

Serosurvey for Pseudorabies (Aujeszky's Disease) in Free-range Wild Boars (*Sus scrofa*) of Brazil

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ABSTRACT: Free-ranging wild boars (*Sus scrofa*) from two different Brazilian biomes were sampled. Only one of 36 (2.8%) animals tested from central-western Brazil were positive for antibodies against pseudorabies virus (PRV) by enzyme-linked immunosorbent assay. Peccaries and other wildlife species can be exposed to PRV in these areas. In addition, wild boars could have impact on livestock and PRV eradication.

Pseudorabies or Aujeszky's disease, caused by the pseudorabies virus (PRV), has been considered a notifiable and contagious livestock disease (Lee and Wilson 1979). This disease is often associated to high mortality in piglets and abortion (Lee and Wilson 1979). Domestic pigs (*Sus scrofa domesticus*) and wild boars (*Sus scrofa*), members of family Suidae, have been recognized as natural hosts of PRV (Müller et al. 2011).

The pseudorabies virus can affect other wildlife species, including the Tayassuidae family, such as collared peccaries (*Pecari tajacu*) and white-lipped peccaries (*Tayassu pecari*; De Castro et al. 2014). Pseudorabies is also known to cause high mortality in mammalian carnivores (Zhang et al. 2015). Although no report has been made in free-ranging wildlife, cases of PRV infection caused mortality in a Iberian lynx (*Lynx pardinus*) in Spain (Masot et al. 2016), a Florida panther (*Felis concolor coryi*) in Florida, US (Glass et al. 1994), a red fox (*Vulpes vulpes*) in Denmark (Bitsch and Munch 1971), and even a wild mouse (*Peromyscus* sp.) in Illinois, US (Weigel et al. 2000).

In Brazil, wild boars have been classified as an exotic invasive species, originated from Eurasian wild boars and their hybrids, and nationwide hunting is officially permitted as a strategy for population control and eradication (Kmetiuk et al. 2019). Due to their highly adaptive capacity, wild boars have invaded all six Brazilian biomes, including the Atlantic Forest and Cerrado of central-western, south-western, and southern Brazil (Kmetiuk et al. 2019). Although PRV infection is reportedly established in wild boars elsewhere, no study has been conducted in free-ranging wild boars of Brazil. Our aim was to determine the presence of PRV antibodies in wild boars from different areas of two Brazilian biomes.

The study was conducted in preserved and degraded areas in the Atlantic Forest biome of southern Brazil, including the Vila Velha State Park (25°12'34"S, 50°03'37"W) and the nearby developed Campos Gerais region in southern Brazil, and in degraded areas in the Cerrado biome of central-western Brazil, at the Aporé municipality (18°57'54"S, 51°55'33"W).

Free-ranging wild boars from degraded areas were sampled following hunting by firearm, under the Brazilian hunting laws, performed by legally registered hunters at the Brazilian Institute of the Environment and Renewable Natural Resources. Free-ranging wild boars from a natural area in the Vila Velha State Park were baited, photomonitoring, trapped, and shot, following previous authorization by the Brazilian Environmental

Biodiversity System (SISBIO license 61805-2/2017).

Blood samples were collected from 94 animals by intracardiac puncture immediately after death from October 2016 to May 2018. In addition, blood samples of 16 fetuses (eight each) from two pregnant sows were also collected after slaughter. The presence of antibodies against PRV was performed at the Biological Institute, a National Livestock Reference Laboratory (São Paulo, Brazil) with wild boar serum samples were tested in duplicate by a commercially available enzyme-linked immunosorbent assay (IDEXX PRV/ADV gB Ab Test[®], IDEXX Laboratories, Westbrook, Maine, USA) and results interpreted as positive or negative. Enzyme-linked immunosorbent assay sensitivity has been considered greater than serum neutralization and has been recommended as the international gold standard serological method for PRV diagnosis and notification by the World Organization for Animal Health (Cunha et al. 2006; World Organization for Animal Health 2018).

Antibodies were detected in 2.8% (1/36) adult free-ranging wild boars from Cerrado biome of central-western Brazil. None of the 20 adult free-ranging wild boars sampled from natural area in the Vila Velha State Park and none of the 38 adult free-ranging wild boars sampled from the Atlantic Forest biome degraded areas of southern Brazil were seropositive to PRV antibodies. Also, none of the 16 fetal wild boars that we sampled were seropositive to PRV. There has not been a previous report of PRV in free-ranging wild boars in Brazil. The seroprevalence to PRV that we report was lower than that reported in Brazilian farmed wild boars, 19.3% (69/358) seropositive animals (Cunha et al. 2006). In addition, although restricted to the Brazilian Pantanal biome (floodplains), feral pigs, classified as *Sus scrofa* have a higher seroprevalence to PRV, with 47.3% (88 of 186) positive animals (Paes et al. 2013). Globally, in free-living wild boars, the seroprevalence of PRV has been reported in Austria, Belgium, Croatia, the Czech Republic, Yugoslavia, France, Germany, Italy, Lithuania, the Neth-

erlands, Poland, Romania, Russia, Slovenia, Spain, Sweden, Switzerland, and the US, ranging from 0.0% (0/260) in France to 60.5% (98 of 162) in Austria (Müller et al. 2011).

The single male wild boar seropositive found in this study might indicate PRV circulation in degraded areas of central-western Brazil, although at a much lower prevalence than in the Pantanal biome. Nonetheless, geographical overlap of native peccaries and invasive wild boars has been described in both Brazilian biomes, the Atlantic Forest and Cerrado (Silveira and Pacheco 2018; Kmetiuk et al. 2019).

Although a concomitant study in Colombia sampling 58 collared peccaries and 15 wild boars, showed no positive cases (Montenegro et al. 2018), De Castro et al. (2014) detected PRV by PCR in spleen samples of all 10 collared peccaries and all three white lipped peccaries sampled from central-western and northern Brazil, using primers designed against swine pseudorabies variant. Such overlap has been confirmed in the Atlantic Forest biome by wild boars infested with the adult and nymph stages of ticks (*Amblyomma brasiliense*), which use collared peccaries as primary hosts in humid forests (Kmetiuk et al. 2019). In the Cerrado biome, peccary habitat destruction and fragmentation has been related to wild boar occurrence (Silveira and Pacheco 2018). Because PRV transmission to secondary hosts can occur by secretions and excretions of infected suids (Sawitzky 1997), the seropositive wild boar in this study suggests the PRV circulation in degraded areas of central-western Brazil, a large area of susceptible wildlife, livestock, and domestic animal coexist. Despite serological PRV detection herein, further studies should be performed to molecularly characterize and isolate wild swine PRV, as well as to fully establish the risk of PRV spillover infection to Brazilian native peccaries and domestic pigs.

Our report of a seropositive free-ranging wild boars in Brazil suggests that PRV is circulating at low levels in the Cerrado of Brazil. This virus has the potential to impact in peccaries and other Brazilian wildlife

species and negatively affect pseudorabies eradication programs in commercial swine farms nationwide.

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Submitted for publication 23 October 2019.

Accepted 23 December 2019.