Leptospira spp. and Brucella ovis seroprevalence in sheep: preliminary results of one year surveillance program

F. BERTELLONI, B. TURCHI, D. CERRI, P. PINZAUTI, F. FRATINI

doi: 10.12681/jhvms.16053

Copyright © 2018, F BERTELLONI, B TURCHI, D CERRI, P PINZAUTI, F FRATINI

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0.

To cite this article:
**ABSTRACT.** In order to assess the diffusion of brucellosis by *Brucella ovis* and *leptospirosis* in sheep flocks in Tuscany, 410 blood samples were collected from males of 76 breeding farms from January to December 2015. All sera resulted negative for *B. ovis*. A percentage of 17.80% sera was found positive for *Leptospira* spp. Among all breeding farms, 34.21% resulted positive. The most represented serovars were Pomona (6.34%), Hardjo (4.14%), Grippotyphosa (3.17%) and Bratislava (1.70%). The highest antibody titers were detected for serovar Hardjo (1:25600) and serovars Pomona and Grippotyphosa (1:1600). These results confirm the role of sheep as maintenance host for serovar Hardjo and highlight the spreading of serovar Grippotyphosa in the study area. Constant field investigation, especially on farm animals, could be useful to determine trends and diffusion of some occupational re-emerging diseases, such as leptospirosis.

**Keywords:** *Brucella ovis*, *Leptospira* spp, sheep, serology, Tuscany.
INTRODUCTION

Brucellosis caused by *Brucella ovis* is a non-zoonotic infection characterized by a clinical or subclinical disease in sheep, subfertility in rams due to epididymis, testicle and accessory sexual glands alterations and, less frequently, placentitis and abortions in ewes. Infected ewes may excrete *B. ovis* through vaginal discharges and milk (López et al. 2006). Brucellosis transmission occurs through passive venereal infection or direct contact, in this case the ram role as an infection spreader is decisive. Rev 1 vaccination program could have contributed to the reduction of the occurrence of *B. ovis* (De Bagues et al., 1995). Since several years vaccination is no longer mandatory in Italy; this could represent a promoting factor for *B. ovis* spreading for which an active serological surveillance program is not planned.

Ovine leptospirosis is an underdiagnosed disease, usually silent, and its effects on livestock are often underestimated (Martins and Lilenbaum, 2014). Even if sheep are usually considered less susceptible to leptospirosis than other livestock species, reproductive disorders and failures due to *Leptospira* and serological positivity have been reported in several countries including Italy (Ciceroni et al., 2000, Cerri et al., 2003, Melo et al., 2010, Tonin et al., 2015). Rarely, leptospirosis in small ruminants may occur in an acute form characterized by anorexia, fever, depression and occasionally anemic or hemorrhagic syndromes (Adler and de la Pena Moctezuma, 2010), but most frequently ovine leptospirosis occurs in a chronic form presented with subfertility, neonatal deaths, abortions, and decreased milk yield, causing substantial economic losses for farmers (Lilenbaum et al., 2009).

On the basis of these observations, considering the presence of numerous sheep farms in Tuscany, a serological survey on *Leptospira* and *B. ovis* prevalence was carried out on the male population of some of the breeding farms involved in the national brucellosis surveillance program (DGR 1204/2009), from January 2015 to December 2015.

MATERIAL AND METHODS

From January 2015 to December 2015, blood samples of 410 rams were collected. All subjects were adult, used as breeding animals and no symptoms were observed at sampling time. Overall, 76 farms were investigated. Fifty-four were located in the northeast of Tuscany, 16 in the southeast and 6 in northwest area of the region. Farms located in the north raised mainly Massese sheep herds of 30 up to 100 animals, while farms located in the south raised Sarda sheep with herds of more than 100 animals. Blood was collected during the routine surveillance program for *Brucella* spp..

The obtained sera were tested for the presence of *B. ovis* and *Leptospira* antibodies.

For the detection of *B. ovis* antibodies, the complement fixation (CF) test was employed. The antigen was a hot saline extract of *B. ovis* strain 63/290. Titers corresponding to ≥ 50 CF test international units/ml were considered positive (Cerri et al., 2000).

Microscopic agglutination test (MAT) was used to detect *Leptospira* antibodies. A panel of 8 serovars was used as live antigen: Icterohaemorrhagiae (strain Bianchi), Canicola (strain Alarik), Pomona (strain Mezzano), Tarassovi (strain Johnson), Grippotyphosa (strain Moscow V), Bratislava (strain Riccio 2), Ballum (strain Castellon 3) and Hardjo (strain Hardjoprajitno). The cultures were grown in Ellinghausen-MacCullough-Johnson-Harris (EMJH—Difco, Detroit, Michigan, USA) at 30 °C for 4–14 days and checked for purity, mobility and agglutination ability. MAT was performed following the procedure previously reported by Cerri et al. (2003). Titers of 1:100 were considered positive; 2-fold serial dilutions were tested to determine the endpoint titer.

Statistical analysis on the distribution of positive sera, farms and serovars related to geographical area was performed using Chi-square test (*P* ≤ 0.05 was considered statistically significant).

RESULTS

All sera resulted negative for *B. ovis*.

Seventy-three out of 410 (17.80%) sera were found positive for *Leptospira* antibodies. In particular, 24/170 sera collected in Northwest of Tuscany, 39/187 collected in Southwest and 10/53 collected in Northeast resulted positive (Table 1).

Among the 76 considered farms, 26 (34.21%) presented at least one seropositive animal. Thirteen of them were located in northwest Tuscany, 10 in southwest and 3 in Northeast (Table 1).
### Table 1. Tested sera and breeding farms in relation to geographic area.

<table>
<thead>
<tr>
<th>Provenience</th>
<th>Tested</th>
<th>Positive to <em>Leptospira</em> (%)</th>
<th>Tested</th>
<th>Positive to <em>Leptospira</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northwest</td>
<td>170</td>
<td>24 (14.11)</td>
<td>54</td>
<td>13 (24.07)</td>
</tr>
<tr>
<td>Southwest</td>
<td>187</td>
<td>39 (20.85)</td>
<td>16</td>
<td>10 (62.50)</td>
</tr>
<tr>
<td>Northeast</td>
<td>53</td>
<td>10 (18.86)</td>
<td>6</td>
<td>3 (50.00)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>410</td>
<td>73 (17.80)</td>
<td>76</td>
<td>26 (34.21)</td>
</tr>
</tbody>
</table>

### Table 2. Number of positive sera and antibody titers detected for each *Leptospira* serovar

<table>
<thead>
<tr>
<th>Serovars</th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
<th>1:800</th>
<th>1:1600</th>
<th>1:3200</th>
<th>1:25600</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Icterohaemorrhagiae</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 (1.46)</td>
</tr>
<tr>
<td>Pomona</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>26 (6.34)</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>13 (3.17)</td>
</tr>
<tr>
<td>Tarassovi</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (0.48)</td>
</tr>
<tr>
<td>Bratislava</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (1.70)</td>
</tr>
<tr>
<td>Hardjo</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>17 (4.14)</td>
</tr>
<tr>
<td>Ballum</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (0.48)</td>
</tr>
</tbody>
</table>
Table 3. Distribution of *Leptospira* serovars seropositivity in relation to their provenience

<table>
<thead>
<tr>
<th>Provenience</th>
<th>Ih</th>
<th>Po</th>
<th>Gr</th>
<th>Ta</th>
<th>Br</th>
<th>Ha</th>
<th>Ba</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northwest</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Southwest</td>
<td>3</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>39</td>
</tr>
<tr>
<td>Northeast</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>TOT</strong></td>
<td>6</td>
<td>26</td>
<td>13</td>
<td>2</td>
<td>7</td>
<td>17</td>
<td>2</td>
<td>73</td>
</tr>
</tbody>
</table>


Twenty-six sera resulted positive for serovar Pomona, 17 for serovar Hardjo, 13 for serovar Grippotyphosa, 7 for Bratislava, 6 for Icterohaemorrhagiae and 2 for serovars Tarassovi and Ballum. No sera resulted positive for serovar Canicola. Table 2 reports the MAT titers for each sample.

Table 3 shows the distribution of positive sera in relation to provenience.

**DISCUSSION**

During routine *Brucella* spp. surveillance program, blood samples from rams were collected in order to extend serological investigation to *B. ovis* and *Leptospira*. Males were selected for the survey because of their involvement in the venereal transmission of these microorganisms (Cerri et al., 1999, Magajevski et al., 2005, Director et al., 2014). Based on our results, *B. ovis* seroprevalence in Tuscany appears to be zero. In accordance with our findings, *B. ovis* infection was not reported in Italy in recent years, even if the disease occurred recently in East Europe and France (Spicić et al., 2009, Petrović et al., 2014, Picard-Hagen et al., 2015).

Concerning *Leptospira* seroprevalence in rams, our results are in accordance with a previous survey carried out on the same geographical area (Cerri et al., 2003). In relation to tested farms, high percentage of positivity was detected. This finding confirms the epidemiological role of the male animals in *Leptospira* spreading and, consequently, their primary function as “detectors” of the presence of infection in the farm. Concerning *Leptospira* serovars, Pomona was the most represented (6.34% positive subjects). The high percentage detected for this serovar, which is unusual for sheep, could be probably explained by the presence of wild boars in the areas where the sampling was conducted, which represent maintenance hosts for Pomona (Żmudzki et al., 2016). The second most detected serovar was Hardjo (4.14%). These results were expected because sheep represent the second maintenance host for this serovar (Farina et al., 1996). A high percentage of seropositive animals (3.17%) for serovar Grippotyphosa was also detected. This serovar was reported all over Europe in the past years, in both wild and domestic animals. Also, infection in sheep with Grippotyphosa serovar could be related to the breeding farm management, as suggested...
by other Authors (Andreoli et al., 2014, Ayral et al., 2014, de Carvalho et al., 2014, Żmudzki et al., 2016). In particular, the presence of this serovar in Tuscany could be related to hares’ importation from eastern Europe for repopulation and hunting purposes (Treml et al., 2007, De Massis et al., 2012). In this study, antibody titers of 1:100 were considered as threshold; titers of 1:100 or 1:200 may correspond to the early stage of the infection, to a previous infection or to vaccinal antibodies, whereas, titers of 1:400 could be representative of infection in endemic areas (Picardeau, 2013). The highest antibody titers were obtained for serovars Hardjo, with values ranging from 1:100 to 1:25600, and for serovars Grippotyphosa and Pomona, with values ranging from 1:100 to 1:1600. These results suggest some probable leptospirosis outbreaks. Concerning other serovars, the sporadic and low antibody titers detected show the spreading of these serovars as well. Grippotyphosa serovar was mainly represented in northwest Tuscany and Pomona serovar in the southwest, while for other serovars no differences were observed in relation to the geographical distribution.

CONCLUSION

In conclusion, *B. ovis* infection seems to be absent in the investigated area, however, in light of recent spreading of this disease in some European countries, continuous surveillance would be useful. Our results highlight the occurrence and spreading of *Leptospira* in sheep in Tuscany. Moreover, infection by serovar Hardjo in sheep is probably underestimated and spreading of serovar Grippotyphosa and Pomona could represent a potential hazard for other animals, such as dogs and cattle. Further serological and bacteriological investigations should be carried out on a higher number of samples from wild and domestic animals, in order to determine more accurately the prevalence of leptospirosis in Tuscany and clarify its potential health risk.

REFERENCES


de Bagüés JMP, Barberán M, Marin CM, Blasco JM (1995) The 
Brucella abortus RB51 vaccine does not confer protection against 
de Carvalho SM, Mineiro AL, Castro V, Genovze ME, Azevedo SS, 
Costa FA (2014) Leptospirosis seroprevalence and risk factors 
for sheep in Maranhão state, Brazil. Trop Anim Health Prod 
46(2):491-4.
De Massis F, Di Provvvido A, Di Sabatino D, Di Francesco D, Zilli K, 
Ancora M, Tittarelli M (2012) Isolation of Brucella suis biovar 2 
from a wild boar in the Abruzzo Region of Italy. Vet Ital 48(4):397-
404.
DGR 1204/2009: Delibera della Giunta Regionale N° 1204 del 21-12-
2009: Modifica delle linee guida per la profilassi e l’eradicazione 
della tubercolosi bovina, brucellosi bovina, brucellosi ovi-caprina 
e leucosi bovina enzootica, Bollettino Ufficiale Della Regione 
Toscana, N. 52, 30/12/2009 (Italian).
Director A, Penna B, Hamond C, Loureiro AP, Martins G, Medeiros 
MA, Lilenbaum W (2014) Isolation of Leptospira interrogans 
Hardjoprajitno from vaginal fluid of a clinically healthy ewe 
suggests potential for venereal transmission. J Med Microbiol 
63(Pt 9):1234-6.
Farina R, Cerri D, Renzoni G, Andreani E, Mani P, Ebani V, Pedrini 
A, Nuvoloni R (1996) Leptospira interrogans in the genital tract 
of sheep. Research on ewes and rams experimentally infected with 
Lilenbaum W, Vargas R, Ristow P, Cortez A, Souza SO, Richtzenhain 
carriers among seroreactive goats and sheep by polymerase chain 
antibodies to Brucella ovis in sheep milk using B. ovis and B. canis 
Magajevski FS, Girio RJS, Mathias LA, Myashiro S, Genovze MÉ, 
and urine of bulls serologically reactive to Leptospira interrogans 
Martins G and Lilenbaum W (2014) Leptospirosis in sheep and goats 
Melo LDSS, Castro MBD, Leite RC, Moreira EC, Melo CRD (2010) 
Main aspects of Leptospira sp infection in sheep. Cienc Rural 
40(5):1235-1241.
Petrović M, Špičić S, Potkonjak A, Lako B, Kostov M, Cvetnić Ž 
(2014) First evidence of Brucella ovis infection in rams in the 
Picarbeau M (2013) Diagnosis and epidemiology of leptospirosis. Med 
Contagious epididymitis due to Brucella ovis: relationship between 
sexual function, serology and bacterial shedding in semen. BMC 
of Brucella ovis infection in Republic of Croatia. Dtsch Tierarztl 
Tonin AA, Martins B, Zago RVMS, Tochetto C, Azenha NP, Schaefer 
PC, Badke MRT (2015) Outbreak of leptospirosis: reproductive 
brown hare as a potential source of zoonotic agents. Veterinarni 
Medicina-Praha 52(10):451-456.
Żmudzki J, Jabłoński A, Nowak A, Żębek S, Arent Z, Bocian L, Pejsak 
Z (2016) First overall report of Leptospira infections in wild boars 