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Epidemiologic Survey for *Brucella suis* Biovar 2 in a Wild Boar (*Sus scrofa*) Population in Northwest Italy

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ABSTRACT: In 2001, antibodies to *Brucella* spp. were detected in the wild boar (*Sus scrofa*) population of Regional Park in the Piedmont, northwest Italy. This was the first report of swine brucellosis in Italian wildlife, and in response, we conducted a survey on wild boars culled in the park to evaluate the presence and epidemiologic pattern of *Brucella*. In total, 2,267 serum samples and 1,841 tissue samples were collected and tested from 2001 to 2007. Differences in antibody and infection prevalence among sampling years were statistically significant ($\chi^2=28.2$, $P<0.0001$ and $\chi^2=27.8$, $P<0.0001$, respectively). Serologic and culture results also differed between gender and age groups, and for serology, a positive age trend was observed in both male and female age classes ($\chi^{2(\text{trend})}=90.9$, $P<0.00005$ OR: 1, 2.5, 4.9, 7.5; $\chi^{2(\text{trend})}=43.1$, $P<0.00002$, OR: 1, 2.5, 2.9, 4.8, respectively).

Key words: *Brucella suis* 2, Italy, swine brucellosis, wild boar.

Apart from classical swine fever, swine brucellosis, caused by *Brucella suis* biovar 2, is one of the most important endemic diseases in the wild boar (*Sus scrofa*) populations in Central Europe (Garin-Bastuji et al., 2000; Godfroid, 2002; Godfroid and Käsbohrer, 2002; Cvetnic et al., 2003; Al Dahouk et al., 2005; Vengust et al., 2006; Leuenberger et al., 2007). In Italy, *B. suis* biovar 2 was detected in hares (*Lepus europaeus*) that were imported from Hungary in 1995, but in spite of an increased sampling effort, brucellosis was not detected in endemic wildlife until the end of the 1990s (Quaranta et al., 1995; Ebani et al., 2003). In northwest Italy, brucellosis has been reported in the Piedmont in alpine ibex (*Capra ibex*; Ferroglio et al., 1998) and in chamois (*Rupicapra rupicapra*; Ferroglio et al. 2003). In 2001, antibodies to *Brucella* spp. were detected in wild boars in the "Mandria di Venaria Reale" Regional Park (Piedmont, northwest Italy 45°8′7″N, 7°37′31″E; Gennero et al., 2006). This park was established in 1978, includes 3,600 ha of fenced area, and is characterized by plain forest with open meadows and streams; it is inhabited by red deer (*Cervus elaphus*, 200–500 animals) and an estimated 500 wild boar. In this study, we conducted a survey of wild boars culled in the park in order to evaluate the presence and epidemiologic pattern of *Brucella* in the wild boar population.

Blood samples were collected from 2,267 culled wild boars from 2001 to 2007. Blood was allowed to clot, and serum was separated by centrifugation and stored at −20 C until testing. After 2002, tissue samples (spleen and genital organs) also were collected from 1,841 animals. Gender and age were determined for all animals. Age was estimated based on tooth eruption pattern; four age categories were used (A: <10 mo; B: 11–18 mo; C: 19–24 mo; D: >25 mo; Briedermann, 1986).

Serum samples were tested by rose Bengal test (RBT) and complement fixation test (CFT) according to Alton et al. (1988), and 20 g of tissue (uterus, spleen, and testicles) were macerated and inoculated onto *Brucella* medium according to Farrell (1974). Samples were inoculated

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onto modified Thayer Martin Medium (Martin et al., 1996), and media plates were enriched with 10% horse serum and selective supplements; cultures were incubated for at least 10 days. If *Brucella* was suspected during Stamp's staining, colonies were identified to genus by classical techniques (Alton et al., 1988) and typed by means of biochemical (Alton et al., 1988) and molecular tests (Corbel et al., 1983; Bricker and Halling, 1994; Cloeckaert et al., 1995) by the laboratories of the National Reference Centre of Brucellosis (Teramo, Italy). Epi Info™ 6 for DOS Current Version 6.04d (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) was used to test for associations between serologic and culture results with year of sampling, season, gender, and age by chi-square tests. Differences were considered significant when $P<0.05$. Agreement between serology and bacteriology test results were calculated using WinEpiscope 2.0 (N. de Blas, C. Ortega, K. Frankena, J. Noordhuizen, M. Thrusfield: http://www.clive.ed.ac.uk/winepiscope/).

Antibodies to *Brucella* spp. were detected with RBT and CFT in 448 of 2,267 serum samples. Of 1,841 animals tested by culture, 448 tested positive (Table 1). All isolates were classified as *Brucella suis* biovar 2.

Differences observed in antibody prevalence among sampling years were statistically significant ($\chi^2=28.2$, $P<0.0001$); prevalence of infection as determined by culture differed by years ($\chi^2=27.8$, $P<0.0001$). For both serology and culture, prevalence decreased significantly in 2005 and 2006 ($\chi^2=12.1$, $P<0.0005$ and $\chi^2=12.6$, $P<0.0005$, respectively) and increased in 2007 (compared to 2004 and 2005; $\chi^2=9.9$, $P<0.002$ and $\chi^2=13.3$, $P<0.0003$, respectively). Differences in the prevalences of antibody- and culture-positive animals also were detected between some gender and age groups. An increased antibody prevalence with age was observed in both males and females ($\chi^{2(\text{trend})}=90.9$, $P<0.00005$ OR: 1, 2.5, 4.9, 7.5 and $\chi^{2(\text{trend})}=43.1$, $P<0.00002$, OR: 1, 2.5, 2.9, 4.8, respectively). These age differences were not observed with culture results. Differences among antibody prevalences in males and females were only detected within the C age class ($\chi^2=8.9$, $P<0.003$).

Antibody prevalence was significantly lower ($\chi^2=10.3$, $P=0.0003$) from August (8%) to November and did not significantly vary during the other months of the year. Prevalence estimates based on culture were significantly lower ($\chi^2=38.9$, $P=0.00000$) from July to November, and the lowest prevalence occurred in August (2%).

In comparisons of serology and culture results for the 1,762 wild boars for which both serum and tissue samples were available, the test agreement was low (Kappa=0.37), and 26.8% (51/190) of samples positive on bacteriology were negative on both serologic tests.

Our data confirm the presence of *B. suis* 2 in wild boar in Italy, and it appears that the prevalence based on serology (19.8±1.6%) and culture (10.8±1.4%) is high. These prevalence estimates are consistent with estimates from traditionally (Godfroid et al., 1994; Al Dahouk et al., 2005) and nontraditionally endemic areas (Hars et al., 2000; Ruiz-Fons et al., 2006). Annual prevalence estimates (antibodies and culture) suggest that *B. suis* 2 was maintained in this population from 2001 to 2004 at a relatively constant level. In 2005 and 2006, prevalence significantly decreased, and in 2007, it returned to previous 2001–2004 levels. The cause of this variation is not known, and data related to wild boar density are not available to evaluate potential population-dependent causes. Considering the low agreement among serologic and culture results, it is recommended that both be used for future epidemiologic surveys.

The increased antibody prevalence with age observed with both males and females may be attributable to sexual maturity. In

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TABLE 1. Serologic and bacteriologic results (positives/examined; prevalence with 95% confidence interval [CI95%] in parentheses) on sera and organs from wild boar (*Sus scrofa*) collected from 2001 to 2007 in the "Mandria di Venaria Reale" Regional Park, Piedmont, Italy (RBT = rose Bengal test; CFT = complement fixation test; M = male; F = female; n.d. = not done).

Years[a]	Sex	RBT/CFT	Bacteriologic test
2001	M	11/180 [6.11% CI95%: 2.61–9.61]	n.d.
	F	8/201 [3.98% CI95%: 1.28–6.68]	n.d.
2002	M	44/151 [29.14% CI95%: 21.89–36.39]	17/141 [12.06% CI95%: 6.68–17.43]
	F	25/134 [18.66% CI95%: 12.06–25.25]	9/124 [7.26% CI95%: 2.69–11.83]
2003	M	42/130 [32.31% CI95%: 24.27–40.35]	20/144 [13.89% CI95%: 8.24–19.54]
	F	45/187 [24.06% CI95%: 17.94–30.19]	25/205 [12.20% CI95%: 7.72–16.68]
2004	M	39/144 [27.08% CI95%: 19.83–34.34]	22/148 [14.87% CI95%: 9.13–20.60]
	F	43/136 [31.62% CI95%: 23.80–39.43]	22/148 [14.87% CI95%: 9.13–20.60]
2005*	M	43/214 [20.09% CI95%: 14.73–25.46]	19/220 [8.64% CI95%: 4.92–12.35]
	F	42/250 [16.08% CI95%: 12.17–21.43]	14/254 [5.51% CI95%: 2.71–8.32]
2006*	M	25/167 [14.97% CI95%: 9.56–20.38]	8/135 [5.93% CI95%: 1.94–9.91]
	F	32/193 [16.58% CI95%: 11.33–21.83]	12/155 [7.74% CI95%: 3.54–11.95]
2007	M	31/104 [29.81% CI95%: 21.02–38.60]	19/98 [19.39% CI95%: 11.56–27.22]
	F	18/76 [23.68% CI95%: 14.13–33.24]	11/69 [15.94% CI95%: 7.30–24.58]
Subtotal	M	235/1090 [21.56% CI95%: 19.12–24.00]	105/886 [11.85% CI95%: 9.72–13.98]
	F	213/1177 [18.10 CI95%: 15.90–20.30]	93/955 [9.74% CI95%: 7.86–11.62]
Total		448/2267 [19.76% CI95%: 18.12–21.40]	198/1841 [10.75% CI95%: 9.34–12.17]

[a] Asterisk denotes significant values ($P<0.05$).

females, antibody prevalence increased sharply from class A (<10 mo) to class B (>11 mo; $\chi^2=9.6$, $P<0.002$), while it did not increase significantly from class B to class C. Difference in antibody prevalence between genders was only detected in age class C ($\chi^2=8.9$, $P<0.003$), while differences in other age classes were not significant. No significant differences were observed between males and females in bacteriology, even if a positive correlation seemed to emerge for class C (19–24 mo; $\chi^2=3.3$, $P=0.06$). The higher prevalence of antibody- and culture-positive animals observed from December to June could be related to reproductive season; estrus generally starts in late autumn until February, while partum occurs mainly from February until June (Durio et al., 1995).

Brucella suis has not been previously detected in Italy, from either wild boar or domestic swine, and it is possible that it was introduced by hares imported from European countries where it is endemic (Dedek, 1983; Hubalek et al., 1993; Quaranta et al., 1995; Pilaszek et al., 1996). As suggested by Lanfranchi et al. (2003), wildlife disease management must be based on avoiding introduction of new pathogens, and a health risk assessment should be included in all future translocation plans.

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