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Abstract: Leptospirosis is a re-emerging bacterial zoonosisCentral Italy is characterized by a geographic area that promote Leptospira circulation.: Data on seroepidemiological survey carried out from 2002 to 2016 in Central Italy were reported and discussed. Overall, 709 out of the 8488 (8.35%) tested sera were positive for Leptospira at the cut-off titer (1:100) and 218 (2.57%) at higher titer (≥1:400). The highest percentages of positivity was recorded for coypus (22.86%), swine (19.74%) and bovine (, 13.03%). Pomona and Bratislava resulted the serovars more often detected, followed by Harjo and Icterohaemorragiea; while, a low number of positive sera was detected for serovars Ballum, Canicola and Tarassovi.. .. Percentage of positive sera for each year slightly decreased from 2002 to 2008 and rose from 2009., a rise in this; particularly, high percentages of positive reaction were recorded in 2014 (17.23%), 2015 (19.61%) and 2016 (38.05%).

Bertelloni et al., *Epidemiology of Leptospirosis in North-Central Italy: fifteen years of serological data (2002-2016)*.

Highlights

- Fifteen years of serological data on *Leptospira* in animals were presented;
- An increase positivity to *Leptospira* in last years of investigation was recorded;
- Rise or fall of some serovars were observed;
- Some changes in hosts/serovars relations were detected;

# Epidemiology of Leptospirosis in North-Central Italy: fifteen years of serological data (2002-2016)

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**Running Title**: *Leptospira* in North-Central Italy 2002-2016

**Abstract (ridurre a 150 parole)**

Leptospirosis is a re-emerging bacterial zoonosisCentral Italy is characterized by a geographic area that promote *Leptospira* circulation.: Data on seroepidemiological survey carried out from 2002 to 2016 in Central Italy were reported and discussed. Overall, 709 out of the 8488 (8.35%) tested sera were positive for *Leptospira* at the cut-off titer (1:100) and 218 (2.57%) at higher titer (≥1:400). The highest percentages of positivity was recorded for coypus (22.86%), swine (19.74%) and bovine (, 13.03%). Pomona and Bratislava resulted the serovars more often detected, followed by Harjo and Icterohaemorragiea; while, a low number of positive sera was detected for serovars Ballum, Canicola and Tarassovi.. .. Percentage of positive sera for each year slightly decreased from 2002 to 2008 and rose from 2009., a rise in this; particularly, high percentages of positive reaction were recorded in 2014 (17.23%), 2015 (19.61%) and 2016 (38.05%).

**Key words**: *Leptospira*, epidemiology, seroprevalence; surveillance; zoonosis.

## 1. Introduction

Leptospirosis represents one of the most important widespread re-emerging bacterial zoonosis [1–3]. Several outbreaks accounting for thousands of deaths worldwide highlight the importance of leptospirosis as a severely neglected infectious disease [4,5]. Leptospirosis has a wide distribution and occurs overall in tropical, subtropical and temperate zones, favoured by a large variety of both wild and domestic mammals which can play the role of natural reservoirs of *Leptospira* [1,6]. Some animals are asymptomatic renal carriers of this bacterium and they contribute to maintain the infection in a particular environment by constantly shedding *Leptospira* with urine [7,8]. Accidental contact with *Leptospira* infected urine causes the incidental infection and produces clinical diseases. While, specific *Leptospira* serovars which show close relationship with particular animal species develop host-maintained infection. The maintenance host generally does not develop symptoms, except after long time, but it acts as a natural source of a specific serovar [6,8]. In fact, *Leptospira* epidemiology is strictly related to the presence and widespread of the maintenance hosts species [9]. In recent years, some serovars seem to be prevalent and emerging, especially among wild animals, but also in domestic species. This occurrence suggests that the epidemiology of leptospirosis may change over time in animals as well as in humans [10].

Central Italy, and in particular Tuscany, is a geographic area characterized by some peculiarities which promote the presence and persistence of *Leptospira* in hosts and in environment: a) presence of wild animals which could represent potential reservoirs; b) presence of domestic animals raised in semi-extensive or extensive farms, which promotes contact with wild species; c) a significant presence of hunting activity; d) abundance of wetlands such as marshes, ponds and irrigation canals.

The main purpose of the present work was to refer data on a sero-epidemiological survey carried out in Central Italy, particularly in Tuscany, on serum samples collected from 2002 to 2016 in order to assess the prevalence of leptospirosis in domestic and wild animals and to compare it with the data from a previous epidemiological surveillance investigation carried out in the same area between 1995 and 2001 [9].

## 2. Material and Methods

From January 2002 to December 2016, 8488 serum samples were analysed for *Leptospira*. Sera were collected from healthy and ill animals belonging to 23 different species: sheep (*Ovis aries*, 2682 sera), swine (*Sus scrofa*, 1332 sera), bovine (*Bos Taurus*, 1328 sera), dog (*Canis lupus familiaris*, 1144 sera), wild boar (*Sus scrofa*, 479 sera), goat (*Capra hircus*, 327 sera), european brown hare (*Lepus europaeus*, 162 sera), red fox (*Vulpes vulpes*, 94 sera), horse (*Equus caballus*, 74 sera), roe deer (*Capreolus capreolus*, 74 sera), coypus (*Myocastor coypus*, 70 sera), fallow deer (*Dama dama*, 65 sera), donkey (*Equus asinus*, 2 sera), ferret (*Mustela putorius furo*, 1 serum), cat (*Felis catus*, 4 sera), red deer (*Cervus elaphus*, 56 sera), wolf (*Canis lupus*, 43 sera), rats (*Rattus norvegicus*, 34 sera), mouflon (*Ovis musimon*, 8 sera), mouse (*Mus musculus*, 8 sera), Guinea pig (*Cavia porcellus*, 2 sera), alpaca (*Vicugna pacos*, 1 serum) and bear (*Ursus arctos*, 1 serum). Furthermore, 329 sera were of human origin and collected from men with clinical symptoms referable to leptospirosis. All sera were collected in North-Central Italy. Serological investigation was carried out with Microscopic Agglutination Test (MAT) [11]. The following serovars were used as live antigens: Icterohaemorrhagiae (strain Bianchi), Canicola (strain Alarik), Pomona (strain Mezzano), Tarassovi (strain Mitis Johnson), Grippotyphosa (strain Moscow V), Bratislava (strain Riccio 2), Ballum (strain Castellon 3) and Hardjo (strain Hardjoprajitno). Titers of 1:100 were considered positive; 2-fold serial dilutions were tested to determine the endpoint titer.

## 3. Results

Overall, 709 out of the 8488 (8.35%) sera resulted positive for *Leptospira* at the breakpoint titer (1:100). All samples collected from the following species resulted negative: alpaca, donkey, guinea pig, mouse, rat, ferret, cat, fallow deer, red deer, roe deer, mouflon, bear and wolf. Two hundred and eighteen sera (2.57%) resulted positive at high titer (≥1:400). Table 1 reports the number of positive sera grouped by animal species. The highest percentages of positive sera were recorded for coypus, swine and bovine at low (22.86%, 19.74% and 13.03%, respectively) and high titer (5.61%, 6.31% and 3.16%, respectively). None horse sera showed positivity to titer of 1:400 or higher. Coypus, wild boar, fox and hare resulted the unique wild species that showed positive reactions. In particular, 22.86%, 8.56%, 3.04% and 1.85% of sera resulted

positive at titer ≥1:100 for coypus, wild boar, fox and hare, respectively. Among the 329 human sera examined, 10 (4.26%) resulted positive and 3 (1.06%) showed a titer of 1:400 or higher.

Table 2 shows the distribution of positive sera grouped by serovars and animal species. Pomona and Bratislava resulted the serovars more often detected, with 267 and 224 positive reactions (titer 1:100), respectively. Moreover, for the same serovars, 68 and 67 sera, respectively showed positivity to high titer (≥1:400). A low number of positive sera was detected for serovars Ballum, Canicola and Tarassovi (13, 28 and 32 sera, respectively). Hare was the only species in which positivity to serovar Icterohaemorrhagiae was never found. Similarly, goat resulted the only species in which serovar Bratislava was never detected. Positivity to serovars Icterohaemorrhagiae and Canicola was found mainly in dog sera. Serovars Pomona and Bratislava resulted more often associated with swine. Most of the sera positive to Tarassovi were from wild boar. Positivity to serovars Grippotyphosa and Hardjo was detected especially in bovine sera.

Among human sera, positivity to six serovars was recorded: Icterohaemorrhagiae, Canicola, Pomona, Grippotyphosa, Bratislava and Ballum. Icterohaemorrhagiae was the most detected serovar. Positive samples were never found among human sera from 2004 to 2012. In 2013, 2014 and 2016 positive sera were 1/20, 4/9 and 1/11, respectively; all these sera showed an antibody titer <1:400.

Percentage of positive sera for each year slightly decreased from 2002 to 2008; in particular in 2008 only 1 out of 597 (0.17%) sera resulted positive at titer of 1:100. From 2009, the percentage of positive sera increased; particularly, in 2014, 2015 and 2016 a high percentage of positive sera was recorded: 17.23%, 19.61% and 38.05%, respectively. Figure 1 reports the percentage of positive sera to *Leptospira interrogans* and to each serovar detected in each year of investigation.

Table S1 (supplementary material) reports the distribution of positive sera at titer ≥ 1:100 and ≥1:400 in relation to animal species, *Leptospira* serovars and year of detection.

## 4. Discussion

Leptospirosis is a worldwide public health and veterinary problem, frequently underestimated, characterized by a downward trend [12]. Climatic changes, modifications of ecological niches, emergence

of new potential maintenance-hosts could represent the most important factors involved in *Leptospira* epidemiology. The environmental and geographic features of North-Central Italy area can be considered as the optimal conditions for *Leptospira* spreading [13–21]. Tuscany, the main region involved in this serological survey, is characterized by some peculiarities which promote the presence and persistence of *Leptospira* in hosts and in the environment. Increasing presence of wild animals, potential reservoirs, presence of animals raised in semi-extensive or extensive farms, hunting activity and presence of wetlands such as marshes, ponds, lakes and irrigation canals are the main factors involved in enzootic trend of leptospirosis in Italy.

In this study, serological results obtained whit a panel of eight *Leptospira* serovars were reported. Strains employed as live-antigens was chosen considering the serovars more often detected, by isolation or serology, in Italy [10,13,17,18,22–24]; indeed, positivity to other serovars were rarely reported [25]. Moreover, these strains were employed routinely in our Laboratory for research and diagnosis, and, for this reason, all serum samples considered were tested with same MAT antigens.

In this survey, the total apparent prevalence of antibodies against *Leptospira* registered during 2002-2016 was 8.65%. In a previous investigation, carried out in the same area during 1995-2001, the total apparent prevalence was 6.81% [9]. It is not possible to exclude that this increase could be due to the different number of available samples and the proportion and representation of the different animal species included in the study. However, the increase could be also related to a changing of some environmental conditions (rainfall, temperature, ecc…) which promoted the rise and spreading of new serovar/strains or the re-emerging of endemic strains. The observed increase of positive sera was mainly related to the last years of this investigation (2013-2016) and could be due to an increase of rainfall in the investigated area, especially in wetlands.

Serological investigations highlighted different apparent prevalence trends for each of the eight *Leptospira* serovars tested. Apparent prevalence of positive sera decreased from 1995-2001 to 2002-2016 for Icterohaemorrhagiae (from 22.24% to 13.78%), Bratislava (from 55.04% to 24.69%) and Hardjo (from 22.08% to 14.22%). Furthermore, apparent prevalence increases for Canicola (from 0% to 3.08%), Pomona

(from 0.64% to 29.43%), Grippotyphosa (from 0% to 9.81%), Tarassovi (from 0% to 3.52%) and Ballum (from 0% to 1.43%) [9]. The results obtained from previous and from this investigation could be different for some reasons. In the previous work [9], carried out in the same investigated area, employing the same *Leptopsira* serovars and strains as antigens, the threshold titer was 1:400, conversely in this investigation the threshold titer used was 1:100. Furthermore, the number of tested sera for each animal species could have influenced the detected total apparent prevalence.

Considering the distribution of positivity detected year by year, it is possible to observe a decrease in the percentage of positive sera detected from 2002 to 2008 and an increase starting from 2009. In particular, from 2014 it was registered an impressive increase of *Leptospira* positive samples (Figure 1a).

Annual fluctuations in *Leptospira* spreading and seropositivity is well documented [26] and could be related to many factors as climate changing, rains and drought springs. Fluctuations in observed data could be related not only to environmental modifications, but also to hosts changing: variations in exposure to *Leptospira* by animal, introduction of new wild animal species, changing in herds management. As concerns this last point, in lasts years breeding management changed from indoor intensive to extensive or semi-extensive with outdoor access in order to improve animal welfare. Furthermore, it could be related to modifications of *Leptospira* epidemiology: introduction of new serovars/strains or change in host specificity range by classical serovars/strains.

As for serovar Icterohaemorrhagiae, seropositivity reflected more or less the observed global trend. However, positivity to this serovar was constantly detected, even if during some years with only few positive samples (Figure 1b). This is an expected result, indeed, Icterohaemorrhagiae is maintained by rats and it is the serogroup most often involved in animal and human infection in many parts of the world [27].

Only few samples scored positive to serovar Canicola during the first years of investigation and no positive sera were detected between 2006 and 2012 (Figure 1c). It seems that this serovars re-emerged from 2013. In nature, Canicola is maintained by dog and vaccination programs carried out for about 50 years in this species led to its disappearance [28]. Data obtained in this investigation showed in recent years an increase

of positivity to Canicola in animals different to dogs. This finding could suggest a possible change in host range of this serovar.

Serovar Pomona seemed to be almost disappeared from the investigated area for more than fifteen years (Figure 1d). Ten positive reactions were recorded from 2002 to 2013, furthermore, a previous investigation conducted in the same geographical area [9], reported an apparent prevalence of 0.64% (4 out of 9885 sera) between 1995 and 2001. From 2014, the number of positive sera increased, and Pomona was the most detected serovar in 2016. In the last years of the investigation, a large number of swine sera were analysed, and this could have influenced the improved detection of positivity to Pomona. However, a considerable number of positive reactions was found also in sera of species other than pig, supporting the hypothesis of the rise of this serovar in investigated area.

Circulation of serovar Grippotyphosa in the investigated area was slightly documented during this investigation. This is in accordance with previous reports conducted in the same region and in Italy [9,10]. From 2013, a slight increase in the detected number of positive reactions was registered (Figure 1e). This trend is in accordance with many studies conducted in Europe, where Grippotyphosa is considered an emerging serovar [29–32]. Considering the studied area, it is not possible to exclude that this occurrence could be related to the import from East Europe of wild animals for hunting purposes, in particular hares [33,34].

Positivity to serovar Tarassovi was never detected from 2002 to 2013 (Figure 1f). This trend is in line with other National surveys [9,10], indeed, in past years, Tarassovi showed a very limited diffusion in Italy. In 2014, a peak of positivity was registered which could resemble an epidemic event. Positive reactions were found mainly in wild boar, but also in domestic animals. In 2015 and 2016, the number of positive sera decreased and probably it will return to zero. These results suggest that Tarassovi is not disappeared from our territory, and infections could occur, since Tarassovi is probably maintained in the environment by *reservoirs* other than swine, as suggested by previously reported data in Italy [10].

As regard serovar Bratislava, its trend reflects the global trend observed. Every year, positivity to this serovar was detected, with exception of 2008 and 2011 (Figure 1g). This finding could probably be expected

189 considering the high number of positive *reservoir* hosts present in investigated area, as hedgehog and wild
190 boar [35,36]. Generally, Bratislava is reported as an emerging serovar, but considering the obtained data it
191 could be considered as endemic in investigated area.
192 Considering the abundance of cattle and, especially, sheep herds in the studied area, the low number of
193 positivity for serovar Hardjo for many years was unexpected. Indeed, this serovar were frequently detected
194 in Italy and sometimes it was involved in clinical outbreaks [9,10,25]. As for the other serovars, after a silent
195 period of about 12 years, from 2014, an increase of positive sera for Hardjo was registered, suggesting a
196 restart of the circulation of this serovar in investigated area. More focused investigations should be
197 probably required to better understand this finding. It may be supposed it could be related to the increase
198 of outdoor herds, especially for dairy cows, related to the increased attention for animals welfare.
199 Furthermore, animal species different to domestic ruminants could be infected and this could contribute to
200 the rise of serovar Harjo.
201 During the investigated period, serovar Ballum showed a very limited diffusion. In 2014, a slight increase of
202 seropositivity was registered, but it was a limited event. In Italy, this serovar was rarely detected in
203 serological investigations [9,10,13,18,22], even if it was recently isolated from small mammals in Tuscany
204 [24]. These observations could suggest a low virulence for animals and man of Ballum strains circulating in
205 our territory.
206
207 4.1. Bovine
208 The total apparent prevalence in bovine increase from 0.005% (1995-2001) [9] to 13.03% (2002-2016). In
209 cattle, higher prevalence of serovar Hardjo has been detected: 7.08%8 (94/132) and 2.26% (30/1328) sera
210 scored positive at titer ≥ 1:100 and ≥ 1:400, respectively. These data confirm that bovine represents the
211 main maintenance-host for serovar Hardjo (serogroup Sejroe) [7]. Relative high number of positive
212 reactions was also detected for serovars Pomona and Grippotyphosa in cattle sera. A percentage of 4.15%
213 (55/1328) and 0.23% (3/1328) were positive for Pomona at titer ≥ 1:100 and ≥ 1:400, respectively. Severe
214 infections in cattle due to this serovar is uncommon and usually occur in young animals [7]. Nevertheless,

based on a recent National survey, Pomona resulted the second representative serovar in cattle in Italy [10]. The relative high number of positivity could be related to the semi-extensive or extensive farms. These types of breeding promote the contact with wild animal, in particular wild boars. As concern serovar Grippotyphosa, 3.61% (48/1328) and 0.38% (5/1328) sera scored positive at titer ≥ 1:100 and ≥ 1:400, respectively. Positivity to this serovar resulted higher than that observed in previous investigations conducted in Italy [9,10]. However, in recent years, in other European countries, serovar Grippotyphosa was often detected in cattle and occasionally involved in clinical leptospirosis outbreaks [26,29,30]. In accordance with these Authors, our data seem to identify Grippotyphosa as an emerging serovars in cow. Low level of positivity was scored for serovars Icterohaemorrhagiae, Canicola, Tarassovi, Bratislava and Ballum, in accordance with other studies [9,10].

4.2. Dog

The total apparent prevalence in dog increased from 5.42% (1995-2001) to 8.65% (2002-2016) [9]. Icterohaemorrhagiae and Bratislava resulted the most represented serovars, with 5.07% (58/1144) and 4.19% (48/1144) sera positive at titer ≥ 1:100, respectively. These results are in accordance with data available in literature [7,9,10]. Percentages of 1.40% (16/1144) and (0.17% 2/1144) of sera scored positive for serovar Pomona at titer ≥ 1:100 and ≥ 1:400, respectively. Infection by serovar Pomona in dog produce a severe disease characterized by lethargy, fever, inappetence, diffuse haemorrhage, renal and liver failure [37,38]. In Europe, infections in dogs caused by this serovar are rare and reported only in few countries of East-Europe, such as Romania [7]. For this reason serovar Pomona was not included in dog vaccines [39]. Our data seem to suggest an increasing incidence of this serovar in dog during last years (Table S1) [9,10,13]. Taking into account the increase of Pomona positivity in dog and the severe symptoms, the research on this serovar could be intensify. As regards serovar Canicola, 1.22% (14/1144) and 0.09% (1/1144) sera resulted positive at titer ≥ 1:100 and ≥ 1:400, respectively. This result confirms the decreasing of this serovar in many European countries during the last years due to the use of vaccines [7,40]. Low number of positive sera was observed for Grippotyphosa and Tarassovi with 0.35% (4/1144) and 0.09%

241 (1/1144) of positive sera at titer ≥ 1:100, respectively. In Europe, Grippotyphosa is considered an emerging
242 serovar in dogs [13,29,31,32] and was included in leptospirosis dog vaccine. Contrary to what is generally
243 observed in Europe, data obtained by this investigation suggest a limited spreading of Grippotyphosa in
244 dogs among investigated area. In Europe, Tarassovi is rarely reported in dog [10,17]. This is in accordance
245 with our data, since only one serum was found positive to this serovar, even if at high titer (1:800)
246 suggesting a recent and probably acute infection. No positivity was encountered for serovars Hardjo and
247 Ballum, which are rarely detected in dog as suggested by several investigations [7,10,40].
248
249 4.3. Swine
250 The total apparent prevalence in swine increased considerably from 9.16% (1995-2001) [9] to 19.74%
251 (2002-2016). It is known that swine is the maintenance host for serovar Pomona (serogroup Pomona),
252 serovar Tarassovi (serogroup Tarassovi) and serovar Bratislava (serogroup Australis) [7]. As expected,
253 Pomona and Bratislava were the serovars more often detected in this species. For Pomona, 12.01%
254 (160/1332) and 3.38% (45/1332) of swine sera have been detected positive at titer ≥ 1:100 and ≥ 1:400,
255 respectively. While for Bratislava, 9.83% (131/1332) and 2.33% (311332) of sera scored positive at titer ≥
256 1:100 and ≥ 1:400, respectively. Bratislava is considered an emerging serovar and it could be the cause of
257 abortion and other reproductive disorder in swine. However, some strains become “pig-adapted”, causing
258 subclinical infections [41]. In different European countries, during last years, Bratislava was the more
259 detected serovar in pig sera samples [10,42]. Despite the fact that swine represents the *reservoir* host for
260 Pomona, in last years, the seroprevalence of this serovar in pig was low [9,10,42]. It is noteworthy that, in
261 this investigation, the seroprevalence of Pomona in swine was increasing, in contrast with other surveys.
262 On the other hand, only 0.15% (2/1332) and 0.08% (1/1332) of sera were detected positive for Tarassovi at
263 titer ≥ 1:100 and ≥ 1:400, respectively. These data confirm that this serovar seems to disappear, as
264 suggested by other investigations [9,10,42]. The cause could be the wide use of vaccination program in
265 swine farm [43,44]. As regard serovar Canicola, 0.45% (6/1332) of sera scored positive at titer 1:100/1:200
266 (3 at titer of 1:100 and 3 at titer of 1:200). Some studies demonstrated that swine could be infected by

267 serovar Canicola and that intraspecies transmission is possible [42,45]. For this reason, pigs are considered
268 a new possible potential maintenance host for serovar Canicola, although its real epidemiological role is not
269 still clarified [46,47]. The results of this investigation confirm the circulation of this serovars among swine in
270 Italy too. No positive sera were detected for serovars Grippotyphosa and Ballum, and only 0.15% (2/1332)
271 and 0.08% (1/1332) of sera resulted positive for Icterohaemorrhagiae and Hardjo, respectively. These data
272 are in accordance with other studies [9,10,42].
273
274 4.4. Horse
275 The total apparent prevalence obtained in horse was characterized by a remarkable decrease from 11.08%
276 (1995-2001) [9] to 2.89% (2002-2016). This species is susceptible to a wide range of incidental infections,
277 that are often characterized by absence of clinical symptoms. Bratislava is the most common serovar
278 detected in horses, but also Grippotyphosa, Pomona, Icterohaemorrhagiae, Autumnalis, Sejroe, Canicola,
279 and Ballum serogroups are occasionally reported [7,48]. The results of this investigation confirm this
280 evidence; indeed, positive reactions were detected only for Icterohaemorrhagiae (1.24% - 3/242 sera),
281 Bratislava (0.83% - 2/242 sera), Pomona (0.41% - 1/242 sera) and Hardjo (0.41% - 1/242 sera). The low
282 number of positive sera detected in horse could be related to few clinical samples or few specimens with
283 clinical manifestations. Also, the sera could have been collected after equine uveitis manifestation. Equine
284 uveitis, also known as “moon blindness”, is an ocular disease consequent to *Leptospira* infection in horse,
285 that occurs after the acute phase of leptospirosis when the antibody titer decrease [48].
286
287 4.5. Sheep and Goat
288 The total apparent prevalence observed in sheep decreased from 12.13% (1995-2001) [9] to 3.13% (2002-
289 2016). These results could seem unexpected. Indeed, in investigated area sheep flocks are breed
290 extensively or semi-extensively and this could promote contact with wild animals. However, our data are in
291 line with a recent survey conducted by the Italian reference center for leptospirosis [10]. Despite sheep
292 represent the second maintenance host for serovar Hardjo [49], infection by this serovar could induce

293 subclinical or clinical disease [7], with abortion, stillbirth, birth of weak lambs, agalactia and infertility [50–
294 52]. Although the results of this investigations do not show a high number of positive samples (0.71% -
295 19/2682) for serovar Hardjo, almost all have a titer ≥ 1:400 (12 sera out of 19 positive). These data could
296 suggest the occurrence of clinical infection in sheep confirming the high virulence of serovar Hardjo for this
297 animal species. Sheep could be infected also by other serovars [7]. In this investigation, relative high
298 number of positivity was reported for serovars Pomona (1.12% 30/2682), Icterohaemorrhagiae (0.75% -
299 20/2682) and Grippotyphosa (0.75% - 20/2682) at titer ≥ 1:100. These results are in disagreement with
300 previous investigations carried out in the same area [9] and in Italy [10,25]. Pomona and
301 Icterohaemorrhagiae could cause disease in sheep, in particular they have been associated with
302 reproductive disorders [53,54]. Furthermore, other positive reactions were found in sheep sera for
303 Bratislava (0.52%), Tarassovi (0.30%), Ballum (0.19%) and Canicola (0.04%) at titer≥ 1:100. Seropositivity for
304 these *Leptospira* serovars was previously reported in sheep where they could represent the cause of
305 accidental infections [55]. Even if the presence of these serovar was reported in other studies, the reactivity
306 to all tested serovars detected in sheep during this investigation seems unusual. It could be probably
307 explained by the presence of wild boars, hares, hedgehogs, rodents (as mice and rats) and other wild
308 animals, which represent maintenance hosts for different serovars, in the areas where the sampling was
309 conducted [23,29,33,40,56–58].
310 For goat sera, only 2.75% (9/327) of samples resulted positive for Icterohaemorrhagiae at titer ≥ 1:100,
311 among them 6 were positives at titer ≥ 1:400. Our data confirm that goats are not very susceptible to
312 *Leptospira* infection, as reported in literature by other study, where seropositivity was described for
313 serovar Hardjo [51,59,60], Icterohaemorrhagiae [60,61] and Poi [25].
314
315 4.6. Wild boar
316 The total apparent prevalence in wild board increased from 2.39% (1995-2001) [9] to 8.56% (2002-2016).
317 The highest prevalence was detected for serovars Tarassovi and Bratislava. Similarly to swine, wild boar is a
318 maintenance host for serovars Pomona, Tarassovi and Bratislava [7]. For Tarassovi, 3.76% (18/479) and

319 0.63% (3/479) of sera scored positive at titer ≥ 1:100 and titer ≥ 1:400, respectively. This result disagrees
320 with other studies previously conducted in the same area of investigation and in Italy. In Tuscany, from
321 1995 to 2001 no positive sera for Tarassovi has been detected in wild board [9,14]. In Italy, recent surveys
322 indicate the same trend: no Tarassovi seropositivity or few positive samples (2 out of 1987 sera) were
323 found in wild boar [10,22,62]. However, high seroprevalence for this serovar has been reported in different
324 European Countries [63–65]. Wild boar could represent the *reservoir* host of this serovar, disappeared in
325 other domestic animal, and it could contribute to maintain Tarassovi strains in environment. Furthermore,
326 the Tarassovi high prevalence in investigated area could be connected to the import in Tuscany of wild boar
327 from East Europe Countries for hunting [34,63,64]. The second most representative serovar in wild boar
328 was Bratislava: 2.51% (12/479) and 0.21% (1/479) of positive sera have been detected at titer ≥ 1:100 and
329 titer ≥ 1:400, respectively. Bratislava is one of the most worldwide spread *Leptospira* [26], consequently,
330 this result was expected. Indeed, this serovar is frequently detected in wild boar in Italy [9,10,14,22,62] and
331 in Europe [63–67]. Unexpected data has been recorded concerning positivity to Pomona, considering that
332 wild boar is a potential *reservoir*, as reported by other studies carried out in Europe [66]. Only 0.84%
333 (4/479) and 0.21% (1/479) of sera resulted positive for this serovar at titer ≥ 1:100 and titer ≥ 1:400,
334 respectively. These data were unexpected, but similar to those reported by other Authors in Italy
335 [10,22,62]. As concerns serovar Hardjo, 2.92% (14/479) and 1.46% (7/479) of sera scored positive at titer ≥
336 1:100 and titer ≥ 1:400, respectively. This serovar is generally associated to cattle and sheep [7] and its
337 detection in wild boar seems singular. In Italy, no Hardjo seropositivity has been founded previously in wild
338 boar [10,22,62]. However, Hardjo seems to be the most prevalent serovar in East Europe in wild boar [66].
339 For this reason, our finding could be related to the import of animals from East Europe for hunting
340 purposes. Nevertheless, in investigated area there are a lot of free-range farms of cattle and sheep and it is
341 plausible to assume that wild boar became infected after direct or indirect contact with these animals.
342 Moreover, half of positive sera showed an antibody titer higher than 1:400, suggestive of recent and
343 possible acute infection. For all these reasons it could not be excluded a possible involvement of wild boar
344 in epidemiology of *Leptospira* serovar Hardjo, as maintenance or incidental host. Furthermore, positivity to

345 other serovars was detected at titer ≥ 1:100:0.62% (3/479) of sera for serovar Icterohaemorrhagiae, 0.21%
346 (1/479) of sera for serovar Canicola, 0.21% (1/479) of sera for serovar Grippotyphosa and 0.21% (1/479) of
347 sera for serovar Ballum . These animals could easily come in contact with *Leptospira* due to their lifestyle
348 and positivity to many different serovars is sporadically, but constantly reported [10,22,63–67].
349
350 4.7. Other wild animals
351 Excluding wild boar, seropositivity in wild animals was recorded only for hares, foxes and coypus. It is well
352 documented that hare could be infected by different *Leptospira* serovars, especially Grippotyphosa
353 [22,33,56,68]. Only 1.85% (3/162) of hares sera scored positive in this survey, 0.62% (1/162) for Bratislava
354 (titer 1:200) and 1.23% (2/162) for Ballum (titer 1:100 and 1:200, respectively).
355 Fox was reported as incidental host for different serovars, such as Icterohaemorrhagiae, Ballum and
356 Bratislava [10,58,69]. Low number of fox sera examined in this investigation resulted positive for
357 *Leptospira*, in particular for serovar Icterohaemorrhagiae (1.06% - 1/94) and Bratislava (3.19% - 3/94). Only
358 1.06% (1/94) of sera resulted positive at titer ≥ 1:400, for serovar Bratislava.
359 For both these species, a low percentage of positive sera was detected compared to the other reported
360 surveys. This could suggest a weak involvement of these two animal species in *Leptospira* epidemiology in
361 investigated area.
362 As regard coypus, some studies highlighted the circulation of the same serovars among these animals and
363 its possible role as reservoir host [18,70–72]. In accordance with these studies, an high percentage of sera,
364 32.86% (23/70), analyzed in this investigation scored positive to *Leptospira*. Positivity was recorded for
365 Grippotyphosa (20.00% - 14/70), Bratislava (11.43% - 8/70) and Icterohaemorrhagiae (1.43% - 1/70).
366 Furthermore, 4.29% (3/70) and 2.86% (2/70) of sera exhibited a titer ≥ 1:400 for serovars Bratislava and
367 Grippotyphosa, respectively; these data could suggest a recent infection, confirming the circulation of these
368 serovars in coypus. Positivity to Bratislava and Icterohaemorrhagiae was frequently reported, while
369 positivity to Grippotyphosa, which is considered an emerging serovar in Europe, could open new interesting
370 epidemiological scenarios.

No positive reactions were detected in sera from wild ruminants: roe deer, red deer, fallow deer and mouflon. Our data confirm the marginal role that these animals have in the epidemiology of *Leptospira* according to the low number of positivity reported in literature [9,10,17,73,74].

No positivity was detected in sera collected from other wild animals (wolves, rats, bear and ferret). However, small number of samples were analyzed for these species and it is not possible to advance robust epidemiological hypothesis.

4.8. Human

The total apparent prevalence observed in man slightly decreased from 5.60% (1995-2001) [9] to 4.26% (2002-2016). Humans are incidental hosts for *Leptospira* and they could be infected by serovars maintained by animals in a particular geographical region. In past years in Europe, human leptospirosis had a fluctuating trend, but different confirmed cases were always registered. In particular in Italy about 40 human cases were annually recorded from 2008 to 2015 [75,76]. According to the present survey, Icterohaemorrhagiae remained the most detected serovar from human sera. However, positivity to all tested serovars, with exception of Tarassovi and Hardjo, was recorded. This finding could suggest a change also in human leptospirosis related to a modification of *Leptospira* epidemiology in investigated area.

## 5. Conclusion

Leptospirosis is probably the most widespread, (re-)emerging and prevalent zoonotic disease in the world. However, due to the difficult to exactly diagnose the disease clinically and by laboratory test, sometime, it could be not recognized and consequently severely neglected. For this reason, the true spread and increase of leptospirosis remains probably unknown [12]. Considering that many domestic and wild mammals represent natural carriers of pathogenic leptospires, acquire epidemiological information on animal leptospirosis could be helpful for both human and breeding animal health. Despite that isolation and strains characterization should have an highest diagnostic value, serology represent for some disease, such as leptospirosis, the best instrument for epidemiological purpose. The results of this investigation provide

information on *Leptospira* epidemiology in a defined geographical area involving many different animal species, maintenance end accidental hosts, and humans. Moreover, a long period of time was considered and this allowed to put in evidence a fluctuation in *Leptospira* positivity recovery, as also suggested by other Authors; this trend involved both the total percentage of positive animals registered year by year and the serovars encountered. Our results seem to highlight an increase of *Leptospira* in North-Central Italy and a change in serovars potentially involved in animal and human infection.

Several animals resulted infected by unusual *Leptospira* serovars and this finding could suggest a change in host range for some serovars, that may promote the adaptation to new hosts. Constant serological monitoring results essential to control the evolution of the dynamics of *Leptospira* epidemiology and it could represent the basis to lead future investigations focused on specific animals and that must include both serological that isolation or molecular techniques.

**Conflict of interest statement**

All authors declare no conflict of interests.

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635 **Figure**

636

637 **Figure 1:** Percentage of positive sera detected for *L. interrogans* and for each *Leptospira* serovars year by
638 year.

639 Note: Ic: Icterohaemorrhagiae; Ca: Canicola; Po: Pomona; Tar: Tarassovi; Gri: Grippotyphosa; Br: Bratislava; Ba: Ballum; Har: Har

**Table 1:** Number of positive sera to *Leptospira* at low (≥ 1:100) and high titers (≥ 1:400).

| | | Titer | | | |
|---|---|---|---|---|---|
| **Species** | **Examined** | **≥1:100** | **%** | **≥1:400** | **%** |
| **Bovine** | 1328 | 173 | 13.03 | 42 | 3.16 |
| **Dog** | 1144 | 99 | 8.65 | 27 | 2.36 |
| **Goat** | 327 | 9 | 2.75 | 6 | 1.83 |
| **Horse** | 242 | 7 | 2.89 | 0 | 0.00 |
| **Wild boar** | 479 | 41 | 8.56 | 13 | 2.71 |
| **Hare** | 162 | 3 | 1.85 | 0 | 0.00 |
| **Coypus** | 70 | 16 | 22.86 | 4 | 5.71 |
| **Sheep** | 2682 | 84 | 3.13 | 38 | 1.42 |
| **Swine** | 1332 | 263 | 19.74 | 84 | 6.31 |
| **Fox** | 94 | 4 | 3.04 | 1 | 0.91 |
| **Human** | 329 | 10 | 4.26 | 3 | 1.06 |
| **Total** | 8488 | 709 | 8.35 | 218 | 2.57 |

**Table 2:** Numbers of positive serological reactions detected for the different *Leptospira* serovars at low (≥ 1:100) and high titers (≥ 1:400) in relation to animal species.

| Animal species | Titer | Serovar | | | | | | | | Total | Positive to more serovars |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ic | Ca | Po | Gri | Tar | Br | Har | Ba | | |
| **Bovine** | **≥ 100** | 21 | 4 | 55 | 48 | 3 | 3 | 94 | 4 | 232 | 44* |
| | **≥ 400** | 4 | 1 | 3 | 5 | 1 | 1 | 30 | 2 | 47 | 3* |
| **Dog** | **≥ 100** | 58 | 14 | 16 | 4 | 1 | 48 | 0 | 0 | 141 | 33* |
| | **≥ 400** | 16 | 1 | 2 | 0 | 1 | 19 | 0 | 0 | 39 | 11* |
| **Goat** | **≥ 100** | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 |
| | **≥ 400** | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 |
| **Horse** | **≥ 100** | 3 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 7 | 0 |
| | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Wild boar** | **≥ 100** | 3 | 1 | 4 | 1 | 18 | 12 | 14 | 1 | 54 | 11* |
| | **≥ 400** | 1 | 0 | 1 | 0 | 3 | 1 | 7 | 1 | 14 | 1 |
| **Hare** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 3 | 0 |
| | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Coypus** | **≥ 100** | 1 | 0 | 0 | 14 | 0 | 8 | 0 | 0 | 23 | 7* |
| | **≥ 400** | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 5 | 3 |
| **Sheep** | **≥ 100** | 20 | 1 | 30 | 20 | 8 | 14 | 19 | 5 | 117 | 21* |
| | **≥ 400** | 2 | 0 | 17 | 6 | 3 | 1 | 12 | 1 | 42 | 3* |
| **Swine** | **≥ 100** | 2 | 6 | 160 | 0 | 2 | 131 | 1 | 0 | 302 | 40* |
| | **≥ 400** | 0 | 0 | 45 | 0 | 1 | 41 | 0 | 0 | 87 | 3 |
| **Fox** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 4 | 0 |
| | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| **Human** | **≥ 100** | 7 | 2 | 1 | 2 | 0 | 2 | 0 | 1 | 15 | 2* |
| | **≥ 400** | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 |
| **Total** | **≥ 100** | 125 | 28 | 267 | 89 | 32 | 224 | 129 | 13 | 907 | 158 |
| | **≥ 400** | 31 | 2 | 68 | 14 | 9 | 67 | 49 | 4 | 244 | 24* |

Note: Ic: Icterohaemorrhagiae; Ca: Canicola; Po: Pomona; Tar: Tarassovi; Gri: Grippotyphosa; Br: Bratislava; Ba: Ballum; Har: Hardjo;

*Some sera resulted positive to more than 2 different serovars

**Table S1**

Bertelloni et al., *Epidemiology of Leptospirosis in North-Central Italy: fifteen years of serological data (2002-2016)*

Supplementary material

**Table S1:** Distribution of positive sera at titer ≥ 1:100 and ≥1:400 in relation to animal species, *Leptospira* serovars and year of detection

| Animal species | | | Year | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Serovars | Titer | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | |
| **Bovine** | **Ic** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 18 | 0 | 0 | 21 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 45 | 55 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | **Gr** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 34 | 48 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 5 |
| | **Br** | **≥ 100** | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 10 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 7 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 88 | 0 | 94 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 27 | 0 | 29 |
| **Dog** | **Ic** | **≥ 100** | 10 | 8 | 6 | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 12 | 8 | 3 | 4 | 58 |
| | | **≥ 400** | 5 | 1 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 16 |
| | **Ca** | **≥ 100** | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 2 | 5 | 13 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 10 | 16 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | **Gr** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 4 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 6 | 9 | 6 | 2 | 2 | 1 | 0 | 1 | 2 | 0 | 1 | 6 | 3 | 4 | 4 | 47 |
| | | **≥ 400** | 2 | 1 | 5 | 1 | 1 | 1 | 0 | 01 | 0 | 0 | 0 | 2 | 0 | 4 | 0 | 18 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Goat** | **Ic** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 9 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 6 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Gr** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Horse** | **Ic** | **≥ 100** | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Gr** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Wild boar** | **Ic** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 3 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 4 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 5 | 1 | 18 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| | **Gr** | **≥ 100** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 5 | 12 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 8 | 14 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 5 | 7 |
| **Hare** | **Ic** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Gr** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ba** | **≥ 100** | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Coypus** | **Ic** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Gr** | **≥ 100** | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| | | **≥ 400** | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | **Br** | **≥ 100** | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| | | **≥ 400** | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Sheep** | **Ic** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 7 | 0 | 20 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 23 | 29 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 17 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 8 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 1 | 0 | 22 |
| | **Gr** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 14 | 0 | 18 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 6 |
| | **Br** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 2 | 0 | 14 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 5 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | **Har** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 11 | 0 | 19 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 10 | 0 | 12 |
| **Swine** | **Ic** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 6 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 98 | 62 | 160 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 12 | 45 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | **Gr** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 99 | 32 | 131 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 38 | 33 | 71 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Fox** | **Ic** | **≥ 100** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ca** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Gr** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Br** | **≥ 100** | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | | **≥ 400** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Human** | **Ic** | **≥ 100** | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 6 |
| | | **≥ 400** | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | **Ca** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Po** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Gr** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |
| | | **≥ 400** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | **Br** | **≥ 100** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Ba** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Har** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **Ic** | **≥ 100** | 14 | 11 | 6 | 1 | 0 | 2 | 1 | 1 | 4 | 10 | 1 | 13 | 43 | 12 | 6 | 125 |
| | | **≥ 400** | 5 | 3 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 6 | 0 | 2 | 6 | 2 | 1 | 31 |
| | **Ca** | **≥ 100** | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 7 | 9 | 5 | 28 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | **Po** | **≥ 100** | 0 | 1 | 0 | 0 | 0 | 6 | 0 | 2 | 0 | 0 | 0 | 1 | 9 | 103 | 145 | 267 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 34 | 33 | 68 |
| | **Tar** | **≥ 100** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 11 | 1 | 32 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 3 | 0 | 9 |
| | **Gr** | **≥ 100** | 3 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 18 | 15 | 34 | 89 |
| | | **≥ 400** | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 6 | 2 | 14 |
| | **Br** | **≥ 100** | 12 | 17 | 10 | 2 | 2 | 2 | 0 | 1 | 2 | 0 | 1 | 6 | 17 | 111 | 41 | 224 |
| | | **≥ 400** | 3 | 3 | 6 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 43 | 5 | 67 |
| | **Ba** | **≥ 100** | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 1 | 1 | 13 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 4 |
| | **Har** | **≥ 100** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 18 | 100 | 8 | 129 |
| | | **≥ 400** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 38 | 5 | 49 |

Note: Ic: Icterohaemorrhagiae; Ca: Canicola; Po: Pomona; Tar: Tarassovi; Gri: Grippotyphosa; Br: Bratislava; Ba: Ballum; Har: Hardjo

**Figure**

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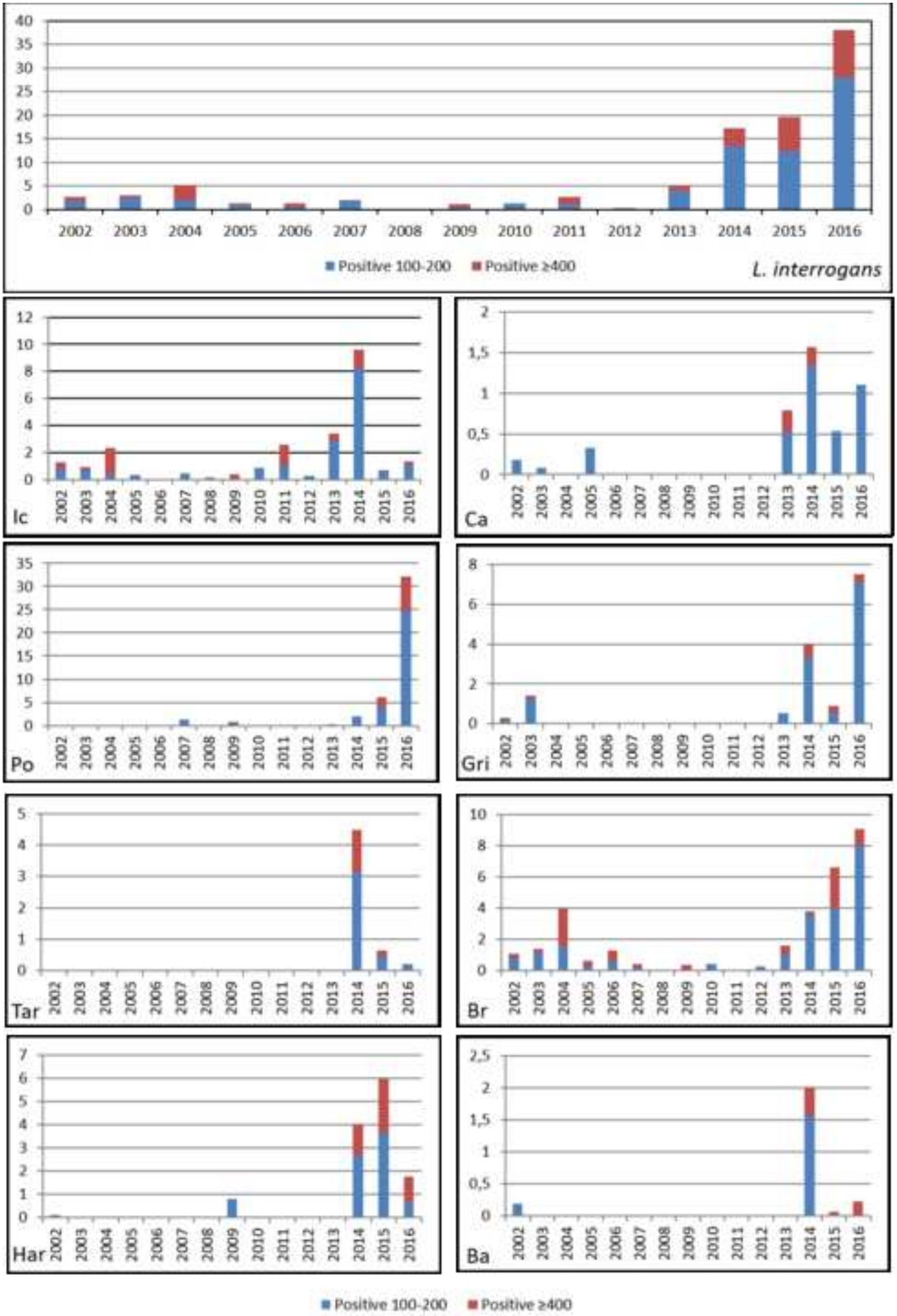

***Conflict of Interest**

Bertelloni et al., *Epidemiology of Leptospirosis in North-Central Italy: fifteen years of serological data (2002-2016)*.

Conflict of interest statement

All authors declare no conflict of interests.