Serum samples collected from Iberian red deer (Cervus elaphus hispanicus; n = 472), fallow deer (Dama dama; n = 293) and European wild boar (Sus scrofa; n = 174) in Asturias, Northern Spain, from 1999 to 2005 were examined for antibodies against a reference panel of 14 Leptospira spp. serovars. Positive antibody titres at a microscopic agglutination test cut-off of 1:80 were detected against serovars Pomona (1.6%, 5.8%, 5.2%), Bratislava (1.1%, 0.7%, 4.7%), Grippotyphosa (0.7%, 2.4%, 1.7%), Muenchen (2.6%, 0%, 0%), Pyrogenes (0.4%, 2.4%, 1.2%), Panama (1.2%, 1.7%, 0%), Copenhageni (0%, 0.7%, 0.6%), Autumnalis (0.4%, 0%, 0.6%) and Icterohaemorrhagiae (0%, 0%, 0.6%) in Iberian red deer, fallow deer and European wild boar, respectively.

The population sizes were 7000 Iberian red deer in 2003, an average of 1070 fallow deer in 1999–2003 (SIAPA, 2008) and 30628 European wild boar (C. Nores, personal communication). Blood samples were collected from 472 Iberian red deer and 293 fallow deer from 1999–2005 and from 174 European wild boar from 2004–2005 (Supplementary Table 1), all of which had been hunted. Sera were screened against a reference panel of 14 leptospiral serovars using the microscopic agglutination test (MAT) (Cole et al., 1973). MAT titres were recorded as the reciprocal of the highest dilution showing 50% antigen agglutination. Samples giving titres of ≥ 1:80 were considered to provide evidence of exposure to Leptospira spp. avoiding cross-reactions at lower cut-off titres. The panel represented the following serovars of Leptospira interrogans: Bratislava (strain Jez Bratislava), Autumnalis (Akiyami A), Bataviae (Van Tienen), Icterohaemorrhagiae (RGA), Pyrogenes (Salinem), Hardjo (Hardjoprajitno), Pomona (Pomona), Grippotyphosa (Moskva V), Copenhageni (M 20) and Muenchen (Munchen C90); Leptospira borgpetersenii: Ballum (S 102) and Castellonis (Castellon 3); and Leptospira noguchii: Panama (CZ 214K) and Louisiana (LSU 1945) genospecies. These reference strains were provided by the Royal Tropical Institute – WHO/FAO Collaborating Centre for Reference and Research on Leptospirosis, Amsterdam, and by the Aggete’s Central Veterinary Laboratory.

Positive antibody titres at a MAT cut-off of 1:80 were detected against serovars Pomona (1.6%, 5.8%, 5.2%), Bratislava (1.1%, 0.7%, 4.7%), Grippotyphosa (0.7%, 2.4%, 1.7%), Muenchen (2.6%, 0%, 0%), Pyrogenes (0.4%, 2.4%, 1.2%), Panama (1.2%, 1.7%, 0%), Copenhageni (0%, 0.7%, 0.6%), Autumnalis (0.4%, 0%, 0.6%) and Icterohaemorrhagiae (0%, 0%, 0.6%) in Iberian red deer, fallow deer and European wild boar, respectively (Table 1). Serovar Pomona was predominant...
among fallow deer (5.8%) and European wild boar (5.2%), while serovar Muench was most frequent among Iberian red deer (2.6%). Titres of antibodies for different leptosiral serovars ranged from 1:80 to 1:2560; Iberian red deer had the highest titres (1:2560) for serovars Muench and Grippotyphosa. Pomona, the most prevalent serovar in this study, also showed high titres (1:1280 in 27.3% of positive reactions) (Supplementary Table 2).

The MAT is specific for the infecting serovar or closely antigenically related serovars. Sera giving positive reactions against serovar Pomona, the most frequent serovar detected in our study, were also tested against serovar Mozdok, since both serovars belong to serogroup Pomona, may cross-react and have been identified in Portugal (Rocha, 1998). All sera showed higher titres against Pomona than Mozdok.

Although many serological surveys of wildlife have been carried out, information on the seroprevalence of Leptospira spp. is limited for most species of Spanish wildlife. Differences in the prevalence of Leptospira spp. infections in different regions may reflect true variations or may result from differences in study designs, including the method of sampling, serovars included or MAT cut-off values. Pomona, one of the most prevalent leptosiral serovars in fallow deer, European wild boar and Iberian red deer in our study, has been reported in 12% of wild boar (1:400 cut-off) in South-Central Spain (Vicente et al., 2002) and was one of the most prevalent serovars in red deer and wild boar in Croatia (Slavica et al., 2008), but was not detected in wild boar from Tuscany (Italy) (Ebani et al., 2000).

In this study, we found the highest prevalence of seropositive samples (titre >1:80) in the microscopic agglutination test by serovar and year for 14 Leptospira spp. serovars in wild ungulates from Asturias (Spain).

Table 1
Prevalence of seropositive samples (titre >1:80 in the microscopic agglutination test) by serovar and year for 14 Leptospira spp. serovars in wild ungulates from Asturias (Spain).

<table>
<thead>
<tr>
<th>Species/year</th>
<th>Bratislava</th>
<th>Autumnalis</th>
<th>Icterohaemorrhagiae</th>
<th>Pyrogenes</th>
<th>Pomona</th>
<th>Grippotyphosa</th>
<th>Copenhageni</th>
<th>Muenchen</th>
<th>Panama</th>
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</tbody>
</table>

* Number positive/number tested (% positive).

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data


References


