First Detected Case of *Anaplasma phagocytophilum* in a dog in Bulgaria

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SUMMARY

A clinical case of *Anaplasma phagocytophilum* infection in an 8-year-old male American Cocker Spaniel is described. Clinical, haematological, blood biochemical, serological, parasitological and cytological examinations supporting the diagnosis have been performed.

Keywords: *Anaplasma phagocytophilum*, dog, clinical sign, haematological and biochemical findings.

**Introduction**

*Anaplasma phagocytophilum* is a Gram-negative, obligate, intracellular, pleomorphic rickettsial pathogen, inducing granulocytic blood infections in man, dogs, cats and horses [1, 6, 8, 23]. The target cells of *A. phagocytophilum* are neutrophils and occasionally, eosinophils [21]. *Ixodes ricinus* has been found to be the main vector of *A. phagocytophilum* in Europe [26]. Granulocytic anaplasmosis in dogs (known until recently as granulocytic ehrlichiosis) is sporadically detected in several European countries: Sweden [9, 10], Norway [1], Switzerland [20], Italy [11], Austria [15, 16], UK [2, 24], Slovenia [27], Czech Republic [18] and Germany [14]. The clinical signs of the disease are not specific − fever (up to 41°C), anorexia, lethargy, depression, vomiting, polyarthritis, splenomegaly, hepatomegaly, lymphadenopathy, anaemia, thrombocytopenia [9, 10, 12, 19, 22]. Haematological disorders include leukopenia, more rarely leukocytosis, normocytic-normochromic anaemia and a mild thrombocytopenia [15, 17]. In 2001, a clinical *A. phagocytophilum* infection in a man was detected for the first time in Bulgaria [4].

**Case history**

In February 2008, a male 8-year old American Cocker Spaniel was referred a private veterinary clinic in Stara Zagora town. The dog had been born in Bulgaria and had never left the country. The owner reported that it was frequently infested with ticks in the summer and autumn of 2007. The owner had observed an extensive bilateral ocular discharge and impaired orientation. The same signs were also present a year ago, when a surgical intervention for retinal revascularization had been performed.

The dog was clinically examined and a blood sample from the cephalic vein was obtained for haematological (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT) and biochemical analysis (urea, creatinine, ALAT and ASAT); another blood sample was obtained after 21 days. A blood smear from the buffy coat, stained according to Romanovski/Giemsa was made for cytological investigation. A serological study for vector-borne infections - *Ehrlichia canis*, *Borrelia burgdorferi*, *Dirofilaria immitis* and *Anaplasma phagocytophilum* was done by means of ELISA test (IDEXX Snap® 4DXTM, Maine, USA).

The clinical examination of the dog revealed an apparently good general condition − preserved vitality and appetite, normal body temperature. The ophthalmologic examination showed an enhanced discharge from both eyes with purulent catarrhal conjunctivitis. On the cornea, ingrowth of blood vessels was observed. The serological result was positive only for *Anaplasma phagocytophilum*. The microscopy of blood smears revealed clearly oval cytoplasmic bodies in 12% of neutrophils, identified as *Anaplasma phagocytophilum* (Fig. 1). Other infectious agents were not identified on the blood smear. The first haematological and biochemical analysis showed mild increase in HGB, MCH, MCHC, ALAT, ASAT and lymphopenia, and the second one – also mild increase of HGB, HCT, MCH, MCHC, ALAT, ASAT.

On the basis of performed examination, a diagnosis of granulocytic anaplasmosis was made. A doxycycline therapy...
Discussion

Anaplasmosis in domestic animals is a long-known disease. In 1930 it is described for the first time as “tick-borne fever” in Scottish sheep. The first cases of human granulocytic ehrlichiosis (anaplasmosis) date back to 1994 in the USA [7]. Soon after its discovery in man, several countries initiated extensive research. In Bulgaria, serosurveillance demonstrated a 9% infection rate in people bitten by ticks; more than 30% of infected ticks were shown to be infected with Anaplasma phagocytophilum [4]. Wild rodents may play an important role as a reservoir of granulocytic anaplasmosis in Bulgaria where 8% of wild rodents were infected with this pathogen [5]. The clinical detection of the infection in Bulgaria in a patient bitten by a tick, gave us another reason to direct our attention to the incidence of this disease in dogs as well.

It is known that the clinical picture of canine granulocytic anaplasmosis is not specific [10,12]. In most cases, the disease starts as an acute systemic disease with fever [25]. The detection of agent’s morules is possible only in the acute phase of the infection [16] (as is our case), and that is why we assume this form of clinical manifestation in the described case. The observed good general condition – preserved vitality and appetite, normal body temperature and the purulent-catrhal conjunctivitis, have not been mentioned as clinical signs from other authors up to now. A specific finding in the described Cocker spaniel was the bilateral ingrowth of blood vessels on the sclera. Affection of eyes with scleral haemorrhages and retinal petechial bleedings are reported by BEXFIELD et al. (2005) in a 10-year-old dog in England.

The haematological changes in granulocytic anaplasmosis are characterized with erythroptenia, decreased haemoglobin content, haematocrit, thrombocytopenia [2], thrombocytopenia and neutrophilia [16]. In other reports, changes in HGB, HCT, MCH, MCHC have not been observed [18]. The changes in the present case – increased HGB, HCT, MCH, MCHC, during both blood samplings, could be possibly related to a permanent systemic tissue hypoxia, dehydration and intra-vascular hemolysis that is known to be accompanied with such changes, without be able to explain its mechanism.

Lymphopenia, similarly to our findings, is also reported by BJOERSDORFF [2002] and MELTER et al. [2007]. Also, elevated activities of liver transaminases have been described [3] but the mechanism of this phenomenon was not discussed. In our patient, the increase in enzyme activities was very slight and thus could not be accepted as significant.

Morula of Anaplasma phagocytophilum were present in 12% of neutrophils. This is within the known range of 1 to 37% [15, 18, 28]. The relatively low percentage of infected neutrophils was probably the reason for the less severe clinical manifestation of the disease. The detection of Anaplasma morulas is only possible during the acute stage of infection [16].

The ELISA-based SNAP 4Dx test has a high sensitivity (99.4%) and specificity (100%). It detects IgM and IgG antibodies against Anaplasma phagocytophilum. The qualitative positive result corresponded at least to 1:80 by IFA [13], that is indicative for the disease.

The performed doxycycline therapy and the good clinical outcome of the treatment confirmed the literature fact for the excellent antiehrlichial and anti-anaplasma effect of tetracyclines [6,25].

Finally, A. phagocytophilum is a zoonotic pathogen and dogs may play an important epidemiology role as a reservoir of the human granulocytic anaplasmosis in Bulgaria.

Conclusion

This is the first incidence of granulocytic anaplasmosis (Anaplasma phagocytophilum) in a dog in Bulgaria.

References

ANAPLASMA PHAGOCYTOPHILUM IN A DOG IN BULGARIA


