

## Serological study of leptospirosis among dairy cattle in Bosnia and Herzegovina

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**Abstract:** A total of 75,206 blood serum samples from dairy cattle and quarantined heifers was collected between 2001 and 2007 and analyzed for bovine leptospirosis by the microscopic agglutination test (MAT). Serovar Hardjo was used for testing of all sera, with the addition of 2 or 3 randomly chosen other serovars (Grippotyphosa, Icterohaemorrhagiae, Pomona, Bataviae, Canicola, and/or Saxcoebing). From the total number of sera tested, 1197 (1.59%) were serologically positive, with a decreasing tendency over the years of research. The most prevalent serovar was Pomona (1.32%), followed by Hardjo (0.52%) and Grippotyphosa (0.37%). The differences among the studied regions in terms of the distribution of serovars and seroprevalence values may be attributed to different farm management approaches and climatic conditions. It is believed that the decrease in seroprevalence toward the last years of investigation may be due to the application of permanent control strategies against leptospirosis and antibiotic therapy for all seropositive animals with an antibody titer equal to or higher than 1:100.

**Key words:** Leptospirosis, dairy cattle, seroprevalence, Bosnia and Herzegovina

Leptospirosis is one of the most widespread zoonoses in the world. Bovine leptospirosis causes abortions, stillbirths, infertility, and loss of milk production (1,2). The disease is caused by a bacterium belonging to the genus *Leptospira*, which is classified into several species. Within these species, over 200 serovars are recognized (3). Bovine infection can be caused by different serovars, depending on the region and maintenance host. Serovar Hardjo is one of the most common causes of leptospirosis among cattle throughout the world (2). Cattle appear to be the primary maintenance host of this serovar (1).

In Bosnia and Herzegovina, the control of bovine leptospirosis includes serological investigation of all dairy cattle from farms and traditional smallholders

that produce milk for dairy companies, as well as cattle from quarantines. The aims of this study were to investigate the seroprevalence and serovar distribution of *Leptospira* spp. among dairy cattle and quarantined heifers and to evaluate the possible correlation of seroprevalence with farm management and climate conditions.

Blood sera from 75,206 cattle were collected and analyzed for bovine leptospirosis during the period from 2001 to 2007. Samples were obtained from dairy cows (64,716) in 4 regions and from quarantined dairy heifers imported into the country (10,490). All of the samples were tested for leptospirosis just after collection, without freezing. The dominant farming system in regions C and D was traditional, with not

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more than 3 cows per herd, while farms in regions A and B had 10-15 dairy cows per herd (Figure 1). In 3 regions (A, B, and C), there is a continental climate with an average annual temperature of 12-13 °C and annual precipitation of 1000-1200 mm. In region D, there is a Mediterranean climate with an average temperature of 14-16 °C and annual precipitation of 1500 mm.

Each serum sample was diluted according to the method described by Alston and Broom (4) and was tested by the microscopic agglutination test (MAT). Live *Leptospira* cultures of different serovars (provided by the WHO/FAO/OIE/RIVM Leptospirosis Reference Laboratory of the Royal Tropical Institute, Amsterdam, the Netherlands) were used as antigens. All of the serovars were grown for between 3 and 10 days in modified Ellinghausen and McCullough medium (5) at 29 °C. Before analysis, antigens were diluted until the turbidity was 0.2 according to McFarland standards, determined with a spectrophotometer (Densimat, bioMerieux).

According to previous monitoring of bovine leptospirosis, serovar Hardjo (Hardjoprajitno) was obligatory for testing all sera. Other serovars were chosen randomly: Bataviae (Swart), Canicola (Hond Utrecht IV), Grippotyphosa (Duyster),

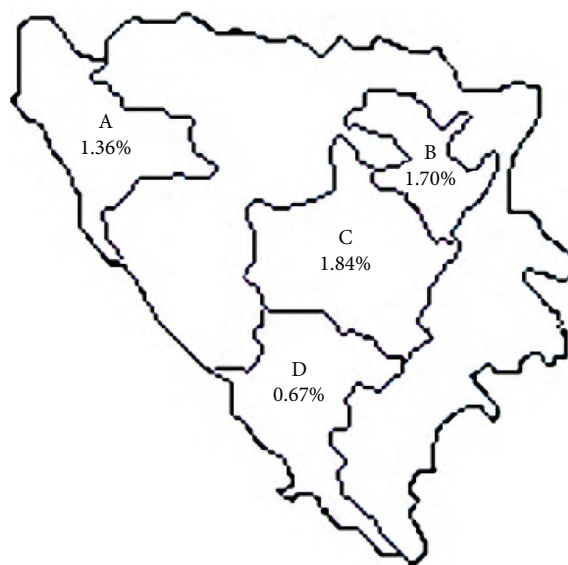


Figure 1. Seroprevalence of leptospirosis in regions of Bosnia and Herzegovina, 2001-2007.

Icterohaemorrhagiae (Kantorowicz), Pomona (Pomona), and/or Saxcoebing (Mus 24) (Table). Initially, all sera were examined at a dilution of 1:100 with chosen antigens. The sera, with some degree of agglutination, were retested at dilutions ranging from 1:30 to 1:30,000. Any serum with approximately 50%

Table. Seroprevalence and distribution of specific antibody titers of different serovars of *Leptospira* spp. in cattle in Bosnia and Herzegovina, 2001-2007.

Serovars	No. tested	Distribution of antibodies titer						No. (%) of positive sera
		1:30	1:100	1:300	1:1000	1:3000	1:≥10,000	
Hardjo	75,206	133	158	75	20	6	1	393 (0.52)
Grippotyphosa	63,647	91	91	34	10	4	3	233 (0.37)
Pomona	40,459	283	184	57	9	1	2	536 (1.32)
Icterohaemorrhagiae	33,350	18	8	1	-	-	-	27 (0.08)
Bataviae	4805	1	-	-	-	-	-	1 (0.02)
Canicola	2205	-	-	-	-	-	-	-
Saxcoebing*	1330	3	2	1	1	-	-	7 (0.53)
TOTAL	75,206	529	443	168	40	11	6	1197 (1.59)

\*Serovar Saxcoebing was used only in 2007.

or less of free leptospiras at a dilution of 1:30 was considered as positive (14). The highest dilution of serum with 50% of free leptospiras was considered as the final titer. Negative controls were conducted routinely.

A chi-square test was used for statistical analysis of seroprevalence and serovar distribution. Of the 75,206 sera tested, 1197 (1.59%) were positive for leptospirosis. While the highest seroprevalence was obtained in region C (1.84%), the lowest was found in region D (0.67%) (Figure 1). The difference between the regions was statistically significant ( $P < 0.001$ ). Seroprevalence of leptospirosis in dairy cows was close to that in quarantined heifers (1.59% and 1.58%, respectively).

The year of investigation had a significant effect ( $P < 0.05$ ) on the seroprevalence of leptospirosis. The highest seroprevalence was observed during 2003 in all regions except for region A, where the seroprevalence was the highest (4.88%) in 2006. No sample was collected from this region in 2007. The decrease in seroprevalence after 2003 was particularly evident for serovar Pomona ( $P < 0.001$ ) (Figure 2). This serovar had the highest seroprevalence (1.32%), followed by Hardjo (0.52%) and Grippytyphosa (0.37%). The difference between the seroprevalence values of serovars was statistically significant ( $P < 0.001$ ). Antibodies to serovar Canicola were not detected in any sera, and serovar Bataviae was detected in only one serum (Table).

Titers as high as 1:1000 or more were detected in 57 samples (4.76% of the positive sera). Serovar Hardjo was the most frequent, with 27 samples (Table),

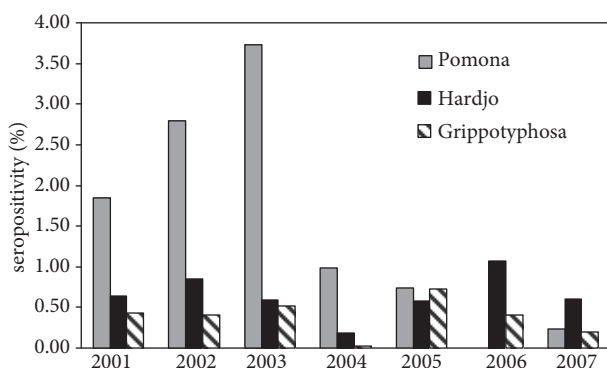


Figure 2. Distribution of the 3 most frequent *Leptospira* serovars in Bosnia and Herzegovina by year.

followed by Grippytyphosa, Pomona, and Saxcoebing (17, 12, and 1, respectively), but these differences were not statistically significant ( $P = 0.620$ ). When the results were taken into consideration by region, it was determined that serovar Pomona was dominant in regions C and D (59.6% and 55.6% of seropositive cattle) and in quarantines (53.6% of seropositive heifers), and serovar Hardjo was the most frequent in regions A and B (41.4% and 50% of seropositive cattle). Grippytyphosa was detected in all regions except region D, and the highest proportion of seropositive animals with this serovar was obtained in region A (38.25% of seropositive cattle). Other serovars were less frequent in all regions. The three serovars detected in quarantined heifer were Pomona, Hardjo, and Grippytyphosa.

The results of this study showed relatively low seroprevalence (1.59%) of bovine leptospirosis in Bosnia and Herzegovina. This is lower than rates reported from some other countries (6-11), as well as rates from previous investigations in Bosnia and Herzegovina (12). The most frequent serovar detected in this research was Pomona, followed by Hardjo and Grippytyphosa. This is contrary to previous investigations in Bosnia and Herzegovina, where Hardjo and Grippytyphosa were the most frequent (12). However, after 2003, the frequency of Pomona significantly decreased, and Hardjo and Grippytyphosa were the most frequent. Generally, serovar distributions may be related to farm management (7). Typically, traditional farming systems, with not more than 3 cows fed on pasture most of the year, have been applied in Bosnia and Herzegovina. These farming systems allow more direct contact between cattle and the free-living animals that are the maintenance host for many serovars. During the last several years, after the largest dairy companies were established in regions A and B, farm management changed, with an increasing number of farms possessing 10-15 dairy cows per herd. This system allows for more frequent contact among cattle themselves, including cattle that are maintenance hosts for serovar Hardjo, and this could be a possible explanation for the domination of serovar Hardjo in regions A and B.

The differences in serovar distribution and seroprevalence among regions may be related

to different regional mean temperatures, mean precipitation amounts, the presence of wildlife populations, and soil types (13). For instance, the lowest seroprevalence (0.67%) was obtained in region D, with a Mediterranean climate, contrary to the other regions, which have continental climates. Region D also has dominant rocky limestone soil and the lowest annual relative humidity.

It is interesting that 44.19% of positive sera had a titer of 1:30. Since vaccination is not a part of the control program of bovine leptospirosis in Bosnia and Herzegovina, we think that all of these titers present a natural infection.

A low titer is common for adapted serovars like Hardjo, but unusual for accidental serovars like Pomona (7). In this research, 53.8% of cattle with antibodies against Pomona had a titer of 1:30. This low titer could be explained by a possible longer duration of immune response induced by this serovar. Similar results were reported for serovars Bratislava

and Grippotyphosa (14). In addition, the low titer of Pomona could be the result of cross-reactions with some other closely related serovars.

The results of the present study have shown that the seroprevalence of leptospirosis among dairy cattle in Bosnia and Herzegovina is relatively low, with a tendency toward decrease over time. This is probably due to the permanent serological monitoring of all dairy cattle and antibiotic therapy for all seropositive animals with an antibody titer equal to or higher than 1:100. In addition, the results indicate a relationship between seroprevalence in cattle and farm management approaches or climatic conditions.

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