Infection with *Anaplasma phagocytophilum* in a young dog: a case report

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**ABSTRACT:** An 11-months-old male Golden Retriever occasionally found to have *Ixodes ricinus* ticks attached to the skin developed the acute onset of fever, lameness and inappetence followed by rapidly progressive depression, ataxia and reluctance to move. Inclusions (morulae) were observed in granulocytes. The blood analysis revealed severe thrombocytopenia, lymphopenia, eosinopenia, elevation of alkaline phosphatase and hypercholesterolaemia, mostly suggestive of an *Anaplasma phagocytophilum* infection. The amplification of a DNA sequence specific for *Anaplasma phagocytophilum* and detection of specific antibodies supported the diagnosis.

*Borrelia burgdorferi*, another tick-borne pathogen, or specific antiborrelial IgG antibodies were not detected. The dog was treated with oral doxycycline for 14 days: clinical symptoms resolved within six days.

**Keywords:** anaplasmosis; infection; dog
Infection with *B. burgdorferi* (Cohn, 2003). Twenty-five percent of dogs healthy or suspected of borreliar or anaplasmal infection (*n* = 731) had significant titres for both infections. The co-infection with *A. phagocytophilum* and *B. burgdorferi* is more likely to induce illness in the dogs as compared to the infection with either organism alone (Beall et al., 2006).

Infections with other tick-borne agents – *Babesia* spp. and tick-borne encephalitis (TBE) virus – with typical clinical symptoms are also reported sporadically in dogs in the Czech Republic (Klimes et al., 2001).

We present a case report of a young dog that fully recovered after developing febrile illness, lameness, depression and ataxia as the acute onset of suspected tick-borne disease.

**Case history**

An 11-months-old pet male Golden Retriever found to have several *Ixodes ricinus* adult female ticks attached to the skin between March and June developed acute signs of infection at the beginning of June. On admission to the veterinary clinic (Day 0), the dog showed the severe acute onset of pyrexia (39.6°C), lethargy, inappetence and ataxia. It was not able to either stand or walk. The general clinical examination including the inspection and palpation of leg bones and joints did not reveal any additional physical abnormalities.

The baseline biochemistry screening (Spotchem, SP-4430, Arkray) showed hypercholesterolaemia and elevation of alkaline phosphatase level (Table 1). The remaining parameters tested (urea, creatinine, ALT, AST, GMT) were within the reference ranges. Intravenous fluid therapy with saline solution (60 ml/kg/24 h) was immediately initiated. Intravenous metamizol (25 mg/kg, Vetalgin inj., Intervet) and subcutaneous amoxicillin/clavulanate (12.5 mg/kg, Synulox, RTU, Pfizer) were given to control fever, pain and suspected infection.

On Day 2, the fever (40.5°C) and depression increased. Intravenous fluid therapy with saline solution was given again (80 ml/kg/24 h). Haematological (AL Cell Counter 871, AL Systeme) and biochemical (Vitalab Selectra, Merck; EasyLyte Plus, Medica) analyses revealed some values outside the reference ranges (Table 1). Severe thrombocytopenia, lymphopenia and elevated erythrocyte sedimentation rate (+83/h, adjusted for hematocrit +77/h, refer-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At baseline</th>
<th>Day 2</th>
<th>Week 3</th>
<th>Month 5</th>
<th>Reference range**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocyte (count)</td>
<td>ND</td>
<td>29*</td>
<td>287</td>
<td>299</td>
<td>200–500 × 10⁹/l</td>
</tr>
<tr>
<td>Leukocyte (count)</td>
<td>ND</td>
<td>10.1</td>
<td>10.6</td>
<td>12.3</td>
<td>6–17 × 10⁹/l</td>
</tr>
<tr>
<td>Band neutrophil (count)</td>
<td>ND</td>
<td>0.05*/505*</td>
<td>0</td>
<td>0</td>
<td>rel. 0–0.03/abs. 0–300 mm³</td>
</tr>
<tr>
<td>Segmented neutrophil (count)</td>
<td>ND</td>
<td>0.87*/8 787</td>
<td>0.57*/6 042</td>
<td>0.62*/7626</td>
<td>rel. 0.60–0.77/abs. 3 000–11 500 mm³</td>
</tr>
<tr>
<td>Lymphocyte (count)</td>
<td>ND</td>
<td>0.03*/303*</td>
<td>0.30/3 180</td>
<td>0.34/4182</td>
<td>rel. 0.12–0.30/abs. 1 000–4 800 mm³</td>
</tr>
<tr>
<td>Monocyte (count)</td>
<td>ND</td>
<td>0.05/505</td>
<td>0.06/636</td>
<td>0.02*/246</td>
<td>rel. 0.03–0.10/abs. 150–1 350 mm³</td>
</tr>
<tr>
<td>Eosinophil (count)</td>
<td>ND</td>
<td>0*</td>
<td>0.07/742</td>
<td>0.02/246</td>
<td>rel. 0.02–0.10/abs. 100–1 250 mm³</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>ND</td>
<td>1.33</td>
<td>ND</td>
<td>1.62*</td>
<td>up to 1.50 mmol/l</td>
</tr>
<tr>
<td>Calcium</td>
<td>ND</td>
<td>2.82*</td>
<td>ND</td>
<td>2.70</td>
<td>up to 2.77 mmol/l</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>11.0*</td>
<td>10.51*</td>
<td>ND</td>
<td>10.91*</td>
<td>2.74–7.65 mmol/l</td>
</tr>
<tr>
<td>Total protein</td>
<td>ND</td>
<td>64.0</td>
<td>ND</td>
<td>68.6</td>
<td>58–76 g/l</td>
</tr>
<tr>
<td>Albumin</td>
<td>ND</td>
<td>36.75</td>
<td>ND</td>
<td>39.09*</td>
<td>23–36 g/l</td>
</tr>
<tr>
<td>ALP</td>
<td>4.29</td>
<td>5.04*</td>
<td>ND</td>
<td>2.30</td>
<td>up to 2.60 μkat/l</td>
</tr>
<tr>
<td>Creatinine kinase</td>
<td>ND</td>
<td>4.25*</td>
<td>ND</td>
<td>1.16</td>
<td>up to 2.50 μkat/l</td>
</tr>
</tbody>
</table>

ND = not determined; *the value outside the reference range, **based on Bush (1996)
ence range up to 10/1/h) were observed. Eosinophils could not be detected and the total number of leukocytes was within the reference range (Table 1).

The other haematological parameters, i.e. haemoglobin, hematocrit and calculated characteristics, were within reference ranges (MCV 72.1 fl; MHC 14.5 fmol and MCHC 32.7g/dl). Giemsa-stained blood smears revealed the presence of isolated bacteria or inclusions (morulae) (Figure 1) in 36% of three hundred neutrophils.

Hypercholesterolaemia and elevation of creatinine kinase and alkaline phosphatase were observed (Table 1). Amylase activity was reduced (7.10 μkat/l, reference range 10–30 μkat/l). The other biochemical parameters tested (urea, creatinine, phosphorus, calcium, sodium, potassium, chloride, glucose, total protein, albumin, bilirubin, ALT, AST, GMT, and lipase) were in reference ranges. PCR analyses of *A. phagocytophilum* and *B. burgdorferi sensu lato* were performed as described by Jackson et al. (2002) and Marconi and Garon (1992), respectively. Template DNA was isolated from EDTA-collected blood specimens of the infected animal and five clinically healthy dogs as negative controls by a QiaAmpTissue kit (Qiagene). DNAs from the strain EHR 02 of *A. phagocytophilum* (Hulinska et al., 2004) and strain M192 of *B. garinii* (Hulinska et al., 1999) were used as positive controls. The 293 bp amplicon specific to *A. phagocytophilum* was detected only in the infected dog and the positive control (Figure 2) but it was not found in the healthy dogs. No amplicon specific to *B. burgdorferi* was detected in the blood specimens from either the infected animal or the five clinically healthy dogs. The ELISA test using whole cell *B. afzelii* antigen (Test-Line, Brno, Czech Republic) detected IgM to *B. burgdorferi* (index of positivity 1.40; positive result higher than 1.15) and IgG negativity in the blood of the infected dog. Since *A. phagocytophilum* and borrelial infection was suspected, the antibiotic was converted to 10 mg/kg doxycycline (Ronaxan, Merial) given orally twice daily for 14 days.
On the following day the temperature decreased to 39.0°C. Intravenous glucose in a 5% solution (20 ml/kg/24 h) and fluid therapy with vitamins (Duphalyte, Fort Dodge, 5 ml/kg/24 h) were administered.

On Day 5, the temperature further decreased to 38.5°C and the dog showed only slight lethargy. The clinical examination on Day 6 indicated full clinical recovery, with the temperature falling to 38.2°C. The therapy with doxycycline was continued for another 14 days.

Three weeks after the first examination all haematological parameters were within reference ranges. The recovered dog was followed up at monthly intervals.

Five months later, the laboratory analysis confirmed haematological and biochemical parameters to be in reference ranges. The dog was IgM positive and IgG negative again. Specific IgG antibodies to A. phagocytophilum were detected in the convalescent serum by the indirect immunofluorescence assay (IFA) at a titre of 1:320 five months after the entry examination.

DISCUSSION

Canine granulocytic anaplasmosis usually presents as an acute febrile systemic illness (Stiles, 2000). Specific diagnostic tests include visualisation of specific morulae, anti-\textit{Anaplasma} IgM and IgG antibody detection and PCR analysis, which is most reliable for early diagnosis (Engvall et al., 1996; Bjoersdorf, 2002).

The dog presented the most typical systemic clinical symptoms, i.e. fever, ataxia, depression, lameness, and laboratory results, i.e. – thrombocytopenia, eosinopenia, lymphopenia and occurrence of band neutrophils (Bjoersdorf, 2002), suggestive of acute granulocytic anaplasmosis. However, other reported symptoms such as lymphadenopathy, splenomegaly and hepatomegaly (Neer, 1998) were not detectable by palpation.

The inspection and palpation of leg bones, muscles and joints did not reveal any physical abnormalities in the limbs. The reactivity to pain during palpation indicated that the nervous system was not involved. Even if no special neurological and musculoskeletal examinations were performed, the reluctance to move, typically reported in acutely infected animals (Engvall et al., 1996), seemed to be due to deep depression known to result from stress as a consequence of homeostasis imbalance due to inflammation.

Inclusions were observed in 36% of neutrophils: this proportion was close to the highest reported figure, i.e. 37% (Kirtz et al., 2000). The persistence of the infection was screened microscopically and by the amplification of specific \textit{A. phagocytophilum} DNA in two consecutive blood samples collected three weeks and five months after the acute attack with negative results. However, Egenvall et al. (2000) detected \textit{A. phagocytophilum} infection in three dogs for six months but caution would be advised in comparing their results obtained in the experimentally infected and immunosuppressed dogs with ours found in the naturally infected animal.

The amplification of the specific fragment of \textit{A. phagocytophilum} and not of that of \textit{Borrelia} spp. confirmed the infection with \textit{A. phagocytophilum}. The positivity to antiborrelial IgM antibodies indicated either an exposure to \textit{Borrelia} spp. or cross-reactivity with anaplasmal or other gram-negative bacterial surface antigens. The aetiological role of \textit{Borrelia burgdorferi} in the case presented is disputable as no DNA and specific IgG antibodies were detected on admission and in convalescent specimens, while ELISA antibodies to whole cell or recombinant \textit{Borrelia burgdorferi} antigens were reported to remain constant for nearly two years (Straubinger, personal communication).

The elevation of hepatic transaminase activity is presented in the literature (Bjoersdorff, 2002) rather than increased alkaline phosphatase (ALP), creatinine kinase (CK) and cholesterolemia as described in our case history. The precise mechanisms for the elevations remain unknown. Elevated ALP and CK could probably be connected with septicaemia because of the effects of large numbers of multiplying bacteria and endotoxaemia. Bacterial endotoxins released in the blood could evoke liver and cardiac cell damage (Bush, 1996). The reasons for stable elevated cholesterol levels remain unclear. Increased lipolysis at the time of acute stress or starvation could be omitted because the level persisted five months after the successful therapy. An idiomatically increased serum chole-
terol level in the dog could be one reason explaining the phenomena (Bush, 1996).

The therapy with doxycycline proved as effective as found by others (Stiles, 2000; Cohn, 2003). The temperature started to decrease on day 2 and all clinical symptoms resolved on day 6 after the initiation of the therapy. At 3-week and 5-month follow-ups no clinical or laboratory changes except for elevated cholesterol were observed.

It is not likely that the dog was coinfected with another tick-borne agent. Babesia spp. was not seen microscopically in red blood cells and most of the typical clinical symptoms of babesios (anaemia, icterus, haemoglobinuria, dyspnoea and a low body condition index) did not appear in the dog (Taboada, 1998). The coinfection with TBE virus was possible but unlikely as the primary TBE symptoms typical of TBE infection (shyness and unwillingness, periods of excitement and irregular movements and high fever in the course of the disease) (Svoboda et al., 2001) were not observed and the dog responded quickly (within 24 hours) to the specific antibiotic therapy suggesting bacterial infection.

All findings summarized above, particularly the presence of specific morulae and DNA in neutrophils, negative laboratory or clinical results for other tick-borne agents, thrombocytopenia and the fact that a high titre of specific IgG antibodies was detected in the convalescent serum suggest that *A. phagocytophilum* was the single causative agent of the acute infection. Other authors also reported the detection of specific IgG antibodies by IFA from 2 weeks up to more than six months after infection in dogs suffering from anaplasmosis (Kirtz et al., 2000, 2005; Poitout et al., 2005). To the author’s knowledge this is the first confirmed case of canine anaplasmosis caused by *A. phagocytophilum* to be reported in the Czech Republic, where the infection was already suspected (Huml et al., 1996).

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REFERENCES


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