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Serologic Study on Leptospirosis Infection in Cattle in Main Albanian Coastline Districts

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A B S T R A C T

In this study we tested 550 sera blood samples in order to investigate seroprevalence of leptospiral infection in cattle managed in coastline area from Shkodër to Vlora in Albania. The sera samples were tested by means of the microagglutination test (MAT). Sera were stored at -20°C until use. They were initially screened at serum dilution of 1:100 against 9 strains of pathogenic serovars of *Leptospira*: icterohaemorrhagiae, canicola, copenhageni, pomona, hardjo, bratislava, tarassovi, grippotyphosa and ballum. The samples were judged as positive if ≥ 50% of agglutination of leptospira in a dilution test serum of ≥ l:100 were observed. Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from l:100 to 1:3200. Antibodies against one or more serovars were detected in 65 (11.9%) sera at dilution ≥1:100. Antibodies against more than one serovar were found in 5 (0.9%) positive sera. Among the positive sera, antibodies were most frequent to serovar Icterohaemorrhagiae 36.9%, Hardjo 30.8%, Grippotyphosa 16.9%, Pomona 9.2%, Copenhageni 3.1%, Bratislava 3.1%. The results of this survey indicate that leptospiral infection is common in dairy cattle.

Introduction

Leptospirosis is bacterial infection disease that affects all farm animal species and human. It is a re-emergent global zoonotic disease (Bharti *et al.*, 2003). The genus *Leptospira* is quite diverse and to date there are known 17 genospecies. Based on serological methods there are identified more than 250 serovars. Many serovars are adapted in specific hosts, while animals and humans may be affecting by several serovars.

Both direct and indirect transmissions are well described. A range of hosts, such as wildlife animals, pigs, horses cattle and rats are identified as main and or alternative reservoirs for pathogenic leptospires in animals. Clinical signs in infected cattle vary from subclinical to severe disease. There are described milk drop syndrome, abortions, hemoglobinuria, jaundice, fever, and rarely meningitis. Most commonly serovars involved in cattle are, *L.* Pomona, *L.*

Grippotyphosa, *L*. Icterohaemorrhagiae (Prescott *et al.*, 1988; Mughini-Gras *et al.*, 2014; Garvey *et al.*, 2014).

The epidemiology and diagnosis of infection with leptospires by isolation of pathogen is difficult and problematic (Lilenbaum and Martins, 2014). In many countries, diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, cost and complexity of the isolation method, and long incubation period. Serological methods are extensively used in both human and veterinary medicine (Wynwood *et al.*, 2016). Despite there are described and used a range of serological tests, two tests are more commonly applied in veterinary diagnosis, 1) the microscopic agglutination test (MAT) and 2) enzyme-linked immunosorbent assay (ELISA) (Cumberland *et al.*, 1988 (OIE, 2014).

In Albania, the cattle livestock sector represents an important part of the household economy. The cattle management is based on traditional system where co-grazing with other species is a common practice. The prevalence and circulation of *Leptospira* serovar Hardjo, for which bovine represents the maintain host, as well as the occurrence of infections caused by other pathogenic serovars of *Leptospira* are not well documented, although typical clinical signs indicate presence of infection. Furthermore, several human cases of leptospirosis are reported in Albania, involving cattle as recognised source of infection.

The aim of the study was the evaluation of the leptospirosis seroprevalence in Albanian cattle population. The serum samples were tested by means of the microagglutination test (MAT) using a panel of 9 strains of pathogenic serovars of *Leptospira*: icterohaemorrhagiae, Canicola, Copenhageni, Pomona, Hardjo, Bratislava, Tarassovi, Grippotyphosa and Ballum. The sera screening dilution was 1: 100, then two-fold serial dilution were prepared to obtain the titre.

Materials and Methods

In total we tested 550 bovine sera blood samples taken from cattle managed in four districts, 15 villages and 220 farms located on Albania coastline. The samples were tested at accredited laboratory of Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy.

The serum samples were tested by means of the microagglutination test (MAT) using a panel of 9 strains of pathogenic serovars of *Leptospira*: Icterohaemorrhagiae, Canicola, Copenhageni, Pomona, Hardjo, Bratislava, Tarassovi, Grippotyphosa and Ballum. The sera screening dilution was 1:100, then two-fold serial dilution were prepared to obtain the titre.

Results and Discussion

The MAT results are presented on Table 1-3.

The overall sero-prevalence found was 11, 9%; the leptospira antibodies for at list one serovar were found in four districts (100%), 10 of 11 villages (91%) and 50 (22.7%) of 220 herds (Table 1).

Distribution of leptospiral infection in cattle in various districts was significantly different (Table 3).

The highest positive herds were in found in Durrës 40%, and lowest was in Lezha (15.3%), whereas in Shkodër was 16.9% and Vlora 20.7% (Figure 1).

The reported results of seroprevalence of leptospiral infection in cattle are different from country to country.

These differences may be the consequence of environmental factors and control efforts. The environmental factors have been shown to have influential effects on development of leptospiral infection in animal and human beings. Long-term survival of pathogenic leptospires outside the host requires a warm, moist environment with a near neutral pH (Miller *et al*., 1991).

Out of 9 serotypes there were detected specific antibodies to six leptospira serovars. Antibodies were most frequent to serovar Icterohaemorrhagiae 36.9%, Hardjo 30.8%, Grippotyphosa 16.9%, Pomona 9.2%, Copenhageni 3.1%, Bratislava 3.1% (Table 2).

The majority of titer levels were 1:100 for all serovars and the frequency of 1:100, 1:200, 1:400 and 1:800, 1:1600 and 1:3200 were 67.7, 4.6, 9.2, 10,7, 6.2 and 1.5%, respectively.

Considering the cut-off 1:400, suggestive of acute infection, only 27.7% of samples showed the significant titres. Antibodies to *L*. Icteroha-emorrhagiae were at low titers, at cutoff level 1:100, and only one sample was at 1:400 titer (Table 2).

All positive samples for *L*. Copenhageni were at 1:100 titers. The antibody titers for *L*. Pomona, for which cattle serve as main and alternative reservoirs of infection, was quite diverse and 50% of them were at 1:400 and 1:800, even for 16.7% the titer was as highest 1:3200 (Table 2, and Figure 2).

Table.1 The serological evidence of leptospirosis infection based on MAT results according, districts and villages at farms level

Districts	No of tested farms	Positive farms	Negative farms	Positive percentage
Durrës	**55**	**22**	**33**	**40**
Lagjia 15	31	15	16	48.4
Ramilli	1	1	0	100
Sukth	23	6	17	26.1
Lezhë	**59**	**9**	**50**	**15.3**
Gjadër	15	2	13	13.3
Gocaj	5	2	3	40
Tale	39	5	34	12.8
Shkodër	**77**	**13**	**64**	**16.9**
Bërdice e Madhe	24	6	18	25
Dheu i Lehtë	26	5	21	19.2
Shelqet	17	2	15	11.8
Vlorë	**29**	**6**	**23**	**20.7**
Nartë	24	6	18	25
Shkozë	5	0	5	0
Total	**220**	**50**	**170**	**22.7**

Table.2 Distribution of serovar specific anti-leptospirosis antibodies and their titration in seropositive samples.

Serovars\titers of *Leptospires*	**1:100**	**1:200**	**1:400**	**1:800**	**1:1600**	**1:3200**	**Total**
Icterohaemorrhagiae	23 (95.8)		1(4.2)				**24** (36.9)
Copenhageni	2 (100)						**2** (3.1)
Pomona	2 (33.3)		1 (16.7)	2 (33.3)		1 (16.7)	**6** (9.2)
Hardjo	8 (40)	2 (10)	3 (15)	4 (20)	3 (15)		**20** (31.1)
Bratislava	1 (50)	1 (50)					**2** (3.1)
Grippotyphosa	8 (72.7)		1 (9.1)	1 (9.1)	1 (9.1)		**11** (16.9)
Total	**44 (67.7)**	**3 (4.6)**	**6 (9.2)**	**7 (10.7)**	**4 (6.2)**	**1 (1.5)**	**65**

Table.3 Seroprevalence of leptospira infection based on MAT

Districts	Samples tested	Positive
Vlorë	51	6 (11.8%)
Lezhë	99	11 (11.1%)
Durrës	271	34 (12.6%)
Shodër	129	14 (10.9%)
Total	550	65 (11.9%)

Fig.1 Percentage of farms positive for leptospirosis infection in different districts of Albania

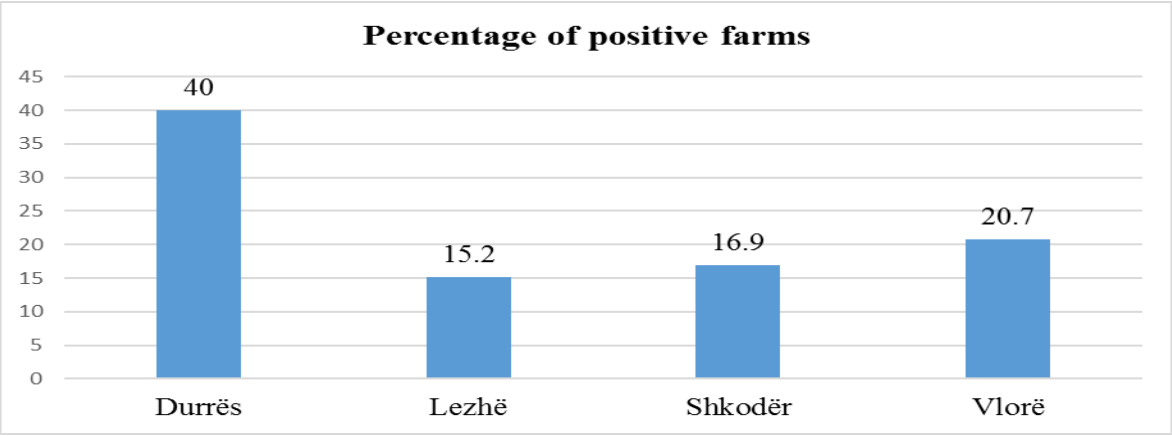
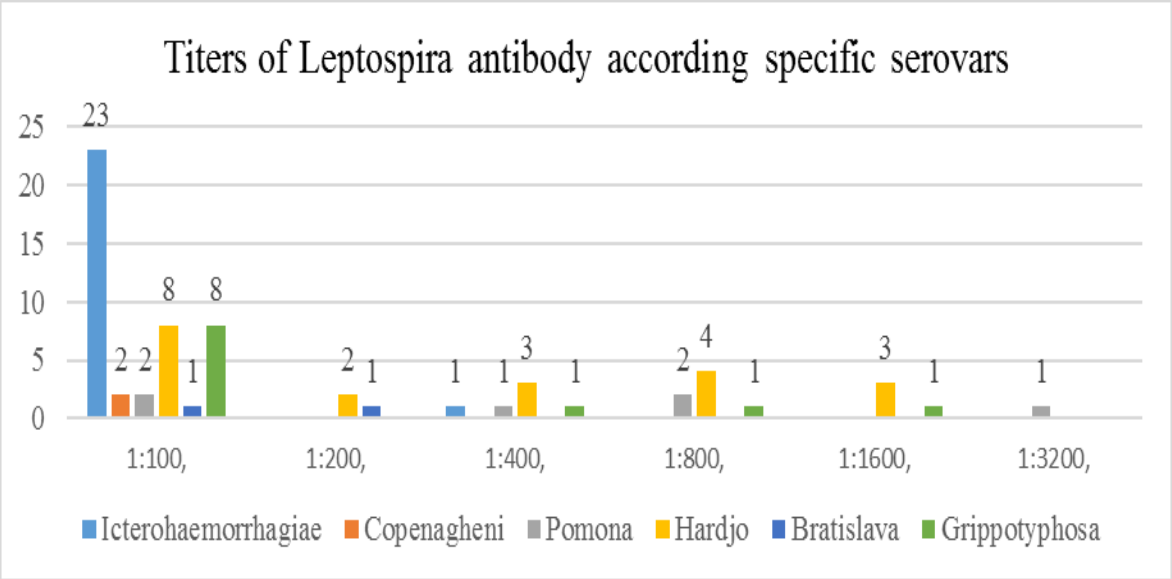


Fig.2 Different leptospira serovars detected by MAT in different districts of Albania



The antibody titers for *L. hardjo*, for which cattle are the main reservoirs of infection, was as follow: 40% of samples belong to titer 1:100, 10% at level 1:200, 15% were at level 1:400, 20% at level 1:800 and 15% at level 1:1600 (Table 2).

The antibody titers for *L.bradislava* were at level 1:100 and 1:200 respectively. The antibody titers for *L.* Grippotyphosa range from 1:100 (72.7%) to 1:1600 (27.3%) (Table 2). Antibodies against one or more serovars were detected in 65 (11.9%) cattle (Table 1).

Antibodies against two serovars were found in five (7.7%) sera samples. Mix infection was evident in Durrës in 3 samples and coinfection of *L. ichterohaemorhagiae*, and *L. hardjo*, *L. grippotyphosa* and *L. copenhageni* respectively was serologically identified.

The presence of coinfection was found in Lezhë, and 2 samples shown positive results for serovars Ichterohaemorhagiae and Pomona and other for Ichterohaemorhagiae and Grippotyphosa, respectively.

The presence of infection and dominant titre of 1:100 reveal that leptospiral infection in cattle in Shkodra, Lezhë, Durrës and Vlorë is endemic and occurs mostly in subclinical form, however our study show that acute infection is active in some farms.

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