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Free-Range wild Boars (*Sus scrofa*) and Rabies in Brazil: Absence of Molecular Detection in the Central Nervous System of Seropositive Animals.

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HIGHLIGHTS

- First molecular survey of Rabies lyssavirus (RABV) in wild boars of Brazil.
- Although seropositivity, all wild boars were negative for RABV herein.
- Wild boars should be systematically tested for RABV in Brazil.

Abstract: Samples of brain cortex of 12 free-range healthy wild boars from Southern Brazil were tested for RABV by dFAT and qPCR, all negative. The negative results in seropositive healthy wild boars may be associated with exposure of non-viable virus particles, virus proteins by bat saliva or consumption of contaminated carcasses.

Keywords: exotic species; invasive species; rabies cycle; *Sus scrofa.*

INTRODUCTION

Rabies is a lethal zoonosis affecting mammals worldwide. In Brazil, sylvatic cycle has been maintained by free-living animals, mainly hematophagous (vampire) bats, particularly *Desmodus rotundus* species, as maintenance host. In this scenario, native and exotic species have been susceptible to the virus and participate blood source in bat growth as for [1].

In Brazil, a study has shown free-range wild boars (*Sus scrofa*) as blood meal for vampire bats in Atlantic Forest and Pantanal biomes [2]. Wild boars have been recognized as exotic invasive species originated by Eurasian species in Brazil, with nationwide control officially permitted (Normative Instruction 03/2013) as a strategy for population management [3]. In a previous study in natural and rural Brazilian areas, free-range and non-vaccinated wild boars fed upon by vampire bats have shown serum titers against rabies virus [4].

Although this exotic invasive species has been found in all six Brazilian biomes and may play a role in the sylvatic rabies cycle in Brazil, no molecular survey of *Rabies lyssavirus* (RABV) detection has been conducted to confirm the original serological findings. Thus, the present study aimed to molecularly assess *Rabies lyssavirus* (RABV) in seropositive and seronegative wild boars from Vila Velha State Park, southern Brazil, as a complement of previous study [4].

MATERIAL AND METHODS

This study has been approved by the Ethics Committees of Animal Use (059/2017). Free-range wild boars from a conservation unit of Vila Velha State Park, southern Brazil, were baited, photo-monitored, trapped and euthanized from November 2016 to May 2018 [4]. Brain cortex samples of wild boars were assessed immediately after death, placed in sterile tubes and stored at -20ºC until processing. The samples were tested for RABV by direct fluorescent antibody test (dFAT) [5] and quantitative polymerase chain reaction (RT-qPCR) with PowerSYBR®Green (Thermo-Fisher) and primers as previously described [6].

RESULTS

A total of 12 free-range apparently healthy wild boars were sampled. All 12 free-range were negative for RABV by dFAT and RT-qPCR. As previously reported, all 12 wild boars tested herein have been fed upon by vampire bats, and 4/12 (33.3%) presented serum titers for rabies exposure (≥0.10 IU/mL) when tested by modified rapid fluorescent focus inhibition test [7], which detects neutralizing antibodies to rabies virus [8], as previously published [4] (Table 1).

Table 1. Serological and molecular analysis for RABV in wild boars from Southern Brazil.

sample ID	dFAT (UI/mL)	RT-qPCR
60	0,02	Negative
61	0,01	Negative
62	0,09	Negative
71	0,009	Negative
72	0,04	Negative
74	0,07	Negative
75	0,09	Negative
76	**0.16***	Negative
77	**0.13***	Negative
78	0,03	Negative
79	**0.13***	Negative
80	**0.35***	Negative

*seropositive animals with serum titers ≥0.10 IU/mL

DISCUSSION

In the present, seropositive wild boars were negative to RABV by dFAT and RT-qPCR. The lack of RABV detection in seropositive healthy wild boars may be speculated as associated with exposure of non-viable rabies virus particles or to rabies virus proteins by contaminated bat saliva during blood feeding on wild boars or by wild boar consumption of contaminated carcasses from other animals [9].

The wild boars herein have shown low titers (0.13, 0.13, 0.16 and 0.35 IU/mL) when compared with vaccinated wild boars (≥0.50 UI/mL), as previously reported [10, 11], which could indicate non-specific neutralizations. The serosurvey considered 0.50 UI/mL as cut off, following the World Health Organization (WHO) recommendation [10, 11]. Such titer has been also shown to be immune-protective in some wildlife species after vaccination, with a titer higher than 0.5 IU/mL having more than 95% survival likelihood following infection [12]. Despite frequently used as evidence of exposure in serosurveys of unvaccinated wildlife, this cutoff has been validated in response to vaccination in few wildlife species, not in unvaccinated individuals [13].

In Brazil, titers from 0.1 to 0.5 IU/mL have been tentatively related to natural exposure of RABV to native free-range wildlife species [9]. Whether these titers were accidentally acquired or indicate active infection in wild boars still on a phase of the incubation in which rabies virus had not yet reached the brain cortex should

be further investigated. In addition, virus neutralization cut-off levels of natural and experimental RABV exposure remain to be fully established.

Specific antibodies against rabies virus have been detected in human, domestic dog and wildlife serum samples of healthy and unvaccinated individuals, potentially indicating nonlethal exposure to rabies virus in rabies endemic areas [13]. Varying in methodology and cutoff titles, with different specificity and sensitivity, rabies serological tests may lack the ability of detecting nonlethal exposures in unvaccinated individuals [13]. As RFFIT (but not ELISA) has presented false positives on a study conducted in a rabies-free island, ELISA may be more specific for detection of nonlethal exposures [14].

To the author's knowledge, there is no molecular report of Rabies lyssavirus in wild boars worldwide to date. Comparatively, few studies have reported Rabies lyssavirus infection in domestic swine, and clinical signs vary according to dog, bat or wildlife viral variant [15]. Thus, lesions and viral antigen in brain cortex samples remain to be fully characterized in swine species.

In addition, potential cross reactivity with other lyssaviruses may also result in rabies false positives, impairing reliable estimative of true prevalence of nonlethal rabies exposures [13]. In such cases of nonlethal exposure, rabies virus may be suppressed before the onset of recognizable clinical symptoms, as the most likely alternative course of defeated infection [13].

As previously shown, rabies transmission from bats to wild boars was 5.3-fold higher in Atlantic Forest than Pantanal studied areas, associated with higher frequency of encounters between two species [2]. Since oral vaccination have not been conducted in wild boars in Brazil, future studies should considerer systematic test for RABV to check wild boar role in rabies cycle and spillover in both natural and rural areas.

Finally, better estimates of rabies exposure in wildlife, livestock, domestic and laboratory species may be used as a tool for disease surveillance and models [13].

CONCLUSION

Finally, as similarly performed with the passive nationwide surveillance of native wildlife species in Brazil, post-mortem central nervous system samples of wild boars should be systematically tested for RABV to check whether wild boars play a role in rabies cycle.

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Conflicts of Interest: The authors declare no conflict of interest.

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