# *Serological surveillance of Leptospirosis in Italy: two-year national data (2010-2011)*

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Animal species,
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Leptospirosis,
Microscopic agglutination test,
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**Summary**
Nowadays, leptospirosis is a re-emerging widespread infectious disease often underestimate worldwide. The National Reference Centre for Leptospirosis (NRCL), at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia (Italy), with the cooperation of all the other Istituti Zooprofilattici Sperimentali (IIZZSS), evaluated the distribution of such important zoonosis in Italy. Serological data obtained between 2010-2011 by each laboratory were collected by the NRCL and discussed. Serum samples collected from 43,935 animal specimens were analysed by the Microscopic Agglutination Test (MAT), using a panel of 8 serogroups as antigens (Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi). A MAT cut-off of 1:100 was used to identify the serological positivities, 6,279 sera showed positive titers. Bovine (46.9%), swine (27.5%), ovine and goat (7.4%), dog (6.9%), and wild boar (4.5%) samples were delivered to the Laboratories more frequently than equine and other species sera. Data analysis showed that the most common serogroups in Italy are: Australis present in dogs, wild boars, horses, hares, swine, foxes, and rodents; Sejroe detected in cattle, sheep, goats, and buffaloes; Icterohaemorrhagiae present in dogs, goats, and foxes; Pomona detected in swine, cattle, and wild species; Grippotyphosa reported in hares.

## *Indagine sierologica sulla presenza di* Leptospira *spp. in Italia: dati nazionali 2010-2011*

**Parole chiave**
Dati nazionali,
Italia,
*Leptospira interrogans*,
Leptospirosi,
Sierosorveglianza,
Specie animali,
Tecnica di agglutinazione microscopica.

**Riassunto**
La leptospirosi è una malattia infettiva riemergente diffusa in tutto il mondo la cui prevalenza è spesso sottostimata. Il Centro Nazionale di Referenza per la Leptospirosi (NRCL), presso l'Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia (Italia), con la collaborazione di tutti gli altri Istituti Zooprofilattici Sperimentali (IIZZSS), ha valutato la diffusione di tale importante zoonosi in Italia. I dati sierologici, ottenuti tra il 2010 e il 2011, da ciascun Istituto, sono stati raccolti dal NRCL e valutati. I campioni di siero raccolti da 43.935 soggetti di varie specie animali sono stati analizzati con la tecnica di agglutinazione microscopica (MAT), utilizzando un pannello di 8 antigeni di diverso sierogruppo (Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi). Per identificare le positività sierologiche è stato stato utilizzato il valore soglia di 1:100. Sono stati rilevati titoli positivi per 6.279 sieri. I campioni da bovini (46,9%), da suini (27,5%), da ovini e caprini (7,4%), da cani (6,9%) e da cinghiali (4,5%) sono stati più numerosi rispetto a quelli da equini e da altre specie. L'analisi dei dati ha mostrato che i sierogruppi più comuni in Italia sono: Australis presente in cani, cinghiali, cavalli, lepri, suini, volpi e roditori; Sejroe rilevato in bovini, ovini, caprini e bufali; Icterohaemorrhagiae presente in cani, caprini e volpi; Pomona rilevato in suini, bovini, e specie selvatiche; Grippotyphosa è stato riportato nelle lepri.

## *Introduction*

Leptospirosis is a zoonotic infectious disease, caused by pathogenic serovars of *Leptospira*. It is a worldwide public health and veterinary problem, difficult to diagnose and, consequently, frequently underestimated (Chappel and Smythe 2012, Hartskeerl *et al.* 2011, WHO 2011). A large spectrum of domestic and wild animals are involved as renal carrier state in the maintenance of leptospirosis in the environment. It has been reported in cattle, pigs, equine, sheep, goat, dogs, wild boars (*Sus scrofa*), rodents, hedgehog, hares, deer, as well as other species. *Leptospira* infection can be incidental or host-maintained. In the first case, infection results from direct or indirect contact with infected urine; a maintenance hosts is defined as an animal which is capable of acting as a natural source of leptospiral infection for its own species and associated to specific *Leptospira* serovars (Faine *et al.* 1999, Levett 2001). In recent years, some serovars have become prevalent and emerging among both wild and domestic animals. This situation indicates that the epidemiology of leptospirosis may change over time in animals as well as in humans. Having knowledge of the trend of these changes could be useful to improve the diagnosis and the surveillance systems of human and veterinary leptospirosis, which are still inadequate (Chappel and Smythe 2012, Hartskeerl *et al.* 2001, WHO 2011).

The aim of the present study is to update the current epidemiological situation of leptospirosis in Italy, on the basis of serological data obtained from sera of domestic and wild animals, examined by all 10 Italian Istituti Zooprofilattici Sperimentali (IIZZSS) between 2010-2011. All data have been collected and elaborated by the National Reference Centre for Animal Leptospirosis (NCRL) at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia (Italy).

## *Materials and methods*

From January 2010 to December 2011, 43,935 serum samples were collected by 10 IIZZSS from domestic and wild animal species suspected of infection or to be controlled. They were examined by microscopic agglutination test (MAT), as described by the OIE guidelines (OIE 2008).

The panel of antigens was composed by 8 serogroups, which are representative of those known to exist in the Italian area: Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi. The two serovars, Copenhageni and Icterohaemorrhagiae, included in the serogroup Icterohaemorrhagiae are generally in use. The former serovar is used by all IIZZSS, while the latter is used by 4 of them (Table I). Samples showing titers equal or higher than the MAT cut-off of 1:100 against one or more serovars were considered positive. Serological data were also processed considering a cut-off 1:400, in order to exclude possible cross-reactions and vaccinal interferences.

Results were grouped on the basis of low (1:100-1:200), medium (= 1:400), and high titers (≥ 1:800) to show the most significant positivities.

**Table I.** *Leptospira antigens used in the microscopic agglutination test.*

| Serogroup | Serovar | ID |
|---|---|---|
| Australis | Bratislava | AB |
| Ballum | Ballum | BB |
| Canicola | Canicola | CC |
| Grippotyphosa | Grippotyphosa | GG |
| Icterohaemorrhagiae | Copenhageni | IC |
| | Icterohaemorrhagiae | II |
| Pomona | Pomona | PP |
| Sejroe | Hardjo | SH |
| Tarassovi | Tarassovi | TT |

## Results

The results of serological test are reported in Tables II and III. Among the 43,935 tested sera, 6,279 (14.3%) were positive against one or more serovars at MAT.

A group of 275 samples was collected from not-identified animal species and 80 samples from humans (Table II). In this study, the data processing was focused on the 5,035 samples positive to a single serovar (Table III), while seropositivity against two or more serovars (1,220 sera samples) were disregarded and no statistical analysis was done. Antibody titers has been detailed for each species and described in Figures 1-13.

### Cattle and buffaloes

A total of 2,250 (10.9%) out of 20,592 cattle tested sera reacted as MAT positive (Table II). Serovar Hardjo (serogroup Sejroe) was confirmed as the most prevalent agent of *Leptospira* infection in cattle (79.6%) often at titers ≥ 1:400 (85.5%), followed by serovars Pomona (serogroup Pomona) (14.4%), and Bratislava (serogroup Australis) (3.4%) (Table III, Figure 1).

In buffalo, positivities were detected in 21 (6.8%) samples out of 310 examined (Table II). All samples were positive to serovar Hardjo (100%) at cut-off ≥ 1:400 (Table III, Figure 2).

### Swine

Positive reactions were detected in 2,243 (18.6%) out of 12,068 swine sera (Table II). Antibody titers against serovar Bratislava (serogroup Australis) were prevalent (83.9%), followed by titers against serovar Pomona (serogroup Pomona) (13.5%). Only 14 samples reacted positive against serovar Tarassovi (serogroup Tarassovi) (0.8%). If the cut-off ≥ 1:400 was considered, 48.9% of samples was positive against Bratislava, 45% was positive against Pomona, and 4.2% was positive against Tarassovi (Table III and Figure 3).

### Dogs

Serum samples from 3,028 dogs were examined and 904 (29.9%) showed positive reactions (Table II). Serovars Bratislava (serogroup Australis) (39.3%), Copenhageni (serogroup Icterohaemorrhagiae) (32.1%), Icterohaemorrhagiae (serogroup Icterohaemorrhagiae) (16.1%) and Canicola (serogroup Canicola) (10.5%) were detected (Table III). Several cross-reactions among different serovars were also observed. If the cut-off ≥ 1:400 was considered, 57.6% of samples was positive against Bratislava, 18.8% was positive against Copenhageni, and 4.9% was positive against Canicola (Table III and Figure 4).

### Horse

A total of 747 sera were analysed and 222 (29.7%) scored positive (Table II). Serovars Bratislava (serogroup Australis) (78.5%), Copenhageni (serogroup Icterohaemorrhagiae) (7.0%), and Icterohaemorrhagiae (serogroup Icterohaemorrhagiae) (5.8%) were prevalent. Five samples (2.9%) reacted positive to Grippotyphosa (Table III). If the cut-off ≥ 1:400 was considered, 87.9% of samples was positive against Bratislava (Table III and Figure 5).

### Wild boars

A total of 1,987 sera were examined and 188 (9.5%) were positive (Table II). Serovars Bratislava

**Table II.** *Numbers of serum samples of domestic and wild animals examinated by microscopic agglutination test with cut-off of ≥ 1:100.*

| Species | Tested samples | |
|---|---|---|
| | Total | Positive |
| Cattle | 20,592 (46.9%) | 2,250 (10.9%) |
| Swine | 12,068 (27.5%) | 2,243 (18.6) |
| Dog | 3,028 (6.9%) | 904 (29.9%) |
| Sheep | 2,466 (5.6%) | 105 (4.3%) |
| Wild boar | 1,987 (4.5%) | 188 (9.5%) |
| Goat | 765 (1.7%) | 37 (4.8%) |
| Horse | 747 (1.7%) | 222 (29.7%) |
| Hare | 709 (1.6%) | 23 (3.2%) |
| Wild ruminants | 333 (0.8%) | 9 (2.7%) |
| Buffalo | 310 (0.7%) | 21 (6.8%) |
| Rodents | 151 (0.3%) | 46 (30.5%) |
| Fox | 220 (0.5%) | 50 (22.7%) |
| Other species | 204 (0.5%) | 71 (34.8%) |
| Not identified | 275 (0.6%) | 89 (32.4%) |
| Humans | 80 (0.2%) | 21 (26.3%) |
| Total | 43,935 | 6,279 |

**Table III.** *Italian serological data according to low (≥ 1:100) and high titers (≥ 1:400) in the microscopic agglutination test.*

| Species | Positive samples to a single serovar with low (≥ 1:100) and high titer (≥ 1:400) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Titers | AB | BalBal | CC | GG | IC | II | PP | SH | TT | Total |
| Cattle | ≥ 1:100 | 73 (3.4%) | 3 (0.1%) | 1 (0.05%) | 3 (0.1%) | 9 (0.4%) | 28 (1.3%) | 311 (14.4%) | 1,716 (79.6%) | 12 (0.6%) | 2,156 |
| | ≥ 1:400 | 13 (1.1 %) | - | - | - | 1 (0.1%) | 2 (0.2 %) | 161 (13.2%) | 1,042 (85.5%) | - | 1,219 |
| Swine | ≥ 1:100 | 1,537 (83.9%) | 1 (0.1%) | 3 (0.2%) | - | 13 (0.7%) | 3 (0.2%) | 247 (13.5%) | 14 (0.8%) | 14 (0.8%) | 1,832 |
| | ≥ 1:400 | 153 (48.9%) | - | - | - | 2 (0.6%) | - | 141 (45%) | 4 (1.3%) | 13 (4.2%) | 313 |
| Dog | ≥ 1:100 | 154 (39.3%) | - | 41 (10.5%) | 3 (0.7%) | 126 (32.1%) | 63 (16.1%) | 2 (0.5%) | 3 (0.7%) | - | 392 |
| | ≥ 1:400 | 83 (57.6%) | - | 7 (4.9%) | 1 (0.7%) | 27 (18.8%) | 22 (15.3%) | 2 (1.4%) | 2 (1.4%) | - | 144 |
| Horse | ≥ 1:100 | 135 (78.5%) | 1 (0.6%) | 2 (1.7%) | 5 (2.9%) | 12 (7%) | 10 (5.8%) | 2 (1.7%) | 3 (1.7%) | 2 (1.7%) | 172 |
| | ≥ 1:400 | 29 (87.9%) | - | - | - | 2 (6.1%) | - | 2 (6.1%) | - | - | 33 |
| Wild boar | ≥ 1:100 | 149 (90.8%) | - | - | 1 (0.6%) | 2 (1.2%) | - | 10 (6.1%) | - | 2 (1.2%) | 164 |
| | ≥ 1:400 | 59 (89.4%) | - | - | 1 (1.5%) | - | - | 4 (6.1%) | - | 2 (3.0%) | 66 |
| Sheep | ≥ 1:100 | 9 (9.1%) | - | - | - | 1 (1.01%) | 3 (3.03%) | 1 (1.01%) | 85 (85.8%) | - | 99 |
| | ≥ 1:400 | - | - | - | - | - | - | - | 53 (100%) | - | 53 |
| Fox | ≥ 1:100 | 33 (71.7%) | - | - | 1 (2.2%) | 12 (26.1%) | - | - | - | - | 46 |
| | ≥ 1:400 | 13 (76.5%) | - | - | - | 4 (23.5%) | - | - | - | - | 17 |
| Rodents | ≥ 1:100 | 32 (91.4%) | - | - | - | 1 (2.8%) | - | 2 (5.7%) | - | - | 35 |
| | ≥ 1:400 | 15 (93.8%) | - | - | - | - | - | 1 (6.3%) | - | - | 16 |
| Goat | ≥ 1:100 | 2 (7.1%) | - | - | - | - | 11 (39.3%) | 3 (10.7%) | 12 (42.8%) | - | 28 |
| | ≥ 1:400 | 1 (8.3%) | - | - | - | - | - | 1 (8.3%) | 10 (83.3%) | - | 12 |
| Hare | ≥ 1:100 | 10 (43.5%) | 2 (8.7%) | - | 7 (30.4%) | 1 (4.3%) | - | 1 (4.3%) | 2 (8.7%) | - | 23 |
| | ≥ 1:400 | 5 (50%) | - | - | 3 (30%) | 1 (10%) | - | 1 (10%) | - | - | 10 |
| Buffalo | ≥ 1:100 | 4 (20%) | - | - | - | 2 (10%) | - | - | 14 (70%) | - | 20 |
| | ≥ 1:400 | - | - | - | - | - | - | - | 9 (100%) | - | 9 |
| Wild ruminants | ≥ 1:100 | 1 (33.3%) | - | - | - | - | - | 2 (66.6%) | - | - | 3 |
| | ≥ 1:400 | - | - | - | - | - | - | 1 (100%) | - | - | 1 |
| Other animal species | ≥ 1:100 | 10 (15.4%) | - | - | - | 1 (1.5%) | 1 (1.5%) | - | - | 53 (81.5%) | 65 |
| | ≥ 1:400 | 8 (15.4%) | - | - | - | 1 (1.9%) | - | - | - | 43 (82.7%) | 52 |
| Total | ≥ 1:100 | 2,149 (42.7%) | 7 (0.1%) | 47 (0.9%) | 20 (0.4%) | 180 (3.6%) | 119 (2.4%) | 581 (11.5%) | 1,849 (36.7%) | 83 (1.6%) | 5,035 |
| | ≥ 1:400 | 379 (19.5%) | - | 7 (0.4%) | 5 (0.3%) | 38 (1.6%) | 24 (1.2%) | 314 (16.2%) | 1,120 (57.7%) | 58 (3%) | 1,945 |

AB = Australis Bratislava; BalBal = Ballum Ballum; CC = Canicola Canicola; GG = Grippotyphosa Grippotyphosa; IC = Icterohaemorrhagiae Copenhageni; II = Icterohaemorrhagiae Icterohaemorrhagiae; PP = Pomona Pomona; SH = Sejroe Hardjo; TT = Tarassovi Tarassovi.

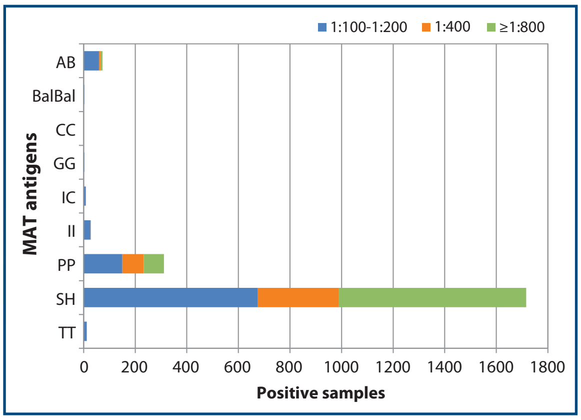


**Figure 1.** *Serological titers detected with microscopic agglutination test (MAT) in cattle positive serum samples.*

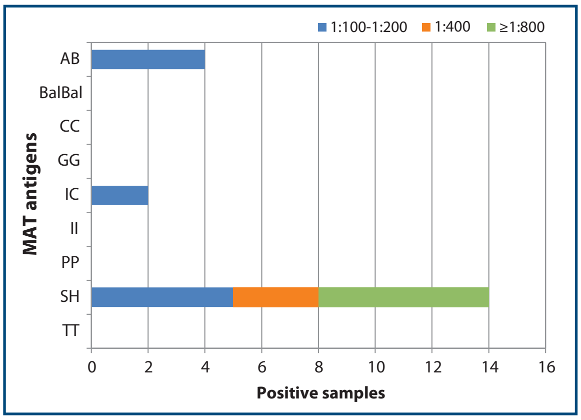


**Figure 2.** *Serological titers detected with microscopic agglutination test (MAT) in buffalo positive serum samples.*



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(serogroup Australis) (90.8%) and Pomona (serogroup Pomona) (6.1%) were prevalent (Table III). If the cut-off ≥ 1:400 was considered, 89.4% of samples was positive against Bratislava, 6.1% was positive against Pomona, and 3% was positive against Tarassovi (Table III and Figure 6).

## Sheep and goats

Two thousand four hundred sixty-six sheep and 765 goat sera were tested. Positive reactions were detected in 105 (4.3%) sheep and 37 (4.8%) goat samples (Table II). In both cases, serovar Hardjo was prevalent (85.8% and 42.8%, respectively). If the

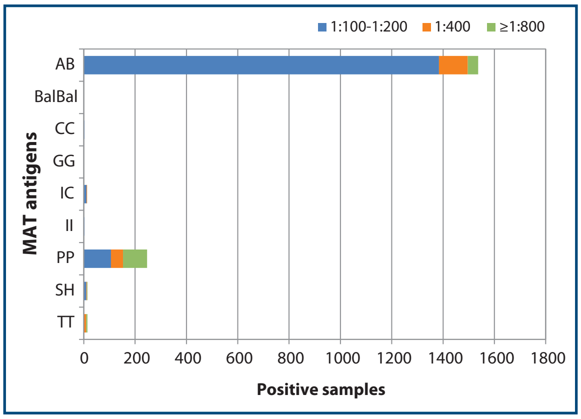


**Figure 3.** *Serological titers detected with microscopic agglutination test (MAT) in swine positive serum samples.*

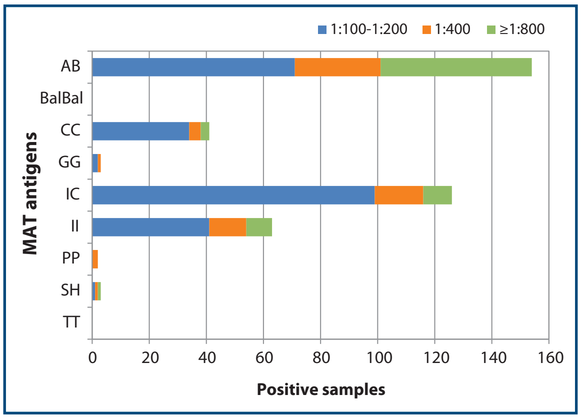


**Figure 4.** *Serological titers detected with microscopic agglutination test (MAT) in dog positive serum samples.*

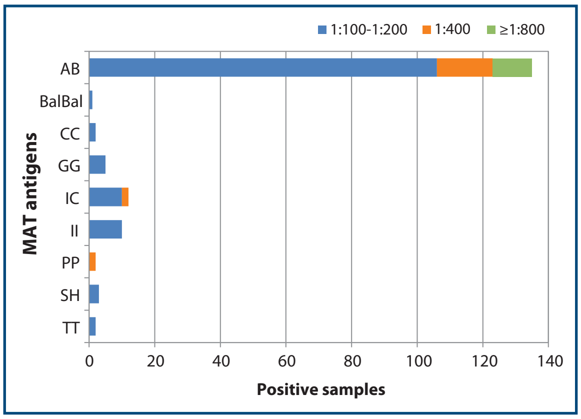


**Figure 5.** *Serological titers detected with microscopic agglutination test (MAT) in horse positive serum samples.*

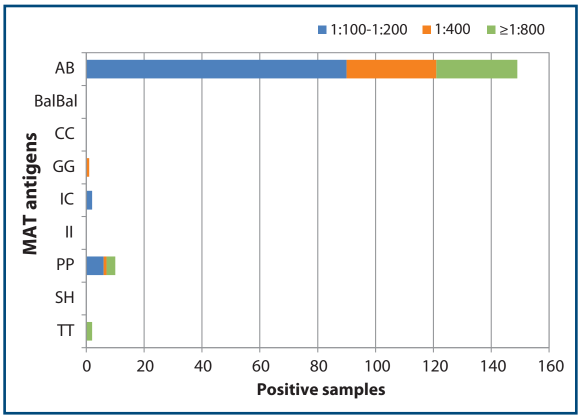


**Figure 6.** *Serological titers detected with microscopic agglutination test (MAT) in wild boar positive serum samples.*

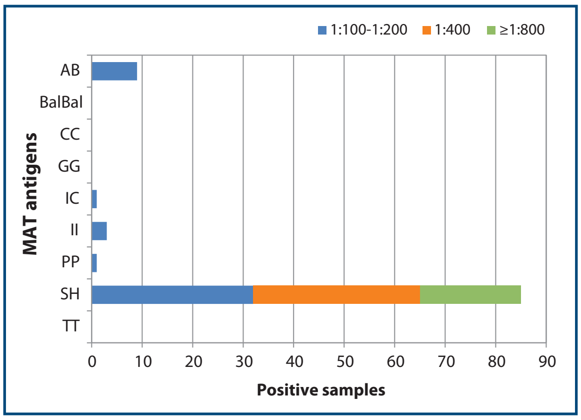


**Figure 7.** *Serological titers detected with microscopic agglutination test (MAT) in sheep positive serum samples.*

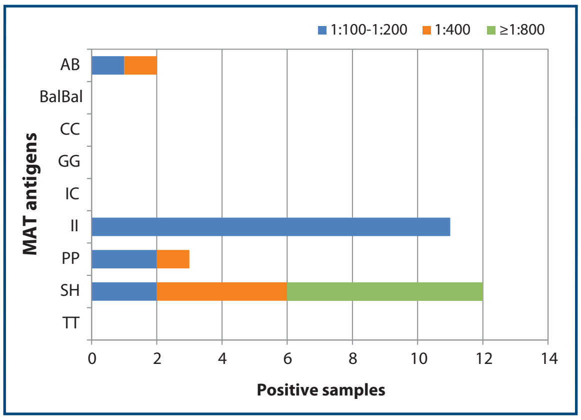


**Figure 8.** *Serological titers detected with microscopic agglutination test (MAT) in goat positive serum samples.*

cut-off ≥ 1:400 was considered, 100% of samples was positive against Hardjo in sheep, while 83.3% in goats (Table III; Figure 7 and Figure 8).

### Wild animals

One hundred and ninety-nine out of 1,617 sera, collected from different wild animal species (foxes, rodents, hares, wild ruminants and other species), as described more in details in Table II, showed to be positive.

If the cut-off ≥ 1:400 (Table III, Figure from 9 to 13) was considered, 93.8% of rodents, 76.5% of foxes, 50% of hares and 15.4% of other species were positive against Bratislava; 23.5% of samples was positive against Copenhageni in foxes. Reactions have also been detected against Grippotyphosa in hares (30%) and Copenhageni in foxes (23.5%). The high percentage of positivities against Tarassovi (82.7%) is related to samples collected from turtles.

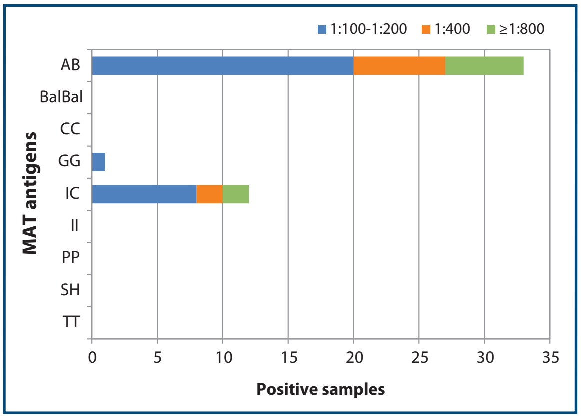


**Figure 9.** *Serological titers detected with microscopic agglutination test (MAT) in fox positive serum samples.*

## Discussion

### Cattle

Cattle are the maintenance hosts of serovar Hardjo. Infection by serovar Hardjo generally results in no clinical signs, which are not always detected by the MAT. Nevertheless, these animals may be infected

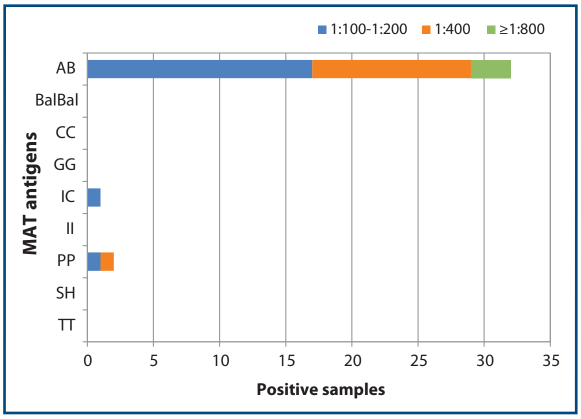


**Figure 10.** *Serological titers detected with microscopic agglutination test (MAT) in rodents positive serum samples.*

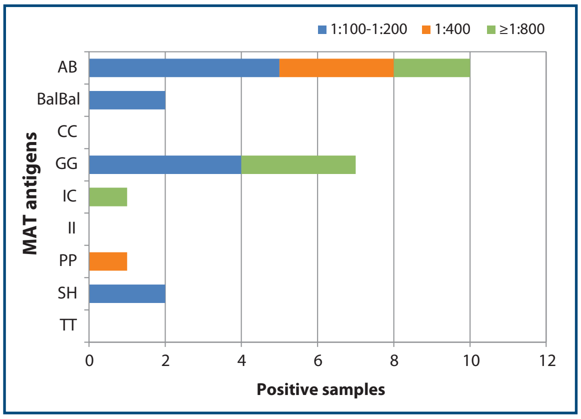


**Figure 11.** *Serological titers detected with microscopic agglutination test (MAT) in hare positive serum samples.*

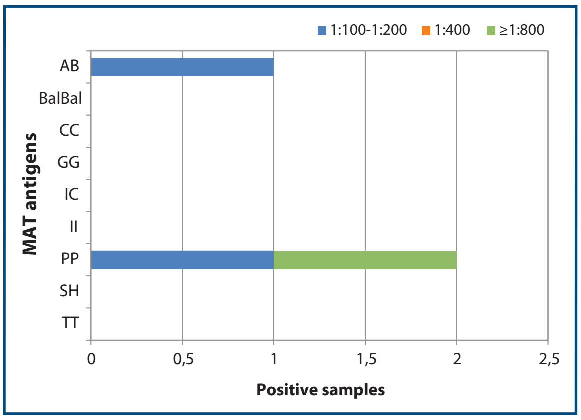


**Figure 12.** *Serological titers detected with microscopic agglutination test (MAT) in wild ruminants positive serum samples.*

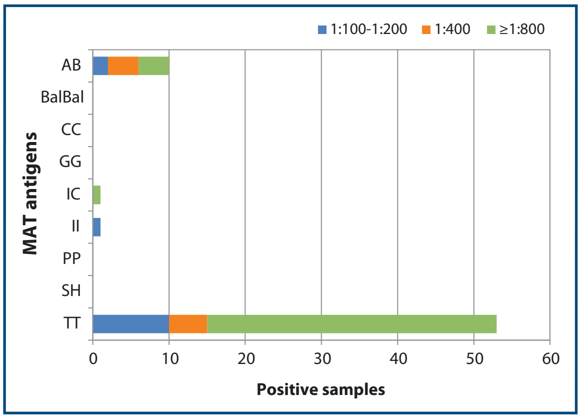


**Figure 13.** *Serological titers detected with microscopic agglutination test (MAT) in other species positive serum samples.*

and shed leptospires through urine (Bolin 2003). In Italy, some strains isolated from urine belong to genotype hardjobovis (Tagliabue and Farina 1995, Tagliabue 1990).

Serovar Pomona is generally associated to abortion and it has been isolated from dairy cattle in Italy (Luini *et al.* 1993). The seroprevalence detected against serogroup Australis suggests the adaptation of this *Leptospira* to domestic animals, confirmed also by the isolation of the serovar Lora from cattle in Italy (Autorino *et al.* 1994).

## Swine

Swine are the reservoir of serovars Pomona and Tarassovi, which were isolated from kidneys in Italy (Tagliabue 1990). Outbreaks caused by serovar Tarassovi are decreasing (Tagliabue and Farina 1995), while seropositivity against serovar Bratislava is widely spread. The high prevalence of seropositivity with a cut-off ≥ 1:100 should raise concern because strains belonging to serogroup Australis (serovars Bratislava, Muenchen, and Lora) have been associated to abortion in pregnant sows and have also been isolated in seronegative pigs and aborted foetuses in Europe and USA (Ellis *et al.* 1986, Ellis and Thiermann 1986, Ellis 1989, Ellis *et al.* 1991, Hartman *et al.* 1975). However, strains belonging to serogroup Australis have not been yet isolated in Italy (Tagliabue and Farina 1995, CRNL data 2010-2011).

## Dogs

Leptospires belonging to serogroup Icterohaemorrhagiae (serovars Copenhageni and Icterohaemorrhagiae) and Canicola are typically involved in canine leptospirosis and controlled with the use of vaccines. A decreasing seroprevalence to serogroup Icterohaemorrhagiae has been reported (Cerri *et al.* 2003). This study shows a low number of positivities for Canicola. The wide use of vaccination is responsible of the decrease of positivities against this serovar in Europe (André-Fontaine 2006) and in Italy (Scanziani *et al.* 1994, Scanziani *et al.* 2002). The high prevalence of Bratislava reported in this study is consistent with previous studies (Cerri *et al.* 2003, Tagliabue and Farina 1995) and it is confirmed by the isolation of the strain from infected urine in Europe (Van den Broek *et al.* 1991, Nielsen *et al.* 1991). Multiple positivities to two or more serovars are usually detected in dogs, as it was the case also in this study. This can be due to cross-reactions between antigens of different serovars or mixed infections. In this case, the MAT was not able to identify the real aetiological agent (Cerri *et al.* 2003, Tagliabue and Farina 1995).

## Horse

Horses are frequently infected by pathogenic *Leptospira* spp., confirmed by serological evidences, but the disease is rare.

Isolation of Bratislava from foetuses and kidneys in Northern Ireland may explain the role of horses in the maintenance of this serovar (Ellis *et al.* 1983). However, the virulence of Bratislava is not clear, due to the seropositivities detected in apparently healthy horses (Tagliabue and Farina 1995). In a serological study on healthy slaughtered horses coming from Italy and Poland, serogroup Australis was detected in the former and Grippotyphosa in the latter (Soldati *et al.* 1995). The present study confirms the prevalence of serovar Bratislava also at high titres as recorded in the existant literature (Cerri *et al.* 2003, Tagliabue and Farina 1995); while serovar Grippotyphosa, maintained in *Microtus arvalis* widespread in East Europe (Codazza *et al.* 1990), was detected in few cases. The serogroup Icterohaemorrhagiae, previously related to the presence of rodents (Hashimoto *et al.* 2007), was detected in horses in this study although in a low number of sera.

## Wild boars

Wild boars are known as animal hosts for *Leptospira* spp. that are important in the epidemiology of infection. Pomona and Bratislava are the most frequently detected serovars in Europe. The increase of population density of wild boars and the consequent migration to agricultural and urban areas have been noticed in many European countries and also in Italy. The infection is endemic and so the risk of interaction between wild boars, humans, and domestic animals could rise making possible the spreading of new serovars (Ebani *et al.* 2003, Figarolli *et al.* 2012, Jensen *et al.* 2006, Jensen *et al.* 2007, Mason *et al.* 1998, Vengust *et al.* 2008). Positive reactions against Icterohaemorrhagiae, Copenhageni, Sejroe, Saxkoebing, Tarassovi, and Canicola have been also detected, frequently at low titers, such as Bratislava and Pomona (Farina and Andreani 1970, Montagnaro *et al.* 2010, Piredda *et al.* 2011, Vengust *et al.* 2008).

## Sheep and goats

Although sheep and goats are less susceptible to *Leptospira* infection than cattle, outbreaks have been recorded in countries where their breeding is commonly performed, like Italy (Cerri *et al.* 1996, Ciceroni *et al.* 2000).

Hardjo, genotype hardjobovis is the most involved serovar in *Leptospira* infection. It has been isolated from urine of infected sheep, which are considered maintenance hosts and carriers (Fusi *et al.* 1994).

### Wild animals

The main serovar in the species included in this study, especially in carnivors and in rodents, is Bratislava. The detection of serovar Grippotyphosa in hares is in agreement with other studies and it is due to the import of such animals from East Europe, where this serovar is endemic (Tagliabue and Farina 1995, Zanni *et al.* 1992, Zanni *et al.* 1995). The serovar Copenhageni (serogroup Icterohaemorrhagiae) is detected in foxes. Of interest is the high prevalence of Tarassovi in turtels. It seems that this serovar is widespread in the United States, although few data are available (Glosser *et al.* 1974).

## Conclusions

The purpose of this study was to update the knowledge about the prevalence and the distribution of leptospirosis in Italy.

The data obtained showed that leptospirosis is present in a range of domestic and wild species in Italy.

The epidemiological situation in domestic and wild animals reported in this study does not appear particularly different from that described in previous serological findings (Tagliabue and Farina 1995, Cerri *et al.* 2003). Serovar Hardjo confirms its role in determining infection in ruminants, while serovar Bratislava is prevalent in other species.

The evaluation of MAT titres should take into account the vaccinal antibody interferences.

Low titres against Bratislava, Pomona, and Tarassovi in swine could be of vaccinal origin. Similarly, in dogs low titres are obtained against the serogroups traditionally included in vaccines, Icterohaemorrhagiae and Canicola. Given that the vaccine protection in dogs is serovar – specific, when new canine vaccine formulations including *e.g.* Bratislava and Grippotyphosa – the antibody dynamic of these serovars has to be considered.

In dogs is very frequent the occurrence of multipositive sera, sometimes due to cross-reactions, and this makes difficult to identify the real circulation of leptospires in this species. Since dogs are considered as sentinel of human zoonoses, they should be regularly monitored. It is also advisable to accentuate surveillance in wild animals with a targeted monitoring for some species, *e.g.* those considered in this study, since the low number of samples does not allow for drawing conclusive considerations.

In order to have a quick and continuous availability of data, the National Reference Centre for Leptospirosis is developing a computerised system for collecting and processing the data of the sera including multipositive ones (*e.g.* selecting the serogroup with the higher titre).

The creation of a network able to collect all national laboratory data should be useful for the surveillance of leptospirosis in Italy and to enable the exchange of information between the Italian and International Health Institutions.

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## References


André-Fontaine G. 2006. Canine leptospirosis - Do we have a problem? *Vet Microbiol*, **117**, 19-24.

Autorino G.L., Salvati F., Ammadeo D., Korver H. & Ellis W.A. 1994. Identification of leptospires belonging to the Australis group isolated in central Italy. *In* Proc. 8th Meeting of European Leptospira Workers, Anzio (Rome), July 11th-13th 1994, 15.

Bolin C.A. 2003. Diagnosis and control of bovine leptospirosis. *In* Proc. 6th Western Dairy Management Conference, March 12-14, Reno, NV, 155-159.

Cerri D., Ebani V.V., Fratini F., Pinzauti P. & Andreani E. 2003. Epidemiology of leptospirosis: observations on serological data obtained by a "diagnostic laboratory for leptospirosis" from 1995 to 2001. *New Microbiol*, **26**, 383-389.

Cerri D., Nuvoloni R., Ebani V., Pedrini A., Mani P., Andreani E. & Farina R. 1996. *Leptospira interrogans* serovar *hardjo* in the kidneys and genital tracts of naturally infected sheep. *New Microbiol*, **19**, 175-178.

Chappel R.J. & Smythe L.D. 2012. Leptospirosis-importance of a One Health approach. *Microbiol Australia*, **4**, 154-156.

Ciceroni L., Lombardo D., Pinto A., Ciarrocchi S. & Simeoni J. 2000. Prevalence of antibodies to *Leptospira* serovars in sheep and goats in Alto Adige-South Tyrol. *J Vet Med B*, **47**, 217-223.

Codazza D., Sala V., Tagliabue S., Piccinini R. & Cugini F.P. 1990. Indagini sieroepidemiologiche in equini importati dall'Europa Orientale a scopo macellazione. *Atti Soc Ital Sci Vet*, **44**, 1071-1074.

Ebani V., Cerri D., Poli A. & Andreani E. 2003. Prevalence of *Leptospira* and *Brucella* antibodies in wild boars (*Sus scrofa*) in Tuscany, Italy. *J Wildl Dis*, **39** (3), 718-722.

Ellis W.A., McParland P.J., Bryson D.G. & Cassels J.A. 1986. Prevalence of *Leptospira* infection in aborted pigs in Northern Ireland. *Vet Rec*, **118**, 63-65.

Ellis W.A. & Thiermann A.B. 1986. Isolation of *Leptospira interrogans* serovar *bratislava* from sows in Iowa. *Am J Vet Res*, **47** (7), 1458-1460.

Ellis W.A. 1989. *Leptospira* Australis infection in pigs. *Pig Vet J*, **22**, 83-89.

Ellis W.A., Montgomery J.M. & Thiermann A.B. 1991. Restriction endonuclease analysis as a taxonomic tool in the study of pig isolates belonging to the Australis serogroup of *Leptospira interrogans*. *J Clin Microbiol*, **29** (5), 957.

Ellis W.A., O'Brien J.J., Cassells J.A. & Montgomery J.M. 1983. Leptospiral infection in horses in Northern Ireland: serological and microbiological findings. *Equine Vet J*, **15** (4), 317-320.

Faine S., Adler B., Bolin C. & Perolat P. 1999. *In Leptospira* and leptospirosis. 2nd ed. MediSci, Melbourne, Australia.

Farina R. & Andreani E. 1970. Leptospirosi degli animali selvatici in Italia. *Arch Vet Ital*, **21** (2-3), 127-141.

Figarolli B.M., Gaffuri A., Alborali G.L., D'Incau M., Tagliabue S. 2012. Survey on leptospirosis in wild boars (*Sus scrofa*) in Lombardy, Northern Italy. *In* Proc. European Meeting of Leptospirosis, Eurolepto, 31 May-2 June 2012, Dubrovnik (Croatia), 63-64.

Fusi P., Savio M.L., Tagliabue S., Amaddeo D., Autorino G.L. & Pacciarini M.L. 1994. Genomic variability of *L. borgpetersenii* serovar *hardjo* type hardjobovis isolates in Italy. *In* Proc. 8th Meeting of European Leptospira Workers, Anzio (Rome), July 11th -13th 1994, 65.

Glosser J.W., Sulzer C.R., Eberhardt M. & Winkler W.G. 1974. Cultural and serologic evidence of *Leptospira interrogans* serotype Tarassovi infection in turtles. *J Wildl Dis*, **10** (4), 429-35.

Hartman E.G., Brummelman B. & Dikken H. 1975. *Leptospirae* of serotype Lora of the serogroup Australis isolated for the first time from swine in the Netherlands. *Tijdschr Diergenesk*, **100**, 421-425.

Hartskeerl R.A., Collares-Pereira M. & Ellis W.A. 2011. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin Microbiol Infec*, **17**, 494-501.

Hashimoto V.Y., Gonçalves D.D., da Silva F.G., de Oliveira R.C., Alves L.A., Reichmann P., Muller E.E. & de Freitas J.C. 2007. Occurence of antibodies against *Leptospira* spp. in horses of the urban area of Londrina, Paraná, Brazil. *Rev Inst Med Trop S Paulo*, **49** (5), 327-330.

Jensen A., Luge E., Guerra B., Wittschen P., Gruber A.D., Loddenkemper C., Schneider T., Lierz M., Ehlert D., Appel B., Stark K. & Nöckler K. 2007. Leptospirosis in urban wild boars, Berlin, Germany. *Emer Infect Dis*, **13** (5) 739-742.

Jensen A., Nöckler K., Schönberg A., Luge E., Ehlert D. & Schneider T. 2006. Wild boars as possible source of hemorrhagic leptospirosis in Berlin, Germany. *Eur J Clin Microbiol Infect Dis*, **25**, 544-546.

Levett P.N. 2001. Leptospirosis. *Clin Microbiol Rev*, **14** (2), 296-326 .

Luini M., Scanziani E., Giusti A.M., Tagliabue S., Bignazzi R., Bovera C. 1993. Leptospirosi da *Leptospira interrogans* sierogruppo Pomona in bovine da latte. *Praxis Vet*, **14** (4), 5-6.

Mason R.J., Fleming P.J.S., Smythe L.D., Dohnt M.F., Norris M.A. & Symonds M.L. 1998. *Leptospira interrogans* antibodies in feral pigs from New South Wales. *J Wildl Dis*, **34** (4), 738-743.

Montagnaro S., Sasso S., De Martino L., Longo M., Iovane V., Ghiurmino G., Pisanelli G., Nava D., Baldi L. & Pagnini U. 2010. Prevalence of antibodies to selected viral and bacterial pathogens in wild boars (*Sus scrofa*) in Campania region, Italy. *J Wildl Dis*, **46** (1), 316-319 .

Nielsen J.N., Cochran G.K., Cassels J.A. & Hanson L.E. 1991. *Leptospira interrogans* serovar Bratislava infection in two dogs. *J Am Vet Med Assoc*, **199**, 351-352.

Piredda I., Palmas B., Noworol M., Canu M., Fiori E., Picardeau M., Tola A., Pintore A. & Ponti N. 2011. Indagine sulla prevalenza della leptospirosi nei cinghiali del centro-nord Sardegna. Atti del XIII Congresso Nazionale SIDILV-Trani, 12-14 Ottobre 2011, 63-64.

Scanziani E., Calcaterra S., Tagliabue S., Luini M., Giusti A. M. & Tomba M. 1994. Serological findings in cases of acute leptospirosis in the dog. *J Small Anim Pract*, **35**, 257-260.

Scanziani E., Origgi F., Giusti A.M., Iacchia G., Vasino A., Pirovano G., Scarpa P. & Tagliabue S. 2002. Serological survey of leptospiral infection in kennelled dogs in Italy. *J Small Anim Pract*, **43**, 154-157.

Soldati G., Tagliabue S., Fontana M. C., Iotti G. & Bertaglia L. 1995. Leptospirosi: monitoraggio sierologico in equini della provincia di Modena e di importazione. *Prog Vet*, **50** (22), 743-746.

Tagliabue S. & Farina R. 1995. Inchiesta epidemiologica sulla diffusione delle leptospirosi tra gli animali domestici ed alcune specie selvatiche. *Sel Vet*, **36** (11-12), 941-952.

Tagliabue S. 1990. Nuove acquisizioni nella diagnosi di leptospirosi. *Atti Soc Ital Sci Vet*, **44**, 963-967.

Van den Broek A.H.M., Thrusfield M.V., Dobbie G.R. & Ellis W.A. 1991. A serological and bacteriological survey of leptospiral infection in dogs in Edinburgh and Glasgow. *J Small Anim Pract*, **32**, 118-124.

Vengust G., Lindtner-Knific R., Zele D. & Bidovec A. 2008. *Leptospira* antibodies in wild boars (*Sus scrofa*) in Slovenia. *Eur J Wildl Res*, **54**, 749-752.

World Organisation for Animal Health (OIE). 2008. Leptospirosis. *In* Manual of diagnostic test and vaccines for terrestrial animals. Chapter 2.1.9, 251-264. Paris, OIE.

World Health Oraganisation (WHO). 2011. Leptospirosis: an emerging public health problem. *Weekly Epidemiol Rec*, **86** (6), 45-52.

Zanni M.L., Capucci L., Fabbi M., Giovannini S., Lavazza A., Marzadori F., Tagliabue S., Poglayen G., Roda R. & Tasselli A. 1992. Programma di monitoraggio sanitario della lepre (*Lepus europeus* Pallas) in provincia di Ravenna. *Sel Vet*, **33** (10), 1103-1108.

Zanni M.L., Poglayen G., Marzadori F., Benassi M.C., Capucci L., Carnapé E., Fabbi M., Magnino S., Tagliabue S., Roda R., Tasselli A., Serra R., Venturi L., Bartolucci M., Galuppi R. & Lavazza A. 1995. Monitoraggio sanitario della lepre (*Lepus europeus* Pallas) in provincia di Ravenna. *Sel Vet*, **36** (1), 1-26.