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SHORT COMMUNICATION

Serological survey of bovine viral diarrhea (BVDV-1), brucellosis, and leptospirosis in captive white-lipped peccaries (*Tayassu pecari*) from the Midwest region in Brazil

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ABSTRACT. The present study was conducted to assess the occurrence of anti-*Brucella* sp., anti-BVDV-1, and anti-*Leptospira* spp. antibodies from captive white-lipped peccary (*Tayassu pecari*). A cross-sectional survey was performed testing 100 serum samples collected in a commercial breeding herd. All samples were submitted to the acidified antigen test (AAT), virus neutralization test (VNT) and microscopic agglutination test (MAT) with live antigens. None of the samples tested agglutinated in the AAT screening test. In the VNT, 28 samples presented a cytotoxic effect and were excluded from the evaluation. For BVDV-1, only one sample (1/72; 1.38%) was positive, with antibody titers of 40. For leptospirosis, 9% (9/100) of the samples reacted to at least one of the 24 serovars tested, with 8% (8/100) positive for serovar Patoc and 1% (1/100) for serovar Grippotyphosa. The maximum titer observed was 100. The identification of antibodies against the serovars Patoc and Grippotyphosa suggests that the sampled individuals have been exposed to the pathogen at some point during their lifetime. Regarding BVDV-1, this may be the first serological survey to describe seropositive samples in tayassuids.

Key words: infectious disease, tayassuidae, zoonosis.

INTRODUCTION

Swine and peccaries belong to the *Artiodactyla* order and to the *Suidae* and *Tayassuidae* families, respectively. Two species of peccaries are found in Brazil, the White-lipped peccary (*Tayassu pecari*) and the Collared peccary (*Tayassu tajacu*) (Keuroghlian and Eaton 2008). These two species of tayassuids are widely hunted in tropical forests to avoid their entry into certain regions and for the consumption by local communities (Bodmer *et al* 1996, Cullen, Bodmer and Valladares-Padua 2000, Altrichter and Boaglio 2004). Predatory hunting of peccaries has been motivated by their gregarious and occasional aggressive behaviour, besides food habits that promote the damage of grain and horticultural plantations (Oliver 1993). The reduction of ecological impact occasioned by predatory tayassuid hunting may be possible by implementing commercial, sustainable, conservationist alternatives for income generation for communities living in areas inhabited by those species (MMA 2001).

Studies regarding health aspects of tayassuids are important because they provide essential information considering the development and widespread captive breeding of peccary species in several Latin American countries (Nogueira and Nogueira-Filho 2011), taking into account the risks attributed and the environmental impact. Serological surveys conducted on *T. pecari* and *T. tajacu* populations revealed antibodies against *Leptospira* spp. (Ito *et al* 1998, Mayor *et al* 2006, Freitas *et al* 2010, Navas-Suárez *et al* 2017), vesicular stomatitis virus and pseudorabies virus (Corn 1987), *Orbivirus* spp. (Gerber *et al* 2012) and *Brucella* spp. (Corn 1987, Gruver and Guthrie 1996, Ito *et al* 1998, Mayor *et al* 2006) which are important agents causing infections in domestic pigs. Some molecular studies in *T. pecari* and *T. tajacu* have also detected antigens of *Erysipelothrix rhusiopathiae* (Coutinho *et al* 2012), *Trypanosoma evansi* e *T. cruzi* (Herrera *et al* 2008), porcine circovirus type 2, herpesvirus suis type 1, *Mycoplasma hyopneumoniae* and *Pasteurella multocida* (Castro *et al* 2014, Navas-Suárez *et al* 2017). To date, antibodies and molecular detection of bovine viral diarrhoea virus (BVDV) have been described exclusively in wild boars (Sedlak, Bartova and Machova 2008, Weber *et al* 2016).

Evidence has pointed to the risks of transmission of pathogenic agents among domestic swine, tayassuids and wild boars. Based on this assumption, the tayassuids may serve as susceptible hosts and potential reservoirs of agents that can infect domestic and wild pigs in addition to other species (Nava and Cullen 2003, Herrera *et al* 2008, Freitas *et al* 2010, Coutinho *et al* 2012). Freitas *et al* (2010) demonstrated the presence of anti-*Leptospira interrogans* antibody titers in *T. pecari* that maintained close interaction with local communities and other domestic animals, indicating that the close relationship among humans, domestic animals, and tayassuids resulted in the contact with the pathogen. In this scenario, the present study assessed the occurrence of antibodies against *Brucella* sp., BVDV-1 and

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Leptospira spp. in white-lipped peccary (*Tayassu pecari*) from a commercial breeder located in the Cerrado area, Central-Western region of Brazil.

MATERIAL AND METHODS

STUDY SITE

The study was conducted in a commercial farm (process Ibama nº 02001.004190/1999-54) composed of a set of properties located in the Cerrado area which has about 330 km^2 bordering the state of Minas Gerais, state of Goiás and state of Bahia. The farm is dedicated to the commercial breeding of *T. pecari*, *T. tajacu*, Ema (*Rhea Americana*) and red-footed tortoise (*Chelonoidis carbonaria*). The tayassuids breeding on the farm began in 2001. In 2008, the facilities were restructured and animal nutrition was reformulated by increasing the capacity and number of animals within the picket in an intensive breeding system. Each animal species was bred at a different site and each site consisted of 180 m^2 pickets, which housed groups of 6 to 10 animals. The pickets of the tayassuids consisted of an earthen floor without vegetation, surrounded by walls and fences over the walls. The pickets were located next to each other on both sides of a long corridor, and within them, there were feeders and water fountains (figure 1). This study was approved by the Ministry of the Environment (SISBIO nº 56725-1).

ANIMALS

In total, the property had 365 *T. pecari*. The family groups and groups with defined hierarchy were considered the selection criteria for sampling and a total of 100 animals were sampled, 72 females and 28 males.

Animals from 14 pickets were collected and, of those, four pickets with ten animals per group (two pickets with animals between three and six months of age and two pickets with animals between seven and 10 months of age) and ten pickets of families with six animals per group (all ages). The number of animals sampled per group according to sex and age is presented in table 2.

SAMPLES

In July 2017, the samples were collected in a single time point. For the blood sample collections, each group of animals was transferred to the management area. All

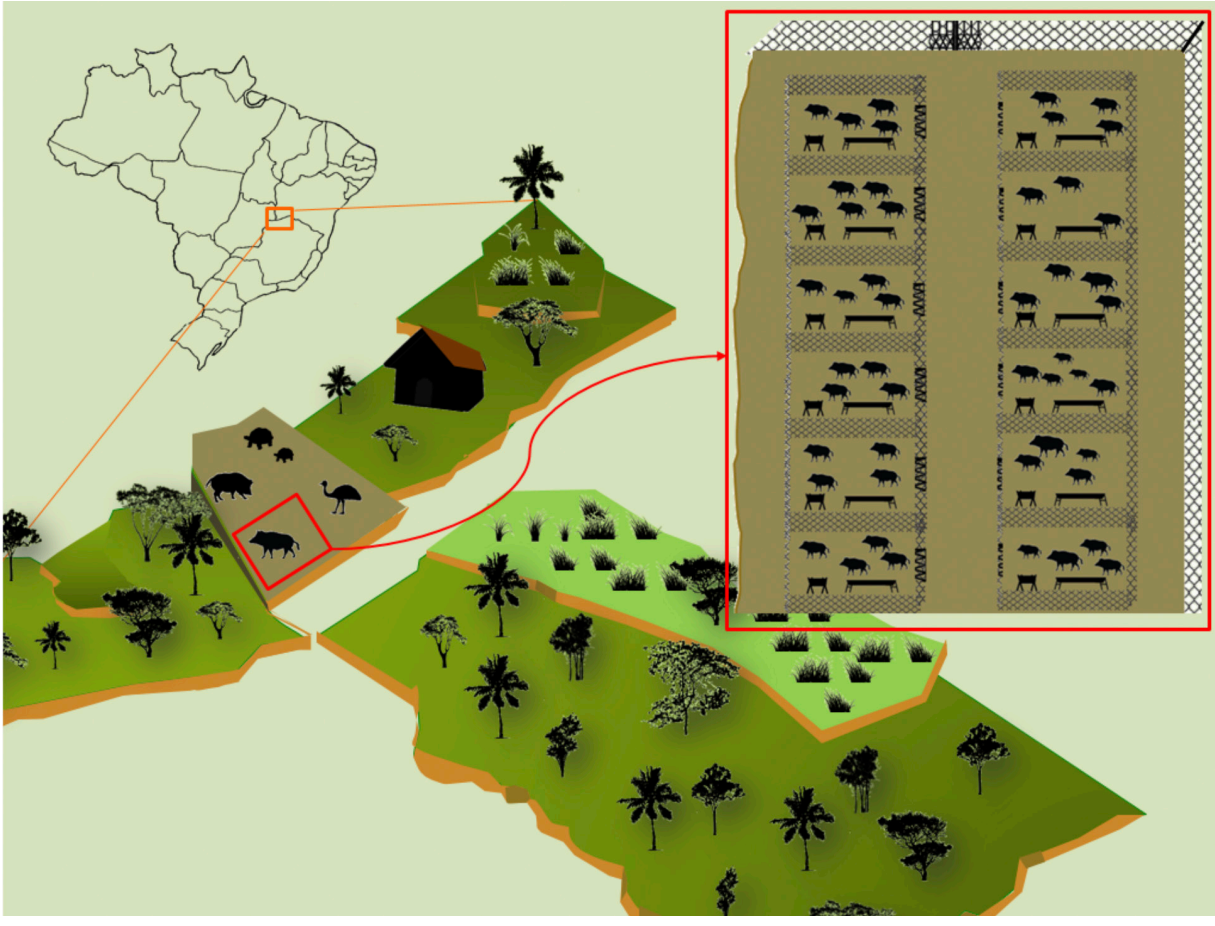

Figure 1. Schematic representation of the study site, surrounded by Cerrado vegetation and bordering the state of Minas Gerais, state of Goiás, and state of Bahia. In detail, the spatial organisation of *Tayassu pecari* pickets arranged side by side.

Table 1. Frequency of anti-*Leptospira* agglutinins by microscopic agglutination test (MAT) in 100 *Tayassu pecari* and risk factor analysis for positive reactions by sex by Fisher's exact test (*P*>0.05).

Serogroup		Total positive reactions (%)	
		CI** 95(%)	Maximum titer
Patoc	8 (8%)	7.94 to 8.05	100
Grippotyphosa	1 (1%)	0.17 to 5.44	100
Icterohaemorrhagiae	4 (4%)	1.56 to 9.83	50*
Shermani	1 (1%)	0.17 to 5.44	50*
Butembo	1 (1%)	0.17 to 5.44	50*
Pyrogenes	1 (1%)	0.17 to 5.44	50*
Bratislava	2 (2%)	0.55 to 7	50*
Patoc	16 (16%)	15.92 to 16.07	50*
Overall	100 (100%)		
Sex as a risk factor	Odds ratio	CI 95(%)	*P*-value
Male (3|28)*** Female (6|72)	1.28	0.19 to 6.51	0.71

*Reagents in the screening test (titer <100).
**Confidence interval
***Positive for any serovar| total individuals by sex

Table 2. Distribution of males and females of *Tayassu pecari* collected in 14 pickets with three groups, submitted to serodiagnosis of brucellosis, bovine viral diarrhea and leptospirosis.

Picket No.	Individuals by gender		Group
	Male	Female	
1	4	6	3 to 6 months
2	3	7	
3	4	6	7 to 10 months
4	4	6	
5	2	4	Families
6	1	5	
7	1	5	
8	2	4	
9	1	5	
10	2	4	
11	1	5	
12	1	5	
13	1	5	
14	1	5	

animals had a microchip and earrings with a registration number for tracking information. In the management area, the animal was placed inside of a structure similar to a cattle chute, developed in an appropriate size for the mechanical restraint of *T. pecari*. The cage had side openings for accessing to the punctured region. A total of 10 ml of blood of which animal was collected from the jugular, or cephalic or saphenous veins, prioritizing the easy access for each situation, in an attempt to avoid the animal excessive or prolonged stress. The blood sampling lasted on average two minutes per animal. The serum samples were identified and maintained at –20 °C, for further serological tests.

LABORATORY PROCEDURES

Acidified antigen test (AAT). The AAT was performed as recommended in the Manual of the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (Brazil 2006). The method consists of

placing 0.03 mL of the serum in contact with 0.03 mL of the antigen in a checkered glass plate followed by slight homogenisation, then keeping the plate in rotational and under constant movements until the moment of the reading, which was done four minutes after the reaction using a box with light (or agglutinoscope) to observe the formation of agglutination lumps. The antigen used in this technique was prepared with a *Brucella abortus* sample 1119/3 at 8.0% cell volume, stained with Bengal rose, pH 3.65. The AAT tests, as well as the following tests, were applied following the recommendations for domestic pigs (*Sus scrofa domesticus*) and they were not standardised for *T. pecari*.

Viral neutralization test (VNT). The samples were submitted to the VNT for the detection of antibodies against BVDV-1a (Singer strain), as recommended by the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE 2015) with modifications. All seropositive samples at screening were duplicated for repeatability of the result. On each plate, negative and positive controls were added. A sample was considered positive when the total neutralisation of 100 $TCID_{50}$ occurred in the serum and no cytopathic effect (CPE) was observed in the cell layer in serum dilutions higher than 1:10 (OIE 2015) with modifications. Antibody titers were expressed as the reciprocal of the highest dilution at which viral neutralisation was verified, and the final titer was the geometric mean of the titers found in the duplicates.

Microscopic agglutination test (MAT). The MAT was performed for the diagnosis of leptospirosis using a collection of live antigens that included 24 serological variants of pathogenic leptospires of the following serogroups (serotype representatives): Australis (Australis, Bratislava), Autumnalis (Autumnalis, Butembo), Ballum (Castellonis), Batavie (Batavie), Canicola (Canicola), Celledoni (Whitcombi),Cinoptery (Cinoptery), Grippotyphosa (Grippotyphosa), Hebdomadis (Hebdomadis), Icterohaemorrhagie (Copenhageni, Icterohaemorrhagiae), Javanica (Javanica), Panama , Pomona (Pomona), Pyrogenes (Pyrogenes), Sejroe (Hardjo, Wolffi), Shermani (Shermani), Tarassovi (Tarassovi), Djasiman (Sentot) and two saprophytic leptospires: Andamana (Andamana) and Seramanga (Patoc) (Lee *et al* 2017).

The screening was performed at 1:50 and 1:100 dilutions. In the presence of agglutination, the sera was titrated in two-fold serial dilutions. The titer was given as the reciprocal of the highest dilution, titers of 1:100 to 1:200 are considered low positive, interpreted as exposure to *Leptospira* spp., Titer ≥1:400 are considered high positive, indicating active or recent infection (Faine *et al* 1999, Boqvist *et al* 2002). Only cultures from 4 to 14 days, which did not present contaminants or autoagglutination, were used as antigens.

STATISTICAL ANALYSIS

A descriptive analysis of the frequency results was carried out, with the calculation of the respective confidence intervals. The association between sex and the number of seropositive for *Leptospira* spp. was analysed using the Fisher exact test ($P<0.05$).

RESULTS AND DISCUSSION

In this study, none of the 100 serum samples of *T. peccary* tested had antibodies detectable by AAT for *Brucella* sp., thus, it was not necessary to perform confirmatory tests. Similar results were obtained in the northern and northeastern regions of Brazil, where researchers reported the absence of positive serological results for *Brucella abortus* in a group of 11 different species, including *T. peccary* (Minervino *et al* 2018). Previous data in the north of Brazil described 4.9% (2/41) of serum samples of *T. tajacu* reactive for *Brucella* sp., in a nursery with a history of infertility and mortality of the offspring, with the seropositive animals being euthanised due to sanitary issues (Mayor *et al* 2006, 2007). Therefore, epidemiological surveys studies regarding disease in tayassuids breeding are not clear regarding the possibility of such species serving as reservoirs or carriers of *Brucella* sp.

BVDV is a pestivirus that shows genetic and antigenic similarities to classical swine fever and other pestiviruses and has an important impact on cattle and pigs (Brodersen 2014). Out of the 100 samples submitted to VNT, 28 showed a cytotoxic effect and were excluded from the evaluation. For BVDV-1a, one animal was reactive 1/72 (1.38%, CI 95% 0.24 to 4.45), with a titer of 40. This animal was male, 48 kg, belonging to the G8 group. In the literature, there is no evidence of the detection of antibodies against BVDV in *T. pecari* or *T. tajacu*. In the present study, only one sampled animal showed anti-BVDV antibodies.

In Brazil, Gatto *et al* (2016) and Gatto *et al* (2018) identified seropositivity for BVDV-1 and BVDV-2 in pig serum samples from intensive breeding from several Brazilian states. Almeida *et al* (2017) also detected BVDV-1 and BVDV-2 seropositive samples in non-technified rearing farms at the northeast of the state of São Paulo. Wild animals infected with BVDV are described as indicative of the presence of the virus in nearby herds located within a given region, although the role of tayassuids in the epidemiology of BVDV is unknown (Milićević 2018).

Serological diagnosis of leptospirosis showed that 9% of the *T. pecari* samples were reactive to several serovars at low titers (> 1/50): Patoc, Grippotyphosa, Itereohaemorrhagiae, Shermani, Butembo, Pyrogenes, and Bratislava. However, titers higher that 1/100 were only found for serovar Patoc 8% (8/100) and 1% (1/100) for serovar Grippotyphosa, and no association was observed between sex and the occurrence of *Leptospira* spp. antibodies ($P>0.05$). The

occurrence of antibodies against *Leptospira* spp. in *T. pecari* was described by Ito et al (1998), in animals from Pantanal, the southern region of the state of Mato Grosso, which were reagents for the serovars Copenhageni, Icteratohaemorrhagiae IV, Panama, Patoc, and Autumnalis. In fragments of the Atlantic Forest located in the state of São Paulo, serum samples of *T. pecari* were seropositive to serovars Pomona and Icterohaemorrhagiae IV (Nava 2008). Freitas *et al* (2010) described the occurrence of the serovars Autumnalis, Pomona, Copenhageni, Canicola, Hardjo, Grippotyphosa, Icterohaemorrhagiae, Bataviae, Tarassovi, and Hebdomadis in *T. pecari* from an ecosystem interacting with cattle.

In Pantanal (southern region of the Mato Grosso state), Ito *et al* (1998) reported the occurrence of serovar Patoc. Seroreactivity for Butembo and Automnallis serovar was detected in pigs samples from Amazonas state (Brazil) (Mayor 2006, Mendoza *et al* 2007). In the same region, Amazonas state, a serological survey identified positive samples for Australis, Hebdomadis, Autumnalis, Bataviae, Djasiman, Grippothyphosa, Balum, Canicola, Mini, Tarassovi, and Icterohaemorragiae (Nava 2008). Pyrogenes and Patoc were described as the most prevalent in *T. tajacu*. In the Peruvian Amazon, seropositivity was reported for Iquitos, Australis, Hebdomadis, Icterohaemorrhagiae, Autumnalis, Tarassovi, Cynopteri, and Ballum (Jori *et al* 2009).

In the present study, serovar Patoc was the predominant serovar detected. The occurrence of antibodies against serovar Patoc was also described in Pantanal, southern region of the state of Mato Grosso in *T. pecari* and *T. tajacu* (Ito *et al* 1998) and only in *T. tajacu* by Nava (2008). Also, this serovar was described in wild boars in the state of São Paulo and Paraná (Marchiori Filho *et al* 2002). Only 1% of the animals were seropositive for Grippothyphosa, a result similar to that found by Freitas *et al* (2010) in *T. pecari* and by Mendoza *et al* (2007) in *T. tajacu.*

The emergence of human leptospirosis cases has been reported in several countries. The increase of human infections has been associated with increased exposure of the host to the pathogen, particularly during outdoor activities, sports practices or contact with wild animals. Thus, the sylvatic cycle of the disease is a growing concern for public health, justifying epidemiological surveillance studies regarding leptospirosis in different species of wild animals including tayassuids (Morgan *et al* 2002).

Reagent serum samples were identified for BVDV-1a and *Leptospira* spp. in *T. pecari* from a commercial breeder in the midwest of Brazil. As far as we know, this is the first serological survey to describe describe the presence of BVDV-1 (strain Singer) antibodies in *T. pecari*. Yet, we must also take into account that the diagnostic methods carried out are not standardised for *Tayassu* sp. species, and there may be false negatives and even false positives. To date, there is no consistent evidence supporting that BVDV can infect those wild species. Monitoring captive-reared wildlife is important to detect pathogen excretion which could affect human health and or other animals (wild or domestic) under production.

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