TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2019, vol. 4, No 1(6): 51–55

ANAPLASMOSIS IN STRAY DOGS FROM SOFIA DISTRICT, BULGARIA

Iliyan Manev[1], Victoria Marincheva[2], Polina Ilieva[2], Krasimira Genova[1], Roman Pepovich[1]

[1]University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
[2]Animal Rescue, Sofia, Bulgaria
E-mail: doc_man08@abv.bg

ABSTRACT

Anaplasmosis is a vector-borne zoonosis which affects different domestic and wild species, including humans. The available data for the distribution of the disease in stray dogs from different parts of Bulgaria is quite insufficient. The aim of the study is to introduce the results of the serological examination of 159 stray dogs from Sofia district for 2017. Thirty of them (18.87%) were positive for specific antibodies determined through chromatographic immunoassay. Forty percent of the positive dogs were accompanied by some hematological or biochemical alterations typical of anaplasmosis.

Key words: anaplasmosis, vector-borne diseases, zoonosis, canine.

Introduction

Anaplasmosis is an important tick-borne disease with a worldwide distribution. Two bacterial species from genus *Anaplasma*, *Anaplasmataceae* family can affect canidae (Dumler et al., 2001). The first one is *Anaplasma phagocytophilum*, which infects neutrophils and rarely eosinophils and is the causative agent of ***Canine granulocytic anaplasmosis***. The other one – *Anaplasma platys* – affects platelets and causes ***Canine cyclic thrombocytopaenia***. They are Gram-negative, non-motile aerobes which are obligate intracellular bacteria. There exists a serological cross-reactivity between both bacteria species. Tick-transmission is the predominant means of infection and *Ixodes ricinus* is the main vector in Europe. *A. phagocytophilum* causes infection and potential illness in dogs, horses, cattle, sheep, goats, cats, rodents, wild animals, birds and humans while *A. platys* affects dogs (Sainz et al., 2015). Clinical signs are unspecific – fever, lethargy, anorexia and many of the naturally infected dogs remain asymptomatic as indicated by the high number of healthy seropositive dogs relative to dogs with the clinical disease (Kohn et al., 2008). Several laboratory alterations seem to be predominant and aid the diagnosis – lymphopenia, thrombocytopenia, hypoalbuminemia, elevated liver enzymes (Kohn et al., 2008).

Nowadays there is a continuously increasing data for the infection epidemiology in canine population in Europe. The seroprevalence depends on the area and used laboratory technique and varies between 2.7% for France to 56.5% for Austria (Sainz et al., 2015). The most commonly used assays for detection of anti-Anaplasma antibodies are rapid tests, based on ELISA and immunochromatography, or indirect immunofluorescence antibody test (IFAT). Several surveys which included comparison between PCR detecting anaplasma antigen and IFAT detecting antibodies determined considerable mismatch. The survey of Jensen et al. (2007) with dogs from Germany (n=111) established positive results with IFAT and PCR 43.2% / 6.3% respectively. Even worse are the results from Portugal (n=55) – 55% / 0% (Santos et al., 2009) and from Italy (n=215) – 15.0% / 0.02% (Ebani et al., 2013). Thus positive serological assays may overestimate the real existence of infection but at the same time can be used as an epidemiological indicator. In endemic

areas antibody titer may be a result of previous infection and may not be indicative of acute infection (ESCCAP Guideline, 2012).

The first canine anaplasmosis case in Bulgaria was presented a decade ago (Tsachev et al., 2008) and nowadays some epidemiological data are already available (Tsachev, 2009; Pantchev et al., 2015).

The aim of this study is to determine the seroprevalence to *Anaplasma phagocytophilum/ Anaplasma platys* among stray dogs from Sofia district and to perform comparison with the available epidemiological data from Bulgaria and the neighboring countries. Further we performed blood examination of the antibody positive animals in order to evaluate the presence of typical for anaplasmosis hematological and biochemical alterations.

Materials and methods

Study area and animals – the survey was carried out in Sofia district (west part of Bulgaria) Study included 159 stray dogs from different parts of the city and neighbored villages. All animals were mix breed, from both sexes (male – n=83, female – n=76), age from app. 6 months to app. 10 years old.

Clinical materials – blood samples were collected from *v. saphena lateralis* or *v. cephalica antebrachii* through standard technique in tubes with EDTA for whole blood and with clot activator for serum separation. Immunological, hematological and biochemical assays were performed *ex tempore*.

Immunodiagnostic assay – the used test was *Anigen Rapid CaniV-4Test Kit* (BioNote Inc., South Korea) – a chromatographic immunoassay which includes the qualitative detection of antibodies against *Anaplasma phagocytophilum / Anaplasma platys*. The test was performed with whole blood.

Hematological and biochemical tests – the blood examination included complete blood count (CBC) supplied in SI units by Mindray BC-2800 Vet automatic blood counting analyzer. Biochemical profile included albumin (Alb), alkaline phosphatase (AP) and alanine aminotransferase (ALT) performed on semiautomatic analyzer Mindray BA- 88A using reagents by Giesse Diagnostics, Italy. The results of the main CBC and biochemical parameters are presented as mean value with standard deviation.

Statistical analysis – the hematological data are presented as mean value with standard deviation and were analyzed with Microsoft Excel 2010.

Results

Immunodiagnostic assay – the samples from 30 dogs were positive for specific antibodies against Anaplasma spp. The seroprevalence was 18.87%.

Hematological and biochemical tests – the results from the main CBC and some biochemical parameters are presented in Table 1.

Table 1: Some hematological and biochemical parameters in anti-Anaplasma antibodies positive dogs

	Unit	Mean ± SD	Range	Ref. range
Le	x 10^9/L	15.31 ± 5.05	7.7 – 26.9	6.0-16.9
Gr	x 10^9/L	12.05 ± 4.79	6.5 – 21.9	3.3-12
Ly	x 10^9/L	2.94 ± 1.43	0.9 – 7.0	1.1-6.3
RBCs	x 10^{12}/L	6.37 ± 1.13	3.89 – 8.82	5.6-8.7
Hb	g/L	148.4 ± 33.12	75 – 235	120-180
Plt	x 10^9/L	360.3 ± 148	33 – 639	175-500
Alb	g/L	30.83 ± 7.36	15.8 – 37.8	22-39
AP	U/L	190.87±178.7	23 – 516	23-212
ALT	U/L	119.5 ± 83.1	23 – 516	10-90

The mean results of the hematological parameters showed no deviations from the standard reference range and only slight elevation of the ALT activity. Still the individual results presented specific deviations which can be linked to anaplasmosis. Total 12 animals (40%) showed at least one alteration in some of the laboratory indeces. Six of the dogs (20%) were lymphopenic, five of them (16.67%) – with anemia, one dog was with severe thrombocytopenia (3.3%), single with hypoalbuminemia (3.3%), four of the examined (13.33%) – with elevated alkaline phosphatase and four (13.33%) with elevated alanine aminotransferase activity. Five of the animals (16.67%) showed more than one alteration.

Discussion

It's a well-known truth that the qualitative detection of specific immunoglobulins against some infectious agents should not be interpreted as a diagnosis. The seroconversion in Anaplasmosis is achieved after two weeks and some antibody titer can be ascertained months and years later (Gaunt et al., 2010). The results from the blood tests determined that 40% of the antibody positive dogs have some deviations. At the same time there is a serious disadvantage of their significance because of the low specificity. Based on the results we may ascertain that a large number of the seropositive animals in our study are not with active Anaplasmosis at the time of sampling. It should not be underestimated that discrepancy between antibody titers and clinical presentation is also a possible scenario and some actively infected dogs may have a low titer (Carrade et al., 2009).

The seroprevalence for Anaplasmosis in stray dogs from Sofia district have to be considered in comparison with studies with similar design from other parts of Bulgaria and southeastern Europe. The epidemiological survey of Tsachev (2009) through IDEXX Snap® 4DXTM Test, USA included samples from different parts of the country and established positive assays only in South Bulgaria. The seroprevalence for Plovdiv (n=57) and Stara Zagora (n=29) region was 3.5% and 17.24% respectively. Small number of tested dogs (n=6) from Sofia showed no positive results. Another research based on the same ELISA test and IFAT from Stara Zagora (n=167) detected anti-Anaplasma antibodies in 41.6% (Pantchev et al., 2015). Recent study among pet dogs from Sofia (n=160) determined seroprevalence of 8.75% in this canine population (Borisov et al., 2017). The applying of a regular antiparasitic treatment against ticks in pet dogs can serve as an explanation for the difference in the percent of the seropositive stray and house-dogs in Sofia (18.87% vs. 8.75%).

Because of the zoonotic potential of the infection correlations can be made with available data for Anaplasma distribution in tick and human population from Sofia region. A study based on

detection of Anaplasma specific primers LA1/LA6 through PCR technique presented that 19% (n=166) of the adult ticks and 4% (n=80) of the nymphs harboured *A. phagocytophilum* (Gladnishka et al., 2005). The percent of positive human sera (n=200) after tick biting was 9% and 2.9% among healthy controls (n=70) (Christova and Dumler, 1999).

The epidemiology of Anaplasmosis in canine population from other regions of southeastern Europe indicated severe diversity in prevalence – from only 2.1% in Romania (n=1446) (Mircean et al., 2012), 15.5% in Serbia (n=84) (Potkonjak et al., 2015), to 50% in Albania (n=30) (Hamel et al., 2009).

Conclusion

The contribution of the rapid antibody assays in the diagnosis of canine Anaplasmosis is undoubted but their use separately is insufficient especially in endemic areas. The alterations in some blood parameters can have auxiliary function. Based on the results of this first serological survey we may conclude that there is a considerable seroprevalence to Anaplasmosis in stray dogs in Sofia district.

References

1. Borisov B., Marinov G., Panayotov P., Zlateva N. (2017). *Vector born diseases in dogs – Dirofilariosis and Anaplasmosis. A clinical study.* Tradition and modernity in Veterinary Medicine, vol. 2, No 1(2), pp. 59–66.
2. Carrade D., Foley J., Borjesson D., Sykes J. (2009). *Canine Granulocytic Anaplasmosis: A Review.* J Vet Intern Med; 23, pp. 1129–1141.
3. Christova I., Dumler J. (1999). *Human granulocytic ehrlichiosis in Bulgaria.* Am J Trop Med Hyg; 60, pp. 58–61.
4. Dumler J., Barbet A., Bekker C., Dasch G., Palmer G., Ray S., Rikihisa Y., Rurangirwa F. (2001). *Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila.* Journal of Systematic and Evolutionary Microbiology, 51, pp. 2145–2165.
5. Ebani V., Bertelloni F., Turchi B., Cerri D. (2013). *Serological and molecular survey of Anaplasma phagocytophilum in Italian hunting dogs.* Ann Agric Environ Med. 20(2): 289–92.
6. ESCCAP Guideline. (2012). *Control of vector-borne diseases in dogs and cats.* Second edition, pp. 31–33.
7. Gaunt S., Beall M., Stillman B., Lorentzen L., Diniz P., Chandrashekar R., et al. (2010). *Experimental infection and co-infection of dogs with Anaplasma platys and Ehrlichia canis: hematologic, serologic and molecular findings.* Parasit Vectors. 3(1), pp. 33.
8. Gladnishka T., Tasseva E., Christova I., Nikolov M., Lazarov S. (2005). *Detection of Borrelia burgdorferi sensu lato, Anaplasma phagocytophilum and Spotted Fever Group Rickettsiae in ticks from the region of Sofia, Bulgaria (Acari: Parasitiformes: Ixodidae).* European Arachnology 2005 (Deltshev, C. & Stoev, P., eds) Acta zoologica bulgarica, Suppl. No. 1, pp. 339–343.
9. Hamel D., Silaghi C., Knaus M., Visser M., Kusi I., Rapti D., et al. (2009). *Detection of Babesia canis subspecies and other arthropod-borne diseases in dogs from Tirana, Albania.* Wien Klin Wochenschr. 121 Suppl 3, pp. 42–5.
10. Jensen J., Simon D., Murua Escobar H., Soller J., Bullerdiek J., Beelitz P., et al. (2007). *Anaplasma phagocytophilum in dogs in Germany.* Zoonoses Public Health. 54(2), pp. 94–101.

11. Kohn B., Galke D., Beelitz P., Pfister K. (2008). *Clinical Features of Canine Granulocytic Anaplasmosis in 18 Naturally Infected Dogs.* J Vet Intern Med, 22, pp. 1289–1295.
12. Mircean V., Dumitrache M., Gyorke A., Pantchev N., Jodies R., Mihalca A. et al. (2012). *Seroprevalence and geographic distribution of Dirofilaria immitis and tick-borne infections (Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato, and Ehrlichia canis) in dogs from Romania.* Vector Borne Zoonotic Dis. 12(7), pp. 595–604.
13. Pantchev N., Schnyder M., Globokar Vrhovec M., Schaper R., Tsachev I. (2015). *Current Surveys of the Seroprevalence of Borrelia burgdorferi, Ehrlichia canis, Anaplasma phagocytophilum, Leishmania infantum, Babesia canis, Angiostrongylus vasorum and Dirofilaria immitis in Dogs in Bulgaria.* Parasitol Res 114, pp. S111–S124.
14. Potkonjak A., Vračar V., Savić S., Lako B., Radosavljević V., Cincović M., Suvajdžić L., Jurišić A., Petrović A. (2015). *The seroprevalence of Anaplasma phagocytophilum infection in dogs in the Autonomous Province of Vojvodina, Serbia.* Veterinarski arhiv 85 (4), 385–394.
15. Sainz A., Roura X., Miró G., Estrada-Peña A., Kohn B., Harrus S., Solano-Gallego L. (2015). *Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe.* Parasites & Vectors, 8, pp. 75.
16. Santos A., Alexandre N., Sousa R., Nuncio M., Bacellar F., Dumler J. (2009). *Serological and molecular survey of Anaplasma species infection in dogs with suspected tickborne disease in Portugal.* Vet Rec. 164(6), pp. 168–71.
17. Tsachev I. (2009). *Exotic zoonoses among dogs in Bulgaria (monocytic ehrlichiosis, granulocytic anaplasmosis, visceral leishmaniasis).* Thesis for DSc, p. 40.
18. Tsachev I., Petrov V., Flaming K., Brown C. (2008). *First Detected Case of Anaplasma phagocytophilum in a dog in Bulgaria.* Revue Méd. Vét., 159, 11, 562–564.