

## Part 2. Veterinary medicine

UDC 619:616.98-078:57.083.34: 576.893.192.6:636.7(497.113)

### SEROEPIDEMIOLOGICAL RESEARCH OF BABESIOSIS IN DOGS IN THE AREA OF NOVI SAD, AUTONOMOUS PROVINCE OF VOJVODINA, REPUBLIC OF SERBIA

Potkonjak A., Vračar V.\*, Novakov N., Stevančević O., Stojanac N.

University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine,  
Novi Sad, Serbia, E-mail: vuk.vracar@polj.uns.ac.rs

Savić S.

Scientific Veterinary Institute 'Novi Sad', Novi Sad, Serbia

Petrović A., Jurišić A., Rajković D.

University of Novi Sad, Faculty of Agriculture, Department of Environmental and Plant Protection, Novi Sad, Serbia

Bursać A.

JKP 'Zoo Higijena i veterina Novi Sad', Novi Sad, Serbia

Lukić B.

Veterinary ambulance 'Moj veterinar', Kać, Serbia

Gerilovych A.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine

**Summary.** Canine babesiosis is emerging tick-borne disease of dogs. Due to the expansion of the vector, increase in the incidence of this disease in Europe is evident. The aim of this research is to conduct a seroepidemiological cross-sectional study and to establish the value of seroprevalence of canine babesiosis caused by *B. canis*.

The study included population of dogs from the area of Novi Sad. Blood sera from 191 dogs were serologically tested using indirect immunofluorescent antibody test. The dogs were divided into three groups as following: hunting dogs, companion dogs and stray dogs.

The determined overall seroprevalence of *B. canis* infection in the examined dog population was 26.17%. The highest seroprevalence of this infection was evident in the group of stray dogs (35.0%), followed by seroprevalence — in the group of hunting dogs (32.75%), and the lowest — in the group of companion dogs (13.7%). Divergence in the results obtained in our research and previous studies conducted in Serbia indicates the necessity of more extensive seroepidemiological and molecular studies of canine babesiosis.

**Keywords:** canine babesiosis, *Babesia canis*, seroprevalence, dogs, indirect immunofluorescent antibody test, Serbia.

**Introduction.** Canine babesiosis is one of the most important transmitted by ticks infectious diseases of dogs. Although known since 1893, this disease remains a challenge for diagnosis and treatment to the veterinarians worldwide (Penzhorn, 2011).

In Europe the predominant causative agent of canine babesiosis is *Babesia canis*, videlicet its two subspecies: *B. c. canis* transmitted by *Dermacentor reticulatus* ticks and *B. c. vogeli* transmitted by *Rhipicephalus sanguineus* (Hamel et al., 2009; Matijatko et al., 2014). The disease has enzootic character and occurs seasonally in spring and summer when ticks are most active (Bourdoiseau, 2006). The clinical presentation of canine babesiosis is diverse and implies a combination of a febrile syndrome and a hemolytic syndrome, which can be fatal when complicated (Matijatko, Torti and Schetters, 2012; Solano-Gallego and Baneth, 2011).

Recently canine babesiosis, the disease considered to be endemic in Southern Europe, has been reported as an autochthonous in several more northern European countries such as Holland, Latvia, and Norway (Berzina et al., 2013; Matjila et al., 2005; Øines, Storli and Brun-Hansen, 2010). With significant variations amongst countries and regions, caused by the vector distribution, Halos et al. (2014) have determined the overall annual incidence of 0.7% of clinical babesiosis in the investigated dog population.

For quite some time presence of *B. canis* has been confirmed in Serbia both in several tick species (*R. sanguineus*, *D. marginatus*, *D. reticulatus*, and *H. conncina*) and in dogs. Determination of babesiosis in dogs was based mostly on microscopic examination of blood smears, still Potkonjak et al. (2014) recently confirmed the presence of specific antibodies to the *B. canis* antigens in hunting dog sera using IFA test (Mihaljica et al., 2012; Tomanović et al., 2013; Pavlović et al., 2009).

---

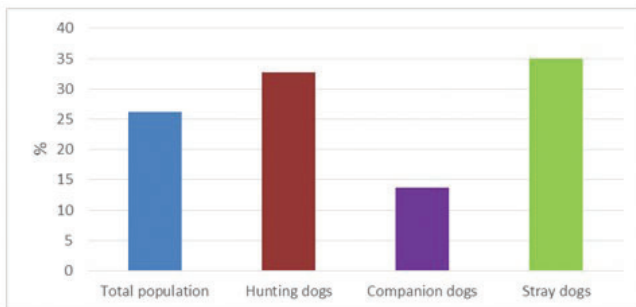
\* Corresponding author

**The aim** of this research is to conduct a seroepidemiological cross-sectional study and to establish the value of seroprevalence of canine babesiosis caused by *B. canis*.

**Materials and methods.** The study included population of dogs from the area of Novi Sad. Blood sera from 191 dogs were tested. The dogs were divided into three groups as following: hunting dogs, companion dogs and stray dogs. From the samples of the full venous blood, after the retraction of blood clot in vacationers with coagulation activator, samples of blood serum were separated by centrifugation at 3 000 rpm for 10 min and frozen at  $-20^{\circ}\text{C}$  till the indirect immunofluorescence was carried out.

Immunofluorescent antibody test 'MegaScreen@FLUOBABESIA canis' manufactured by 'MEGACOR Diagnostik' (Austria) was used for the serological investigation. Concerning specific geographical area, only the titer of 1:128 was used, and all reactions positive for this and higher titers were declared as positive. Positive reaction was characterized by the appearance of sharply defined apple-green fluorescent merozoites within the cytoplasm of the infected erythrocytes.

**Results.** Anti-Babesia canis antibodies of class G were detected using indirect immunofluorescent antibody test in 50 samples from the 191 examined dog blood sera. The determined overall seroprevalence of infection caused by *B. canis* in examined dog populations was 26.17% (Fig. 1).



**Figure 1.** Comparative review of the determined values of seroprevalence of canine babesiosis among different dog groups.

In the examined population of 58 hunting dogs seropositive were 19 dogs. Seroprevalence of *B. canis* infection determined in this group was 32.75% (Fig. 1).

In the group of companion dogs 10 from the 73 examined dogs were seropositive. Seroprevalence of *B. canis* infection determined in this group was 13.7% (Fig. 1).

In the group of stray dogs from the 60 examined dogs specific antibodies to *B. canis* antigens were found in sera of 21 dogs. Seroprevalence of *B. canis* infection determined in this group was 35.0% (Fig. 1).

**Discussion and conclusions.** The determined overall seroprevalence of *B. canis* infection was 26.17% in the examined dog population. The highest seroprevalence of this infection was evident in the group of stray dogs (35.0%), followed by seroprevalence — in the group of hunting dogs (32.75%), and the lowest — in the group of companion dogs (13.7%).

In accordance with the increase in the number of the vectors and the proven presence of *B. canis* both in the vectors and in dogs, serological confirmation of the pathogen in dogs was expected (Potkonjak et al., 2014). During the period 1999–2001 in the area of Belgrade, by microscopic examination of stained blood smears, Pavlović et al. (2002) determined the prevalence values of *B. canis* infection among dogs ranging from 71,7 to 75,5%. These seroprevalence values were significantly higher than the value obtained in this research. In addition, very high seroprevalence of *B. canis* in dogs has been reported in Italy (70%) (Pennisi et al., 2012).

The first data on seroprevalence of canine babesiosis in Hungary in 2006 announced Hornok, Edelhofer and Farkas (2006). They determined the seroprevalence of 5.7% using the indirect immunofluorescent antibody test. This is notably lower value than obtained in this study, as well as results reported in Albania (9.9–13.0%) and Sicily (5.17%) (Hamel et al., 2009; Lazri et al., 2008; Torina and Caracappa, 2006). In Slovakia reported seroprevalence ranges from 4.8% in Nové Zámky to 28.8% in Komárno (Kubelová et al., 2013) that corresponds to our results (from 13.7 to 35.0%).

Similar results to ours were reported by Imre et al. (2013); in dogs in Romania where seroprevalence value was 19.8%. Furthermore, they found that the seroprevalence of infection caused by *B. canis* was significantly higher in hunting dogs and dogs from rural areas compared to the population of dogs that live in different conditions, in kennels and the owners houses respectively. Looking at the values of the seroprevalence of this infection in different populations of dogs, it can be concluded that they are similar in hunting dogs and dogs that live on the street, which corresponds to observations by the authors from Romania, considering that a large part of the sera in a group of stray dogs from our research originate from rural areas. That dogs in shelters and street dogs, who are in close contact with ticks are more likely to become infected with babesias compared to dogs kept as companion animals indicates Cassini et al. (2009). This observation corresponds to our findings; lowest seroprevalence of canine babesiosis (13.7%) was in the population of companion dogs, which mainly live in the house and have a known owner due to the timely removal of ticks from the body of dogs and the application of repellents.

Greater exposure to the vectors of the hunting and stray dogs is a possible reason for the higher values of seroprevalence of canine babesiosis infections obtained in our research in comparison with the companion dogs.

Furthermore, divergence in the results obtained in our research and in previous studies conducted in Serbia indicates the necessity of more extensive seroepidemiological and molecular studies of canine babesiosis.

**Acknowledgements.** This research was funded by a Provincial Secretary for Science and Technological Development of AP Vojvodina (name of the project: Research of Lyme disease and other vector borne zoonoses in Vojvodina, project No 114–451–1293/2014–03).

## References

- Berzina, I., Capligina, V., Baumanis, V., Ranka, R., Cirule, D. and Matise, I. (2013) 'Autochthonous canine babesiosis caused by *Babesia canis canis* in Latvia', *Veterinary Parasitology*, 196(3–4), pp. 515–518. doi: 10.1016/j.vetpar.2013.03.015.
- Bourdoiseau, G. (2006) 'Canine babesiosis in France', *Veterinary Parasitology*, 138(1–2), pp. 118–125. doi: 10.1016/j.vetpar.2006.01.046.
- Cassini, R., Zanutto, S., di Regalbono, F. A., Gabrielli, S., Calderini, P., Moretti, A., Tampieri, M. P. and Pietrobelli, M. (2009) 'Canine piroplasmiasis in Italy: epidemiological aspects in vertebrate and invertebrate hosts', *Veterinary Parasitology*, 165(1–2), pp. 30–35. doi: 10.1016/j.vetpar.2009.06.044.
- Halos, L., Lebert, I., Abrial, D., Danlois, F., Garzik, K., Rodes, D., Schillmeier, M., Ducrot, C. and Guillot, J. (2014) 'Questionnaire-based survey on the distribution and incidence of canine babesiosis in countries of Western Europe', *Parasite*, 21(13), 7 pp. doi: 10.1051/parasite/2014015.
- Hamel, D., Silaghi, C., Knaus, M., Visser, M., Kusi, I., Rapti, D., Rehbein, S. and Pfister, K. (2009) 'Detection of *Babesia canis* subspecies and other arthropod-borne diseases in dogs from Tirana, Albania', *Wiener klinische Wochenschrift*, 121(S3), pp. 42–45. doi: 10.1007/s00508-009-1234-3.
- Hornok, S., Edelhofer, R. and Farkas, R. (2006) 'Seroprevalence of canine babesiosis in Hungary suggesting breed predisposition', *Parasitology Research*, 99(6), pp. 638–642. doi: 10.1007/s00436-006-0218-8.
- Imre, M., Farkas, R., Ilie, M., Imre, K., Hotea, I., Morariu, S., Morar, D. and Dărăbuș, G. (2013) 'Seroprevalence of *Babesia canis* infection in clinically healthy dogs from western Romania', *Journal of Parasitology*, 99(1), pp. 161–163. doi: 10.1645/GE-3129.1.
- Kubelová, M., Sedlák, K., Panev, A. and Šíroky, P. (2013) 'Conflicting results of serological, PCR and microscopic methods clarify the various risk levels of canine babesiosis in Slovakia: a complex approach to *Babesia canis* diagnostics', *Veterinary Parasitology*, 191(3–4), pp. 353–357. doi: 10.1016/j.vetpar.2012.09.016.
- Lazri, T., Duscher, G., Edelhofer, R., Bytyci, B., Gjino, P. and Joachim, A. (2008) 'Infektionen mit arthropodenübertragenen Parasiten bei Hunden im Kosovo und in Albanien unter besonderer Berücksichtigung der Leishmanieninfektionen', *Wiener klinische Wochenschrift*, 120(S4), pp. 54–58. doi: 10.1007/s00508-008-1076-4.
- Matijatko, V., Torti, M. and Schetters, T. P. (2012) 'Canine babesiosis in Europe: how many diseases?', *Trends in Parasitology*, 28(3), pp. 99–105. doi: 10.1016/j.pt.2011.11.003.
- Matijatko, V., Torti, M., Ki, I., Šmit, I., Štokovic, I., Vranješ-Đuric, S., Milanović, S., Mrljak, V. and Brkljačić, M. (2014) 'Serum cortisol and insulin concentrations in dogs naturally infected with *Babesia canis*', *Veterinarski Arhiv*, 84(6), pp. 551–562. Available at: <http://www.vef.unizg.hr/vetarhiv/papers/2014-84-6-1.pdf>.
- Matijala, T. P., Nijhof, A. M., Taoufik, A., Houwers, D., Teske, E., Penzhorn, B. L., de Lange, T. and Jongejan, F. (2005) 'Autochthonous canine babesiosis in The Netherlands', *Veterinary Parasitology*, 131(1–2), pp. 23–29. doi: 10.1016/j.vetpar.2005.04.020.
- Mihaljica, D., Radulovic, Z., Tomanovic, S., Cakic, S., Penezic, A. and Milutinovic, M. (2012) 'Molecular detection of *Babesia* spp. in ticks in northern Serbia', *Archives of Biological Sciences*, 64(4), pp. 1591–1598. doi: 10.2298/abs1204591m.
- Øines, Ø., Storli, K. and Brun-Hansen, H. (2010) 'First case of babesiosis caused by *Babesia canis canis* in a dog from Norway', *Veterinary Parasitology*, 171(3–4), pp. 350–353. doi: 10.1016/j.vetpar.2010.03.024.
- Pavlović, I., Milutinovic, M., Petkovic, D., Terzin, D. and Terzin, V. (2002) 'Epizootological research of canine babesiosis in the Belgrade district', *The Journal of Protozoology Research*, 12(1–2), pp. 10–15.
- Pavlović, I., Petković, D., Terzin, V., Terzin, D. and Vojinović, D. (2009) 'Babesiosis of dogs at Belgrade area in period 2004–2008' [Babezioza pasa na području Beograda u periodu 2004–2008. godine]. In: XI Epizootology Days with International Participation [XI simpozijuma epizootološki dani sa međunarodnim učešćem]. Apatin, Banja Junaković, 1–4.04.2008. pp. 165–166. [in Serbian].
- Pennisi, M.-G., Capri, A., Solano-Gallego, L., Lombardo, G., Torina, A. and Masucci, M. (2012) 'Prevalence of antibodies against *Rickettsia conorii*, *Babesia canis*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* antigens in dogs from the Stretto di Messina area (Italy)', *Ticks and Tick-borne Diseases*, 3(5–6), pp. 315–318. doi: 10.1016/j.ttbdis.2012.10.026.
- Penzhorn, B. L. (2011) 'Why is Southern African canine babesiosis so virulent? An evolutionary perspective', *Parasites and Vectors*, 4(1), pp. 51. doi: 10.1186/1756-3305-4-51.
- Potkonjak, A., Savić, S., Spasojević Kosić, Lj., Vračar, V., Novakov, N., Bursać, A., Stevančević, O. and Stojanac, N. (2014) 'Finding of *Rickettsia conorii* and *Babesia canis* antibodies in hunting dogs in Vojvodina' [Nalaz antitela na uzročnike *Rickettsia conorii* i *Babesia canis* kod lovačkih pasa u Vojvodini]. In: 25 Serbian Veterinary Meeting [25 Savetovanje veterinara Srbije]. Zlatibor, 11–14 sept. 2014. pp. 327–332. Available at: <http://niv.ns.ac.rs/StariSajt/tr31084/fajlovi/14/sredjene/82.2014.pdf>. [in Serbian].
- Solano-Gallego, L. and Baneth, G. (2011) 'Babesiosis in dogs and cats—Expanding parasitological and clinical spectra', *Veterinary Parasitology*, 181(1), pp. 48–60. doi: 10.1016/j.vetpar.2011.04.023.
- Tomanović, S., Chochlakis, D., Radulović, Ž., Milutinović, M., Čakić, S., Mihaljica, D., Tselentis, Y. and Psaroulaki, A. (2012) 'Analysis of pathogen co-occurrence in host-seeking adult hard ticks from Serbia', *Experimental and Applied Acarology*, 59(3), pp. 367–376. doi: 10.1007/s10493-012-9597-y.
- Torina, A. and Caracappa, S. (2006) 'Dog tick-borne diseases in Sicily', *Parassitologia*, 48(1–2), pp. 145–147.