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Molecular detection of *Mycoplasma suis* in captive white-lipped peccaries (*Tayassu pecari*) and wild boars (*Sus scrofa*) in Brazil

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ABSTRACT

Mycoplasma suis, the etiological agent of swine hemoplasmosis, is an epicellular bacterium that adheres to the surface of pig erythrocytes leading to deformations of the target cells. Little is known about the occurrence of *M. suis* in wild swine populations around the world, its economic impact on swine herds, and the risk of human infection. The aim of this study was to investigate, by quantitative real-time PCR (qPCR) based on the 16S rRNA gene, the occurrence of *M. suis* in a captive population of white-lipped peccaries (100 *Tayassu pecari*) and in free-living wild boars (14 *Sus scrofa*) in Brazil. None of the white-lipped peccaries were positive for *M. suis*, whereas seven (50%) wild boars were positive in qPCR assays. The quantification of *M. suis*-16S rRNA copies/μL ranged from $1.42 \times 10^{\circ}$ to 3.906×10^{1} in positive animals, indicating a low bacteremia and a chronic carrier status in free-living wild boars. In conclusion, *M. suis* might be a non-frequent pathogen in wild suids maintained in captivity. Despite the low bacteremia, the prevalence of *M. suis* in wild boar population in Brazil seems to be high.

1. Introduction

Mycoplasma suis, the etiological agent of swine hemoplasmosis, is an epicellular bacterium belonging to the Order Mollicutes, inside the hemotrophic mycoplasmas group [1]. The pathogen adheres to the swine erythrocytes and causes structural changes, inducing lyse of the infected cells by the reticuloendothelial system [2]. The acute phase of the disease is characterized by severe hemolytic anemia, high fever, and hypoglycemia, which can lead to death. The chronic phase of the disease may cause production deficits, such as weight loss, low feed conversion, and reproductive problems [3–5]

The agent is spread in countries that play a major role in the pork production sector, such as Brazil [6,7] and China [8,9], considered two of the most important producers of pork meat [10]. Besides, this agent has also been detected in swine from Argentina [11], the USA [5,12], Hungary [13], Germany [14–16], Switzerland [15], and Japan [17]. The zoonotic potential of *M. suis* has been reported in China, where the pathogen was detected in veterinarian practitioners [9].

In addition to the importance of this disease in pig farms, swine hemoplasmosis also affect wild boars [16]. These generalist, opportunistic animals are widely distributed throughout Eurasia and north of Africa, being adapted to several environmental conditions. Wild boars are one of the most successful invasive mammal species, occurring in all continents but Antarctica [18]. In Brazil, wild boars were introduced in all Central-Southern part of the country, with negative consequences on the native fauna and flora by predation and rooting [19]. As these invasive mammals also act as diseases reservoirs, the impact of their introduction in the Neotropical region can be relevant both to commercial pig farms and wildlife.

Molecular evidence of circulation of this agent in boars in Brazil have been reported [6], even though there is no report so far of the occurrence of *M. suis* in the native Tayassuidae [20].

In this context, an investigation around the dynamic of the transmission of *M. suis* between wild and farm pigs is needed, in order to assess the real situation of swine hemoplasmosis in Brazil. Therefore, the present study aimed to detect *M. suis* in white-lipped peccaries and

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wild boars in Brazil, using molecular techniques.

2. Materials and methods

2.1. Sampling areas, blood collection and DNA extraction

In July 2016, blood samples were collected by jugular vein puncture from 100 white lipped-peccaries (71 females and 29 males). The animals used in the present study were sampled in a conservationist farm located between the states of Minas Gerais, Goiás and Bahia. DNA extraction from 250 µL of each blood sample was performed using a protocol previously described by [21].

Additionally, between August and November 2017, 11 wild boars were hunted down by professional hunters in the region of Monte Azul Paulista, São Paulo state, Brazil. Tissue samples (spleen and/or liver) were collected from 14 wild boars, respectively. DNA extraction was performed from tissue samples (10 mg spleen and/or 25 mg liver samples) using a QIAamp DNA Blood Mini-kit (QIAGEN®, Valencia, California, USA), following manufacturer's instructions.

DNA concentration and quality (260/280 nm ratio) were measured using a Thermo Scientific NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific®, Wilmington, Delaware, USA). In order to avoid contamination, DNA extraction and PCR reactions were performed in separated rooms. The DNA was stored at −20 °C until the time of cPCR and qPCR testing.

Conventional PCR for the endogenous gene gapdh

In order to rule out the presence of inhibitors in extracted DNA samples and thereby avoid false-negative results in qPCR for *M. suis*, all of the DNA samples were submitted to a previously described conventional PCR (cPCR) based on *gapdh* gene [22]. The amplified products were submitted to horizontal agarose gel electrophoresis stained with ethidium bromide. Amplicons were visualized using an ultraviolet transilluminator (Chemi-Doc, Bio-Rad®, Hercules, California, USA) coupled to a computational program of image analysis (Image Lab, Bio-Rad, Hercules, California, USA).

2.2. Mycoplasma suis-qPCR based on the 16S rRNA gene

DNA samples exhibiting positive results in conventional PCR for the *gapdh* gene were submitted to a previously described qPCR assay for *M. suis* based on 16S rRNA gene [12]. The standard curve of the qPCR assays was constructed using 10-fold serial dilutions of pIDTSmart plasmids (10^7 to 10°) (Integrated DNA Technologies, Coralville, Iowa, USA) encoding a 16S rRNA *M. suis* sequence (insert containing 156 bp). The number of plasmid copies was determined according to the formula (Xg/ µL DNA/ [plasmid size (pb) x 660]) x 6.022×10^{23} x plasmids copies/µL. Plasmids and ultra pure water were used as positive and negative controls, respectively. The amplification efficiency (E) was calculated from the slope of the standard curve in each run using the following formula: $E = 10^{-1/slope}$. The qPCR assays followed the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) [23].

3. Results

All 114 samples showed to be positive in cPCR based on *gapdh* gene, discarding the presence of inhibitors in DNA samples. All white-lipped peccaries showed to be negative in qPCR assays for *M. suis* based on 16S rRNA gene, whereas 7 wild boars (50%) showed to be positive for *M. suis*. The qPCR assays were performed in four different plates with mean values of reactions efficiency (E), determination coefficient (R^2), slope and y-intercept (y-Int), ranged from 91.1% to 103.5% (mean = 97.6%), 0.992 to 0.996 (mean = 0.994), 3.241 to 3.555 (mean = 3.386) and 30.102 to 42.016 (mean = 38.279), respectively (Table 1).

The positive samples were obtained from adult wild boars: three males and four females. Only one animal showed the presence of *M. suis* DNA in both spleen and liver samples. The remaining positive animals showed the presence of *M. suis* DNA only in spleen samples. The range of Cq and quantification values of positive samples were 34.86–39.2 and $1.42 \times 10^{\circ}$ – 3.906×10^{1} *M. suis-16S rRNA* gene copies /µL of extracted DNA, respectively (Table 2).

Table 1
Parameters of *M. suis* – qPCR assays based on 16S rRNA gene in blood and tissue samples from captive white-lipped peccaries and wild boars.

Reaction	E	R^2	Slope	y-Int
1	95.9%	0.994	3.424	41.301
2	103.5%	0.996	3.241	30.102
3	91.1%	0.992	3.555	42.016
4	99.9%	0.995	3.325	39.698
Mean	97.6%	0.994	3.386	38.279

Efficiency (E), Correlation coefficient (R^2), y-Intercept (y-Int).

Table 2
Quantification cycles (Cq) and quantification values (number of *M. suis*-16S rRNA copies/µL) in qPCR assays from wild boar tissue samples.

Animal identification	Sex	Tissue sample	Cq	Quantification of *M.suis-16S rRNA gene* (number of copies /µL)
21	Male	Spleen	36.03	1.286×10^{1}
25	Female	Spleen	38.045	0.351×10^{1}
27	Female	Spleen	34.975	2.651×10^{1}
28	Male	Spleen	34.86	3.906×10^{1}
29	Male	Spleen	35.58	1.735×10^{1}
30	Female	Spleen / liver	39.2 / 37.255	0.141×10^{1} / 0.548×10^{1}
37	Female	Spleen	35.705	1.593×10^{1}
Mean			36.879	1.526×10^{1}

4. Discussion

Few studies have been conducted regarding the occurrence of *M. suis* in wild swine in the world. In the present study, all blood samples from white-lipped peccaries were negative in qPCR assays for *M. suis*. Similarly, negative results were reported for *M. suis,* based on blood smears and conventional 16S rRNA gene PCR assays, in 22 collared peccary (*Tayassu tajacu*) and six white-lipped peccaries (*Tayassu pecari*) sampled in Bela Vista Sanctuary, Foz do Iguaçu and Curitiba zoo, in the state of Paraná, southern Brazil [20].

The introduction of species beyond their natural geographic distribution is of major concern for humans, animals and ecosystems health. When it comes to this issue, the wild boar *Sus scrofa* and its feral varieties have been incriminated as the most harmful invasive species [24]. In fact, feral pigs have been reported in 472 Brazilian municipalities, which comprised four of the five political regions of the country. The southeast (253 municipalities) region of Brazil has been reported as the most affected region by this alien species [25,26]. Indeed, this animal species has been associated to crop fields damages, livestock attacks, and indirect losses associated to the budget involved control programs. Indeed, the ecological impacts of feral pigs are related with its rooting and wallow behavior, which may reduce the cover and diversity of plant species [27], affect soil properties [28] and also facilitate the spread of diseases to wildlife [29].

Herein, seven out of fourteen (50%) wild boars were positive for *M. suis*. Although the number of sampled wild boars were relatively low for elucidating the real prevalence of this agent in the population of wild boars in Brazil, this preliminary study highlight the circulation of this pathogen in this invasive species. Indeed, the found occurrence was higher than those found in previously studies conducted in populations of wild boars in Germany [16] and in southern Brazil [6]. In the southeastern region of Germany, 10.03% out of 359 sampled wild boars

showed to be positive for *M. suis* based on qPCR assays targeting *msg1* gene [16]. In southern Brazil, *M. suis* DNA was detected in 25% out of four wild boars based on cPCR assays targeting 16S rRNA gene [6]. Interestingly, the low quantification values ($1.42 \times 10^{\circ}$ – 3.906×10^{1} *M. suis*-16S rRNA gene/μL of extracted DNA volume) in positive animals suggests the presence of a low bacteremia in free-living wild boars, which may play a role of chronic carriers for this pathogen. On the other hand, high quantification levels, ranging from 1.64×10^{1} – 6.64×10^{7} *M. suis*-16S rRNA gene/μL of extracted DNA volume was previously reported among pigs sampled in non-technified farms in northeastern Brazil [7]. Further studies aiming at assessing the real risk imposed by this invasive species in extensive pig herds in Brazil are much needed.

Even though the main vector for *M. suis* among swine has not been identified, mosquitoes and lice (*Haematopinus suis*) have been incriminated as probable vectors for the agent [30,31]. This louse species has been observed in a few boars in the study area (Lux Hoppe, personal observation). Future studies aiming at evaluating the role of aggressive interactions (such as territorial or dominance disputes), other arthropod vectors (e.g., ticks), and transplacental transmission in the epidemiological cycle of *M. suis* among wild boars should be performed.

Finally, it is noteworthy to highlight that the zoonotic potential of *M. suis* has been already reported. For instance, *M. suis* DNA was detected in 49% of blood samples collected from 65 veterinarians and agricultural technicians in China [9]. Consequently, the direct contact between infected swine blood with human skin lesions may favor the infection by *M. suis*. Therefore, taking into account the One Health concept, veterinarians, technicians, wild boars hunters, veterinary students, among other people who have direct contact with these animals, may be at risk of infection by *M. suis*.

5. Conclusion

Mycoplasma suis circulates in free-living boars in the state of São Paulo, southeastern Brazil. Further studies should be conducted in order to investigate the presence of this hemoplasma species in larger populations of wild and captive swine, taking into account alternative mechanisms of transmission as well as its zoonotic potential in Brazil.

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