

**Serological survey on *Leptospira* infection in slaughtered swine in North-Central Italy**

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3 1 **Serological survey on *Leptospira* infection in slaughtered swine in North-Central Italy**  
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52 20 Running head: *Leptospira* in swine in North-Central Italy  
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## 22 ABSTRACT

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24 Swine can act as asymptomatic carriers of some *Leptospira* serovars. In this study, 1194 sera from 61 farms  
25 located in five different Regions of North-West Italy were collected from slaughtered healthy pigs. Presence of  
26 antibody against four *Leptospira* serovars was evaluated. Overall, 52.5% of analyzed farms presented at least  
27 one positive animal and 34.4% presented at least one positive swine with titer  $\geq 1:400$ . A percentage of 16.6%  
28 sera was positive and 5.9% samples presented a positive titer  $\geq 1:400$ . Tuscany and Lombardy showed the  
29 highest percentage of positive farms (64.3% and 54.6%, respectively) and sera (28.5% and 13.3%, respectively),  
30 probably due to environmental conditions and potential risk factors, which promote maintenance and spreading  
31 of *Leptospira* in these areas. The main represented serogroups were Australis (21.3% positive farms, 8.2%  
32 positive sera) and Pomona (18.0% positive farms, 8.1% positive sera). In swine, these serogroups are the most  
33 detected worldwide; however, our results seem to highlight a reemerging of serogroup Pomona in pigs in  
34 investigated areas. A low percentage of sera (0.6%) scored positive to Canicola, leaving an open question on the  
35 role of pigs in the epidemiology of this serovar. Higher antibody titers were detected for serogroups Australis  
36 and Pomona. Swine leptospirosis is probably underestimated in Italy and could represent a potential risk for  
37 animal and human health.

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39 **Keywords:** leptospirosis; swine; serology; Pomona; Australis.

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## 41 Introduction

42 Leptospirosis is one of the most widespread zoonotic disease caused by bacteria belonging to genus *Leptospira*.  
43 These bacteria could infect both human and animals. Many wild and domestic animals could act as *reservoir*  
44 hosts; these do not develop symptoms, but contribute to the environmental maintenance of leptospires. The  
45 source of human infection is direct contact with animal infected urine or indirect through contaminated water [1,  
46 2]. Especially in developed countries, leptospirosis represents an occupational disease associated with particular  
47 kind of works (cropper, farmer, veterinarian, and slaughterer) [3, 4, 5, 6, 7].

48 Swine are common *reservoir* host for some *Leptospira* serovars, in particular Pomona, Tarassovi, and Bratislava;  
49 moreover, some other serovars could infect pigs [8, 9, 10].

50 Serovar Pomona is among the most common serovars isolated from pigs worldwide. In recent years, in-door  
51 housing of swine and vaccination led to a decreasing incidence of this serovars in pig herds, especially in  
52 developed countries. Pig infection by serovar Pomona could result in abortion, stillbirth or birth of weak or ill  
53 piglets with any subsequent limitation on reproductive performance. Young animals could be affected by an  
54 acute systemic illness that may be fatal. Adult non-pregnant animals are usually asymptomatic carriers [9, 11,  
55 12].

56 Serovar Bratislava is characterized by a global distribution and can be considered an emerging serovar in many  
57 countries and in several animal species. Epidemiology, ecology, symptoms and lesions related to this serovar in  
58 pig remain poorly understood due to difficulties in culturing these strains, in contrast to the high seroprevalence  
59 reported worldwide. Reproductive failure, abortion stillbirth and infertility is typically associated to this serovar  
60 in swine [9, 12, 13, 14].

61 Pig was previously thought to act as a maintenance host for serovar Tarassovi. However, in recent years it was  
62 observe a declining seroprevalence in this species. The reasons for this remain in some cases unclear. Tarrasovi  
63 does not spread as rapidly in a pig population as Pomona does, but endemic infection is readily maintained,  
64 generally associated with reproductive failure [9, 15, 16].

65 Other *Leptospira* serovars could be responsible of incidental infections in pigs. Both acute and chronic infections  
66 could be observed, but clinical cases are focal, with limited in-contact spread. Serovars involved vary around the  
67 world [9]. In particular, serovar Canicola has been detected from swine in several countries. In this case, it has

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3 68 been observed a long period of urine shedding and the ability to survive for up to 6 days in undiluted urine.  
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5 69 These findings could suggest an intraspecies transmission [16, 17, 18].  
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8 70 In 2010, Italian pig population amounted to approximately 9.6 million animals. Over 50% was represented by  
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10 71 heavy pigs (weighing more than 130 kg) used for the traditional dry-cured hams. Pig breeding is mainly  
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12 72 concentrated in the northern area of Italy [19, 20].

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14 73 The present study aims to provide a serological survey on the prevalence of *Leptospira* in pigs in North-Central  
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16 74 Italy and to highlight the key role of slaughterhouse as an epidemiological observatory for leptospirosis.  
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## 20 21 76 **Materials and Methods**

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23 77 From September to December 2015, 1194 swine sera samples were collected. Blood samples were taken at  
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25 78 slaughterhouse during jugulation. Sixty-one closed-cycle fattening farms were included in the study. When it  
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27 79 was possible, 20 animals for each herd were sampled using systematic randomization method. All selected  
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29 80 animals were heavy pigs slaughtered at 150-160 Kg at the age of about one year. Only non-vaccinated subjects  
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31 81 were included in this survey. All swine resulted healthy before slaughter and during *post mortem* examination of  
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33 82 carcasses, no macroscopic lesion referable to leptospirosis could be detected. Investigated farms were located in  
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35 83 five different regions of Italy, as reported in Table 1. Fourteen herds (256 sera) were located in Central Italy  
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37 84 (Tuscany); 35 (700 sera) in North-West Italy (Piedmont and Lombardy) and 12 herds (238 sera) were located in  
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39 85 North-Est Italy (Veneto and Emilia Romagna).

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41 86 All blood samples were carried to the Laboratory of Infectious Diseases of the Department of Veterinary  
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43 87 Science, University of Pisa, in refrigerated condition in few hours after collection; all sera, obtained by blood  
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45 88 centrifugation at 1000 g for 15 minutes, were maintained at -20°C until they were used for laboratory  
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47 89 examination.

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49 90 *Leptospira* antibodies were detected by Micro Agglutination Test (MAT). Four *Leptospira interrogans* serovars  
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51 91 were employed as live antigen: Canicola (strain Alarik), Pomona (strain Mezzano), Tarassovi (strain Johnson),  
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53 92 and Bratislava (strain Riccio 2). They were grown in Ellinghausen-MacCullough-Johnson-Harris (EMJH—  
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55 93 Difco, Detroit, Michigan, USA) at 30°C for 4–14 days and checked for purity, mobility and agglutination ability.  
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57 94 MAT was performed following the procedure previously reported by Cerri et al. [21]; a serum was considered

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3 95 positive when it showed 50% agglutination, leaving 50% free cells compared with a control culture diluted 1:2 in  
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5 96 phosphate buffered saline at the cut-off titer of 1:100. In case of positive sera, 2-fold serial dilutions were  
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7 97 performed in order to determine the endpoint titer, which is defined as that last serum dilution able to cause 50%  
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9 98 agglutination.

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## 100 **Results**

101 Thirty-two out of the sixty-one farms analyzed (52.5%) presented at least one positive animal at titer of 1:100. A  
102 percentage of 34.4% (21/61) of the herds presented at least one positive swine with titer  $\geq$ 1:400. In particular,  
103 64.3% (9/14) of farms from Central Italy was positive and 57.1% (8/14) had animals with titer  $\geq$ 1:400. A  
104 percentage of 54.3% (19/35) of herd located in North-West Italy resulted positive and 34.3% (12/35) was  
105 positive with titer  $\geq$ 1:400. Four out of twelve (33.3%) flocks located in North-Est Italy resulted positive and 1/12  
106 (8.3%) presented at least one animal positive with titer  $\geq$ 1:400 (Table 1).

107 A percentage of 16.6% (198/1194) sera was positive and 5.9% (70/1194) showed a titer  $\geq$ 1:400. Seventy-three  
108 out of two hundred and fifty-six (28.5%) sera from Central Italy were positive and 38/256 (18.8%) presented a  
109 titer  $\geq$ 1:400. Among sera collected in North-West Italy, 13.6% (95/700) resulted positive and 4.4% (31/700)  
110 showed a titer  $\geq$ 1:400. A percentage of 12.6% (30/238) of sera from North-Est Italy farms showed positive  
111 result, only one serum (0.4%) presented a titer equal to 1:400 and no sera showed higher titers (Table 1).

112 Figure 1 reports the percentage of positive farms and sera detected in each Regions.

113 Table 2 shows the positive farms for each serovar. Positivity to all serovar tested was found in North-West Italy,  
114 while in Central and North-Est farms positivity to serogroups Pomona, Australis and Tarassovi and to  
115 serogroups Pomona and Australis were only observed, respectively. Furthermore, in Tuscany and Lombardy  
116 serological positivity to more than one serogroups in the same herd was detected. Particularly, in Lombardy five  
117 herds resulted positive for two different serogroups and one farm was positive for all four serovars tested.  
118 Moreover, in three farms a sero-positivity with an antibody titer  $\geq$ 1:400 for two serogroups was observed and  
119 one farm was positive for three different serogroups with a titer  $\geq$ 1:400. In Tuscany, only one farm presented  
120 serological positivity to two different serogroups.

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3 121 Considering sera, the highest number of positive reaction was registered for serogroups Australis and Pomona;  
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5 122 the percentage of positive sera were 8.2% (98/1194) and 8.1% (97/1194), respectively. A percentage of 22.7%  
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7 123 (58/256) sera from Central Italy was positive for Pomona, while 5.9% (15/256) showed positivity to Australis. In  
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9 124 sera collected from North-West Italy, a positivity of 8.0% (56/700) and of 5.1% (36/700) were detected for  
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11 125 serogroups Australis and Pomona, respectively. Thirty-seven of 1194 (3.1%) sera scored positive with titer  
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13 126  $\geq 1:400$  for serogroup Australis, in particular 5.5% (14/256) of sera from Central Italy and 3.1% (22/700) from  
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15 127 North-West Italy. A percentage of 2.6% (31/1194) sera was positive to serogroup Pomona with a titer  $\geq 1:400$ ,  
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17 128 among these, 23/256 (8.9%) were collected in Tuscany and 3/700 (0.4%) in Lombardy. A low number of sera  
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19 129 showed positivity to serogroups Canicola and Tarassovi. Five and three sera, respectively from Lombardy and  
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21 130 Tuscany, showed positivity for two different serovars (Table 3).

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23 131 Higher titer value were recorded for serogroup Australis. Serogroups Canicola and Tarassovi never showed titers  
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25 132 higher than 1:400 (Table 4).

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27 133 Eight sera resulted positive to more than one antigens. In particular, 3 sera were positive to serogroups Pomona  
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29 134 and Australis (titer of: 1:400/1:400, 1:400/1:400 and 1:200/1:100, respectively), 2 sera to serogroups Pomona  
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31 135 and Tarassovi (titer of: 1:400/1:200 and 1:200/1:400, respectively) and 3 sera to serogroups Canicola and  
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33 136 Tarassovi (titer of 1:200/1:400), to serogroups Canicola and Pomona (titer of 1:100/1:400) and to serogroups  
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35 137 Canicola and Bratislava (titer of 1:100/1:200).

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### 38 39 139 **Discussion**

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42 140 The purpose of this research was to provide an overview about *Leptospira* spp. prevalence in an Italian swine  
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44 141 population. Authors focused their attention on North-Central Italy due to the primary importance of this area for  
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46 142 swine breeding. Sampling collection at slaughterhouse allowed the selection of homogeneous samples for age,  
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48 143 weight, general health status and to choose animals not subjected to leptospirosis vaccination program .

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50 144 Serovars more often involved in swine infection (Pomona, Tarassovi and Bratislava) were employed in  
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52 145 serological examinations; furthermore, investigation conducted in the past years in Italy showed very low sero-  
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54 146 prevalence to others serogroups in pig population [14, 21]. However, it seemed appropriate to also consider  
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56 147 serovar Canicola for the role of pig as potential maintenance host [16].

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3 148 Antibody titers of 1:100 were considered as threshold; titers of 1:100 or 1:200 may be suggestive of an early  
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5 149 stage of infection or of a previous infection. Titers of 1:400 or higher can be considered distinctive of endemic  
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7 150 infection [22].  
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9 151 Most recent data about total number of swine farms in the investigated area show a decrease of about 75%  
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11 152 between 2000 and 2010 [20]. Based on this trend, we can assume that approximately 1.4% of pig farms of North  
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13 153 Italy and Tuscany was analyzed in this survey.  
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15 154 Consequently, the detected *Leptospira* seroprevalence (52.5%) among analyzed farms seems remarkable.  
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18 155 Lombardy and Tuscany showed the highest percentages of positive farms with 54.6% and 64.3%, respectively.  
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20 156 Moreover, in these regions the highest percentage of farms characterized by subjects with antibody titers  $\geq$  1:400  
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22 157 was detected.  
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25 158 As concerning sera, the highest percentage of positivity was detected in Tuscany (28.5%); in other regions sera  
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27 159 positive percentages ranged from 11.0% (Veneto) to 17.5% (Piedmont).  
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29 160 The higher percentages of positivity of Tuscany and Lombardy compared to the other analyzed regions could be  
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31 161 due to particular environmental conditions, potential risk factors and the abundance of reservoirs in the wild  
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33 162 fauna. In fact, in the last years, the occurrence of *Leptospira* was reported in humans, wild and domestic animals  
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35 163 in these areas [23, 24, 25, 26, 27, 28, 29, 30].  
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37 164 The main represented serogroup was Australis with 21.3% of positive farms and 8.2% of positive sera; followed  
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39 165 by serogroup Pomona with 18.0% and 8.1% of positive farms and sera, respectively. As concerns serogroup  
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41 166 Australis, our results are quite in accordance with other studies previously conducted in Europe. In particular,  
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43 167 Tagliabue et al. [14] referred 12.74% of positivity among Italians swine from 2010 to 2011. Cerri et al. [21]  
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45 168 reported a prevalence of 8.85% in swine sera collected in Italy from 1995 to 2001. However, these data are only  
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47 169 partially comparable to those from the present study since they are referred to antibody titers  $\geq$ 1:400. The  
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49 170 percentage reported by Boqvist et al. [31] in Sweden in 2008 (3.9%) was slightly lower.  
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51 171 As concerns serogroup Pomona, it was remarkable to observe how, after several reports highlighting its decrease  
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53 172 in Italian swine populations [14, 21], this infection seems to reemerge among pigs. Recently, in fact, in Europe  
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55 173 this serovar has been frequently reported in wild and domestic animals [32, 33, 34, 35, 36].  
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3 174 In past years, swine vaccination against *Leptospira* (classical swine vaccines used in Italy contain inactivated  
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5 175 *Leptospira* strains belonged to serogroups Pomona, Tarassovi and Australis) led to a decrease of this infection in  
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7 176 pig population. However, nowadays, many farms tend to vaccinate only breeding animals, in order to reduce the  
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9 177 economic impact of vaccination. This practice could be the main cause of the reemerging of some serovars in  
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11 178 swineherds, especially in areas with a high epidemiological risk, such as those considered in this study. This  
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13 179 hypothesis could be more appropriate for some than other serovars; in fact, for serogroup Tarassovi the  
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15 180 maintenance host is *Sus scrofa*, while for serogroups Pomona and Australis, several potential maintenance hosts,  
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17 181 both among domestic and wild animals, can be present in the environment. Consistently, the detected positivity  
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19 182 percentage for serogroup Tarassovi was very low (0.3%). However, according to a recent study carried out in  
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21 183 Italy [14], the presence of serological positivity for this serovar seems to suggest its ability to persist in certain  
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23 184 ecological niches.

24 185 Regarding serogroup Canicola, few sera resulted positive (0.6%), all collected in Lombardy farms. The role of  
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26 186 swine in epidemiology of leptospires belonging to this serogroup is not yet well clarified, although, other  
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28 187 Authors reported that pigs are commonly infected by this serovar [1]; moreover, serovar Canicola was isolated  
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30 188 from swine in Brazil [18]. Recently, serological positivity to this serogroup was reported in North-West Italy in  
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32 189 wild boar [29] and coypus [30].

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34 190 Some sera showed positivity to more than one serogroups; these findings could be ascribed to a coinfection or,  
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36 191 more likely, to a cross-agglutination.

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38 192 As concerns antibody titers, the highest values were reported for serogroups Pomona and Australis; in particular,  
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40 193 for Australis, eight sera showed an antibody titer of 1:1600 and three sera of 1:3200. This evidence is in  
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42 194 accordance with different surveys carried out by several Authors [14, 21, 31].

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45 195 Based on our results, in Italy, leptospirosis spreading in swine is probably underestimated. If on the one hand  
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47 196 swine leptospirosis outbreaks in Italy are sporadically reported, on the other hand several surveys highlighted a  
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49 197 considerable number of serological positivity. This information suggests a potential risk for animal and human  
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51 198 health, in particular for slaughterhouse workers historically involved in outbreaks of “swineherd's disease”  
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53 199 mainly caused by Pomona and Tarassovi serovars. Moreover, this survey highlights the importance of  
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55 200 slaughterhouse as epidemiological observatory for reemerging infectious diseases characterized by silent  
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57 201 infection, such as leptospirosis.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

For Review Only

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280 **Figures**

281 **Figure 1:** Percentage of positive farms and sera detected in each Regions.

For Review Only

## Tables

**Table 1:** Number of farms and serum samples from different Italian Regions examined and resulted positive at low ( $\geq 1:100$ ) and high titers ( $\geq 1:400$ ) in the microscopic agglutination test.

Area of Italy	Region	Farms		Sera			
		Analyzed	Positive	Analyzed	Positive	Titer $\geq 1:100$	Titer $\geq 400$
			Titer $\geq 1:100$	Titer $\geq 400$		Titer $\geq 1:100$	Titer $\geq 400$
	<b>Lombardy</b>	33	18	11	660	88	30
<b>North-West</b>	<b>Piedmont</b>	2	1	1	40	7	1
	<b>Emilia Romagna</b>	7	3	1	138	19	1
<b>North-East</b>	<b>Veneto</b>	5	1	0	100	11	0
<b>Central</b>	<b>Tuscany</b>	14	9	8	256	73	38
	<b>Total</b>	61	32	21	1194	198	70

**Table 2:** Number of positive farms to serovars Canicola, Pomona, Tarassovi and Bratislava at low ( $\geq 1:100$ ) and high titers ( $\geq 1:400$ ).

		Positive Farms to different serovar with low ( $\geq 1:100$ ) and high titer ( $\geq 1:400$ )										
Area of Italy	Region	Examined farms	Canicola		Pomona		Tarassovi		Bratislava		Positivity for more than one serovars	
			$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$
North-West	Lombardy	33	3	1	10	5	1	1	12	9	6	4
	Piedmont	2	0	0	0	0	0	0	1	1	0	0
North-East	Emilia Romagna	7	0	0	1	0	0	0	2	1	0	0
	Veneto	5	0	0	0	0	0	0	1	0	0	0
Central	Tuscany	14	0	0	7	6	1	1	2	2	1	1
	<b>Total</b>	61	3	1	18	11	2	2	18	13	7	5



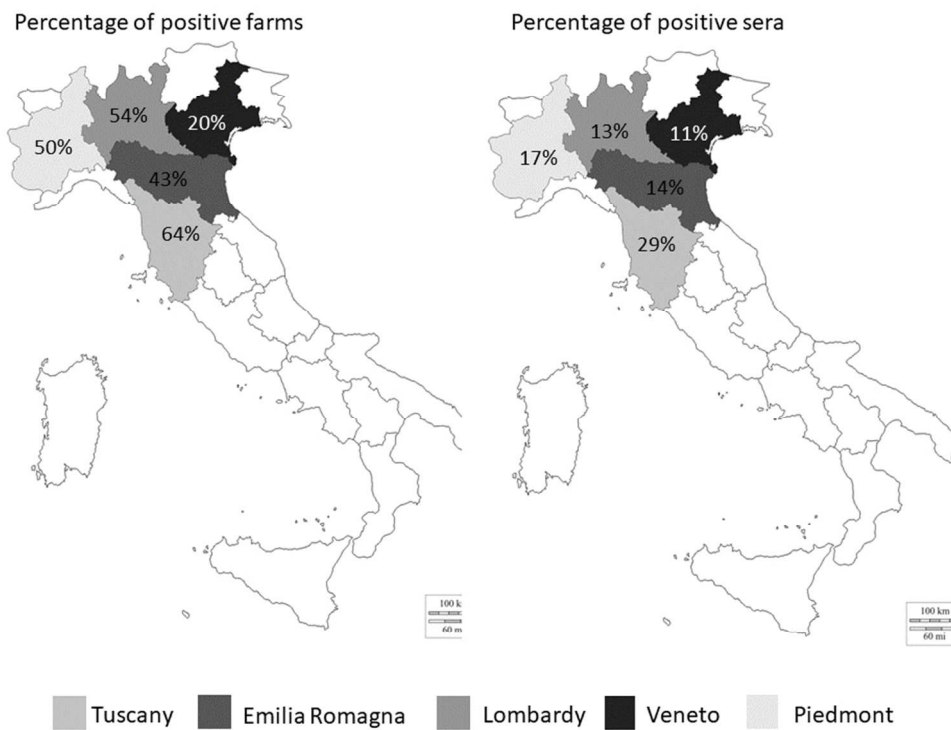
**Table 3:** Number of positive sera to serovars Canicola, Pomona, Tarassovi and Bratislava at low ( $\geq 1:100$ ) and high titers ( $\geq 1:400$ ).

Positive Farms to different serovar with low ( $\geq 1:100$ ) and high titer ( $\geq 1:400$ )												
Area of Italy	Region	Examined Sera	Canicola		Pomona		Tarassovi		Bratislava		Positivity for more than one serovars	
			$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$	$\geq 1:100$	$\geq 400$
North-West	Lombardy	660	7	2	36	8	1	1	49	21	5	2
	Piedmont	40	0	0	0	0	0	0	7	1	0	0
North-East	Emilia Romagna	138	0	0	3	0	0	0	16	1	0	0
	Veneto	100	0	0	0	0	0	0	11	0	0	0
Central	Tuscany	256	0	0	58	23	2	1	15	14	3	0
	<b>Total</b>	1194	7	2	97	31	3	2	98	37	8	2

**Table 4:** Serological titers detected with microscopic agglutination test for serovars Canicola, Pomona, Tarassovi and Bratislava.

Serovar	Titer						Total
	1:100	1:200	1:400	1:800	1:1600	1:3200	
Canicola	3	3	2	0	0	0	8
Pomona	38	28	24	6	1	0	97
Tarassovi	0	1	2	0	0	0	3
Bratislava	23	38	18	8	8	3	98
<b>Total</b>	64	70	46	14	9	3	206*

\*Total number of positive reactions detected, the total number of positive sera were 198, but 8 of them showed positivity for 2 different antigens.



32 Figure 1: Percentage of positive farms and sera detected in each Regions.

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