians and cat owners may result in more frequent recognition of the disease in these animals (21, 22).

As more cases of *Bartonella* infections have been observed recently in cats, there is a continued need to conduct studies on bartonellosis epidemiology, diagnosis, and prevention, and broaden its scope to areas previously not considered endemic for the disease. To successfully prevent bartonellosis, an understanding of the ways the disease spreads, its geographical scope, and the factors that favour its development is necessary.

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