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

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Abstract

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

Introduction

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

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

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

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

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

Other *Leptospira* serovars could be responsible for incidental infections in pigs. Both acute and chronic infections could be observed, but clinical cases are focal, with the limited in-contact spread. Serovars involved vary around the world [9]. In particular, serovar Canicola has been detected from swine in several countries. In this case, it has been observed a long period of urine shedding and the ability to survive for up to 6 days in undiluted urine. These findings could suggest an intraspecies transmission [16–18].

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

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

In 2010, Italian pig population amounted to approximately 9.6 million animals. Over 50% was represented by heavy pigs (weighing more than 130 kg) used for the traditional dry-cured hams. Pig breeding is mainly concentrated in the northern area of Italy [19, 20].

The present study aims to provide a serological survey on the prevalence of *Leptospira* in pigs in North-Central Italy and to highlight the key role of a slaughterhouse as an epidemiological observatory for leptospirosis.

Materials and methods

From September to December 2015, 1194 swine sera samples were collected. Blood samples were taken at slaughterhouse during jugulation. Sixty-one closed-cycle fattening farms were included in the study. When it was possible, 20 animals for each herd were sampled using the systematic randomisation method. All selected animals were heavy pigs slaughtered at 150–160 kg at the age of about 1 year. Only non-vaccinated subjects were included in this survey. All swine resulted healthy before slaughter and during *post-mortem* examination of carcasses, no macroscopic lesion referable to leptospirosis could be detected. Moreover, a retrospective investigation, conducted by Public Veterinary, revealed that no leptospirosis infection occurred among slaughterhouse workers during about the previous 20 years. Investigated farms were located in five different regions of Italy, as reported in Table 1. Fourteen herds (256 sera) were located in Central Italy (Tuscany); 35 (700 sera) in North-West Italy (Piedmont and Lombardy) and 12 herds (238 sera) were located in North-East Italy (Veneto and Emilia Romagna).

All blood samples were carried to the Laboratory of Infectious Diseases of the Department of Veterinary Science, University of Pisa, in refrigerated condition in few hours after collection; all sera, obtained by blood centrifugation at 1000 ***g*** for 15 min, were maintained at −20 °C until they were used for laboratory examination.

Leptospira antibodies were detected by Micro Agglutination Test (MAT). Four *Leptospira interrogans* serovars were employed as live antigen: Canicola (serogroup Canicola, strain Alarik), Pomona (serogroup Pomona, strain Mezzano), Tarassovi (serogroup Tarassovi, strain Mitis Johnson) and Bratislava (serogroup Australis, strain Riccio 2); all employed strains were reference strains. They were grown in Ellinghausen–MacCullough–Johnson–Harris (EMJH—Difco, Detroit, Michigan, USA) at 30 °C for 4–14 days and checked for purity, mobility and agglutination ability. MAT was performed following the procedure previously reported by Cerri *et al.* [21]; a serum was considered positive when it showed 50% agglutination, leaving 50% free cells compared with a control culture diluted 1:2 in phosphate-buffered saline at the cut-off titre of 1:100. In case of positive sera, twofold serial dilutions were performed in order to determine the endpoint titre, which is defined as that last serum dilution able to cause 50% agglutination.

Results

Thirty-two out of the 61 farms analysed (52.5%) presented at least one positive animal at titre of 1:100. Furthermore, 21/61 (34.4%) of the herds presented at least one positive swine with titre ⩾1:400. In particular, 9/14 (64.3%) of farms from Central Italy were positive and 8/14 (57.1%) had animals with titre ⩾1:400. Nineteen out of 35 (54.3%) of herd located in North-West Italy resulted positive and 12/35 (34.3%) were positive with titre ⩾1:400. Four out of 12 (33.3%) units located in North-East Italy resulted positive and 1/12 (8.3%) presented at least one animal positive with titre ⩾1:400 (Table 1).

As concern sera, 198/1194 (16.6%) were positive and 70/1194 (5.9%) showed a titre ⩾1:400. Seventy-three out of 256 (28.5%) sera from Central Italy were positive and 38/256 (18.8%) presented a titre ⩾1:400. Among sera collected in North-West Italy, 95/700 (13.6%) resulted positive and 31/700 (4.4%) showed a titre ⩾1:400. As concern samples from North-East Italy farms, 30/238 (12.6%) sera showed a positive result, only one serum (0.4%) presented a titre equal to 1:400 and no sera showed higher titres (Table 1). Figure 1 reports the percentage of positive farms and sera detected in each region.

Table 2 shows the positive farms for each *Leptospira* serogroup. Positivity to all serovar tested was found in North-West Italy, while in Central and North-East farms positivity to serogroups Pomona, Australis and Tarassovi and to serogroups Pomona and Australis were only observed, respectively. Furthermore, in Tuscany and Lombardy serological positivity to more than one serogroups in the same herd was detected. Particularly, in Lombardy five herds resulted positive for two different serogroups and one farm was positive for all four serovars tested. Moreover, in three farms a seropositivity with an antibody titre ⩾1:400 for two serogroups was observed and one farm was positive for three different

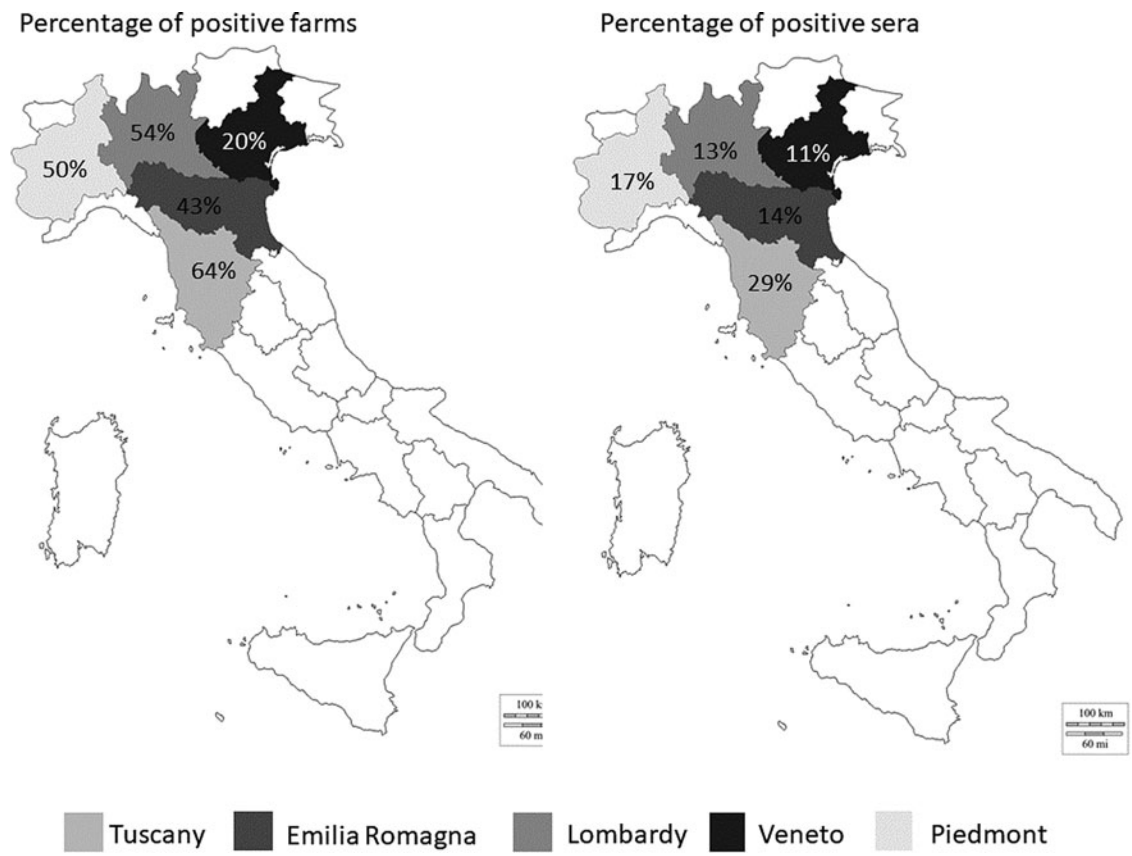

Fig. 1. Percentage of positive farms and sera detected in each region.

serogroups with a titre ⩾1:400. In Tuscany, only one farm presented serological positivity to two different serogroups.

Considering sera, the highest number of positive reactions was registered for serogroups Australis and Pomona; 98/1194 (8.2%) and 97/1194 (8.1%) sera were positive, respectively. As concern sera from Central Italy, 58/256 (22.7%) were positive for Pomona, while 15/256 (5.9%) showed positivity to Australis. Among samples collected from North-West Italy, 56/700 (8.0%) and 36/700 (5.1%) sera showed positivity for serogroups Australis and Pomona, respectively. Thirty-seven of 1194 (3.1%) sera scored positive with titre ⩾1:400 for serogroup Australis, in particular, 14/256 (5.5%) sera from Central Italy and 22/700 (3.1%) from North-West Italy. Thirty-one of 1194 (2.6%) sera were positive to serogroup Pomona with a titre ⩾1:400, among these, 23/256 (8.9%) were collected in Tuscany and 8/660 (1.21%) in Lombardy. A low number of sera showed positivity to serogroups Canicola and Tarassovi. Five and three sera, respectively from Lombardy and Tuscany, showed positivity for two different serogroups (Table 3).

Higher titre value was recorded for serogroup Australis. Serogroups Canicola and Tarassovi never showed titres higher than 1:400 (Table 4). Eight sera resulted positive to more than one antigen. In particular, three sera were positive to serogroups Pomona and Australis (titre of 1:400/1:400, 1:400/1:400 and 1:200/1:100, respectively), two sera to serogroups Pomona and Tarassovi (titre of 1:400/1:200 and 1:200/1:400, respectively) and three sera to serogroups Canicola and Tarassovi (titre of 1:200/1:400), to serogroups Canicola and Pomona (titre of 1:100/1:400) and to serogroups Canicola and Australis (titre of 1:100/1:200).

Discussion

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

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

Antibody titres of 1:100 were considered as a threshold; titres of 1:100 or 1:200 may be suggestive of an early stage of infection or of a previous infection. Titres of 1:400 or higher can be considered distinctive of endemic infection [22].

Most recent data about a total number of swine farms in the investigated area show a decrease of about 75% occurred between 2000 and 2010 [20]. Based on this trend and on data reported by Italian national database of Zoothechnical Registry Office (Banca Dati Nazionale (BDN) dell'Anagrafe Zootecnica) [23], we can assume that approximately 1.4% of pig farms of North Italy and Tuscany was analysed in this survey. Concerning the percentage of analysed farms, the detected *Leptospira* seroprevalence

Table 2. Number of positive farms to serogroups Canicola, Pomona, Tarassovi and Australis at low (⩾1:100) and high titres (⩾1:400)

			Positive Farms to different serogroup with low (⩾1:100) and high titre (⩾1:400)									
			Canicola		Pomona		Tarassovi		Australis		Positivity for more than one serogroups	
Area of Italy	Region	Examined farms	⩾1:100	⩾400	⩾1:100	⩾400	⩾1:100	⩾400	⩾1:100	⩾400	⩾1:100	⩾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
	Total	61	3	1	18	11	2	2	18	13	7	5

Table 3. Number of positive sera to serogroups Canicola, Pomona, Tarassovi and Australis at low (⩾1:100) and high titres (⩾1:400)

			Positive sera to different serogroups with low (⩾ 1:100) and high titre (⩾ 1:400)									
			Canicola		Pomona		Tarassovi		Australis		Positivity for more than one serogroups	
Area of Italy	Region	Examined Sera	⩾1:100	⩾400	⩾1:100	⩾400	⩾1:100	⩾400	⩾1:100	⩾400	⩾1:100	⩾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
	Total	1194	7	2	97	31	3	2	98	37	8	2

Table 4. Serological titres detected with microscopic agglutination test for serogroups Canicola, Pomona, Tarassovi and Australis

	Titre						
Serogroups	1:100	1:200	1:400	1:800	1:1600	1:3200	Total
Canicola	3	3	2	0	0	0	8
Pomona	38	28	24	6	1	0	97
Tarassovi	0	1	2	0	0	0	3
Australis	23	38	18	8	8	3	98
Total	64	70	46	14	9	3	206[a]

[a]Total number of positive reactions detected, the total number of positive sera were 198, but eight of them showed positivity for two different antigens.

(52.5%) could suggest a significant diffusion of this pathogen. Lombardy and Tuscany showed the highest percentages of positive farms with 54.6% and 64.3%, respectively. Moreover, in these regions the highest percentage of farms characterised by subjects with antibody titres ⩾1:400 was detected.

Concerning the positivity percentage of all tested sera (16.6%), our result is comparable with data reported by Tagliabue *et al.* [14] during a recent National survey. Due to the fact that Italian swine farms are mainly located in this area, our findings could be representative of the spreading of *Leptospira* in closed-cycle fattening pig farms. The highest percentage of positivity was detected in Tuscany (28.5%); in other regions sera positive percentages ranged from 11.0% (Veneto) to 17.5% (Piedmont). The higher percentages of positivity of Tuscany and Lombardy compared with the other analysed regions could be due to particular environmental conditions, potential risk factors and the abundance of reservoirs in the wild fauna. In fact, in the last years, the occurrence of *Leptospira* was reported in humans, wild and domestic animals in these areas [24–31].

The main represented serogroup was Australis with 21.3% of positive farms and 8.2% of positive sera; followed by serogroup Pomona with 18.0% and 8.1% of positive farms and sera, respectively. As concerns serogroup Australis, our results are quite in accordance with other studies previously conducted in Europe. In particular, Tagliabue *et al.* [14] referred 12.74% of positivity among Italians swine from 2010 to 2011. Cerri *et al.* [21] reported a prevalence of 8.85% in swine sera collected in Italy from 1995 to 2001. However, these data are only partially comparable with those from the present study since they are referred to antibody titres ⩾1:400. The percentage reported by Boqvist *et al.* [32] in Sweden in 2008 (3.9%) was slightly lower.

As concerns serogroup Pomona, it was remarkable to observe how, after several reports highlighting its decrease in Italian swine populations [14, 21], this infection seems to reemerge among pigs. Recently, in fact, in Europe, this serovar has been frequently reported in wild and domestic animals [33–37].

In past years, swine vaccination against *Leptospira* (classical swine vaccines used in Italy contain inactivated *Leptospira* strains belonging to serogroups Pomona, Tarassovi and Australis) led to a decrease of this infection in pig population. However, nowadays, many farms tend to vaccinate only breeding animals, in order to reduce the economic impact of vaccination. It would be possible to speculate that this practice could be a probable cause of the reemerging of same serovars in swineherds, especially in areas with a high epidemiological risk, such as those considered in this study. This hypothesis could be more appropriate for some than other serovars; in fact, for serogroup Tarassovi the maintenance host is *Sus scrofa*, while for serogroups Pomona and Australis, several potential maintenance hosts, both among domestic and wild animals, can be present in the environment. Consistently, the detected positivity percentage for serogroup Tarassovi was very low (0.3%). However, according to a recent study carried out in Italy [14], the presence of serological positivity for this serovar seems to suggest its ability to persist in certain ecological niches.

Regarding serogroup Canicola, few sera resulted positive (0.6%), all collected in Lombardy farms. The role of swine in the epidemiology of leptospirae belonging to this serogroup is not yet well clarified, although, other Authors reported that pigs are commonly infected by serovar Canicola [1]; moreover, this serovar was isolated from swine in Brazil [18]. Recently, serological positivity to this serogroup was reported in North-West Italy in wild boar [30] and coypus [31]. Some sera showed positivity to more than one serogroups; these findings could be ascribed to a coinfection or, more likely, to a cross-agglutination. As concerns antibody titres, the highest values were reported for serogroups Pomona and Australis; in particular, for Australis, eight sera showed an antibody titre of 1:1600 and three sera of 1:3200. This evidence is in accordance with different surveys carried out by several authors [14, 21, 32].

Frequently, slaughterhouses represent an important surveillance station especially for foodborne pathogens, such as *Salmonella*, *Campylobacter* and *Trichinella* [38–40]. Furthermore, slaughterhouse can be useful also to detect some specific swine diseases [41]. For these reasons, slaughterhouse could assume an important epidemiological role in highlighting some important zoonosis difficult to detect at the herd level. Moreover, it is safe to assume that the distribution of serovars in swine at slaughterhouse reflects the distribution of serovars that could be found in pig farms.

Based on our results, in Italy, leptospirosis spreading in swine is probably underestimated. If on the one hand swine leptospirosis outbreaks in Italy are sporadically reported, on the other hand, several surveys highlighted a considerable number of serological positivity. This information suggests a potential risk for animal and human health, in particular for slaughterhouse workers historically involved in outbreaks of ‘swineherd's disease’ mainly caused by Pomona and Tarassovi serovars. Moreover, this survey highlights the importance of slaughterhouse as an epidemiological observatory for reemerging infectious diseases characterised by silent infection, such as leptospirosis.

Conflict of interest. The authors declare that they have no conflict of interest.

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