

Risk factors for the presence of antibodies against *Anaplasma* spp. in dogs in Poznań

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Summary

The aim of the study was to identify the incidence of antibodies against *Anaplasma* spp. in dogs living in Poznań. Canine anaplasmosis is a tick-borne dog disease caused by *Anaplasma phagocytophilum* or *A. platys* infection. To identify the prevalence of antibodies against *Anaplasma* spp. in dogs, we analyzed blood samples collected from 349 client-owned dogs living in Poznań (Poland). Using an immunochromatographic rapid test (Caniv-4, VetExpert, Poland), antibodies against *Anaplasma* spp. were detected in 32 (9.2%) dogs aged over two years. Sex, hair length, and location (region of Poznań) were not identified as statistically significant risk factors for the presence of antibodies against *Anaplasma* spp. The odds ratio for antibodies against *Anaplasma* spp. in large breed dogs vs small breed dogs was 3.76. The results of the study suggest a growing presence of *Anaplasma* spp. in dogs living in big cities in Poland.

Keywords: *Anaplasma* spp., anaplasmosis, dogs, Poland

Canine anaplasmosis is a tick-borne dog disease caused by *A. phagocytophilum* (30) or *A. platys*. The latter pathogen, however, has not been recorded in Poland for years (15, 25). Typical clinical signs of the disease include weakness, lethargy, and anorexia. A common sign observed in anaplasmosis is a reluctance to walk due to polyarthritis and musculoskeletal pain. Some unspecific signs can also be noted, including vomiting, diarrhea, bleeding diatheses, such as epistaxis, and even neurological signs (16, 19, 29). This disease rarely ends in death, but its course could be complicated by other infections (32).

The pathogens are transmitted by ticks (*Ixodidae*), which are common ectoparasites of vertebrates with a worldwide distribution. They are also vectors for many pathogens, including piroplasms, i.e., viruses and bacteria. These pathogens can cause chronic diseases, affecting not only animals, but also humans (12, 17). Ticks become infected by feeding on the blood of infected hosts. From the economic perspective, most alarming is that ticks are vectors of a range of pathogens posing a serious threat to livestock and pets (10, 11, 14).

Tick vectors for canine diseases include *Ixodes ricinus*, *I. hexagonus*, *I. crenulatus*, and *I. rugicollis* (41).

Of these, *I. ricinus* is Poland's most widespread tick species that can transmit *Borrelia burgdorferi* sensu lato, causing Lyme disease, and *Anaplasma phagocytophilum*, causing anaplasmosis (20).

The first case of canine anaplasmosis in Poland was described in 2001 (36). *A. phagocytophilum*, which causes anaplasmosis, was later also observed in other animal species: pigs, cattle, horses, cats, and dogs in northern and eastern Poland (5-7, 26, 34, 37, 38).

The aim of the present study was to determine the incidence of antibodies against *Anaplasma* spp. in dogs living in Poznań in western Poland.

Material and methods

Study area and blood collection. All dogs included in the study were examined in three veterinary clinics in Poznań, Poland, between April 2016 and March 2017. The animals were selected in a retrospective cohort case study manner, meaning that the animal visiting the clinic was included in the study after blood testing and was assigned to one of two groups according to its tick-borne infection status: a group with antibodies or a group without antibodies. Each dog's age, sex, size, hair length, and location (region of Poznań) were recorded to describe its unique characteristics. The following features were considered as

potential risk factors: age (17 levels), sex (male, female), size (large, medium, small), hair length (long, short), date of the test performance (169 levels), and the region of Poznań where the dog lived (5 levels).

Serology of *Anaplasma* spp. The dogs included in the study were client-owned and came from Poznań or the Poznań district. The tests were performed in three veterinary clinics for dogs visiting the clinic for preventive or therapeutic purposes. Blood was collected from each animal into a test tube for collecting serum and then centrifuged at 3,000 rpm/minute to obtain serum. Next, a rapid test Caniv-4 (VetExpert, Poland) detecting antibodies against *Anaplasma* spp. was performed. For this purpose, two drops of test serum were dropped with a disposable dropper into the sample hole, followed by 10 µl into the “Anaplasma Ab” well. Three drops of assay diluents were dropped into each well. After 10-15 minutes, a single band indicated a negative result, while two bands indicated a positive result, i.e., antibodies in the test sample. Since the test detects antibodies against the MSP antigen (major surface protein), it does not distinguish between *A. phagocytophilum* and *A. platys*. This test is a chromatographic immunoassay with a sensitivity of 96.1% and a specificity of 99.3%. It is also used to qualitatively detect *Dirofilaria immitis* antigen and antibodies to *Ehrlichia canis* and *Borrelia burgdorferi* in the serum, plasma, or whole blood of dogs.

Description of statistical methods. The statistical analysis of the risk factors for the presence of antibodies against *Anaplasma* spp. was performed by several different methods. First, data were analyzed in a retrospective cohort case study by calculating the odds ratio (OR) for risk factors such as sex, size, and hair length. The OR indicates how frequently a disease occurs in a group affected by the first level of a risk factor compared to a group affected by the second level of the same risk factor. The analysis is based on computing the odds ratio with the following equation:

$$OR_{dis} = (A/B)/(C/D)$$

where A and B are the numbers of animals that were affected by the first of the two levels of a risk factor, but animals A are infected whereas animals B are not; C and D are several animals that were affected by the second level of the two levels of a risk factor, but animals C are infected whereas animals D are not. The statistical association of the risk factor with the tick-borne infection was estimated by computing the 95% confidence interval on a logarithmic scale for each OR.

Second, logistic regression from the statistical package R (R Core Team, 2013) was performed to further study the risk factors for tick-borne infections. The logistic regression was chosen because it makes it possible to analyze binomial data concerning the outcome of a tick bite (affected by the disease – 0, not affected by the disease – 1) with a simultaneous fit of multiple effects in the same model. In this analysis, all six potential risk factors were tested. The model applied was as follows:

$$\text{Log}(\text{test result}) = \text{age} + \text{sex} + \text{size} + \text{hair length} + \text{location} + \text{date of test}$$

where *test result* was the dependable binomial variable indicating infection or no infection in a dog, *age*, and *date of the test* were fitted as linear variables. In contrast, sex,

size, *hair length*, and *location* of the animal were held as class variables.

Results and discussion

In total, 349 dogs were included in the study and were available to analyze risk factors for the presence of antibodies against *Anaplasma* spp. If an animal showed inconclusive test results for antibodies against *Anaplasma* spp. or the record of any of the risk factors was missing, the observation was excluded from the analysis (Tab. 1). The number of animals in different classes of risk factors is presented in Tab. 2-6. Antibodies against *Anaplasma* spp. were detected in 32 (9.2%) of the 349 dogs examined (Tab. 1). For seven dogs, the results of the test were inconclusive and, therefore, ignored in the analysis (Tab. 1). Among the dogs examined, there were more males (n = 163, 46.7%) than females (n = 145, 41.5%), but information about the sex of 41 animals was missing (Tab. 5). There were also more males (n = 19, 5.4%) than females (n = 12, 2.7%) among dogs with positive results of the test (Tab. 5).

Sex, hair length, and location were not identified as statistically significant risk factors for the presence of antibodies against *Anaplasma* spp. (Tab. 3, Tab. 4).

Tab. 1. Number of tests performed for the evaluation of the prevalence of antibodies against *Anaplasma* spp. and their result in dogs with tick bites

Test result	Number	% in total
Inconclusive	7	2.01%
Negative	310	88.83%
Positive	32	9.17%
Total	349	100.00%

Tab. 2. Number of tests for *Anaplasma* spp. and their results according to dogs' sex. The animal record was excluded from the analysis if the factor or the test results were unknown

Sex	Test result			Total
	Inconclusive	Negative	Positive	
Not known	–	2	1	3
Female	2	145	12	159
Male	5	163	19	187
Total	7	310	32	349

Tab. 3. Logistic regression of risk factors for the occurrence of antibodies against *Anaplasma* spp. in dogs

Risk factor	Linear/Class	Estimate (with SD)	P-value
Age	Linear	–0.151 (0.06)	0.012
Size	Class	–	0.016
Hair length	Class	–	n.s.
Sex	Class	–	n.s.
Region of Poznań	Class	–	n.s.
Date of test	Linear	0.002 (0.003)	n.s.

Tab. 4. Number of tests for *Anaplasma* spp. and their result according to dogs' hair length. If the factor or the test results were unknown, the animal record was excluded from the analysis

Hair length	Test result			Total
	Inconclusive	Negative	Positive	
Not known	1	64	2	67
Long	6	117	10	133
Short	–	129	20	149
Total	7	310	32	349

Tab. 5. 95% confidence intervals on a logarithmic scale for the odds ratio testing dogs' characteristics as a risk factor for a positive result of an *Anaplasma* spp. test

Dog's characteristics	Odds ratio	Lower bound	Upper bound	Association of risk factors with disease
Large/Small	3.758	0.292	2.356	Yes
Medium/Small	0.451	–1.988	0.396	No
Large/Medium	1.695	–0.404	1.460	No
Large/All sizes	2.555	0.163	1.713	Yes
Long hair/Short hair	1.813	–0.204	1.395	No
Males/Females	1.408	–0.414	1.099	No

Tab. 6. Number of tests for *Anaplasma* spp. and their results according to dogs' size. If the factor or the test results were unknown, the animal record was excluded from the analysis

Dog size	Test result			Total
	Inconclusive	Negative	Positive	
Not known	1	64	2	67
Large breed	1	91	18	110
Small breed	5	95	5	105
Medium breed	–	60	7	67
Total	7	310	32	349

The large breed of the dog, however, was a statistically significant risk factor for positive test results (OR = 3.76 vs. small dogs and 2.56 vs. small and medium breeds combined). The age of the animal, as well, seems to be a significant factor for the occurrence of the disease, since antibodies against *Anaplasma* spp. were confirmed only in dogs over two years of age (Tab. 7).

The survival curves for five regions of Poznań differed significantly (Tab. 8). There were no statistical differences between survival curves for sex, hair length, and size.

Anaplasma spp. is the causative agent of anaplasmosis in many animal species, including dogs, horses, cattle, sheep, goats, and humans. There are reports of *Anaplasma* spp. infection in camelids and domestic cats, but these cases are fairly rare (5, 6, 23, 24, 34). In humans, it is an emerging zoonosis transmitted by ticks of growing importance for public health. During the disease, the presence of antibodies or bacterial DNA has been confirmed by serological and molecular

Tab. 7. Number of tests for *Anaplasma* spp. and their results according to dogs' age. If the factor or the test results were unknown, the animal record was excluded from the analysis

Age [years]	Test result			Total
	Not conclusive	Negative	Positive	
> 1	–	8	–	8
1	–	22	–	22
1.5	–	1	–	1
2	–	21	2	23
2.5	–	3	–	3
3	–	24	2	26
3.5	–	1	–	1
4	–	25	4	29
5	1	15	–	16
5.5	–	1	–	1
6	1	24	3	28
6.5	–	4	1	5
7	–	17	1	18
8	2	27	3	32
8.5	–	3	–	3
9	2	34	1	37
9.5	–	2	–	2
10	–	19	3	22
11	1	19	4	24
12	–	14	4	18
12.5	–	3	–	3
13	–	13	3	16
14	–	3	–	3
15	–	4	1	5
16	–	1	–	1
Not known	–	2	–	2
Total	7	310	32	349

Tab. 8. Number of tests for *Anaplasma* spp. and their results according to the region of Poznań, where the dog lived. If the factor or the test results were unknown, the animal record was excluded from the analysis

Region of Poznań	Test result			Total
	Inconclusive	Negative	Positive	
Not known	–	1	–	1
Center	–	13	1	14
South	–	36	3	39
North	2	78	1	81
East	3	93	20	116
West	2	89	7	98
Total	7	310	32	349

methods in many wild and domestic animals worldwide (33).

We analyzed blood samples from dogs for the presence of antibodies against *Anaplasma* spp. to study the prevalence of the pathogen in dogs living in a city.

We sampled 349 dogs, and the results of the immunochromatographic rapid test were positive for 32 (9.2%) dogs. What is of interest is that only 4 dogs positive for *Anaplasma* spp. showed signs of clinical disease, including fever, anemia, lameness, weakness, and cachexia. Clinical diagnosis of canine anaplasmosis is difficult because clinical signs are non-specific and can be mistaken for those of other diseases.

The areas endemic to canine monocytic ehrlichiosis are mainly Mediterranean countries, but it is increasingly reported from other regions, including Poland. Dog breeds particularly vulnerable to ehrlichiosis are German Shepherds and Siberian huskies, in which the disease lasts longer and follows a more severe course (1, 2). Recent epidemiological data show that an average of 13.7% (0-49.1%) of ticks are infected with *B. burgdorferi* in Europe and up to 40% in Poland (2, 3). In Poland, canine tick-borne diseases are a significant problem in veterinary practice. However, these cases are not reported as often as in countries with a warmer climate, such as Mediterranean countries, or the United States. During the period of arachnid activity (spring, autumn), more than half of the patients of veterinary clinics in some regions of Poland suffer from tick diseases (3). The tick-borne diseases endemic to dogs in Poland are babesiosis, dipylidiosis, and subcutaneous dirofilariasis (39). Recently, anaplasmosis has been recognized as the second most common tick-borne disease of dogs after babesiosis (13, 27, 30). Early studies performed in dogs in Poland found the prevalence of *Anaplasma* spp. at a very low level of 0.5-1.0% (37, 40, 41). A study of 400 dogs in eastern Poland showed total seroprevalence to be highest for *B. burgdorferi* (11.0%), followed by *A. phagocytophilum* (8.0%), and *E. canis* (1.5%) (11). Adaszek et al. (4) surveyed 420 dogs of different breeds and sexes (262 males, 158 females) aged 4 months-14 years, referred to veterinary clinics and offices throughout Poland with signs of apathy (n = 420), spleen enlargement (n = 187), and lameness (n = 158), which were found to have thrombocytopenia by hematological examination. The dogs were tested for antibodies to *E. canis*, which were detected by IF reaction in 40 (9.5%) serum samples and by CaniV-4 rapid tests in 34 (8.1%) serum samples. Comparing these data with our results, we can conclude that the number of cases of *Anaplasma* spp. in dogs in Poland is growing. In recent years, we have also seen an increase in anaplasmosis cases in dogs in Europe (18). The prevalence is relatively high in Germany: 6.3-50.1% (28). In Italy, the level of seroprevalence may reach up to 33% (8, 9, 21). Lower levels are observed in Spain: 5-11.5% (17, 35) and in Great Britain: 0.8% (16).

Our results show that antibodies against *Anaplasma* spp. occur more frequently in older and large-breed dogs (older than two years). It may be related to the fact that older dogs have a more prolonged exposure

to tick bites, whereas large breed dogs usually spend more time outdoors. Of course, not all tick bites result in infection with *Anaplasma* spp. but the infection risk is relatively high. The DNA of *A. phagocytophilum* was detected in *Ixodes ricinus*, the most widespread tick in Poland, and *I. hexagonus* ticks. In a study by Zygmier et al. (41), the prevalence of *Anaplasma* spp. in ticks collected from dogs was 2.9%. In a study by Król et al. (22) conducted on ticks collected from dogs, positive results for *Anaplasma* spp. were found in 21.3% of *I. ricinus* and 8.1% of *I. hexagonus*. However, in *I. ricinus* collected in forests and suburban areas, the prevalence was relatively high, at 14-23.7% (20, 31), possibly due to numerous reservoirs of this pathogen. Zygmier et al. (41, 42) studied hard ticks collected from dogs in the Warsaw area. Among 590 ticks they studied, 209 were identified as *I. ricinus* and 381 as *Dermacentor reticulatus*. DNA of *B. canis* was detected in 11% of *D. reticulatus*. We found that 6.2% of *I. ricinus* ticks harbored *B. burgdorferi* s.l.-specific DNA, and 2.9% harbored DNA of *A. phagocytophilum*. Results of Žele et al. (43) show that wild animals are susceptible and naturally infected with *A. phagocytophilum* and are likely to be important reservoirs of *A. phagocytophilum* in Europe. The highest seroprevalence was found in roe deer (84.4%), but it was also elevated in chamois (77.8%), wild boars (69.6%), brown bears (65.2%), and red deer (60%). The increasing number of tick-borne diseases in dogs in Poland makes it advisable to educate pet owners about preventing these diseases. The primary methods of preventing tick-borne diseases are avoidance of ticks in spring and autumn and the prophylactic use of acaricides (1).

In interpreting our results, it is necessary to discuss the method of the analysis. The immunochromatographic test method we used in our study makes it possible to detect antibodies against pathogens and can be performed in every veterinary clinic as "point-of-care-diagnostics". In contrast, DNA extraction and PCR are more sensitive assays for accurate diagnosis of the disease and determination of the exact species of pathogen. A comparison of the results concerning sera positive for *A. phagocytophilum* in the IF test with the results of CaniV-4 and Snap 4Dx tests showed a concordance of 100%. All sera negative for *A. phagocytophilum* in the IF test were also negative in both CaniV-4 and Snap 4Dx tests. Comparison of the sera positive for *Borrelia burgdorferi* in ELISA showed a concordance of 92.5% with the results of the CaniV-4 test and a concordance of 87.5% with the Snap 4Dx test results (5). In a study by Adaszek et al. (4), all serum samples reacting positively in the CaniV-4 assay also responded positively in the immunofluorescence assay. The concordance of the two tests was set at 85%.

In conclusion, this study revealed a significant prevalence of antibodies against *Anaplasma* spp. in dogs living in Poznań. It is, therefore, worth paying

more attention to preventing tick infections and better diagnosing anaplasmosis in veterinary clinics.

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