UDK:636.75

INVESTIGATION OF PRESENCE DIFFERENT SEROTYPES OF LEPTOSPIRA SPP. IN POPULATION OF HUNTING DOGS IN WESTERN SERBIA

Maric, J.¹, Elezovic M.¹, Vojinovic D.², Milovanovic M.³, Zaric I.¹, Djuricic B.¹

Summary: Leptospirosis is an acute, subacute or chronic infectious disease of large number of animals and humans, which is classified as a zoonosis. Pathogens belong to the genus Leptospira, family Leptospiracae. The main vectors for spreading diseases and most common reservoirs are many wild animals, usually rodents. Hunting dogs are in frequent contact with the holders of the bacteria, in which the agent can easily persiste and easily maintain (wet, moisture locations). In these ecological areas dogs are used for tracking and a major supporter of the hunt, and because of that it is easy to contact with pathogens and cause infections. Infected dogs can be with or without the demonstrated clinical symptoms and infection could pass unnoticed by the owner-hunter. A dog may continue to spread infection within the litter, and even on the owner, which is the reason for more often serological control of hunting dogs.

Our study shows the results for population of hunting dogs from several localities in Western Serbia (113 dogs), of which seven were seropositive (6.19%) of the total population. Serological test were performed with agglutination-lysis test.

Stated results are part of broader research, inside of a Project*, and further investigation will include more hunting dogs and ecological studies of small wild game, for which is the dog collector. Further studies are in progress.

Key words: leptospirosis, hunting dogs, seropositive

Introduction

Leptospirosis is an infectious disease panzootic scale, of large number domestic and wild animals and humans . In the epizootiology and epidemiology of this zoonose important place occupied rodents that serve as reservoar species but also as vectors of infection. Pathogens belonging to the genus *Leptospira*, family *Leptospiracae*, and up to this day there are over 200 serovars known [3]. The main representative of the genus *Leptospira* is *L. interrogans* whose serovars *L. icterohaemorrhagiae*, *L. canicola*, *L. hardjo*, *L. bratislava*, *L. australis*, *L.pomona*, *L. grippothyposa*, *L.bataviae* etc. are the most frequent causes of infection in humans and animals. Agents are naturally easily maintain in the water (lakes, ponds, wetlands) and in many vertebrates. Infected animals usually appear clinically healthy, but these pathogens are excreted in urine in the external environment for a long time and in some cases for a lifetime. Hunting dogs are in frequent contact with carriers of pathogens, because they are move freely on the field in which the agent can easily find and easily maintain (wet locations). Infection of dogs mostly occurs peroral or through skin lesion [10, 12].

Leptospirosis in dogs is mostly caused by serovars *L. canicola* and *L. icterohaemorragiae*, in the form of acute, subacute and chronic course. Acute course is characterized by fever, apathy, weakness, with the symptoms that are connected with digestive tract, and numerous petehial hemorrhage. In the subacute course, in addition to general clinical symptoms, there is the appearance of jaundice, ulcers of the oral mucosa, and as kidney damage, occurrence of intense polyuria, and then complete anuria. In some cases, we have an occurrence of conjunctivitis, rhinitis and tonsillitis accompanied by sneezing and coughing. In chronic course, there is a chronic interstitial nephritis or chronic active hepatitis, followed by weight loss, ascites and symptoms of hepatic encephalopathy. Animals with chronic course of illness, which passes without the appearance of visible clinical symptoms with epidemiological-epizootiological aspects, represent a special risk because they are the reservoirs of bacteria in nature and long-term carriers [10, 12].

Diagnosis is based on the epizootiological data, clinical symptoms and laboratory findings. For isolation of agents from blood and urine, it is used Korthof or EJMH liquid medium. Antibodies against leptospira can be proved using the test of microscopic aglutination (MAT), ELISA and PCR techniques. Leptospires are sensitive to most antibiotics and sulfonamides. In addition to antibiotics, it is also conducting long-term symptomatic therapy. The outcome depends on the degree of damage [10, 12].

For immunisation of dogs used inactivated vaccine with serovars *L.canicola* and *L.icterohaemorragiae*, but the immunity is serotype-specific. Since dogs can become infected with the other serotypes, as our research shows, vaccine immunity has not a crucial importance in preventing this disease. The non-specific prophylactic measures include control of rodents as a source of infection and use of zoohygienic measures.

Corresponding author: VM Jovan Maric jovan_vet@yahoo.com;

¹ DVM Jovan Maric, research assistant, DVM Milica Elezovic, research assistant,

Ivan Zaric, student, Dr Vet. sci. Bosiljka Djuricic, Professor,; Faculty of Veterinary Medicine, Belgrade, Bulevar Oslobodjenja 18, Serbia

² Mr Sci. Vojinovic Dragica, Scientific Veterinary Institute of Serbia "Belgrade", Vojvode Toze 14, Belgrade;

³ DVM Marko Milovanovic, Veterinary Ambulance "Pet-Vet", Lazarevac.

Bearing in mind that hunting dogs (hounds) can easily participate in the transfer agent of leptospirosis from wild animals to humans, but also to other animals, the goal of our investigation was to investigate the current epizootic situation in this population of dogs.

Materials and methods

As the material of this study were used blood sera from hunting dogs, collected from four sites: Valjevo, Lazarevac, Gornji Milanovac and Topola. 113 serum samples were examined. From that number of dogs vaccinated against leptospirosis was the 38. From 113 dogs, 63 were females and 49 males. Age of tested dogs ranged from several months to 12 years, and the majority of animals were less than 4 years old. The study included 17 different breeds of dogs. Majority of breeds belonged to the Epagneul Breton (26), Pointer (17), German hunting terrriers (12), Posava's beater (11), and others. The method used in interpreting results was test of microscopic aglutination (MAT), which represents the "gold standard" [12] in the determination of specific antibodies for 7 investigated serovars (L. icterohaemorrhagiae, L. pomona, L grippotyphosa, L. canicola, L.bataviae, L. Sejroe, L.australis). The results are statistically calculated with Microsoft Office Excel 2007.

Results and Discussion

Investigation includes dogs from four sites, in which environment is a large number of hunting grounds. From Valjevo are sampled 20, Lazarevac 12, G. Milanovac 40 and Topola 41 blood serum of hunting dogs. Results.

Table 1. The results of presence of antibodies against Leptospira spp. in dogs blood sera

Place	Number of samples examined	The number of seropositive dogs						
Valjevo	20	3						
Lazarevac	12	0						
Gornji Milanovac	40	2						
Topola	41	2						

Table 2. Titer of antibodies serotypes of *Leptospira spp.* (the highest titer)

Serotype	Pomona	Icterohaemorrhagiae	Grippotyphosa	Sejroe	Canicola	Bataviae	Australis
Titer of antibodies	1:300	0	0	1:100	1:100	1:100	1:100

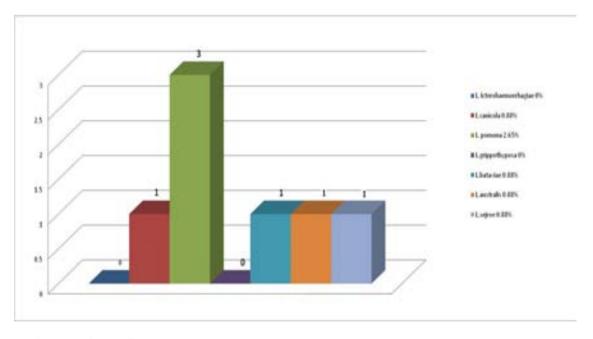


Figure 1. Seroprevalence of *Leptospira spp*.

The number of seropositive dogs was 7 or 6.19% of the total population. The greatest number of seropositive was the serovar *L. pomona* 3 (2.65%) and 1 (0.88%) to: *L. canicola*, *L. bataviae*, *L. sejroe* and *L. australis*. Established antibody titers ranged from 1:100 to 1:300.

Dogs are considered the main host for serovar *canicola*. Previous studies indicate that in dogs from urban areas often occur serovars *L.canicola* and *L. icterohaemorrhagiae*, and studies that included dogs from suburban and rural sites show dominance serovars *pomona* and *grippotyphosa* [2]. In Republic of Serbia, different tests was performed with stray dogs, where was examined 317 samples and leptospirosis was confirmed in 27 cases; mostly registered serovars were *L.icterohaemorrhagiae* and *L.pomona* [4].

All positive animals were positive for one serotype. From 7 positive dogs only one was vaccinated against leptospirosis, in which was established presence of *L. australis*, which is not present in commercial vaccine. As for sex distribution, four were females, and the rest were males. Age of positive dogs ranged from 3 months to 5 years. All 7 dogs belong to different breed. Literature data indicate that the bacteria are often seen in German shepherd [2].

Conclusion

Presented results show significant presence of a causative agent of leptospirosis in the examined areas. Considering that vaccines, which are used in the prevention, containe *L. canicola* and *L. icterohaemorrhagiae*, and seropositivity of tested dogs was registred to a smaller percentage, there might be a reason to use and other strains of leptospira in vaccination. In addition to vaccination, a significant role in the prophylaxis of hunting dogs takes control of rodent populations and education of hunters.

Results indicate a need for further research.

Literature

[1] Michael P. et al. Serovar-specific prevalence and risk factors for leptospirosis among dogs: 90 cases (1997-2002), Journal of the American Veterinary Medical Association, June 15, 2004, Vol. 224, No. 12, Pages 1958-1963, 2004. [2] Kahn, Cynthia M., Line, Scott The Merck Veterinary Manual, Ninth Edition, Leptospirosis in dogs, Merck & Co., INC. Whitehouse Station, N.J., USA, 525-529, 2005. [3] Vinetz JM. Leptospirosis, Curt Opin Infect Dis.; 14:527-38, 2001. [4] Milica Elezovic, Ana Samokovlija, Dragica Vojinovic, Tijana Jovanovic, Miloje Djuric, Jovan Maric, Bojana Bobic Gavrilovic, Vladimir Terzin, Bosiljka Djuricic. Investigation of seroprevalence of antibodies to Leptospira spp. In population of stray dogs in Belgrade area, 17th FECAVA EUROCONGRES, 6th TSAVA Congress, "Moder veterinary practices", Istanbul, Turkey, str. 457, 2011. [5] Fonzar UJ, Langoni H. Geographic analysis on the occurrence of human and canine leptospirosis in the city of Maringá, state of Paraná, Brazil, Rev. Soc. Bras. Med. Trop., Vol 45(1):100-5, 2012. [6] George E. Moore, Lynn F. Guptill, Nita W. Glickman, Richard J. Caldanaro, David Aucoin, Lawrence T. Glickman Canine Leptospirosis, United States, 2002-2004, Emrg. Infect. Dis. 12(3): 501-503, 2006. [7] Elezovic M., Samokovlija A., Djuricic B., Trailovic D. Seroprevalencija antitela na Leptospira spp. U populaciji pasa u prihvatilistima, Kongres veterinara Srbije, Beograd, 104, 2009. [8] Samokovlija Ana, Jovanovic Tijana, Žutic Jadranka, Vojinovic Dragica, Djuricic Bosilika, Nalaz specificnih antitela za Leptospira spp. Kod pasa na teritoriji grada Beograda - "Findings of specific antibodies for Leptospira spp. In dogs in region of Belgrade", , 21. Savetovanje veterinara Srbije, Zlatibor, 186-187, 2010. [9] Samokovlija Ana, Vojinovic Dragica, Jovanovic Tijana, Terzin Vladimir, Djuricic Bosiljka. Ispitivanje seroprevalence antitela za Leptospira spp. U populaciji ulicnih pasa sa teritorije grada Beograda - Investigation of seroprevalence of antibodies to Leptospira spp. In population of stray dogs in Belgrade area, I international epizootiology days-13. serbian epizootiology days, Sijarinska banja, 148-149, 2011. [10] Sonja Radojicic, Miroslav Valcic, Bosiljka Đuricic. Infektivne bolesti zivotinja, Specijalni deo, 195-203, 2011. [11] Samokovlija Ana, Vojinovic Dragica, Jovanovic Tijana, Elezovic Milica, Maric Jovan, Djuricic Bosiljka. Ucestalost pojedinih serotipova Leptospira spp. kod ulicnih pasa na teritoriji grada Beograda, Teslic, 16.savetovanja veterinara Republike Srpske, Veterinarski zurnal, Vol 11, br 1, str 128, Banja Luka, 2011. [12] Miroslava Lolin. Zarazne bolesti zivotinja bakterijske etiologije, Leptospiroze, Zavod za graficku tehniku Tehnoloskometaluruskog fakulteta, Beograd, 109-112, 1991. [13] Manual of Diagnostic Tests and Vaccines of Terrestrial Animals and Bees, OIE, Paris, 6th Edition, Chapter 2.1.9., 2008.