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Prevalence of *Leptospira* and *Brucella* Antibodies in Wild Boars (*Sus scrofa*) in Tuscany, Italy

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ABSTRACT: Five hundred sixty-two blood samples were collected from wild boars (*Sus scrofa*) shot in six districts of Tuscany, central Italy, between 1997 and 2000. Sera were examined for antibodies specific for *Leptospira interrogans* by microagglutination test and *Brucella* spp. by the Rose Bengal test and indirect enzyme-linked immunosorbent assay. Thirty-four (6.0%) samples tested positive for anti-*Leptospira* antibodies, 29 (5.1%) sera were positive for anti-*L. interrogans* serovar bratislava antibodies (titres ranging from 1:100−1:400), and 5 (0.9%) sera were positive for anti-*L.interrogans* serovar icterohaemorrhagiae antibodies (titres 1:100). All the examined sera were negative for anti-*Brucella* antibodies.

Key words: Brucellosis, leptospirosis, serology, *Sus scrofa,* wild boars.

The wild boar (*Sus scrofa*) is widely diffuse in Tuscany, Central Italy. This wild animal can leave its natural habitat to come into contact with domestic animals. For this reason, it is a potential source of infectious diseases for domestic animals sharing the same areas.

Seropositivity to *Leptospira interrogans* infection has been previously demonstrated in wild boars in Italy (Farina and Andreani, 1970; Tagliabue and Farina, 1995; Ponti et al., 1996; Tagliabue et al., 1996). Even though data about leptospirosis in wild boars are not common, significant seroprevalence (37%) to *L. interrogans* serovar bratislava has been observed in recent years (Ponti et al., 1996). Seropositivity to serovars icterohaemorrhagiae, sejroe, saxkoebing, pomona, canicola, copenhageni, and proechimys has also been detected in this species (Farina and Andreani, 1970; Ponti et al., 1996).

Brucella spp. infection is still present among domestic animals living in limited areas of Italy, and brucellosis has been sporadically reported in Tuscany. Wild boar is mainly susceptible to *Brucella suis* biovar 1 and 2, but *B. abortus* and *B. melitensis* infections can occur (Dedek, 1983; Pannwitz, 1984; Wilhem and Zieris, 1987; Robson et al., 1993; Godfroid et al., 1994). In Italy, *B. abortus* and *B. melitensis* rarely have been reported in domestic pigs, and *B. suis* has never been isolated from these animals. *Brucella* spp. were never isolated from wild boars in Italy, but *B. suis* biovar 2 was isolated in southern Italy from a male hare (*Lepus europaeus*) imported from Hungary in 1995 (Quaranta et al., 1995). A study to determine the seroprevalence of brucellosis in hares living in Tuscany provided negative results (Poli et al., 1987). Hares coming from Eastern Europe, where brucellosis by *B. suis* biovar 2 is endemic, have been introduced to restock hunting areas. This species could represent a real source of *B. suis* biovar 2 infection for other animals sharing the same habitats, particularly for wild boars. Furthermore wild boars were also imported from Eastern Europe during the last decade.

A previous serologic investigation was carried out by Giovannini et al. (1988) to determine the presence of anti-*Brucella* spp. seropositivity in 43 fallow deer (*Dama dama)* and 20 wild boars shot in Tuscany. Four wild boars and one fallow deer showed a minimal reaction to the complement fixation test but were negative to the plate agglutination test; these animals were interpreted as non-specific reactors.

The purpose of this study was to investigate the seroprevalence of *Leptospira* sp. and *Brucella* spp. infections in wild boars from Tuscany, Central Italy.

Five hundred and sixty-two serum samples from wild boars shot during the hunt-

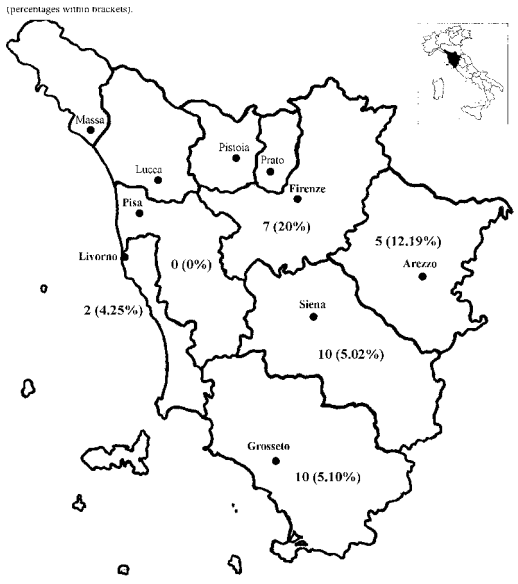

FIGURE 1. Distribution of seropositivity to anti-*Leptospira interrogans* antibodies in the examined areas of Tuscany (percentages within brackets).

ing seasons from 1997 to 2000 were examined. All the animals lived in wooded areas of Tuscany. One hundred ninety-nine of them were shot in the district of Siena (43°19′N–11°18′E), 196 in the district of Grosseto (42°46′N–11°7′E), 47 in the district of Leghorn (43°33′N–10°19′E), 44 in the district of Pisa (43°43′N–10°24′E), 41 in the district of Arezzo (43°28′N–11°35′E), and 35 in the district of Florence (43°46′N–11°16′E). Blood samples were collected intracardially; sera were separated by centrifugation and stored at −20 C until used.

To detect anti-*L. interrogans* antibodies, sera were tested by microagglutination test. Serovars icterohaemorrhagiae (Bianchi strain), canicola (Alarik strain), pomona (Mezzano strain), tarassovi (Jhonson strain), grippotyphosa (Moscow V strain), and bratislava (Riccio 2 strain) cultures were used as live antigens. The cultures were grown in Leptospira Medium Base Ellinghausen-MacCullough-Johnson-Harris (EMJH—Difco, Detroit, Michigan, USA) at 30 C for 4–14 days. The microagglutination test was performed following the procedure previously reported by Faine et al. (1999). Briefly, sera to be tested were diluted 1:50 with sterile saline solution in wells of 96 U-shaped plates. The same volume of antigen suspension was added to each well and mixed by agitation. Plates were incubated at 30 C for 2 hr. A loopful of the suspension in each well was was placed on a slide and examined for agglutination using a darkfield microscope. Sera with antibody titre ≥1:100 were considered positive and 2-fold serially dilutions were tested to determine the end-point titer.

To determine *Brucella* seropositivity, a Rose Bengal test (RBT) and a homemade indirect enzyme linked immunosorbent assay (ELISA) were employed. *Brucella abortus* W99 colored with rose bengal was used as antigen for RBT (Ciuchini and Farina, 1991), while the lipopolysaccharide fraction of *B. abortus* W99 was employed for ELISA. Antigens containing smooth whole cells of *B. abortus* are usually employed to detect antibodies against *B. abortus*, *B. melitensis* and *B. suis*, because the three *Brucella* strains have the same surface lipopolysaccharide (Hendry et al., 1985; Alton et al., 1988).

The ELISA was performed in 96-well plates coated with 50 μl/well of antigen diluted 1:100 in carbonate-sodium bicarbonate buffer (pH 9.6) and incubated at 4 C overnight. Plates were washed three times with phosphate buffered saline at pH 7.4 with 0.04% Tween 80 (PBST) added and dried by tapping firmly upside down on adsorbent paper. Each serum sample was diluted 1:200 in PBST and tested (50 μl/well) in duplicate in antigen coated wells. Plates were incubated at 37 C for 1 hr and washed three times with PBST. Fifty μl of rabbit anti-pig IgG peroxidase labeled secondary antibody (Sigma Chemical Company, St. Louis, Missouri, USA) diluted 1:35,000 in PBST were added to each well. Plates were incubated at 37 C for 1 hr, washed three times with PBST, and once with phosphate-citrate buffer (PCB, pH 5). Colorimetric reaction was developed by adding 50 μl/well of substrate-chromogen

solution (4% o-phenylene-diamine dihydrochloride in PCB and 0.04% hydrogen peroxide) and stopped with 2N H_2SO_4. Plates were read at 492 nm by a spectrophotometer (Titertek Multiskan PLUS MKII ®, Flow Laboratories, Lugano, Switzerland).

Forty serum samples of domestic swine, which had no history of living in areas endemic for brucellosis and were completely healthy by physical examination and laboratory tests, were included as negative controls for the standardization of ELISA test. The optical density (OD) values of the negative controls were used to calculate the cutoff value. It corresponded to three standard deviations above the average OD values given by negative controls in 20 replicates and resulted 0.45. Blood serum samples from five *B. abortus* biovar A3/6-naturally infected domestic sows were employed as positive controls.

Thirty-four (6.0%) of the 562 sera examined scored positive for anti-*L. interrogans* antibodies. Twenty-nine (5.1%) samples were positive for anti-serovar bratislava antibodies (20 samples at 1:100, 5 at 1:200, and 4 at 1:400). Of the 29 positive wild boars, 10 (5.10%) lived in the district of Grosseto, 10 (5.02%) in the district of Siena, seven (20%) in the district of Florence and two (4.25%) in the district of Leghorn. Five (0.9%) sera of wild boars from the district of Arezzo were positive for anti-serovar icterohaemorrhagiae antibodies, all of them with a titre of 1:100. No seropositive reactions to the other serovars were detected.

All serum samples were negative for anti-*Brucella* spp. antibodies when tested with both RBT and ELISA, whereas all positive controls scored positive to RBT and gave OD above the cutoff value (from 0.62 to 0.97).

The results of our study show the presence of *Leptospira*-seropositive wild boars in Tuscany but with a low prevalence and with low antibody titres. These data suggest that *Leptospira* infection is endemic in this wild species in Italy and confirm previous studies performed on sera from domestic and wild animals (Andreani et al., 1995; Cerri et al., 1996; 2000). Most seropositivity to *L. interrogans* was due to the serovar bratislava; whereas positivity to the serovar icterohaemorrhagiae was found only in a lower percentage. The presence of serovar bratislava-seropositive wild boars suggests that this serovar is spreading to other "ecological niches." In fact, leptospires such as *L. interrogans* serovar bratislava, belonging to the serogroup Australis, may be spreading among other species like horses, dogs, and domestic swine as demonstrated by extensive serologic investigations (Little, 1986; Farina, 1998; Cerri et al., 2000). Moreover, serovar bratislava was isolated from horses (Ellis et al., 1983), sheep (Little et al., 1981; Hathaway et al., 1983) and pigs (Little, 1986; Pritchard, 1986). Other serovars of Australis serogroup, such as lora and muenchen, were isolated from domestic swine (Hartmann et al., 1975; Hathaway et al., 1982).

Our data suggest that wild boars might play a role in epidemiology of *L. interrogans* infections because the density of this wild species is high in our areas and there is an increasing possibility of contact with domestic animals.

Results of serologic tests to detect anti-*Brucella* spp. antibodies suggest absence of this infection in wild boars of Tuscany. A previous serologic survey carried out to detect anti-*Brucella* spp. antibodies in 300 sera of fallow (*Dama dama*) deer living in Tuscany in the natural preserve of San Rossore gave similar results (Cerri et al., 1996). Brucellosis in domestic animals is, in fact, drastically reduced in the central part of Italy due to instituted eradication programs.

In conclusion, our study demonstrated that wild boars appear to not play a major role in the epidemiology of *Leptospira* sp. and *Brucella* spp. infections, at least in Italy. But extensive bacteriologic investigations are needed to confirm the presence or absence of these etiologic agents in wild

boar populations. Moreover, the seroprevalence of *B. suis* biovar 2 in hares should be accurately investigated, as these animals may represent a source of infection to domestic pigs and wild boars.

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