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Serological and clinical findings in dogs seropositive for *Anaplasma phagocytophilum* and *Borrelia burgdorferi*

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Abstract

Anaplasma phagocytophilum and *Borrelia burgdorferi* sensu lato are two major tick-borne pathogens of dogs. To gain insight into the clinical findings and possible correlations between clinical signs and seropositivity, a cohort of 254 dogs of different breeds, age, and sex was investigated for serum antibody titers against these pathogens and hematological as well as clinical parameters, focusing on lameness and neurological disorders. Overall, 64.2% of the dogs were seropositive for *Anaplasma* and 61.0% for *Borrelia*, including 40.2% positive for both agents, and a statistically significant association between both pathogens. *Anaplasma*, but not *Borrelia* frequency of infection, was positively associated with age. Of the 94 lame animals, 87% were suspected to suffer from borreliosis or anaplasmosis. Correlations between seropositivity and lameness and between seropositivity and gastrointestinal signs (mostly inappetence) were recorded. Neurological signs, on the other hand, were not associated with positive antibody titers. Hematological changes in hematocrit and thrombocyte count were related to infection, but without a uniform trend. Only a few animals had positive anti-*Borrelia* IgM titers, determined by immunofluorescence antibody test or were positive for *Anaplasma* DNA by PCR. These two tests were not associated with infection in the studied cohort. Lameness was associated with past infections with *Anaplasma* or *Borrelia* (although double infections were not more likely to cause gait abnormalities). With a high background of seropositive but apparently healthy dogs, a set of various diagnostic tools should be applied, in order to reliably link infections with disease and correctly choose the antimicrobial treatment.

Keywords: *Anaplasma*; *Borrelia*; lameness; serology; clinical diagnosis

Abbreviations

A. phagocytophilum: *Anaplasma phagocytophilum*;

B. burgdorferi s.l.: *Borrelia burgdorferi* sensu lato

Introduction

Tick-borne infections of dogs with various pathogens are common in Central Europe [1, 2]. A variety of agents infectious to dogs are transmitted by the castor bean tick, *Ixodes ricinus*, which is the most common tick species in Central Europe, with a wide geographic and host range [3]. Outdoor activities in tick habitats [1, 4, 5], pet travel [6], and the import of dogs from Southern Europe or overseas [7] inevitably leads to an increased contact with autochthonous and non-autochthonous tick vectors.

Dogs can be exposed to a variety of tick-borne pathogens, even when owners apply acaricidal treatment [8]. One of the most common pathogens found in ticks is a gram-negative spirochete, *Borrelia burgdorferi* sensu lato, the etiological agent of Lyme borreliosis, as data from various central and northern European countries with a prevalence of up to 25.7% in *I. ricinus* indicate [9-14]. Small mammals are natural reservoirs for these spirochetes, and both humans as well as dogs [15] can be infected. It has been proposed that dogs may play a sentinel role; in areas where a high prevalence for (zoonotic) tick-borne infections is recorded in dogs, an infection risk must also be assumed for humans [16-18].

Canine (and human) *Borrelia* infections are most often subclinical, but they can cause a variety of clinical signs that can also induce intermittent acute clinical illness and develop into a chronic disease [19]. It is assumed that only a small proportion number of dogs infected with *B. burgdorferi* develops clinical signs [20, 21]. Clinical diseases are characterized by non-specific acute clinical signs, frequently including fever, lymphadenopathy, apathy, and inappetence [18, 20, 22-27]. Cardiovascular and gastrointestinal signs, hematological alterations, and neurological illness have also been described in seropositive dogs, as well as Lyme nephropathy, with sometimes lethal acute or chronic renal failure [19, 26, 28-31]. In chronic diseases, mainly recurrent and variable lameness is described as the principal clinical sign in seropositive dogs [22, 25], which is confirmed in an experimental study [32]. So far, Lyme arthritis is considered as the main clinical consequence of an infection with *B. burgdorferi* in dogs, although experimental and field studies only partially confirm the correlation between seropositivity and disease [20, 27, 33, 34].

Anaplasma phagocytophilum is an intracellular bacterium and the cause of granulocytic anaplasmosis [35]. In Central Europe, it is found frequently in questing *I. ricinus* with its prevalence of up to 24% [36]. In mammalian hosts, however, this pathogen remains mostly undetected, since bacteremia is short and the clinical signs are usually mild and transient. Acute fever may occur in the acute phase, after an incubation time of 1-2 weeks, accompanied by inappetence and lethargy, gastrointestinal signs and hematological changes, and most frequently, thrombocytopenia [35, 37-39]. Frequently, lameness and arthropathy are associated with positive titers to *Anaplasma* [12, 17, 37-42]. High antibody titers seem to correlate, to some extent, with clinical anaplasmosis [40], but during the acute phase serology, can give false negative results because of the time lag in antibody production, which requires re-testing for serum antibodies to confirm active infections [37]. Infections appear to be self-limiting in dogs, although some cases need antibiotic therapy [30, 37, 43, 44].

Routine screens for infections with *Borrelia* or *Anaplasma*, assessing specific antibodies, enable the diagnosis of past infections. Still, very little is known about the course of antibody titers, where correlations between titers and the presence of pathogens and possible relationships between the persistence of antibodies and pathogens can be seen. Due to the high frequency of subclinical infections, determining infections with *B. burgdorferi* or *A. phagocytophilum* as the causes of the often non-specific signs is a challenge in clinical diagnosis. *B. burgdorferi* is notoriously hard to directly detect and difficult to isolate from bodily fluids of the affected patients [20, 45]. In addition, since both are transmitted by the same vector, co-infections are common and hard to differentiate, based on clinical signs [17, 20, 30, 37, 42, 46]. An increased risk to develop clinical signs in co-infected animals compared to mono-infected ones, as well as exacerbation of clinical signs (lameness, thrombocytopenia), have been mentioned [17, 20, 38, 42].

In order to examine a possible association between the positive serum antibody titers against *A. phagocytophilum* and *B. burgdorferi* as well as the clinical signs in naturally infected dogs, we conducted a retrospective study on the canine patients with suspected borreliosis and/or granulocytic anaplasmosis. We evaluated the possible associations of laboratory parameters (serum antibody titers and hematology) with clinical presentations (especially gait abnormalities) to find evidence for a clinical disease of canine borreliosis and anaplasmosis.

Materials and Methods

Animals included in the study

A data set for patients was collected in a private veterinary clinic for companion animals in Heilbronn (49°08`32" N, 9°12`53" O), a city in the north of Baden-Württemberg in the South- West of Germany, in the years 2011-2014.

Overall, 254 dogs of different sex, size, and age (see Table 1) of different breeds (61 mongrels, 193 pedigree dogs from 71 different breeds), were included. Apart from a complete set on these data, the inclusion criteria were (i) a clinical examination for lameness, and (ii) serological examinations for *Borrelia* and *Anaplasma* IgG titers. Subsets were also evaluated for *Borrelia* IgM titers (n=207), PCR for *Anaplasma* in blood (n=41), specific examination of the musculoskeletal system (n=94), as well as the parameters of renal function (n=145) and hematological parameters including leukocytes, thrombocytes, hematocrit, and MCV (n=157).

No dog was reported to be vaccinated against *Borrelia*.

Age/N [%]	<3 years	3-<6 years	6-9 years	9 years and older	Total
Categories					
Male intact	16 [6.3]	29 [11.4]	40 [15.8]	19 [7.5]	104 [40.9]
Male neutered	6 [2.4]	7 [2.8]	16 [6.3]	10 [3.9]	39 [15.4]
Female intact	13 [5.1]	9 [3.5]	15 [5.9]	11 [4.3]	48 [18.9]
Female neutered	14 [5.5]	12 [4.7]	24 [9.5]	13 [5.1]	63 [24.8]
Large hunting dogs	9 [3.5]	16 [6.3]	18 [7.1]	10 [3.9]	53 [20.9]
Small hunting dogs	6 [2.4]	6 [2.4]	12 [4.7]	6 [2.4]	30 [11.8]
Large companion dogs	25 [9.8]	31 [12.2]	57 [22.4]	28 [11.0]	141 [55.5]
Small companion dogs	9 [3.5]	4 [1.6]	8 [3.2]	9 [3.5]	30 [11.8]
TOTAL	49 [19.3]	57 [22.4]	95 [37.4]	53 [20.9]	254 [100]

Table 1. Sex, breed and age distribution of the patients included in the study (n=254). For breeds, animals were summarized into four categories: large (>15 kg) and small (≤ 15 kg) hunting dogs and large (>15 kg) and small (≤ 15 kg) companion dogs. The categorization into these 4 groups was made according to the FCI race breed description (http://www.fci.be/presentation.aspx).

Serology and PCR

Serum or plasma from lithium-heparin blood from the cephalic vein was used for serology. For the determination of antibody titers, immunofluorescence antibody tests (IFAT) were carried out at the Veterinary Laboratory Freiburg (Germany) as follows: For the examination of IgG and IgM antibodies against *Borrelia* spp., in-house prepared or commercially available (Megacor Diagnostik GmbH, Hoerbranz, Austria) pre-made slides were used. For the detection of IgG against *A. phagocytophilum*, slides from Megacor were used. IgG antibody titers ≥1:64 and IgM antibody titers ≥1:32 were considered to be positive for *B. burgdorferi*, and titers ≥1:40 were considered as positive for *A. phagocytophilum*.

To detect *A. phagocytophilum* DNA in the blood samples (lithium-heparin blood), real-time- PCR was performed, targeting the MSP2 gene [47]. Total DNA was extracted from blood samples using a Qiagen blood kit and amplified with an Mx3000p QPCR System (Agilent Technologies, La Jolla, USA). *A. phagocytophilum* DNA was used as a positive control and water was used as negative control.

Statistical Methods

Initially, the prevalence of *Anaplasma* and the prevalence of *Borrelia* (for definition of positive titers: see Materials and Methods section) were tested for independence with a chi-square test. Subsequently, the influence of sex (four categories: male, female, neutered male, neutered female), class of breed (small and large hunting dogs, small and large companion dogs), and age (continuous variable) on the seroprevalences of *Anaplasma* and of *Borrelia* were tested by using a generalized linear model with a binomial family and a logit link function. Furthermore, the influence of explanatory variables (factors: positive for *Anaplasma*/*Borrelia*, gender, breed class; covariate: age) on lameness and gastrointestinal illness was tested as well by using a generalized linear model with a binomial family and a logit link function.

Results

Serology and PCR

Of the 254 examined dogs, 64.2% had a positive *Anaplasma* titer; 61.0% had a positive *Borrelia* titer; 3.2% of the 218 dogs tested for IgM against *Borrelia* were positive, and all of which, except one, had a positive IgG titer as well. *Anaplasma* + *Borrelia* double positive serological results were found in 40.2% of the animals, double negative in 15.0%. *Anaplasma* positive/*Borrelia* negative samples accounted for 24.0%, and *Anaplasma* negative/*Borrelia* positive samples formed 20.9% of all samples.

Only one animal (2.4% of the tested group) was positive in the *Anaplasma* PCR.

While the seroprevalence for *Borrelia* varied only a little between the age groups, the prevalence for *Anaplasma* increased from 36.7% in dogs up to 3 years to 83.2% in animals between 6 and 9 years of age, and declined again slightly in the highest age group. Accordingly, double positives were found most commonly (50.6%) in dogs of 6-9 years and double negative dogs in the youngest group with 32.7% (Figure 1).

Companion dogs had a lower seropositivity for *Borrelia* (53.3% for large and 58.2% for small dogs) than hunting dogs (small: 69.8%, large: 66.7%), while *Anaplasma* seropositivity was lowest in large companion dogs (53.3%), highest in small companion dogs (67.4%), and similar between different groups of hunting dogs (small: 62.3%, large: 63.3%) (Figure 2).

Intact bitches had lower prevalence of both *Borrelia* and *Anaplasma*, and consequently, fewer double seropositivity than the other sex groups (Figure 3).

Clinical evaluation

Gait abnormalities (lameness) were diagnosed in 94 (37.0%) patients in this study, 10 of which (4.7% of all dogs, 12.8% of the animals with gait abnormalities) were lame for other causes (e.g., traumatic, degenerative, tumorous) than for presumed borreliosis or anaplasmosis. Eleven animals (4.3% of all dogs, 11.7% of the lame patients) had one or more swollen joints.

The age distribution of dogs that were lame or not was even, with a mean of 6.4 years (min: <1 year, max: 14 years,

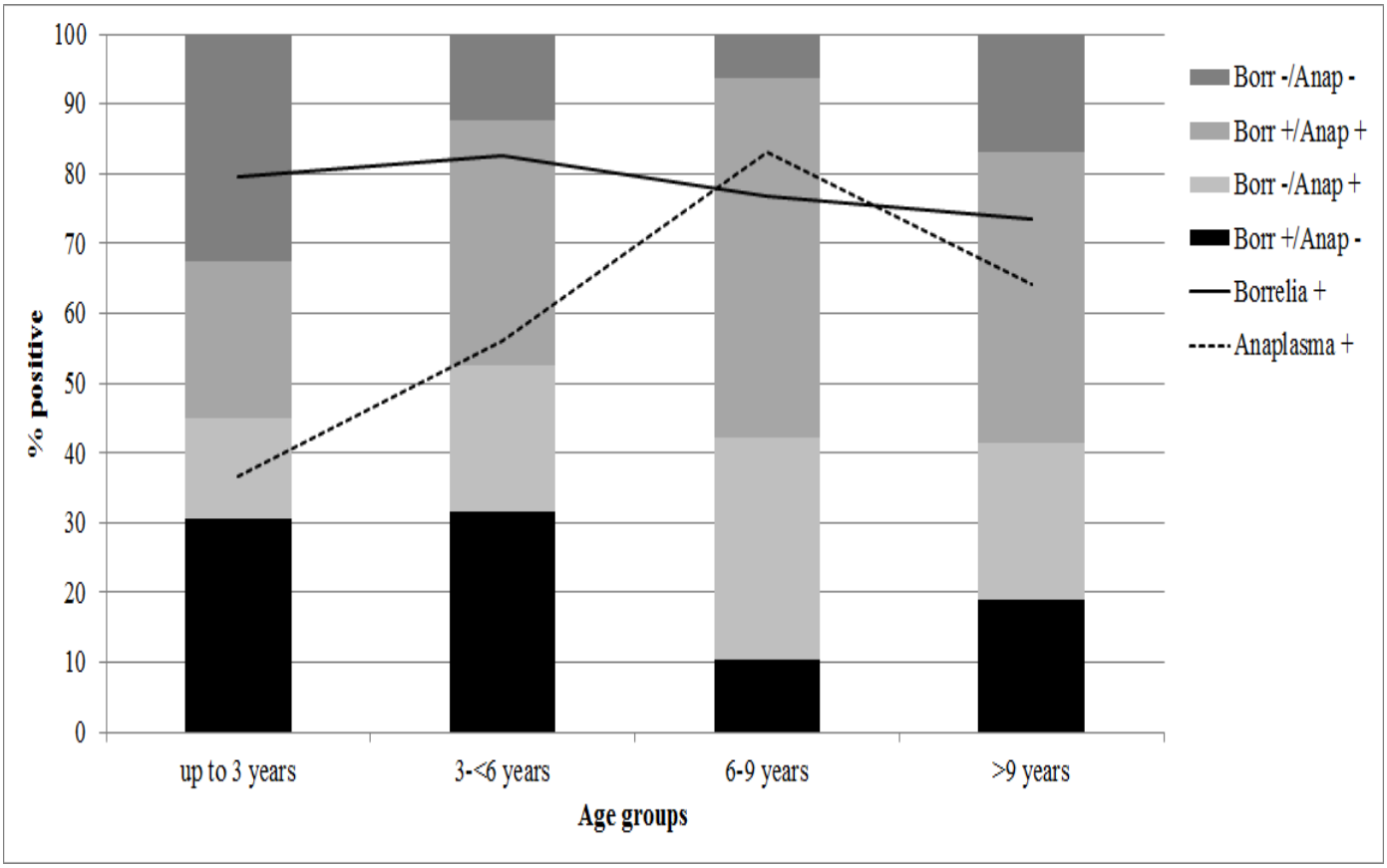

Figure 1. Age and seropositivity for *Borrelia* (Borr) and *Anaplasma* (Anap).

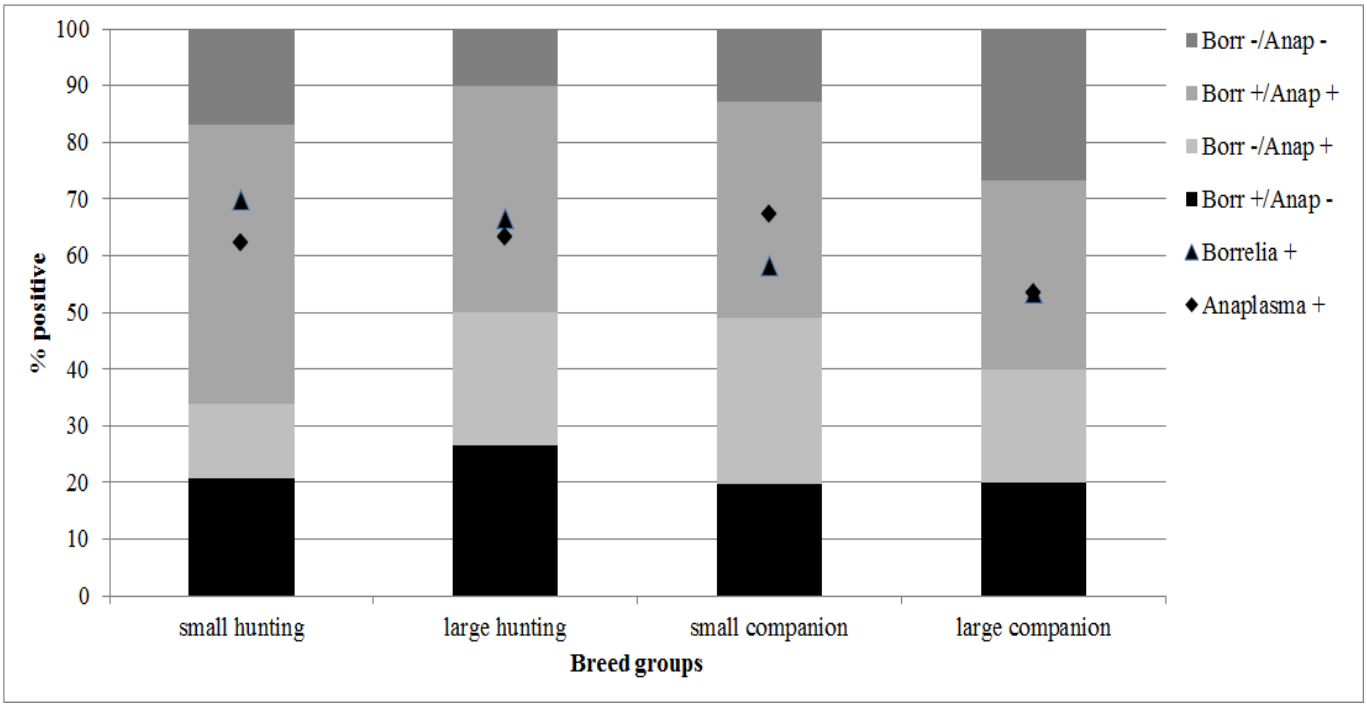

Figure 2. Breed/size and seropositivity for *Borrelia* (Borr) and *Anaplasma* (Anap).

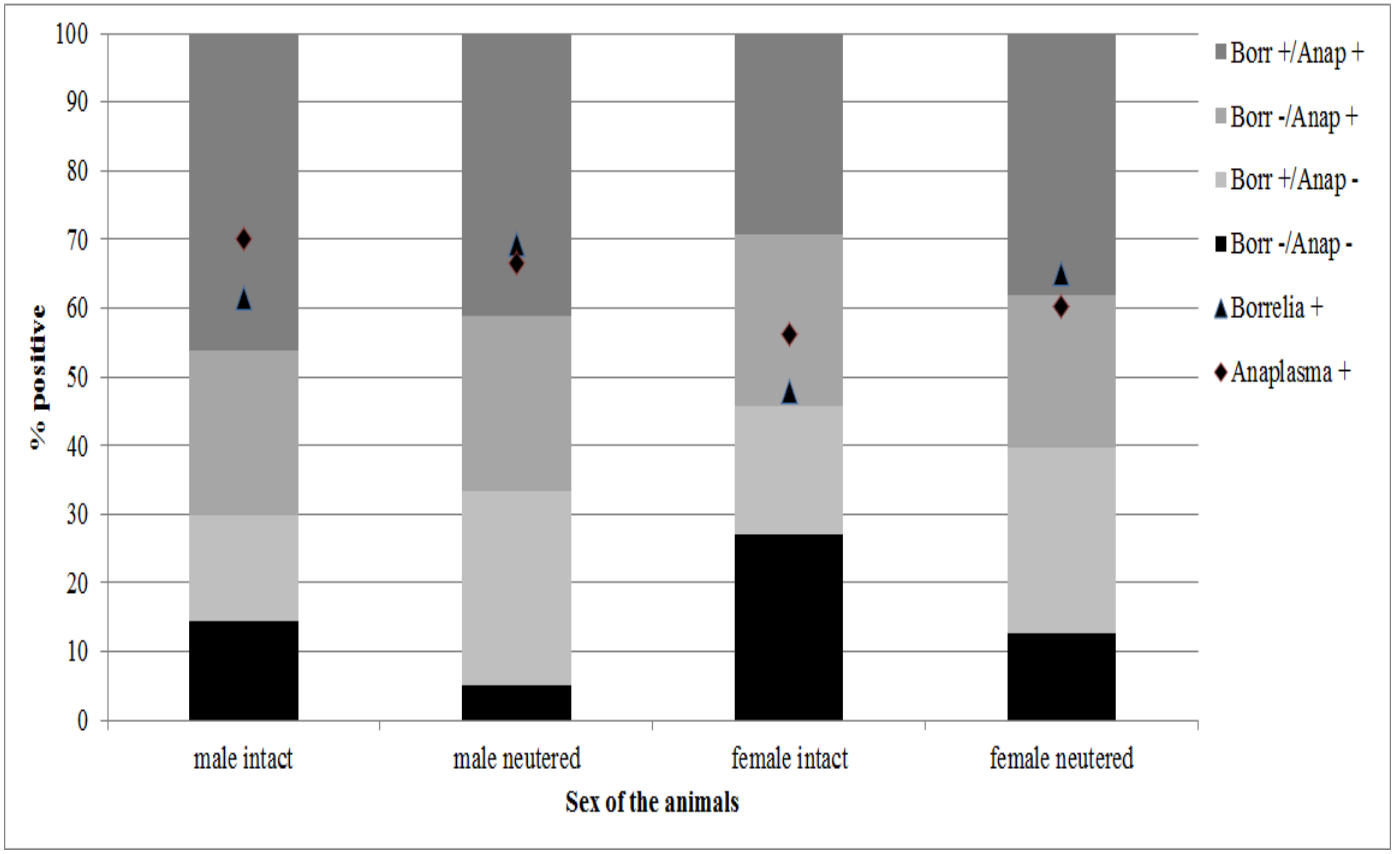

Figure 3. Sex and seropositivity for *Borrelia* (Borr) and *Anaplasma* (Anap).

median: 7 years) for healthy dogs and 6.2 years on an average (min: <1 year, max: 14 years, median: 6.5 years) for lame animals.

Upon neurological examination, 69 dogs (27.2%) showed abnormalities (Table 2), 27 of these were combined with lameness.

Symptoms	n	% (of 69 symptomatic dogs)
Non-specific neurological deviations	61	88.4
Convulsions	29	42.0
Ataxia	8	11.6
Tremor	8	11.6
Lumbar spinal syndrome	7	10.1
Nystagmus	6	8.7
Paralysis/paresis	4	5.8
Opisthotonus	1	1.4

Table 2. Neurological deviations in examined dogs (n=254).

Gastrointestinal signs were seen in 14.2% (n=36) of the 254 dogs, and included abdominal pain (3.5%, n=9), vomiting (2.4%, n=6), diarrhea (1.6%, n=4), inappetence (9.8%, n=25) and weight loss (1.2%, n=3).

Twenty dogs (7.9%) had hyperthermia (>39.5°C), 25 dogs (9.8%) were depressed, and 2.0% of the included dogs had enlarged lymph nodes.

One of 145 tested animals was azotemic.

Hematology (n=157 tested dogs) revealed 85 cases (54.1%) with deviations in leukocytes, thrombocytes, hematocrit, and/or mean corpuscular volume (Table 3).

Parameter	Elevated (%)	Reduced (%)
Leukocytes/µl blood	35 (22.3%)	16 (10.2%)
Thrombocytes/µl blood	14 (8.9%)	23 (14.7%)
Hematocrit (%)	11 (7.0%)	36 (23.9%)
Mean corpuscular volume (fl)	2 (1.3%)	5 (3.2%)

Table 3. Hematological results from 157 tested dogs that were included in the study.

Correlations between anti-*Borrelia/Anaplasma* antibodies and clinical signs

Neurological signs were seen in 27.2% of the dogs, evenly distributed among the groups with different serological results (Figure 4). The overall prevalence of gastrointestinal signs was 14.2% and this increased in animals with a positive *Borrelia* titer (Figure 4). The overall prevalence of lameness was 37.0% in the study group, and it was increased in animals

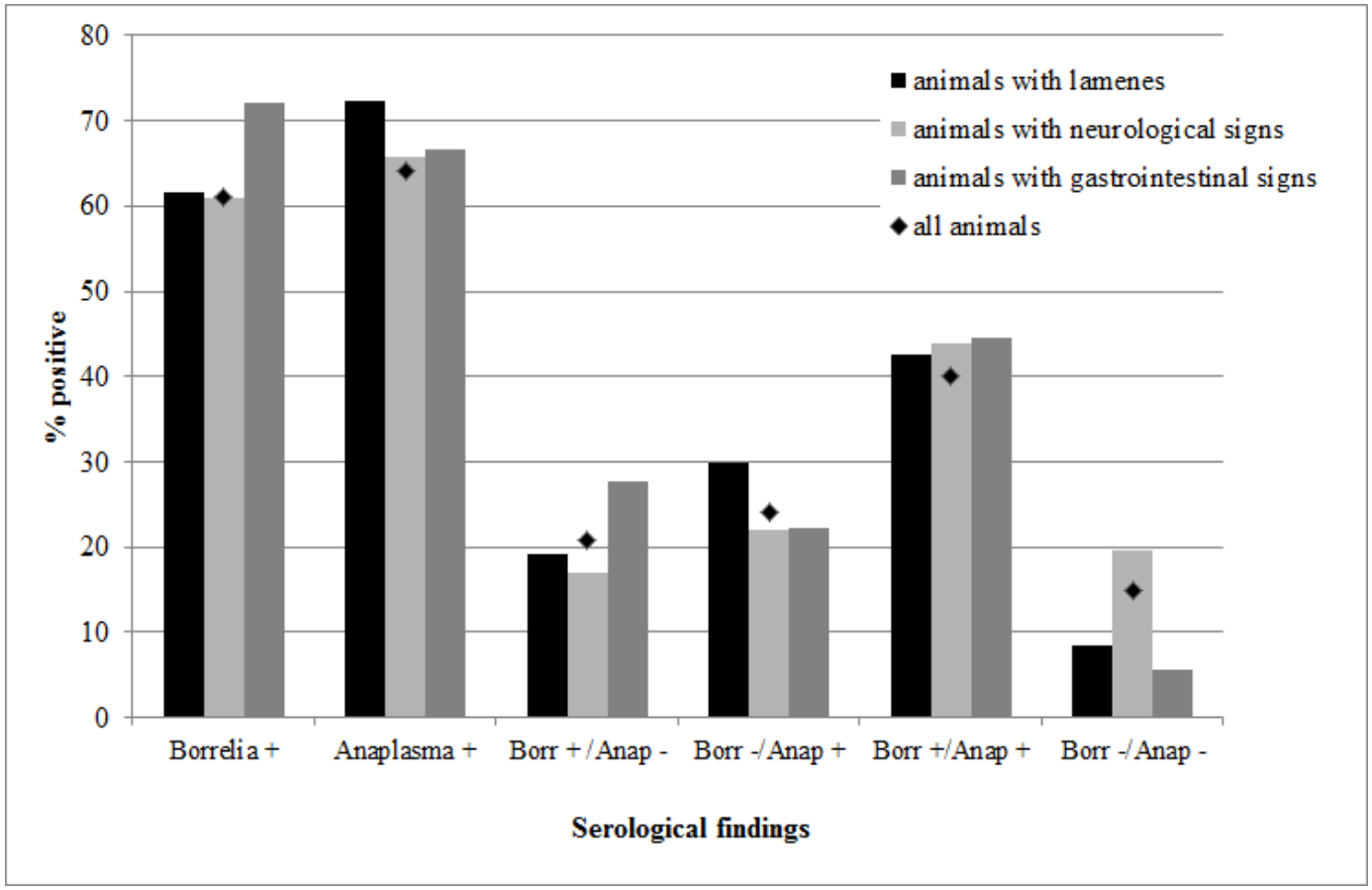

Figure 4. Percentage of clinical signs (neurological signs, gastrointestinal signs, and lameness) in different serological groups. Anap: *Anaplasma,* Borr: *Borrelia*; +: positive, -: negative

infected with *Anaplasma* in single or double infections and absent in animals that were serologically negative for either or both agents (Figure 4).

Statistical Analysis

The prevalence of *Anaplasma* and *Borrelia* infections (defined as positive antibody titer) were associated ($p<0.01$). For positive antibody titers against *Borrelia*, prevalence was not significantly affected by sex, breed, class, or age. For positive antibody titers against *Anaplasma*, older dogs had a significantly higher prevalence than younger dogs ($p<0.001$) and neutered males had a significantly higher prevalence of infections, while no other factor of the life history of dogs had any significant influence.

To evaluate the influence of infections with *Anaplasma*, *Borrelia*, or a combination of both as well as sex, breed, or age on lameness, various models were tested and compared by using the Akaike information criteria (AIC). The model with the lowest AIC, and thus, the best model among those tested, used the presence of infections with *Anaplasma*, *Borrelia*, and the interaction term between both. In this model, the presence of seropositivity for *Anaplasma* had a highly significant positive influence on lameness ($p<0.001$). The presence of seropositivity for *Borrelia* also had a significant positive influence ($p<0.05$), while the concurrent presence of *Anaplasma* and *Borrelia* seropositivity had significant negative influence ($p<0.05$).

Similar to lameness, gastrointestinal signs were positively affected by the seropositivity for *Borrelia* ($p<0.001$), *Anaplasma* ($p<0.05$) and negatively affected by double seropositivity ($p<0.05$), indicating that the latter did not exacerbate the clinical signs of lameness or gastrointestinal disorders.

No influence of the other variables could be detected. In particular, age had no influence on lameness, even though seropositivity for *Anaplasma* significantly increased with it.

Similarly, the influence of seropositivity for *Anaplasma*, seropositivity for *Borrelia*, sex, breed, or age on gastrointestinal signs was evaluated. Here, a model using the presence of both *Anaplasma* and *Borrelia* infections and their interaction term had the lowest AIC. Similar to the above, the effects of presence of both *Anaplasma* and *Borrelia* had a positive influence on gastrointestinal signs (with $p<0.01$ and $p<0.05$, respectively), while the joint effect of both infections had a negative effect, in a way that the prevalence of gastrointestinal signs in the presence of both infections was about that of a single infection ($p<0.05$).

Since hematological data were not available for all dogs, statistical analyses were run separately for this analysis. The best model was the same as for the entire study population. A decrease of hematocrit was associated with the absence of antibodies in *Anaplasma* ($p<0.05$), an increased number of thrombocytes with the absence of *Borrelia* infections

($p<0.01$). Other hematological parameters were not significantly associated, although there was a tendency for an association between the deviations in thrombocyte numbers with seropositivity to either pathogen.

Young animals more often showed leukocytosis when compared to the older ones ($p<0.05$), but changes in leukocytes were not associated with *Borrelia*/*Anaplasma* infections ($p>0.05$).

Decrease and increase of thrombocytes were both positively associated ($p<0.01$ and $p<0.05$, respectively) with gastrointestinal disease. A significant association between gastrointestinal illness and lameness, or hematological deviations and lameness, was not detected.

Neurological alterations were not associated with infections, but significantly increased with age ($p<0.01$) and were considerably more common in small companion dogs ($p<0.05$).

While lameness, gastrointestinal signs, and thrombocyte deviations were associated with the presence or absence of *Anaplasma* or *Borrelia* antibodies, the level of IgG was not associated with any clinical parameter ($p>0.05$). For IgM, no sufficient number of animals was tested for analysis.

Discussion

The examined cohort of 254 dogs had high prevalences of *Anaplasma* and *Borrelia* infections (about 60%), since serological examination was the primary inclusion parameter for this study. This also accounts for the lameness seen in more than one third of the patients.

Infections with *Borrelia* and *Anaplasma* occurred in double infections in 40.2% of dogs and were associated with each other, which is not surprising, as both are transmitted by *I. ricinus* and occur in the same geographical areas [1, 48]. In line with other studies [40, 41, 44], *Anaplasma* infections were significantly more frequent in older dogs, whereas with *Borrelia*, all age groups were infected at similar frequencies. Since the prevalence of *Borrelia* and *Anaplasma* infections were significantly associated, the reason for this difference might be in the dynamics of antibody responses; in *Borrelia* infections, titers may prevail after the infection due to a longer persistence of the pathogen in the host, while for *Anaplasma*, repeated re-infections lead to accelerated responses in older animals upon reinfection, although titers may not last long after the clearance of infection [49].

An alternative explanation might be the higher frequency of *Borrelia* in ticks [9, 10, 12, 15] and an increasing infection frequency of ticks with B. burgdorferi sensu lato [50], leading to earlier and more frequent infections of dogs when compared to *Anaplasma*, which, by contrast, induces less frequent infections and more fluctuating prevalence in different age groups.

Correlations between infection prevalence and age, sex, or dog breed/use are usually not detected for *Borrelia* [18, 51], while increasing prevalence with age were reported previously for *Anaplasma* infections [40, 44, 52]. Differences for breed or use of dogs were not significant; however, use was gauged from the breed and not by the actual use of dogs, as in other studies [40, 44].

Clinically, both infections were associated with lameness as reported before (e.g. [32] for *Borrelia* infections), but this is not a finding always supported by other authors (e.g. [18, 53]). In contrast to the previous reports [17, 20, 38], however, double infections were not increasingly associated with lameness. We conclude that specifically *Anaplasma* infections may lead to lameness, and the effect of *Borrelia* infections is the same but less frequent.

Consequently, after the exclusion of other causes for lameness and arthropathy, such as dislocated patella or cruciate ligament rupture, infections with either *Anaplasma* or *Borrelia* should be considered. As reported previously [22, 39, 54], antibody levels were not associated with the degree of lameness in the examined dogs. IgM seropositivity for *Borrelia* was detected in a small group of dogs solely, and all but one had positive titers for IgG as well, indicating that IgM was poorly associated with infection and disease in the examined cohort.

Correlations between positive antibody titers and gastrointestinal disorders could also be demonstrated (in contrast to the previous findings, e.g. [30, 37, 39, 41]); however, in the present study, these disorders also included inappetence, which might be a sign of general illness as described by [42] for an *A. phagocytophilum* infection, and its association with hematological changes also indicated this. Other clinical signs previously associated with borreliosis, such as anorexia, vomiting, dehydration, polyuria, polydipsia, and wasting [20, 30] were only rarely detected and not associated with infection in our cohort.

Hematological deviations, such as reduced hematocrit, anemia, or thrombocytopenia, were indicative for infections with *Anaplasma* or *Borrelia*. These deviations can also occur during infections with other tick-borne pathogens, such as *Babesia, Ehrlichia, Leishmania*, or some rickettsiae [55-57] and may have skewed the findings in the examined cohort (especially since the changes in hematological parameters were not unidirectional; i.e., deviations towards increase as well as decrease were seen); however, the number of dogs tested was too low to allow for any comparison. We could not statistically confirm the hematological changes previously associated with borreliosis, such as non-regenerative anemia [26], stress-leukogram (leukocytosis, lymphopenia, monocytosis, eosinopenia), or thrombocytopenia [20, 28]. Neither were thrombocytopenia, anemia, or lymphopenia with leukopenia confirmed as a correlate of *Anaplasma* infection [37-38, 41, 42, 58, 59], and unlike the earlier reports [38, 42], thrombocytopenia was not exacerbated by double infection.

Neither hematological deviations nor gastrointestinal signs were related to gait abnormalities, perhaps explained by a changing clinical presentation during the course of infection

– although during the early phase general illness can be seen, lameness is a sign of a more progressive stage of the disease.

Neurological changes, on the other hand, as seen in 27.2% of the dogs, were not associated with positive *Borrelia* or *Anaplasma* antibody titers. Neuroborreliosis is a form that has been described in human patients [60] and discussed in dogs [24, 61, 62]; however, currently, there is no evidence for this [61], and our study also does not support the assumption that dogs can suffer from neuroborreliosis. Interestingly, neurological signs were more frequent with increasing age and seen more in small companion dogs; the reasons for this are not clear. Idiopatic epilepsy, and specifically in older dogs, geriatric vestibular syndrome and discopathy accounted for neurological disorders, but this was not evaluated in detail.

Azotaemia, a sign of Lyme nephropathy [20, 26, 31], was found in only one dog and did not seem to be a frequent sequela of *Borrelia* infections in the examined cohort. Likewise, signs of acute infection, such as fever, apathy, and enlarged lymph nodes were not found frequently in seropositive dogs, which is not surprising, as antibodies are usually produced only after a lag phase of infection, and thus, not directly correlated with acute disease [63], although fever (accompanied by inappetence) is considered to be a main sign of acute anaplasmosis [37, 39].

In the early stage of *A. phagocytophilum* infection, PCR may detect the pathogen (out of 41 dogs that tested negative by serology one was positive by PCR), while antibody titers are still low, so, both tests are useful for clinical diagnosis, depending on the phase of infection [37, 64, 65]. This phenomenon may also explain why correlations between PCR, serology, and clinical illness have previously been described as poor [39, 52, 64, 66]. Occasionally, animals can be positive in both tests [51], indicating that they can become bacteriemic upon reinfection.

In contrast to *A. phagocytophilum*, which can be found in peripheral blood during the acute phase of infection, the detection of *B. burgdorferi* in bodily fluids is not straightforward. It was not detected by PCR or culture in blood and urine samples in a study by Leschnik et al. [34], although the infected dogs seroconverted. By contrast, Skotarczak et al. [22] found a clear correlation between the clinical signs and the results of PCR from peripheral blood. In an experimental study, 17 out of 20 dogs tested positive for *Borrelia* with PCR and ELISA and B. burgdorferi could also be isolated and cultured for eight of them [32]. However, in a clinical setting, the cultivation of this pathogen from EDTA blood does not seem to be a reliable diagnostic technique [24].

Conclusion

In a cohort of clinical patients examined for infections with *Borrelia* and/or *Anaplasma*, lameness and gastrointestinal signs were significantly associated with seropositivity, while other parameters were not. Single blood parameters (or even a combination of these) or the clinical signs cannot reliably diagnose infection in association with a disease. However, a high background of positive titers in healthy animals preclude clinicians from pinpointing lameness to either of the infections, and risk assessments about infections and their clinical presentations have, so far, provided conflicting results. Serum antibody levels were not found to be associated with a disease, although a titer increase upon retesting is indicative of active infection. For *Borrelia* diagnosis, IgM does not appear to be a good diagnostic parameter. Depending on the stage of infection, the animals may present with a variety of clinical signs, and the diagnostic panel available may not equally be well-suited for each phase of infection. Although the percentage of seropositivity was high, there was no correlation with neurological signs in the examined cohort, supporting the idea that neuroborreliosis does not occur in dogs. A combination of both clinical and specific (serological, molecular) diagnostic procedures is proposed in order to determine whether an animal is suffering from borreliosis, anaplasmosis, or both. Seropositivity alone is insufficient to diagnose disease, and clinicians must resist over-diagnosing borreliosis and anaplasmosis on the grounds of serological evidence solely. As previous studies also reported, a poor correlation between serology, PCR, and clinical signs, together with laboratory diagnostic findings, must match clinical signs to enable the correct decisions for treatment, especially when the deviations are non-specific.

Conflict of Interest

The authors declare that they have no conflict of interest.

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