RESULTS OF THE RESEARCH ON BLOOD PARASITES IN CATTLE IN SERBIA

Ivan Pavlović[1]*, Nemanja Zdravković[1], Oliver Radanović[1], Ivan Dobrosavljević[2], Jovan Bojkovski[3], Slavonka Stokić-Nikolić[2], Slobodan Stanojevič[1]

[1]Scientific Institute of Veterinary Medicine of Serbia, J.Janulisa 14, Belgrade 11000, Serbia

[2]Specialistic Veterinary Institute Požarevac, Dunavska 89B, Požarevac 12000, Serbia

[3]Faculty of Veterinary Medicine in Belgrade, Bul. Oslobođenja 18, Belgrade 11000, Serbia

Abstract

The method of semi-intensive pasture breeding, which is the most common in rural households in Serbia, pose a threat for cattle to be infected by ticks and to be infected by pathogens transmitted by ticks, most often by blood parasites such as cattle parasites of genera Anaplasma, Babesia and Theileria. The presence of blood parasites of cattle is insufficiently studied in Serbia, unlike helminths, so there is little data on their origin and prevalence. On the other hand, the examination of the tick fauna has been carried out extensively for several decades, and based on it, it is known that there are many vectors- ticks in these areas that transmit blood parasites of cattle. For these reasons, research on blood parasites of cattle kept in semi-intensive breeding has been initiated in the last few years. In the period 2017-2020, blood tests of cattle from several districts of Serbia were performed for the presence of blood parasites. During the examination, the presence of Anaplasma marginale, A.phagocytophilum, Theileria annulata, Babesia divergens, B. bigemina and B. bovis was established. At the same time, the fauna of ticks, which are vectors of these protozoa, was monitored.

Keywords: *cattle, blood parasites, ticks, Serbia*

1. INTRODUCTION

Modern trends in cattle production have created farms with a large agglomeration of animals. This method of production, in addition to increasing production results, led to a significant reduction of diseases that occur in semi-intensive breeding. This particularly applies to parasitic infections that spread on pasture where vectors and developmental forms of helminths are present, as well as perhaps the one of most dangerous ectoparasites of cattle - ticks. Ticks are obligate hematophagous ectoparasites which have multiple adverse effects on the host organism (Paules et al., 2018). A particular problem is that they spread the tick-borne pathogens to humans, domestic and wild animals, to whom they can be reservoirs, biological vectors and/or transient hosts (Milutinović et al.1998, Taylor et al 2001, Paules et al., 2018).

The method of semi-intensive pasture breeding, which is the most common in rural households in Serbia, presents an opportunity for cattle to be infected by ticks but also to be infected by pathogens transmitted by ticks, most often by blood parasites such as cattle parasites of genera Anaplasma, Babesia and Theileria (Demessie and Derso,2015). The presence of blood parasites of cattle is insufficiently studied in Serbia, unlike helminths, so there is little data on their origin and prevalence. On the other hand, the examination of the tick fauna has been carried out extensively for several decades, and based on it, it is known that there are many vector ticks in these areas that transmit blood parasites of cattle (Petrović et al.1996, Milutinović et al.1997, Pavlović et al.2020). For these reasons, research on blood parasites of cattle kept in semi-intensive breeding has been initiated in the last few years.

This paper presents the results obtained during these preliminary investigations.

2. MATERIALS AND METHODS

2.1. Materials

In the period 2017-2020, from Kolubara, Mačva, Braničevo, Podunavlje and Zaječar districts of Serbia were performed examination for the presence of blood parasites in cattle. In total we examined 572 animals from 61 villages. From examination we used blood from peripheral blood circulation.

During our examination we examined the ticks present on animals. They were collected manually by removing them from cattle with tweezers and were placed in vials with 70% ethanol. We examined 177 cattle from 31 herds. From pastures ticks were collected by the flagging method using $1m^2$ white linen. Ticks were collected at the center of the pasture as well as under scattered vegetation present at the locations.

2.2. Methods

The blood smears were air-dried, fixed in absolute methanol for 1 minute and stained in 10% Giemsa stain for 20-30 minutes (Thrall et al.2012, Mans et al.,2015). Examinations of blood smears we performed with AxioLab A1 microscope with the Axiocam 105 Color microscope camera and Zen Lite software, (Carl Zeiss, Jena, Germany). All occurring blood parasites we were identified by morphological characteristic (Pavlovic and Rogožarski, 2017). The occurrence of *A. phagocytophilum* was investigated by serological (immunofluorescence antibody test (IFAT) and molecular (real-time PCR) methods (Dumler and Brouqui, 2004, Vasić et al.2018).

The tick species and sex/gender were identified by morphometric characteristics. Hard ticks can be easily differentiated by the shape of the basis capitulum and by the form of anal grooves (Pomerancev, 1950, Hillyard 1996, Estrada-Peña, 2004).

3. RESULTS AND DISCUSSION

Blood tests established the presence of *Anaplasma marginale* in 11.9% of examined animals with clinical signs of disease*, A.phagocytophilum* in 1.6%, *Theileria annulata* in 1.4%*, Babesia divergens* in*,* 6.45%, *B. bigemina* in 3,61% and *B. bovis* in 5.76%.

During the examination tick infestation was detected in 39.24% of examined animals and at 59% of pastures contained ticks positively tested for pathogens. All found species of ticks are vectors for anaplasmosis, babesiosis and theileriosis. The most abundant was *Ixodes ricinus* (43.91%), followed by *Dermacentor marginatus* (31.91%), *Rhipicephalus bursa* (18.22%), *R.sanguineus* (11.72%), *Hyalomma savignyi* (*Hy.marginatum*) (7.72%)*, Haemaphysalis punctata* (6.21%) and *D.pictus* (4.72%).

Among tick-borne diseases, bovine anaplasmosis is one of the most important in ruminants worldwide, causing significant economic losses. It is transmitted through the bite of *Ixodes ricinus, Dermacentor sp. Rhipicephalus sp. and Haemophysalis sp.* ticks or tabanid flies. Transmission is highest during tick and fly seasons (Leblond et al.2012). The *Anaplasma* organism invades the red blood cells of infected cattle, and the spleen destroys the infected cells. As a result, infected animals and humans become anemic, weak, and lethargic, go off feed, and run a fever (Stuen et al.2013). The mucous membranes become pale and possibly yellow from the waste products of red blood cell destruction. A characteristic of anaplasmosis, however, is that the urine will not be red or brown as with “red water” or leptospirosis (Capucille, 2008)

Anaplasma marginale is one of the most important tick-borne bacteria of veterinary and public health significance in the family *Anaplasmataceae*. Bovine anaplasmosis is globally distributed tick-borne disease of livestock with great economic importance in cattle industry. First cases we occurred during 2019. at milk cattle on mount Beljanica in eastern Serbia (Braničevo district) (Dobrosavljević et al.2019). The examined cattle are seasonal moved on a mountain pasture. Where in favorable weather conditions (high humidity and temperature) occurs a high prevalence of ticks (transmitters of

A.marginale). In such conditions, the possibility of anaplasmosis occurrence in cattle on pasture is high and during our examination we found it in 11.9% of the examined animals.

Anaplasma phagocytophilum is a zoonotic tick-borne pathogen responsible for granulocytic anaplasmosis, a mild to a severe febrile disease that affects man and several animal species, including cows and horses (Atif, 2016). In Europe, *I. ricinus* is the only proven vector for this pathogen, but studies suggest that other tick genera and species could be involved in its transmission. In Serbia, it was established for the first time in dogs (Pavlović et al., 2012; 2015) and later in cattle in 2013 (Nieder et al., 2013). The absence of clinical sign of ruminants infected with *A. phagocytophilum* highlights the need for molecular techniques to discriminate this species from other species and during our examination we used serological (immunofluorescence antibody test (IFAT) and molecular (real-time PCR) methods (Vasić et al., 2018). In total *A. phagocytophilum* we established in a significantly lower percentage than *A. marginale*, in only 1.6% of suspect cattle.

Theilerioses are a group of tickborne diseases caused by protozoan parasites of the *Theileria* genus. The most important species affecting cattle are *T. parva* and *T. annulata*, which cause acute disease resulting in high levels of mortality (Demessie and Derso,2015).

In the most pathogenic species of *Theileria* (eg., *T. parva* and *T. annulata*), parasite multiplication occurs predominantly within the host WBCs, whereas less pathogenic species multiply mainly in RBCs (Bishop et al, 2004). Development of the schizont stage of pathogenic *Theileria* causes the host WBC to divide; at each cell division, the parasite also divides. Thus, the parasitized cell population expands and, through migration, becomes disseminated throughout the lymphoid system. Later in the infection, some of the schizonts undergo merogony, releasing merozoites that infect RBCs, giving rise to piroplasms. Because the parasite is living inside the RBCs, the body attacks its own infected red blood cells (haemolysis) to destroy the parasite induced anemia of animals (Mans et al.2015).

Theileriosis rarely occurs in cows in Serbia and in about last fifty years only few cases have been reported. Theileriosis can affect cattle of all ages, however heavily pregnant, lactating, and stressed cows tend to be at greatest risk, due to resultant reduction in immunity (Morrison, 2015). In Europe most usually infection is caused by *T. annulata* transmitted by ticks of the genus *Hyalomma*. In Serbia and West Balcan most usually found *Hyalomma marginatum marginatum* (*Hy. savignyi*) and *Hy.detritium* (Milutinović et al., 1996; Pavlović et al., 2014; Pavlović et al., 2020). Theileriosis rarely occurs in cows in Serbia and in about last fifty years only few cases have been reported, last one during 2020 (Pavlović and Dimitrijević, 2020).

Babesiosis is a tick-borne parasitic disease that results in significant morbidity and mortality in cattle. Bovine babesiosis is caused by *Babesia divergens*, *B. bovis*, *B.bigemina* and several other *Babesia* species (Benavides and Sacco, 2007). In Europe including Western Balkan usually occurred *B.divergens.* (Bock et al., 2004, Beugnet and Moreau, 2015). The primary reason for this is a high prevalence of the primary vector ticks from genus *Ixodes,* Rhipicephalus *sp., Hyalomma sp.* and Haemaphysalis *sp*. (Bock et al., 2004). In Serbia and Western Balkan, this type of tick is very often. Research on bovine babesiosis has not been done for more than fifty years in Serbia.

During our examination was established several cases of babesiosis during 2017 and 2020 in a clinical form in the northern part of Serbia. In all cases these were mostly symptoms which characterized of acute form of the disease (Pavlović et al., 2022). Clinical signs consist of elevated body temperature, anorexia, weakness, and hemolytic anemia accompanied by tachycardia and tachypnoea. In affected animals' mucous membranes may be pale or jaundiced, and hemoglobinuria occurs at the peak of the hemolytic crisis. *B.divergens* we found in five herds of cattle and at 37 animals. This disease also known as red water fever. *B. divergens* is transmitted by *Ixodes ricinus*, the most common species of tick in Serbia and the Western Balkans (Petrović et al.1996). B. bigemina and B. bovis occurred at southwest part in Serbia. First, we established in five herd and 21 animals and second in seven herd and 33 cattle. The obtained results indicate the presence of babesiosis in cattle in Serbia and the need for further research into its prevalence, especially if it is known that *B.divergens* has great zoonotic potential.

4. CONCLUSIONS

The obtained results show that in Serbia cattle are infected with blood parasites from the genera Anaplasma, Thaileria and Babesia. They also showed that there are many species of ticks that are vectors of these diseases and indicated the potential epidemiological danger for the spread of these diseases. The obtained results are only screening research that must be carried out in the coming period on the entire territory of Serbia and on a much larger number of animals to get a true picture of the prevalence of these diseases.

ACKNOWLEDGMENTS

The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030).

REFERENCES

1. Atif, AF 2015, "*Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance", *Parasitology Research,* vol.114, no.11, pp.3941-3957.
2. Benavides, MV, Sacco, AM 2007, "Differential *Bos taurus* cattle response to *Babesia bovis* infection", *Veterinary Parasitology,* vol.150, pp.54-64.
3. Beugnet, F, Moreau, Y 2015, "Babesiosis*", Revue scientifique et technique (International Office of Epizootics),* vol.34, no.2, pp.627-639.
4. Bock, R, Jackson, L, de Vos, A, Jorgensen, W 2004, "Babesiosis of cattle", *Parasitology,* vol.129 Suppl:S2, pp.47-69.
5. Bishop, R, Musoke, A, Morzaria, S, Gardner, M, Nene, V 2004, "Theileria: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks", *Parasitology,* vol.129, Suppl.S271, pp.83.
6. Capucille, DJ 2008, "Blackwell's Five-Minute Veterinary Consult: Ruminant", Scott RR (Ed.) pp.50-51.
7. Demessie, Y, Derso, S 2015, "Tick Borne Hemoparasitic Diseases of Ruminants: A Review", *Advances in Biological Regulation,* vol.9, no.4, pp.210-224.
8. Dobrosavljević, I, Pavlović, I , Lazić, M, Stokić-Nikolić, S, Živojinović, M 2019, "Anaplasma marginale – case report", Book of Abstracts 18th International conference life science for Sustainable development, Kluj-Napoca,Romania, pp.437.
9. Dumler, JS, Brouqui, P 2004, "Molecular diagnosis of human granulocytic anaplasmosi Molecular diagnosis of human granulocytic anaplasmosis", *Expert Review of Molecular Diagnostics,* vol.4, no.4, pp.559–569.
10. Estrada-Peña, A 2004, "Ticks of domestic animals in the Mediterranean region: a guide to identification of species", University of Zaragoza,Spain.
11. Hillyard, PD 1996, "Ticks of North-West Europe" The Natural History Museum, London,UK.
12. Kapustin, FU 1955, "Atlas parazitov krovi životnih i klešcei iksodid. Gasudarstvenoeizdetejlstvo seljskohazjajstvenoi literaturi", Moskva,Rusia.
13. Leblond, A, Chastagne,r A, Pradier, S, Bailly, X, Masseglia, S, Vourc'h, G 2012, "La prévalence de l'anaplamose dans le sud de la France", *Bulletin Épidémiologique Santé Animal Alimentation,* pp.30–31.

14. Mans, BJ, Pienaar, R, Latif, AA. 2015, "A review of Theileria diagnostics and epidemiology", *International Journal For Parasitology: Parasites And Wildlife,* vol.4, no.1, pp.104-118.
15. Milutinović, M, Miščević, Z, Ivović, V, Pavlović, I. 1996, "Ecolgical notes of tick (*Acari:Ixodidae*) in the area of East Serbia with emphases on the species Ixodes ricinus and Hyaloma savignyi", *Parassitology*, vol.38, no.1-2, pp.388.
16. Milutinović, M, Aleksić, N, Pavlović, I 1997, "Faunistic and ecological notes on ticks (Acari: Ixodidae, Argasidae) in the extended area of Belgrade", *Magyar Allatorvosok Lapja,* vol.120, pp.434-436
17. Milutinović, M, Aleksić, N, Pavlović,I 1998, "Research of ticks (Acrai: Ixodidae) in natural foci of CCHF and Lyme disease (Serbia)", *Parasitology International*, vol.47, pp.326.
18. Morrison, WI 2015, "The aetiology, pathogenesis and control of theileriosis in domestic animals", *Revuescientifique et technique (International Office of Epizootics),* vol.34, no.2, pp.599- 611.
19. Nieder, M., Bojkovski, J., Pavlović, I., Savić, B., Elezović, M., Silaghi, C 2013, "Studies on the occurence of granulocytic anaplasmosis in cattle and on biodiversity of vectors (ixodid ticks) in Serbia", Zbornik kratkih sadržaja 18 godišnje savetovanje doktora veterinarske medicine Republike Srpske sa međunarodnim učešćem,Teslić, Republika Srpska (BiH), pp.25.
20. Pavlović, I, Rogožarski, D 2017, "Parazitske bolesti domaćih životinja sa osnovima parazitologije i dijagnostike parazitskih bolesti", Naučna KMD, Beograd,Serbia.
21. Pavlović, I, Dimitrijević, A 2020, "Theileriosis in cow - a case report#, -II. International Agricultural, Biological & Life Science Conference E-AGBIOL 2020, Proceeeding, Edirne, Turkey, pp.1219-1221.
22. Pavlović I., Milojkovic N., Curcin Lj., Kovacevic M., Novak N., Ivanovic O 2012, "Prevalence of erlichiosis, anaplasmosis and boreliosis in dogs in Serbia", Abstracts XI European Multicolloquium of Parasitology, Parasites in the Changing World, Cluj-Napoca, Romania, pp.330.
23. Pavlović, I, Jovčevski, S, Jovčevski, St, Kukovska, V, Dimitrić, A 2014, "Tick fauna of sheep and cattle at Kumanovo arae (Macedonia)", *Lucrări Ştiinţifice Medicină Veterinară*, vol.XLVII, no.3, pp.88-95.
24. Pavlović, I, Terzin, V, Petković, D, Terzin, D, Savić, B, Cvetojević, Đ 2015, "Nalaz *Anaplasma phagocytophilum* kod pasa u Beogradu", Zbornik kratkih sadržaja XVII Simpozijum epizootiologa i epidemiologa (XVII Epizootiološki dani), Niš, pp.119-120.
25. Pavlović, I, Ivanović, S, Caro-Petrović, V, Bojkovski, J, Radović, B, Milanović, V, Stefanović, V 2020, "Biodiversity, seasonal dynamics and geographical distribution of sheep and goat ticks in Serbia", Proceeding of 2nd International Symposium: Modern Trends in Agricultural Production and Environmental Protection, Tivat, Montenegro, 109-119.
26. Pavlović, I, Zdravković, N,Vojinović, D, Caro Petrović, V, Bojkovski, J 2022, "Cattle babesiosis in Serbia - case report", Conference Proceeding Books ISPEC 1st International Agricultural Research Congress, Adana, Turkey, pp.82.
27. Paules, IC, Marston, HD, Bloom, ME, Fauci, SA 2018, "Tickborne Diseases — Confronting a Growing Threat", *The New England Journal of Medicine*, vol. 379, pp.701-703.
28. Petrović, Z, Milutinović, M, Pavlović, I (1996) "Istraživanja krpelja (Acari: Ixodidae, Argasidae) u Jugoslaviji", Naučni skup posvećen 100. godišnjici rođenja akademika Čedomira P. Simića, Zbornik,. – SANU, Odeljenje mecidinskih nauka i Jugoslovensko društvo parazitologa, Beograd, pp. 96-101.
29. Stuen, S, Granquist, EG, Silaghi, C 2013, " *Anaplasma phagocytophilum* - a widespread multi-host pathogen with highly adaptive strategies", *Front Cell Infect Microbiology*, vol.3, no.31, pp. 1-33

30. Thrall, MA, Weiser, G, Allison, RW, Campbell,TW 2012, Veterinary Hematology and Clinical Chemistry. 2nd Ed. Wiley-Blackwell.

31. Taylor, LH, Latham, SM, Woolhouse , ME 2001, "Risk factors for human disease emergence", *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol.29, pp.983-989.

32. Vasić, A, Nieder, M, Zdravković, N, Bojkovski, J, Bugarski, D, Pavlović, I, Silaghi, C. 2018, "Tick infestation and occurrence of Anaplasma phagocytophilum and piroplasms in cattle in the Republic of Serbia", *Parasitology Research*, vol.117, no.6, pp.1813–1818