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Seroprevalence of Anti-*Brucella* spp. Antibodies in Wild Boars (*Sus scrofa*), Hunting Dogs, and Hunters of Brazil

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ABSTRACT: All 86 wild boars (*Sus scrofa*), 170 hunting dogs, and 49 hunters sampled from three Brazilian regions were seronegative to *Brucella* spp. by the standard tube agglutination and 2-mercaptoethanol tests, suggesting a low circulation of *Brucella* spp. in wild boars, hunting dogs, and hunters in such areas.

Brucellosis is a zoonosis caused by gram-negative bacteria of the genus *Brucella*, which infects livestock, wildlife, and humans (Godfroid 2002; Ficht 2010). Wild boars (*Sus scrofa*), domestic pigs, and other wildlife species have been indicated as reservoirs of *Brucella suis* and *Brucella abortus* for both livestock and wildlife species (Godfroid 2002; Olsen and Tatum 2017).

In humans, hunting activities are a greater potential source of *Brucella* spp. exposure than are other working and recreational activities (Pereira et al. 2020). Additionally, wild boar hunting has been considered a source of *B. suis* infection in hunting dogs (Mor et al. 2016).

In Brazil, wild boars are classified as exotic invasive species, with nationwide hunting officially permitted for control, generally using hunting dogs for wild boar tracking (IBAMA 2013). Although *B. abortus*, *B. suis*, and *Brucella melitensis* have been recognized as the most prevalent *Brucella* spp. in Brazil and wild boar hunting has been associated with wildlife-dog-human transmission (Pereira et al. 2020), no study has simultaneously assessed *Brucella* spp. exposure in wild boars, hunting dogs, and hunters. Our study aimed to evaluate that.

Our study was conducted October 2016 through May 2018 in preserved and degraded areas in the Atlantic Forest biome of southern and southeastern Brazil, including the Vila Velha State Park in southern Brazil and the Vassununga State Park in southeastern Brazil, and degraded areas in the Cerrado biome of central-western Brazil at the Aporé municipality.

Blood samples were obtained from 86 free-ranging wild boars killed by legally registered hunters. These boars, from natural areas of the Vila Velha and Vassununga state parks, were baited, photo-monitored, trapped, and then killed by firearms; blood samples were collected by intracardiac puncture immediately after death. Blood was collected from 170 hunting dogs from various rural and urban areas using jugular venipuncture, with the approval from the Ethics Committee of Animal Use of the Federal University of Paraná (protocol no. 059/2017). Blood was collected from 49 hunters using cephalic venipuncture with the approval from the Ethical Appreciation at Ethics Committee in Human Health of the Brazilian Ministry of Health (protocol no. 97639017.7.0000.0102).

Testing for antibodies against *Brucella* spp. was performed at the Biological Institute, a National Livestock Reference Laboratory (São Paulo, Brazil). Sera were initially screened with the Rose Bengal test (RBT). Sera positive with the RBT were then tested using anti–*B. abortus* antibodies (which cross-react with *B. suis* and *B. melitensis*) in the standard tube agglutination test (STAT) and the 2-mercaptoethanol test (2MET; Paulin et al. 2002), performed in parallel.

Of the wild boar, only two individuals, one from southern and one from central-western Brazil, were seroreactive by the RBT; all samples from dogs and humans were negative in the RBT, so were not tested further. The two wild boar samples had negative results by both the STAT and 2MET; a sample must be positive in both the STAT and 2MET tests to be considered positive (to increase specificity of the final interpretation; OIE 2018). Thus, all 86 wild boars, 170 hunting dogs, and 49 hunters were considered seronegative to smooth pathogenic *Brucella* spp., suggesting a low circulation of *Brucella* spp. in the wild boars, hunting dogs, and hunters in this area.

The only other study conducted in Brazilian free-range feral pigs, in the central-western Brazil floodplains biome (Pantanal), also found all 105 animals to be seronegative to *Brucella* spp. (Zimmermann et al. 2018). Those authors concluded that, despite widespread brucellosis in bovine herds, sympatric feral pigs may have low environmental exposure to *Brucella* spp. because of low animal density and low cohabitation in such extensive areas.

In addition, a study of domestic pigs (*Sus scrofa domesticus*) from backyard pig farms in southeastern Brazil found only 1/346 (0.29%) pigs were seropositive to *B. abortus*, showing that 1/56 (1.79%) farms were infected (Ricardo et al. 2016), indicating a minor role of domestic pigs inn *Brucella* spp. transmission.

Our results contrast with those of previous studies, which have shown wild boar populations as the main reservoirs for *B. suis* biovar 1 in the US (Leiser et al. 2013). We hypothesize that the lack of brucellosis detection in free-ranging Brazilian wild boars may be due extensive natural and agricultural that do not predispose close contact with livestock.

In Australia, contact between hunting dogs and feral pigs has been associated with a 17-fold increase in canine brucellosis cases and a higher risk of *B. suis* transmission to hunters, household contacts, and livestock (Mor et al. 2016). In contrast, a study in Iran reported only 6/180 (3.3%) seropositive rural dogs, which was considered unrelated to hunting activities (Gharekhani and Sazmand 2019).

Although all hunters analyzed in our study tested negative to *Brucella* spp., hunters are reportedly more exposed to zoonotic pathogens, including *Brucella* spp., when compared with other risk groups, such as veterinarians, farmers, and slaughterhouse workers (Deutz et al. 2003), and particularly associated with feral pig population-control programs involving hunting dogs (Woldemeskel 2013). Our results contrasted with previous epidemiologic studies in the US and Europe, which found association between free-range wild boars and spreading of brucellosis, mostly to domestic pigs and hunters (Leiser et al. 2013). Because RBT is based on the *Brucella* smooth lipopolysaccharide, smooth strains detection of O-polysaccharide (O-PS), false-positive serologic reactions may occur, such as cross-reactions with O-PS bacteria in infected swine (Dieste-Pérez et al. 2015). Additionally, false-positives may occur if immunoglobulin M has been inadequately inactivated (OIE 2018). In contrast, false-negative reactions rarely occur with standard diagnostic methods for *Brucella* spp. (OIE 2018). The two false-positive RBT sera results may indicate *Yersinia enterocolitica* infection, given the similarity between the O-PS component of the smooth lipopolysaccharide of *Brucella* spp. and *Y. enterocolitica*, which have previously associated with false-positive RBT results in free-ranging and captive wild animals of southeastern Brazil (Antunes et al. 2010). Despite the known false-positives found with the RBT because of cross-reactivity with other pathogens or inadequately inactivated immunoglobulin M, the RBT is still useful as a screening test because of its excellent sensitivity, rapidity, feasibility, and low cost.

Because the serologic tests we used only tested smooth *Brucella* spp., further studies on Brazilian hunting dogs should focus on detection of *Brucella canis*, a rough *Brucella* sp. (Hensel et al. 2018).

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