

## RESEARCH ARTICLE



# Sero-prevalence of leptospirosis in two dairy cattle farms in Albania

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## Abstract

Leptospirosis is an important bacterial zoonotic disease caused by *Leptospira interrogans* spp. It occurs worldwide, and is endemic in Albania. Among farm animals, cattle are most affected and it is mostly a herd than individual problem.

**The aim** of this study was to compare the disease seroprevalence in two different dairy cattle and our goal was to identify risk factors and their impact in disease prevalence.

**Materials and methods:** The serological data were drawn from previous microscopic agglutination test carry out on sera blood samples. The statistical analyze was conducted and an association between risk factors and disease prevalence was calculate.

**Results:** The prevalence of Leptospirosis in two dairy farms managed indoors was highly different, respectively 29.3% and 6.6%. The location, flooding, rat control program were identified as risk factors which explain, at list in part, disease prevalence difference.

**Keywords:** Zoonotic disease, Leptospirosis, titre, risk factor.

## 1. Introduction

Leptospirosis is a bacterial zoonotic disease caused by a spirochete called *Leptospira* spp. which affects different animal species and humans [4, 6]. Leptospirosis epidemiology is complex and dynamic, it is characterized by a wide variety of clinical manifestations that often hinder the differential diagnosis. This explains the fact that in some cases there is a lack of diagnosis and in general there are sub-reports of disease [4, 6]. People may be accidentally affected by indirect or direct contact [4]. The incidence of the disease is high in countries with a tropical climate. There is a positive correlation between the amount of rainfall and the incidence of leptospirosis giving it seasonal character in milder climate and a perennial infection in tropical climate. The main source and reservoir of the infection are rodents, mainly rats, domestic animals (cattle, dogs etc), wild animals etc. Leptospirosis is endemic in Albania. The first cases of Leptospirosis in humans have been identified in the Berat [2,3] later it was

reported in Bregui Matit [2, 3] during the epidemic episodes in Lushnje, Shkodër, Delvinë, Sarandë. In 1962, blood samples were collect at the Tirana slaughterhouse and the presence of *Leptospira* spp antibodies in various animals was confirm. In Albania, leptospirosis is a reportable disease since 1960. In recent years, as elsewhere, incidence of leptospirosis shows an increasing pattern, and certain risk factors play an important role. The aim of this study was to compare the seroprevalence of leptospirosis in two dairy cattle farms managed intensively to identify most likely risk factors and their impact.

## 2. Materials and Methods

The seroprevalence was determined based on the serological results obtained. The serological testing results of the two farms managed indoors, located in two geographic regions were used. The MAT test was run at different dilution from 1:100 to 1:3200 for each *Leptospira* serovar, and cut off was determined 1:00. Based on the literature data [7], the risk factors were

identifying and the questionnaires were drafted and filled up by interviewing the owners during field visits. The questionnaire provide data on different aspects: the location, animal and farm ID, the farm size, the history of leptospirosis in the past, the breeding methods, the management system, the frequency of infertility and other related reproduction problem. In addition, the questions were address regarding the mixing with other species, the presence of dogs, irrigation and drainage systems, the presence of swamps, topographical location, age, implementation of the rat control program, vaccination program against leptospirosis. A Chi-square test was conduct to investigate the association between the exposure to most important risk factors and the disease outcome [2]. The odds ratio and its 95% confidence limits were calculated to measure the magnitude of the association. A 5% level of significance was used to evaluate significance of association, i.e. the p-value was considered significant if it was less than 0.05. The

analyses were conducted using Statulator, an online statistical program [2].

### 3. Results and Discussion

Serologic results are present in Table 1 and Table 2. Table 2 shows the serological serum titers according to the farms. The high prevalence of NN farms compared to farm R04 indicates that there are probably other factors that affect the frequency of infection. Interestingly, the prevalence of infection in R04 farm (6.6%) is quite lower compare the NN farm (29.3%). The analysis of risk factors can shed light on these differences. From the questionnaires, we identified that there is a difference between farms regarding specific risk factor: in farm R04 there is in place a rat control program and the farm is not likely to be flooded and to the swamps were not present compare to NN farm, where above mention factor were opposite. The data were organize in a contingency table (Table 3) and analyzed by statulator online software.

**Table 1-** Farm prevalence of *Leptospira* spp infection based on MAT results.

Farm Code and its location	Tested samples	Positive	Prevalence
NN Farm in Lushnja	116	34	6.6
R04 Farm Durrës	167	11	29.3
<b>Total 2016</b>	<b>283</b>	<b>45</b>	<b>15.9</b>

**Table 2–** Frequency of different titres and their respective percentages according to the farms

Farms	Number of samples	Percentage	Number of samples	Percentage
1:100	1	9.1	6	17.6
1:200	2	18.2	7	20.6
1:400	3	27.3	7	20.6
1:800	4	36.4	9	26.5
1:1600	1	9.1	4	11.8
1:3200	0	0.0	1	2.9

The prevalence of leptospira infection was approximately 4.4 times higher in the NN farm, compared with R04 farm. We consider several risk factors, however we are going to present and analyze here the presence of floods and swamps and implementation of control program of rats.

Constructing the contingency table and analyzing it according to the statutory program estimated that this factor had an important role.

A student test, t, was conduct to compare the distribution of mean serological titers for positive animals in two farms. The t values indicate that there

there is a significant difference between farms. The number of animals with different titers is  $2 \pm 1.58$  (mean  $\pm$  standard deviations) for the R04 farm, while for the NN farm tis  $5.7 \pm 2.8$ , and the t statistical value was greater than the critical one ( $P > 0.95$ ).

In addition, there are differences even in terms of circular serovars (data not shown). In the R04 farms were identified as specific antibodies to *Leptospira Hardjo* only, which indirectly means that

animals were expose to only one *Leptospira* serovar. In contrast, at NN farm there were identified specific antibodies for at least three different serovars. This indicates that coinfection with more than one serovar in the same farm is possible. No any animals were identify to have specific antibodies more than one *Leptospira* serovar. This is different compare to extensively management system (data not shown).

Table 3– Exposure to risk factors flooding, swamps and absence of rat control program and diseaseoutcome

<i>Explanatory variable</i>	<i>Categories</i>	<i>Disease outcome</i>		<i>Total</i>	<i>Odds (95% CI)</i>	<i>P-value</i>
		Positive	Negative			
Exposure to risk factor	Positive	34 (29.31%)	82 (70.69%)	116	5.88 (2.83, 12.21)	<0.001
	Negative	11 (6.59%)	156 (93.41%)	167		
<b>Total</b>		<b>45</b>	<b>238</b>	<b>283</b>		

The prevalence of leptospiraspp infection was 4.4 times higher in the NN farm, compared with R04. Three main risk factors were identified that were present in farm NN and animals in farm R04 were not exposed. We analysed and compared the disease prevalence in relation the presence of floods and swamps and misusing the rat control program. Constructing the contingency table and analysing it according to the statutory program estimated that this factor had an important role [1].

Distribution of serological serotypes is similar between the two farms (Table 2). The serological titres have approximately normal distribution in both farms, however there is a significant difference between the two farms, in the R04 farm the number of positive individuals ranged from  $2 \pm 1.58$  (mean  $\pm$  standard deviations) while for the second farm they ranged  $5.7 \pm 2.8$  animals.

There are differences even in terms of circular serovars (data not shown). In the R04 farm were identified positive samples for specific antibodies to *LeptospiraHardjo* only, which indirectly means the

exposure and circulation of only one *Leptospira* serovar. At the NN farm were identified specific antibodies for at least 3 serovars *LeptospiraHardjo* (31 animals), *LeptospiraPomona* (one animal) and *LeptospiraGrippotyphosa* (one animal). This indicates that coinfection of more than one serovaris possible, especially when animals are exposed to other risk factors such as mixing with other animals, or buying from unsafety sources.

The odds ratio indicates that the exposure positive group has 5.88 times the odds of the outcome than the exposure negative group [2]. Also, we are 95% confident that the odds ratio in the population (from where the sample was obtained) would be between 2.83 and 12.21. Since the odds ratio confidence interval does not include the null value (i.e. 1), and the p-value (<0.001) is less than 0.05, the conventionally used criterion to evaluate p-values, the association between exposure and outcome is statistically significant at 5% level of significance [1, 2].

**Conclusion:**The main finding of this study was the relationship between prevalence of leptospirosis and risk factors. There is a significant difference of Leptospira sero-prevalence between farms. The risk factors play an important role in not only in disease prevalence level but also in number of serovars circulating in the farms.

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