The occurrence of *Leptospira* spp. serogroup Pomona infections in wild boars

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Abstract

Leptospirosis is a worldwide zoonosis caused by different pathogenic serovars of the genus *Leptospira*, with very complex aetiology and epidemiology. Recent publications suggest that wild boar (*Sus scrofa*) has an important role in the epizootiology of leptospirosis, representing a potential source of infection. Wild boar populations in urban environments are increasing, posing not only agricultural and ecosystem concerns, but also a public health concern. The aim of this study was to assess the prevalence, temporal patterns and risk factors of Pomona infection in wild boars from areas with pronounced human activity. A total of 159 wild boar serum samples were collected within a health monitoring project in Medvednica Nature Park over a period of six hunting seasons (2012–2016; 2018) and 25.2% samples tested positive. Seropositivity against seven different serogroups was observed, with the highest number of positive reactions to the serogroup Pomona. Annual variation in Pomona reactivity was also detected, and positive reactions were five times more common in females than in males. In most of studies, adults had a higher seroprevalence than younger categories; however, in this study, seroprevalence against Pomona was higher in piglets and subadults. The results indicate that wild boar has the potential of maintaining serovars from the serogroup Pomona in certain natural foci. Due to the expansion of wild boar populations and their intrusion into urban areas, they should be considered a potentially important source of infection for humans and domestic animals.

Key words: *leptospirosis; wild boar; Pomona*

Introduction

Leptospirosis is a worldwide zoonosis caused by different pathogenic serovars of the genus *Leptospira*. The aetiology and epidemiology of leptospirosis are very complex. Until 1989, the genus contained only two species based on their virulence: the pathogenic *L. interrogans* and the non-pathogenic, saprophytic *L. biflexa* (Faine and Stalman, 1982; Kmety and Dikken, 1993; Plank and Dean, 2000).

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Later, based on the same criteria, an intermediate lineage was added (Pérolat et al., 1998). New molecular methods that compare entire genome sequences have revealed the existence of 68 *Leptospira* species grouped into two clades and four subclades (Vincent et al., 2019). Serological classification recognises more than 300 serovars, with some new ones yet to be published (Picardeau, 2017). Each *Leptospira* serovar tends to be maintained by a specific animal host in a certain geographical area. Therefore, serological classification is mainly important from the point of understanding the epizootiology and epidemiology of leptospirosis at the population level. As some serovars are antigenically closely related, for practical reasons and to minimise potential diagnostic bias, serovars were grouped into serogroups (Alexander et al., 2015).

Following the isolation of "new" leptospira in 1936 from the blood of a dairy farmer residing near Pomona (South Queensland, Australia), Derrick (1942) proposed *Leptospira pomona* as a name for this organism. Despite being isolated from humans, certain serovars from serogroup Pomona were soon linked to pig leptospirosis, with pigs considered the main carriers (Faine et al., 1999). Currently, cases of leptospirosis caused by serovars from serogroup Pomona have also been reported in dogs, horses, donkeys, cattle and various wildlife species, including wild boars (Mackintosh et al., 1980; Fairley et al., 1984; Divers et al., 1992; Vicente et al., 2002; Ayanegui-Alcerreca et al., 2007; Prager et al., 2013; Guedes et al., 2021; Žele Vengušt, 2021; Aliberti et al., 2022; Piredda et al., 2023; Tresamol et al., 2023). More specifically, an increasing trend of Pomona infections has been noted in all domestic animals in Croatia (Habuš et al., 2017). Infection with the serogroup Pomona is usually accompanied by high antibody titres and a prolonged presence of bacteria in kidneys, though symptoms may vary from mild to more severe (Bolin, 1994). Serogroup Pomona is composed of seven serovars: Kunming, Pomona, Mozdok, Altodouro, Proechimys, Tsaratsovo and Tropica belonging to five different genomospecies: *L. interrogans, L. kirschneri, L. borgpetersenii, L. noguchii* and *L. santarosai* (Clayton, 1937; Semenova, 1965; Gale et al., 1966; Manev, 1976; Sulzer et al., 1982; Zhang et al., 1987; Pérolat et al., 1990; Bourhy et al., 2012; Paiva-Cardoso et al., 2013).

Several recent publications have discussed the importance of wild boars (*Sus scrofa*) in the epizootiology of leptospirosis (Żmudzki et al., 2016; Cilia et al., 2020a, 2020b; Roquelo et al., 2021; Zamir et al., 2022). Although their exact role has not yet been clarified, some studies suggest that they represent a potential source of leptospirosis. Therefore, the increasing population of wild boar, especially in suburban and urban areas, including recreational areas such as nature and national parks, could not only be a problem for agriculture and the ecosystem (Johann et al., 2020), but also a public health issue.

In Croatia, serological surveys of leptospirosis in wild boars have been carried out in different areas and over different periods of time. The highest percentage of positive wild boar samples was found in the lowland regions of Croatia (Slavica et al., 2010). On the other hand, the lowest number of serologically positive wild boars was found in areas where the predominant characteristics of the habitat are a hilly-karst terrain with an almost complete absence of wetlands, as such conditions prevent the formation of natural leptospirosis foci (Slavica et al., 2010).

The aim of this study was to assess the prevalence, temporal patterns and risk

factors of Pomona infection in wild boars from areas with pronounced human activity.

Materials and methods

Study area

Mt. Medvednica (45°49′00″ to 45°59′00″N and 15°49′45″ to 16°07′45″E) extends in the SW-NE direction above the Croatian capital, with the highest peak (Sljeme, 1035 m) located in the western part of the mountain. Due to its altitude, Medvednica is categorised as a hilly mountain habitat (Anonymous, 2006). It has well-preserved forest communities that cover 81% of the park area. The forest vegetation consists of different communities dominated by sessile oak (*Quercus petraea*), beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*). Unforested park areas are covered by various pastures and meadows, orchards and vineyards, hedges and shrubs. Due to the impermeable or poorly permeable geological subsoil, Mt. Medvednica is rich in streams and springs. In the south-western part, however, the geological surface is dominated by limestone and dolomite, with a steep slope so water does not remain on the surface. The area of the Medvednica Nature Park is characterised by a pronounced biodiversity of flora and fauna, but due to its proximity to the capital, it is also an area of pronounced human activity.

Animals

A total of 159 wild boar serum samples were collected within a health monitoring project in Medvednica Nature Park over a period of six hunting seasons (2012–2016; 2018). Sampling type was non-probability convenience sampling. Blood samples were taken from the animal hearts or jugular veins immediately after shooting, kept in test tubes in a cold place and delivered for testing the same day or the following morning (depending on the time of shooting) to the Faculty of Veterinary Medicine, University of Zagreb. Animals were classified by sex and into five age categories assessed by hunters: (< 1 year), yearlings (1–2 years), subadults (2–3 years), and adults (>3 years). This classification is in accordance with Anonymous (2006). Later, age was corrected at the Faculty of Forestry and Wood Technology, based on criteria provided by Wagenknecht (1984).

Microscopic agglutination test

For antibody detection, microscopic agglutination test was performed as previously described (Dikken and Kmety, 1978; Hartskeerl et al., 2006) using an antigen panel of 12 serovars (Grippotyphosa (strain Moskva V), Sejroe (strain M 84), Bratislava (strain Jež Bratislava), Pomona (strain Pomona), Canicola (strain Hond Utrecht IV), Icterohaemorrhagiae (strain RGA), Tarassovi (strain Perepelitsin), Saxkoebing (strain Mus 24), Ballum (strain Mus 127), Bataviae (strain Swart), Poi (strain Poi) and Hardjo (strain Hardjobovis). The cut-off value was set at 1:50, and presumptive infective serogroups were determined for each sample by identifying the highest titres to one or more serovars belonging to a certain serogroup. Those with the same titres to two or more serogroups were marked as undetermined.

Statistical Analysis

Data were analysed using chi-square ($\chi 2$) test and calculating the odds ratio. Statistical significance threshold was set at a P value ≤ 0.05.

Ethical statement

The sampling for this study was approved by the Veterinary Ethics Commit-

tee at the Faculty of Veterinary Medicine, University of Zagreb, Croatia.

Results

During the observed period, a 159 wild boar blood samples were collected, and 25.2% tested positive. Seropositivity against seven different serogroups was observed, with the highest number of positive reactions (30%; 12/40) to serogroup Pomona. Due to the diversity of serogroups found, the odds of being serologically positive on all other *Leptospira* serogroups was 2.33 (CI 95% 1.1330 to 4.8053) times higher than being seropositive for the Pomona serogroup. This difference is statistically significant (*P*=0.0215). Annual variations of reactivity to serogroup Pomona were also detected; 9.5% (2012; *n*=21), 4.7% (2013; *n*=42), 17.9% (2014; *n*=39), 5.2% (2015; *n*=19) and 0% (2016; *n*=16 and 2018; *n*=22).

All serologically positive reactions were recorded more often in female animals (♂ 20.2%; ♀ 32.3%), and this difference was significant (P=0.0186) when we considered only seroreactivity to serogroup Pomona (♂ 3.2%; ♀ 13.8%). The odds that females will be seropositive to Pomona was about 5 times higher than for males (OR=5.1; CI 95% 1.3143 to 19.8967). Results of the MAT analysis according to season and gender are presented in the Table 1.

Comparisons between different age categories regarding seroreactivity to Pomona were non-significant. The odds ratio of being positive was 2.6 times higher for piglets vs. yearlings (CI 95% 0.3328 to 20.5526), 1.2 times in case of piglets vs. subadults (CI 95% 0.2003 to 7.5635), 1.5 times in case of piglets vs. adults (CI 95% 0.2520 to 9.3920). When comparing yearlings with subadults and adults, the odds ratio was 2.125 (CI 95% 0.3639 to 12.4099) in favour of subadults and 1.700 in favour of adults (CI 95% 0.2931 to 9.8605). Subadults had 1.25 times higher odds of being positive then adults (CI 95% 0.2898 to 5.3922). According to the sex and age, a difference was found for adults, with an odds ratio of 5.787 (CI 95% 0.5499 to 60.8773), though this was non-significant. Pomona positive samples, according to the age category and sex are presented in the Table 2.

Discussion

In general, prevalence of antibodies against *Leptospira* spp. in European wild

Table 1. Results of MAT analysis according to year and gender

		2012	2013	2014	2015	2016	2018	∑
Positive samples (all serogroups)	M	5	6	5	1	0	2	**19**
	F	7	1	7	5	1	0	**21**
	∑ M + F	12	7	12	6	1	2	40
Pomona positive samples	M	1	1	1	0	0	0	**3**
	F	1	1	6	1	0	0	**9**
	∑ M + F	2	2	7	1	0	0	12
Negative samples (all serogroups)	M	5	22	16	7	12	13	**75**
	F	4	13	11	6	3	7	**44**
	∑ M + F	9	35	27	13	15	20	119

Table 2. Pomona positive samples, according to the age category and sex

	Pomona								
	Piglets		Yearlings		Subadults		Adults		∑
	M	F	M	F	M	F	M	F	
	0	2	0	2	2	2	1	3	
∑	2		2		4		4		12

boar populations vary between as low as 2% to up to 65.4% (Vengušt et al., 2008; Montagnaro et al., 2010; Slavica et al., 2010; Boqvist et al., 2012; Vale-Gonçalves et al., 2015). These variations are potentially a result of different climate conditions, density of small mammals (reservoirs) and wild boar populations, but also different MAT cut-off values (1:50, 1:80 or 1:100). Variations in cut-off values greatly influence the interpretation of results, making comparisons between different studies particularly difficult. Previous studies show that the seroprevalence of *Leptospira* in wild boars in different habitats in Croatia ranges from 10 to 30% (Cvetnić et al., 2003; Slavica et al., 2010; Milas, 2013). The overall prevalence of 25.2% found in this study confirms that exposure of wild boars to *Leptospira* is common and indicates the possibility that wild boars play a role in the epidemiology of leptospirosis in this area. Domestic pigs can harbour several pathogenic *Leptospira* serogroups, mainly Pomona, Australis and Tarassovi (Ellis, 2015). Similar serogroups are also associated with infections in wild boars, which is likely a consequence of the relatedness between the species. Older studies in Croatia (Kovačić et al., 1984) reported a prevalence of 9.2% in wild boars with Pomona as the most frequently identified presumptive infective serogroup in the Baranja region.

Cvetnić et al. (2003) reported that among wild boar positive samples, 47.5% belonged to serogroup Pomona, followed by serogroups Australis, Grippotyphosa and Icterohaemorrhagiae. According to Slavica et al. (2010), serogroup Pomona was the second most prevalent in wild boars with a seroprevalence of 21.8%. However, Milas et al. (2013) found only 4.0% seroprevalence for Pomona. High seroprevalence for Pomona were also reported in Spain and Germany (Jansen et al., 2007; Espí et al., 2010). Serovars belonging to serogroup Pomona are relatively common and widespread in both wildlife and livestock, but suids are considered potential maintenance host (Arent et al., 2017). Behaviours typical of wild boars, such as wallowing and rooting, certainly support the spread of infection and perpetuating contamination of the environment. Annual variation of Pomona exposure in wild boars was evident in this study, with the highest prevalence recorded in 2014. These results are in correlation with the increase in the prevalence of leptospirosis in humans and domestic animals recorded in Croatia in the same year, and associated with the significant rainfall and floods that occurred (Habuš et al., 2017).

Analysis of demographic characteristics in this study revealed that the odds of females being seropositive to Pomona were about 5 times higher than in males (OR=5.1; CI 95% 1.3143 to 19.8967; *P*=0.0186). Considering the transmission

patterns of leptospirosis, this can easily be explained by the social behaviour of wild boars. Females live in sounders – larger groups of 6–30 individuals, and they are also more frequently in contact with vaginal discharge or placental fluids during farrowing. Males are mostly solitary animals, seeking out females only during the mating season. Gender as a risk factor has not been confirmed in all previous studies, as some reported no statistically significant difference between the sexes (Vengušt et al., 2008; Slavica et al., 2010), while others found that seropositivity was more common in males (Pedersen et al., 2015). It is important to emphasise that those studies analysed all positive reactions, while analyses in this study focused only those positive for serogroup Pomona.

In most studies dealing with all *Leptospira* serovars, adults had a higher seroprevalence than younger categories. On the contrary, in this study, seroprevalence against Pomona was higher in piglets and subadults, though differences were not statistically significant. Since an odds ratio equal to 1 indicates that there is no association between the observed factor and the outcome, we have to point out that the observed odds are rather low.

The results of this study indicate the potential role of wild boars in maintaining serovars from serogroup Pomona in certain natural foci. Due to the expansion of wild boar populations and their intrusion into urban areas, they should be considered a potentially important source of infection for humans and domestic animals. To fully elucidate the role of wild boars in the epidemiology of leptospirosis, further extensive research is needed aimed at isolating and typing *Leptospira* strains carried by wild boars.

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Seroepidemiološko istraživanje leptospiroze prouzročene serogrupom Pomona u divljih svinja

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Leptospiroza je globalno proširena zoonoza prouzročena različitim patogenim serovarima iz roda *Leptospira*, vrlo složene etiologije i epidemiologije. Novija literatura ukazuje na činjenicu da bi divlje svinje (*Sus scrofa*) mogle imati značajnu ulogu u epizootiologiji leptospiroze i predstavljati

potencijalni izvor infekcije. Ekspanzija populacije divljih svinja u tom smislu, ne predstavlja izazov samo u poljoprivredi i ekologiji, već se može biti i potencijalni javno-zdravstvenim problem. Cilj je ovoga istraživanja bio procijeniti prevalenciju, vremenske obrasce i čimbenike rizika kod infekcije leptospirama serogrupe Pomona u divljih svinja iz područja s izraženom ljudskom aktivnošću. Ukupno 159 uzoraka seruma divljih svinja prikupljeno je u okviru projekta praćenja zdravstvenog stanja u Parku prirode Medvednica u razdoblju od šest lovnih sezona (2012.-2016.; 2018.). Ukupna utvrđena prevalencija bila je 25,2 %, s najvećim brojem pozitivnih reakcija za serogrupu Pomona. Uz izraženu godišnju varijaciju reaktivnosti na serogrupu Pomona, utvrđena je i varijacija s obzirom na spol i dob. Izgledi da će ženke biti seropozitivne bili su oko 5 puta veći nego u mužjaka, a za razliku od većine ostalih studija, seroprevalencija je bila veća u prasadi i subadultnih nego u adultnih jedinki. Rezultati ovog istraživanja pokazali su da divlje svinje imaju moguću ulogu u održavanju serogrupe Pomona u određenim prirodnim žarištima. Zbog ekspanzije populacije divljih svinja i njihovih prodora u urbana područja, treba ih smatrati potencijalno važnim izvorom zaraze i za ljude i za domaće životinje.

Ključne riječi: *leptospiroza, divlja svinja, Pomona*