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SHORT REPORT
Prevalence of *Leptospira* antibodies in wild boars (*Sus scrofa*) from Northern Portugal: risk factor analysis

H. M. VALE-GONÇALVES[1,2,3], J. A. CABRAL[1,2], M. C. FARIA[3], M. NUNES-PEREIRA[3], A. S. FARIA[2,3], O. VELOSO[3], M. L. VIEIRA[4] AND Md. N. PAIVA-CARDOSO[2,3,5]*

[1] *Laboratory of Applied Ecology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[2] *Centre for the Research and Technology of Agro-Environment and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[3] *Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[4] *Group of Leptospirosis and Lyme Borreliosis, Unit of Medical Microbiology, Institute of Hygiene and Tropical Medicine (IHMT), University Nova of Lisboa (UNL), Centre for Microbial Resources (CREM), Faculty of Science and Technology (FCT), Lisboa, Portugal*
[5] *Centre for Veterinary and Animal Science (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*

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SUMMARY

Leptospirosis is a zoonosis of worldwide distribution, caused by infection with pathogenic spirochaetes of the genus *Leptospira*. The wild boar (*Sus scrofa*), an important hunting species in Europe, seems to play a significant role in the epidemiological cycle of leptospirosis. A total of 101 serum samples from wild boar hunted in Northern Portugal were analysed for leptospiral antibodies detection by microscopic agglutination test. Sera were collected during hunting seasons (2011–2013) and tested with 17 different pathogenic serovars of *Leptospira*. Antibodies against nine serovars were detected in 66 (65·4%) of these sera. Serovars Tarassovi and Altodouro exhibited the highest seroreactivity rates (23·8% and 16·8%, respectively), followed by Autumnalis (7·9%) and Bratislava (6·9%). Age and district of origin were found to be risk factors for the presence of leptospiral antibodies in contrast to gender. From a One Health perspective, this study revealed that wild boar should be considered as a potential source of leptospirosis dissemination for humans and animal species (domestic and wild) in shared environments, particularly in the Trás-os-Montes region.

Key words: *Leptospira*, leptospirosis, wild boar, Altodouro, Northern Portugal, serology.

Leptospirosis is a re-emerging zoonosis of worldwide distribution [1]. The infection is caused by pathogenic spirochaetes of the genus *Leptospira* and despite affecting most mammals, domestic and wild, rodents are considered the main reservoirs and shedders [1].

The wild boar (*Sus scrofa*) is widely distributed in Europe, and its populations have increased in the last decades [2]. Their large movements and dispersion can potentially result in the spread of several infectious diseases, namely emerging and re-emerging zoonosis, such as leptospirosis.

* Author for correspondence: Professor M. das Neves Paiva Cardoso, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, PO Box 1013, 5000–801 Vila Real, Portugal.
(Email: maria_neves_cardoso@msn.com)

Studies in European wild boar populations seem to indicate that this species might play an important role in the epidemiological cycle of leptospirosis [3, 4], being a potential transmission source of pathogenic leptospires to humans, livestock and other local sylvatic species, that share the same geographical areas [5, 6].

The goal of this study was to determine the prevalence of specific antibodies against *Leptospira interrogans sensu lato* (*s.l.*) in wild boar from the Trás-os-Montes region, Northern Portugal, in order to improve the understanding of the role of this species in the epidemiology of leptospirosis in this region. Similar studies have been made in other European countries, but never in Portugal.

Wild boar hunts were selected from a list of hunts planned to occur between October and February of the 2011/2012 and 2012/2013 hunting seasons in the Trás-os-Montes region. A total of 27 hunts were attended during the sampling period, taking into consideration the spatial distribution of the hunts in the districts of Vila Real and Bragança, in order to cover as much area as possible. Convenience sampling was performed taking into account the active collaboration of hunters and hunt managers (i.e. by authorizing biological sample collection), and the number of animals shot.

Blood samples were collected from 101 wild boars slaughtered during the above-mentioned hunting seasons. The blood was centrifuged to extract the serum and then stored at −20 °C until serological analysis. Additional animal information was also collected: district of origin, gender and age. Age estimation was based on tooth eruption patterns and according to three age groups: juveniles (<12 months), subadults (12–24 months) and adults (>24 months) [7].

To detect the presence of leptospiral antibodies, serum samples were tested by the microscopic agglutination test (MAT) [8], using the following 17 live serovars (local and reference strains): Altodouro (strain Rim 139-local isolate), Arboreae (Arborea), Autumnalis (Akiyami A), Bratislava (Jêz Bratislava), Bataviae (Van Tienen), Canicola (Hond Utrecht IV), Celledoni (Celledoni), Valbuzzi (Valbuzzi), Hebdomadis (Hebdomadis), Copenhageni (M20), Icterohaemorrhagiae (RGA), Mini (Sari), Panama (CZ 214), Mozdok (5621), Hardjobovis (Lely 607), Wolffi (3705) and Tarassovi (Perepelitsin).

Sera were screened at a 1:50 dilution, and positive samples were titrated in serial twofold dilutions to determine the end-point titre. Samples with titres of 1:50 were considered to provide evidence of exposure to *Leptospira interrogans s.l.*, since higher dilutions may not detect the infection at an early stage or represent a residual level of antibodies years after the exposure to the agent [8].

Statistical analysis was performed using JMP® v. 9.0.1 (SAS Institute Inc., USA). Leptospiral seroreactivity status (positive or negative) was used as dependent variable, and age (juveniles, subadults, adults), gender (males and females), and district of origin (Vila Real or Bragança) as independent variables for χ^2 tests. A P value $<0{\cdot}05$ was considered significant. Risk factors for the presence of antibodies against *Leptospira* were assessed by nominal logistic regression analysis. The strength of association between seropositivity and other variables were estimated by the calculation of odds ratio (OR). An OR value with a lower limit of 95% confidence interval (CI) >1 was taken to indicate a significant association between variables.

The wild boar population analysed encompassed 101 animals categorized by: gender (33 males, 68 females), district of origin (56 from Bragança, 45 from Vila Real), and by age group (27 juveniles, 35 subadults, 39 adults). After preliminary statistical analysis, the animals were regrouped in just two groups: juveniles and adults ('adult' group encompasses both subadult and adult animals). Reorganization of data added robustness to the statistical results, rendering the variable 'age' more explanatory and statistically significant in the context of leptospiral seropositivity in the analysed population. The serological results confirmed the presence of antibodies against nine pathogenic serovars of *Leptospira* (Tarassovi, Altodouro, Autumnalis, Bratislava, Copenhageni, Mozdok, Arboreae, Ballum, Icterohaemorrhagiae) in the wild boar population from the Trás-os-Montes region (Table 1). Seroreactivity or cross-reactions of a serum sample to multiple serovars within a serogroup or different serogroup strains was frequently observed, as stated previously [8]. The serogroup of the strain that had reacted with the highest titre was considered to be the presumptive serogroup of the strain responsible for the infection.

Serological titres ranged from 1:50 to 1:1600, and the majority (63·6%) of positive samples demonstrated low titres ($\leqslant$1:100). This finding is consistent with other serosurvey studies in which low titres were frequently observed [6, 9, 10]. This fact is the reason why, for further statistical analysis, the authors considered sera samples with titres $\geqslant$1:50 as positive, bearing in mind that in epidemiological studies it is much more important to establish the animals' exposure to the aetiological agent independently of

Table 1. *Distribution of positive wild boar from the Trás-os-Montes region (Northern Portugal), according to serogroup and serovar reactivities detected by MAT*

Serogroup	Seropositive animals by serogroup, *n* (%)	Serovar	Seropositive animals by serovar, *n* (%)
Tarassovi	24 (23·8)	Tarassovi	24 (23·8)
Pomona	20 (19·8)	Altodouro	17 (16·8)
Autumnalis	8 (7·9)	Mozdok	3 (3·0)
Australis	7 (6·9)	Autumnalis	8 (7·9)
Icterohaemorrhagiae	4 (4·0)	Bratislava	7 (6·9)
Ballum	3 (3·0)	Copenhageni	3 (3·0)
		Icterohaemorrhagiae	1 (1·0)
		Arboreae	2 (2·0)
		Ballum	1 (1·0)
Total	66 (65·4)	Total	66 (65·4)

MAT, Microscopic agglutination test.

determining if there is any evidence of current infection [8, 11]. The highest titres ⩾1:1600 were obtained for just one sample, against serovar Bratislava. This sample also showed reactivity to multiple serovars at lower titres.

A total of 66 (65·4%) wild boar showed antibodies at a titre of ⩾1:50 and 34·7% at a titre of ⩾1:100, the cut-off titre most currently used by other authors in identical studies [6, 9, 10, 12]. The seropositivity rate obtained in this study is greater than those observed comparatively in other European countries such as Sweden (3·1%) [12], Italy (6%) [10], Spain (14·4%) [13], Germany (18%) [14] and the Czech Republic (16·9%) [9], but lower than the rates reported in Slovenia (45·8%) [6] and Croatia (35·0%) [15].

Titres to Tarassovi and Pomona serogroups were the most common (23·8% and 19·8%, respectively) with titres ranging from 1:50 to 1:400 (Table 1). Seroreactivity to Pomona group was mainly due to serovar Altodouro, which was responsible for 16·8% of the observed reactivity. Serovar Altodouro, a new serovar from the *Leptospira kirschneri* genospecies, was recently isolated from a *Mus musculus* rodent species in the Trás-os-Montes region [1, 16]. Later studies in dogs from Greece [17] have shown that this serovar seems highly reactive, which, in addition to our findings, may indicate the need to include serovar Altodouro as antigen in coming leptospirosis serological studies across Europe, concerning both domestic and wild populations. Several other strains from the Pomona group have been previously isolated in Portugal: serovar Mozdok strains from pigs [18] and small mammals [19], and serovar Tsaratsovo from horses [20]. Pigs are commonly considered reservoirs of strains from serogroup Tarassovi [21] and Pomona [18], and serovars Bratislava and Muenchen (Australis group) were previously isolated in pigs from Northern Ireland [22]. The role of the wild boar is questionable as a potential host for Tarassovi because strains from this serogroup were never isolated from this species, but strains from Pomona, Australis and Icterohaemorrhagiae groups have been isolated from wild boar kidneys in Croatia [3], as well as strains from Pomona serovar in Italy [4].

Several authors have reported seropositivity to different serovars in wild boar populations worldwide [6, 10, 12–15]. Seroreactivity to Tarassovi has been frequently reported [6, 15, 23] and is, similarly to our study, the most reactive serovar observed in wild boar from Slovenia [6]. Antibodies against Pomona is the most frequently reported leptospiral seroreactivity in wild boar populations in Spain [13, 24], and Germany [14], and the second most common in Croatia [15].

In our study, titres to Autumnalis (7·9%), Australis (6·9%), Icterohaemorrhagiae (4%) and Ballum (3%) groups were less frequently observed. Antibodies to serovar Bratislava were less frequently detected than expected, since reactivity to strains of the Australis serogroup is much more often observed, and were the most prevalent in similar studies conducted in Italy [10] and Sweden [12].

Adult wild boars showed a higher seropositivity rate (71·6%) than juveniles (48·2%), and the differences were statistically significant ($P < 0.05$) (Table 2). Age seems to be an important risk factor for the presence of leptospiral antibodies in wild

Table 2. *Wild boar distribution data and results of statistical analysis of serological reactivity to Leptospira strains (Trás-os-Montes region, Northern Portugal)*

Variables	Tested (n)	Positives (n)	Seropositivity rate (%)	χ^2	P value	OR	95% CI
Age				4·813	0·0282	2·72	1·096–6·742
Juveniles	27	13	48·2				
Adults	74	53	71·6				
Total	101	66	65·4				
Gender				1·179	0·2775	–	–
Males	33	24	72·7				
Females	68	42	61·8				
Total	101	66	65·4				
District				7·263	0·0070	3·16	1·349–7·412
Bragança	56	43	76·8				
Vila Real	45	23	51·1				
Total	101	66	65·4				

OR, Odds ratio; CI, confidence interval.

boar from the Trás-os-Montes region (OR 2·72, 95% CI 1·096-6·742), as shown in similar studies from the Czech Republic [9], Germany [14] and Croatia [15]. These results could obviously be anticipated since the possibility of direct or indirect contact with leptospirosis increases over the lifespan.

Gender-wise, males showed a higher seropositivity rate (72·7%) than females (61·8%). Nevertheless, significant statistical differences ($P > 0·05$) were not found (Table 2). These findings corroborate the data presented in studies from Slovenia [6], Germany [14] and the Czech Republic [9].

Regarding district, the seropositivity rate was higher in Bragança (76·8%) than in Vila Real (51·1%) with significant statistical differences ($P < 0·05$) (Table 2). District of origin seems to be an important risk factor for the presence of leptospiral antibodies in wild boar from the Trás-os-Montes region (OR 3·16, 95% CI 1·349-7·412). Other studies in the Trás-os-Montes region corroborate this tendency. Similar results were obtained for indigenous Maronesa cattle from the Trás-os Montes region [25]. Climate and land use distribution seem to explain differences within each of these areas. Bragança is much more rural than the district of Vila Real, with a higher percentage of forested land, especially broad-leaved forest. This type of land cover benefits high humidity in the soil, thus contributing to the maintenance of leptospires in the environment for longer periods of time [26].

In the highly forested region of Trás-os-Montes, hunting activities have great economic impact, putting humans in closer contact with wild animals (reservoirs for these spirochaetes), especially occupational risk groups (hunters, gamekeepers, other forestry-related professionals).

From a One Health perspective, these study results revealed that wild boar should be considered as a potential source of dissemination of pathogenic leptospires for humans, domestic animals and other wild species in shared geographical regions, particularly in the Trás-os-Montes region.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Paiva-Cardoso MdN, *et al.*** Altodouro, a new *Leptospira* serovar of the Pomona serogroup isolated from rodents in northern Portugal. *Infection, Genetics and Evolution* 2013; **13**: 211–212.
2. **Acevedo P, *et al.*** Estimation of European wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment. *Epidemiological and Infection* 2007; **135**: 519–527.
3. **Cvetnic Z, *et al.*** A serological survey and isolation of leptospires from small rodents and wild boars in the Republic of Croatia. *Veterinári Medicína* 2003; **48**: 321–329.
4. **Pintore A, *et al.*** First record of *Leptospira* isolation from wild boars of Sardinia. In: Turk N, Habuš J, eds. *European Meeting of Leptospirosis – Eurolepto 2012*. Dubrovnik: Faculty of Veterinary Medecine, University of Zagreb, 2012, pp. 61.
5. **Little TWA.** Changes in our understanding of the epidemiology of leptospirosis. In: Ellis WA, Little TWA, eds. *The Present State of Leptospirosis Diagnosis and Control*. Dordrecht: Martinus Nijhoff Publishers, 1986, pp. 149–173.
6. **Vengust G, *et al.*** *Leptospira* antibodies in wild boars (*Sus scrofa*) in Slovenia. *European Journal of Wildlife Research* 2008; **54**: 749–752.
7. **Sáenz de Buruaga M, Lucio AJ, Purroy FJ.** The boar. In: Sáenz de Buruaga M, Lucio AJ, Purroy FJ, eds. *Age and Sex Recognition in Game Species* [in Spanish]. Vitoria, Spain: Diputación Foral de Alava, 1991, pp. 31–33.
8. **OIE (Office International des Epizooties).** Chapter 2·1·9. – Leptospirosis. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)*, 7th edn, Paris, 2014 (http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/). Accessed 23 October 2014.
9. **Treml F, Pikula J, Holešovská Z.** Prevalence of antibodies against leptospires in the wild boar (*Sus scrofa* L., 1758). *Veterinári Medicína* 2003; **48**: 66–70.
10. **Ebani V, *et al.*** Prevalence of *Leptospira* and *Brucella* antibodies in wild boars (*Sus scrofa*) in Tuscany, Italy. *Journal of Wildlife Diseases* 2003; **39**: 718–722.
11. **Ellis WA.** Leptospirosis. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, eds. *Diseases of Swine*, 9th edn. New Jersey: Blackwell Publishing, 1992, pp. 691–700.
12. **Boqvist S, *et al.*** The association between rainfall and seropositivity to *Leptospira* in outdoor reared pigs. *Veterinary Journal* 2012; **193**: 135–139.
13. **Espí A, Prieto J, Alzaga V.** Leptospiral antibodies in Iberian red deer (*Cervus elaphus hispanicus*), fallow deer (*Dama dama*) and European wild boar (*Sus scrofa*) in Asturias, Northern Spain. *Veterinary Journal* 2010; **183**: 226–227.
14. **Jansen A, *et al.*** Leptospirosis in urban wild boars, Berlin, Germany. *Emerging Infectious Diseases* 2007; **13**: 739–742.
15. **Slavica A, *et al.*** Detection of *Leptospira* spp. serovars in wild boars (*Sus scrofa*) from continental Croatia. *Veterinarski Arhiv* 2010, **80**: 247–257.
16. **Levett PN, Smythe L.** International Committee on Systematics of Prokaryotes Subcommittee on the Taxonomy of Leptospiraceae: Minutes of the closed meeting. *International Journal of Systematic and Evolutionary Microbiology* 2014; **64**: 1071–1072.
17. **Arent ZJ, *et al.*** Emergence of novel *Leptospira* serovars: a need for adjusting vaccination policies for dogs? *Epidemiology and Infection* 2013; **141**: 1148–1153.
18. **Rocha T.** Isolation of *Leptospira interrogans* serovar mozdok from aborted swine fetuses in Portugal. *Veterinary Record* 1990; **126**: 602.
19. **Collares-Pereira M *et al.*** Analysis of *Leptospira* isolates from mainland Portugal and the Azores islands. *FEMS Microbiology Letters* 2000; **185**: 181–187.
20. **Rocha T, *et al.*** Microbiological and serological study of leptospirosis in horses at slaughter: first isolations. *Research in Veterinary Science* 2004, **76**: 199–202.
21. **Ryan TJ, Marshall RB.** Isolation of a leptospire belonging to the serogroup tarassovi. *New Zealand Veterinary Journal* 1975; **24**: 212–213.
22. **Ellis WA, *et al.*** Prevalence of *Leptospira* infection in aborted pigs in Northern Ireland. *Veterinary Record* 1986; **118**: 63–65.
23. **Montagnaro S, *et al.*** Prevalence of antibodies to selected viral and bacterial pathogens in wild boar (*Sus scrofa*) in Campania region, Italy. *Journal of Wildlife Diseases* 2010; **46**: 16–319.
24. **Vicente J, *et al.*** Antibodies to selected viral and bacterial pathogens in European wild boars from south central Spain. *Journal of Wildlife Diseases* 2002; **38**: 649–52.
25. **Paiva-Cardoso MN,** Epidemiological importance of rodents as reservoirs of *Leptospira* in Maronesa cattle farms in Trás-os-Montes region (thesis). Vila Real, Portugal: University of Trás-os-Montes and Alto Douro, 2009, 272 pp.
26. **Vale-Gonçalves HM,** Analysis and prediction of the occurrence of leptospirosis in wild boars (*Sus scrofa* Linnaeus, 1758) of the Trás-os-Montes region (dissertation). Vila Real, Portugal: University of Trás-os-Montes and Alto Douro, 2014, 93pp.