Risk factors associated with ticks and *Rickettsia* spp. exposure in wild boars (*Sus scrofa*), hunting dogs, and hunters of Brazil

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Abstract

Background and Aim: Wild boars have recently been implicated as the maintainers and carriers of *Amblyomma* spp. ticks, which are essential for *Rickettsia* spp. transmission. Consequently, wild boar hunting may increase the risk of tick exposure and subsequent human tick-borne infection and disease. Therefore, this study was conducted to evaluate the risk factors for ticks and *Rickettsia* spp. exposure in wild boars, hunting dogs, and hunters in Brazilian biomes.

Materials and Methods: The statistical relationship of *Rickettsia* spp. antibodies were evaluated using the Chi-square test in 80 wild boars, 170 hunting dogs, and 49 hunters.

Results: The only statistically significant difference in seropositivity found in this study was between male and female wild boars (p=0.034), probably associated with in-park exposure to *Amblyomma brasiliense* infected with *Rickettsia* spp.

Conclusion: The absence of statistical differences in the associated risk factors for hunting dogs and hunters may indicate a random exposure to *Rickettsia* spp.

Keywords: Brazilian spotted fever, hunting activities, wild boars.

Introduction

In recent years, wild boars have been implicated as the maintainers and carriers of *Amblyomma* spp. ticks in Brazil, which are essential for the transmission of *Rickettsia* spp., particularly Brazilian spotted fever (BSF) [1]. Wild boars are classified as exotic invasive species by Brazilian laws, and their hunting has been permitted nationwide, primarily using hunting dogs (*Canis familiaris*) for tracking and hunting [2,3].

Spotted fever has been considered as the most fatal tick-borne disease worldwide, and wild boars may spread infected ticks with *Rickettsia* spp. from their original habitats to other ecosystems, causing human exposure, particularly in specific human activities such as wild boar hunting [1]. Moreover, hunting dogs may elevate the risk of human infection when bringing infected *Amblyomma* spp. ticks back home [4].

Therefore, this study was conducted to evaluate the risk factors for exposure to ticks and *Rickettsia* spp. in wild boars, hunting dogs, and hunters in two different Brazilian biomes.

Materials and Methods

Ethical approval

This study was approved by the Ethics Committee of Animal Use (protocol number 059/2017) of the Federal University of Paraná, officially included as part of the annual activities of the City Secretary of Health at Ponta Grossa and approved by National Human Ethics Research Committee (number 97639017.7.0000.0102). In-park trapping and tick collection have been authorized by the Environment Institute of Paraná (authorization number 30/17) and by Chico Mendes Institute of Biology (authorization number 61805–2).

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Study period and location

The present study was conducted from November 2016 to May 2018. The risk of BSF was evaluated on preserved and degraded areas in the Atlantic Forest biome of South Brazil, including the Vila Velha State Park, and degraded areas in the Cerrado biome of Central-West Brazil, as reported previously [1].

Sampling

This investigation was a descriptive cross-sectional study of risk factors associated with *Rickettsia* spp. and parasitized ticks infesting wild boars, hunting dogs, and hunters [1].

Blood samples of wild boars, hunting dogs, and hunters were collected from October 2016 through May 2018. Blood samples from free-range wild boars in degraded areas were collected after killing them by a firearm according to Brazilian environmental laws. Furthermore, free-range wild boars from the State Park natural areas of Vila Velha were baited, trapped, and killed by a firearm. Blood samples from all wild boars were collected immediately after death by the intracardiac puncture. Blood samples from hunting dogs were collected by jugular puncture as approved by the Ethics Committee of Animal Use at the Federal University of Paraná (protocol 059/2017). Finally, the blood samples of hunters were collected by cephalic puncture as approved by the Ethical Appreciation at Ethics Committee in Human Health at the Brazilian Ministry of Health (protocol 97,639,017.7.0000.0102). All blood samples were collected in tubes without anticoagulants and maintained at room temperature (25°C) until visible clot retraction, after which they were centrifuged at 1500 rpm for 5 min and the resulting serum was stored at -20°C until processing. In addition, ticks were collected from wild boars, hunting dogs, and hunters as approved by the Chico Mendes Institute of Biology.

Serum samples of wild boars, hunting dogs, and hunters were evaluated for the presence of *Rickettsia amblyommatis* strain Ac37, *Rickettsia bellii* strain CL, *Rickettsia parkeri* strain At24, *Rickettsia rickettsii* strain Taiaçu, and *Rickettsia rhipicephali* strain HJ5 by indirect immunofluorescent antibody assay, as described previously [1]. Sera samples were screened at a 1:64 dilution against each of the rickettsial antigens, and in a seropositive reaction, serial dilutions at 2-fold increments were tested up to the endpoint titer. Ticks were randomly collected from wild boars, hunting dogs, and hunters, subjected to DNA extraction, and individually tested by standard polymerase chain reaction for tick mitochondrial 16S rRNA and rickettsial *gltA* gene, as described earlier [1].

The statistical relationship of *Rickettsia* spp. antibodies were evaluated using the Chi-square test in 80 wild boars, 170 hunting dogs, and 49 hunters. For wild boars, the associations tested were between positivity and sex, age, body size, captured in anthropized and/ or natural area, and biome of capture; for hunting dogs, the evaluated associations were between positivity and sex, age, biome location, mobility, hunting frequency, hunting experience, and animal hygiene; and for hunters, the associations tested were between positivity and sex, age, household location, biome location, occupation, education, hunting method, hunting frequency, hunting experience, frequency of access to forest areas, observation of capybaras (*Hydrochoerus hydrochaeris*) and opossums (*Didelphis* spp.), number of hunters and family members at the household, hunting dog contact, own a hunter dog, ticks collected, previous tick contact, infestation, control and bites, and activities after hunting.

Results

A total of 80 wild boars, 170 hunting dogs, and 49 hunters were sampled. Serological analysis for *Rickettsia* spp. showed that 58/80 (72.5%) wild boars, 24/170 (14.1%) hunting dogs, and 5/34 (14.7%) hunters were seroreagent for *Rickettsia* spp., as previously reported [1].

Wild boar exposure to *Rickettsia* spp. was statistically significant in terms of sex, with females being more likely to be positive (p=0.034); however, this was not associated with other risk factors, including age, area of capture, and between free-range and captured wild boars (Table-1). Hunting dog exposure to *Rickettsia* spp. antibodies showed no statistically significant differences, including sex, age, body score, hygiene, vaccination, limited dog mobility, hunting experience in years, and hunting frequency (Table-1).

Hunter exposure to *Rickettsia* spp. antibodies also demonstrated no statistically significant differences, including age, number of years living in rural areas, hunting experience in years, number of people and dogs in hunter household, occupation, income, education level, household location dog owner, peridomicile and dogs maintained indoors, contact with dogs of other hunters, presence of forest areas nearby the household, and presence of capybaras and opossums in hunting areas (Table-1).

The number of *Amblyomma brasiliense*, *Amblyomma sculptum*, *Amblyomma dubitatum*, and larvae of *Amblyomma* spp. ticks infesting wild boars showed no statistical differences for *Rickettsia* spp. seropositivity when compared between Atlantic Forest with Cerrado biomes and with degraded areas of Atlantic Forest.

Only *Amblyomma aureolatum* ticks were detected in hunting dogs from South Brazil [1], with no statistical significance associated with *Rickettsia* spp. seropositivity. Due to the asymmetric data distribution, the Shapiro–Wilk test (p<0.000001) and the Mann–Whitney U non-parametric test were conducted to confirm the difference in *Rickettsia* spp. seropositivity (20 ± 14 ; 10, 10-40) and seronegativity (21 ± 14 ; 10, 10-40) in groups of dogs with no statistically significant outcome (Table-1).

In the present study, 30/49 (61.2%) sampled hunters reported previous tick contact and 23/49 (46.9%) hunters reported tick bites. In addition, 11/30 (36.6%) hunters reported exposure in all

Table-1: Risk factors for seropositivity of anti-*Rickettsia* spp. antibodies in wild boars, hunting dogs, and hunters.

Ves/total (%) Wild boar variables Sex Fernale 51/80 (63.7) 11/12 9 (58.6) (ref) Ade 29/80 (36.2) 11/12 9 (58.6) (ref) Ade 18/80 (22.5) 13/18 (72.2) (ref) Ade 68 months 18/80 (22.5) 13/18 (72.2) (ref) >64 months 14/80 (17.5) 11/14 (77.6) (ref) >1 year old 44/80 (17.5) 11/14 (78.6) (ref) Capture area 66/80 (82.5) 41/56 (72.8) * Matural 21/80 (26.2) 21/21 (100) (ref) 0.390 Agricultural 45/80 (56.2) 21/21 (100) (ref) 0.371 Degraded Hathatic Forest 21/80 (26.2) 21/21 (100) (ref) 0.371 Degraded Hathatic Forest 21/80 (26.2) 32/49 (65.3) * 0.371 Degraded Hathatic Forest 21/80 (28.7) 32/29 (65.3) * 0.476 No 49/80 (61.2) 32/49 (65.3) * 0.576 0.894	Risk factors of <i>Rickettsia</i> spp.	Total Yes/total (%)	Positive Yes/total (%)	OR (95% CI)	p-value
Wild boar variables Sex Fernale 51/80 (53.7) 41/51 (80.4) 2.89 (1.05-7.96) 0.034 Male 29/80 (36.2) 17/29 (56.6) (ref) 0.034 SG months 18/80 (22.5) 13/18 (72.2) (ref) > SG months-s-41 year old 14/80 (17.5) 10/14 (71.4) 1.04 (0.13-3.48) 0.5955 1 year old 48/80 (60.0) 35/48 (72.9) 1.08 (0.29-4.04) 0.913 Free range(captured 66/80 (82.5) 21/21 (100) (ref) 0.390 Anturopizer area 14/80 (17.5) 11/14 (78.6) + 1.08 (0.29-4.04) 0.913 Natural 21/80 (26.2) 21/21 (100) (ref) 0.390 4.9780 (56.2) 24/45 (57.8) + 1.08 (0.29-4.04) 0.913 Tere range 14/80 (17.5) 11/14 (78.6) + 1.08 (0.29-4.04) 0.422 Capture area 1.4470 (17.5) 11/14 (78.6) + 1.049 0.422 Matin 21/80 (28.7) 21/21 (100) (ref) 0.371 tyte cold <td< th=""></td<>					
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	<6 months	18/80 (22.5)	13/18 (72.2)	(rer)	
$ \begin{array}{c} \text{Solutions} < 1 \text{ year old} & 10/00 (2.5.2) & 10/14 (7.4.2) & 1.04 (0.31, 3.48) & 0.955 \\ \text{s for antis-<1 year old} & 46/80 (60.0) & 35/48 (72.9) & 1.08 (0.294.04) & 0.913 \\ \text{Free range} & 14/80 (17.5) & 11/14 (78.6) & (ref) \\ \text{Captured} & 66/80 (82.5) & 47/66 (71.2) & 0.67 (0.17-2.69) & 0.422 \\ \text{Captured} & 45/80 (65.2) & 21/21 (100) & (ref) & 0.390 \\ \text{Antimutal} & 45/80 (65.2) & 21/21 (100) & (ref) & 0.390 \\ \text{Antimutal} & 45/80 (65.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.371 \\ \text{Degraded Attantic Forest} & 21/80 (26.2) & 21/21 (100) & (ref) & 0.364 \\ -8 \text{ year old} & 27/170 (15.8) & 3/27 (11.1) & (ref) & -18 \\ \text{Solution} & 51/170 (2.9) & 2/5 (40.0) & 1.63 (0.57-4.65) & 0.358 \\ \text{Soly size} & 50/170 (17.6) & 6/30 (20.0) & 1.63 (0.57-4.65) & 0.358 \\ \text{Soly size} & 51/170 (2.9) & 2/5 (40.0) & (ref) & 0 \\ \text{Medium} & 151/170 (18.8) & 22/151 (13.2) & 0.75 (0.02-2.58) & 0.244 \\ \text{Large} & 17/170 (10.0) & 2/110 (14.1) & - & - \\ \text{No} & 0 & (ref) & 0 & (ref) & 0 \\ \text{Vaccination} & 84/170 (49.4) & 15/84 (17.9) & (ref) & 0 \\ \text{No} & 150/170 (28.3) & 7/43 (16.3) & (ref) & 0 \\ \text{Vaccination} & 84/170 (12.2) & 1/43 (14.3) & 0.73 (0.21-2.53) & 0.242 \\ \text{Varge} & 85/170 (51.6) & 9/36 (10.5) & 0.34 (0.22-1.31) & 0.166 \\ \text{No} & 0 & (ref) & 0 & 0 \\ \text{Vaccination} & 84/170 (49.4) & 15/84 (17.9) & (ref) & 0 \\ \text{Vaccination} & 10/170 (18.8) & 22/151 (15.2) & (ref) & 0 \\ \text{Vaccination} & 86/170 (23.5) & 7/43 (16.3) & (ref) & 0 \\ \text{Vaccination} & 163/170 (25.3) & 7/43 (16.5) & 1.16 (0.32-1.3) & 0.212 \\ Va$	Age	18/80 (22 5)	13/18 (72 2)	(rof)	
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$ \begin{array}{cccc} Free range/captured Free range (aptured for the form of the form of$	1 vear old	48/80 (60.0)	35/48 (72.9)	1.08 (0.29-4.04)	0.913
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Biomes Atlantic Forest 21/80 (26.2) 21/21 (100) (ref) 0.371 Degraded Atlantic Forest 23/80 (28.7) 17/23 (73.9) * Tck collection * Yes 31/80 (38.7) 26/31 (83.9) 2.76 (0.89-8.49) 0.070 No 49/80 (61.2) 32/49 (65.3) (ref) Og variables Age Age <1 year old 31/10 (15.8) 3/27 (11.1) (ref)	Degraded Cerrado	36/80 (45.0)	20/36 (55.6)	*	
Audinut Porest 21/60 (28.2) 21/21 (100) (ref) 0.371 Degraded Atlantic Forest 23/80 (28.7) 17/23 (73.9) * Tick collection * 49/80 (61.2) 32/49 (65.3) (ref) 0.070 Dog variables * * * 0.070 * 0.070 Age * 113/170 (65.5) 15/113 (13.3) 2.00 (0.45-8.94) 0.364 > 1 vear old 21/710 (17.6) 6/30 (20.0) (ref) 0.358 Body size * * * 0.364 Small 5/170 (2.9) 2/5 (40.0) (ref) 0.364 Large 14/170 (88.2) 2/14 (14.3) 1.09 (0.23-5.24) 0.913 Hygiene * 14/170 (49.4) 15/44 (17.9) (ref) 0 0 Veccination * * 169/170 (99.4) 24/159 (14.2) 0.859 0 0 0 0 Og mobility 0 0 (ref) 0 0 1/170 (14.1) - - - - No 0 0 0 0 0 <td>Biomes</td> <td>21/80 (26.2)</td> <td>21/21 (100)</td> <td>(10)</td> <td>0.271</td>	Biomes	21/80 (26.2)	21/21 (100)	(10)	0.271
Degrader Atlanter Polest 23/50 (25.7) 17/23 (7.3.7) Teck collection 31/80 (38.7) 26/31 (83.9) 2.76 (0.89-8.49) 0.070 No 49/80 (61.2) 32/49 (65.3) (ref) 0.070 Dog variables - </td <td>Atlantic Forest</td> <td>21/80 (26.2)</td> <td>21/21 (100) 17/22 (72 0)</td> <td>(rer)</td> <td>0.371</td>	Atlantic Forest	21/80 (26.2)	21/21 (100) 17/22 (72 0)	(rer)	0.371
The contention of the second	Tick collection	23/80 (28.7)	1//25 (75.9)		
No 49/80 (61.2) 23/49 (65.3) (ref) 0.010 Dg variables 49/80 (61.2) 32/49 (65.3) (ref) 0.010 Age 21/170 (15.8) 3/27 (11.1) (ref) 0.010 0.0364 >8 years old 30/170 (17.6) 6/30 (20.0) 1.63 (0.57-4.65) 0.358 Body size 5/170 (2.9) 2/5 (40.0) (ref) 0.010 (0.22-5.8) 0.244 Large 14/170 (8.2) 2/14 (14.3) 1.09 (0.23-5.24) 0.913 Hyglene 14/170 (8.2) 2/14 (14.3) 1.09 (0.23-5.24) 0.913 Yes 86/170 (50.6) 9/86 (10.5) 0.54 (0.22-1.31) 0.166 No 84/170 (49.4) 15/84 (17.9) (ref) 0 Ves 169/170 (99.4) 24/169 (14.2) 0.859 0 No 1/170 (10.0) 24/170 (14.1) - - - No 0 0 (ref) 0 0 0 1 - - - - - - - - - - - - - - - <	Yes	31/80 (38 7)	26/31 (83.9)	2 76 (0 89-8 49)	0 070
Dg variables Age (a) (b) (b) (b) Age (1) (1) (ref) (1) (ref) >1 - & year old 113/170 (66.5) 15/113 (13.3) 2.00 (0.45-8.94) 0.364 >8 years old 30/170 (17.6) 6/30 (20.0) 1.63 (0.57-4.65) 0.358 Body size 5mail 5/170 (2.9) 2/5 (40.0) (ref) (ref) Medium 15/170 (88.8) 20/151 (13.2) 0.25 (0.02-2.58) 0.244 Large 14/170 (8.2) 2/14 (14.3) 1.09 (0.23-5.24) 0.913 Yes 86/170 (50.6) 9/86 (10.5) 0.54 (0.22-1.31) 0.166 No 84/170 (49.4) 15/84 (17.9) (ref) 0 Vaccination 7 7 0 0 0 0 0 0 0.59 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1/170 (10.0) 2/170 (14.1) - <td>No</td> <td>49/80 (61.2)</td> <td>32/49 (65.3)</td> <td>(ref)</td> <td>0.070</td>	No	49/80 (61.2)	32/49 (65.3)	(ref)	0.070
Age 27/170 (15.8) 3/27 (11.1) (ref) >1-<8 years old	Dog variables	,	02, 10 (0010)	(101)	
-1 year old 27/170 (15.8) 3/27 (11.1) (ref) >1-<8 years old	Age				
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	<1 year old	27/170 (15.8)	3/27 (11.1)	(ref)	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	>1-<8 years old	113/170 (66.5)	15/113 (13.3)	2.00 (0.45-8.94)	0.364
Body size Small 5/170 (2.9) 2/5 (40.0) (ref) Medium 151/170 (88.8) 20/151 (13.2) 0.25 (0.02-2.58) 0.244 Large 14/170 (8.2) 2/14 (14.3) 1.09 (0.23-5.24) 0.913 Hygiene Yes 86/170 (50.6) 9/86 (10.5) 0.54 (0.22-1.31) 0.166 No 84/170 (49.4) 15/84 (17.9) (ref) Vaccination Yes 169/170 (99.4) 24/169 (14.2) 0.859 No 1707 (0.6) 0 (ref) Deworming Yes 170/170 (100.0) 24/170 (14.1) No (ref) Deg mobility Limited 151/170 (88.8) 23/151 (15.2) (ref) Unlimited 19/170 (11.2) 1/19 (5.3) 0.31 (0.04-2.43) 0.212 Hunting experience <1 year 43/170 (25.3) 7/43 (16.3) (ref) >13 years 40/170 (23.5) 5/40 (12.5) 0.89 (0.29-2.73) 0.842 Hunting frequency (per month) Once 63/170 (37.1) 8/63 (12.7) 2.48 (0.34-17.83) 0.368 4 times 69/170 (4.6) 11/69 (15.9) 1.96 (0.35-11.16) 0.446 8 times 9/170 (5.3) 2/9 (12.2) 1.51 (0.28-8.23) 0.636 Tick collection Tick 0 163/170 (95.8) 23/163 (14.1) (ref) Twice 63/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 No 163/170 (95.8) 23/163 (14.1) (ref) Twice 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) Exercado 23/170 (13.5) 3/23 (13.0) (ref) Tick collection Tex collection Tex collection Tex collection Sex Female 7/49 (14.3) 0.77 (0.0) (ref) Male 4/249 (85.7) 7/42 (16.6) (ref)	>8 years old	30/170 (17.6)	6/30 (20.0)	1.63 (0.57-4.65)	0.358
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Body size				
Medium $151/170$ (88.8) $20/151$ (13.2) 0.25 (0.02-2.58) 0.244 Large $14/170$ (8.2) $2/14$ (14.3) 1.09 (0.23-5.24) 0.913 Hygiene	Small	5/170 (2.9)	2/5 (40.0)	(ref)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Medium	151/170 (88.8)	20/151 (13.2)	0.25 (0.02-2.58)	0.244
Hygiene 86/170 (50.6) 9/86 (10.5) 0.54 (0.22-1.31) 0.166 No 84/170 (49.4) 15/84 (17.9) (ref) Vaccination - 0 0.859 No 1/170 (0.6) 0 (ref) Deworning - - - Yes 170/170 (100.0) 24/170 (14.1) - - No 0 (ref) 0 0 0.2112 Demoming - - - - - - No 0 (ref) -	Large	14/1/0 (8.2)	2/14 (14.3)	1.09 (0.23-5.24)	0.913
No 84/170 (30.0) 9/80 (10.3) 0.34 (0.221131) 0.100 Vaccination 169/170 (99.4) 24/169 (14.2) 0.859 Yes 169/170 (0.6) 0 (ref) Deworming - - - Yes 170/170 (100.0) 24/170 (14.1) - - No 0 (ref) - - Dog mobility 151/170 (88.8) 23/151 (15.2) (ref) 0.212 Unlimited 19/170 (11.2) 1/19 (5.3) 0.31 (0.04-2.43) 0.212 Hunting experience - - - - - <1 year	Vas	96/170 (50 6)	0/96 (10 E)		0 166
Vaccination 13/04 (17.5) 13/04 (17.5) (14) Yes 169/170 (99.4) 24/169 (14.2) 0.859 No 1/170 (0.6) 0 (ref) Deworming - - - Yes 170/170 (100.0) 24/170 (14.1) - - No 0 (ref) 0 0 Dg mobility 1 1/170 (88.8) 23/151 (15.2) (ref) 0 Unlimited 19/170 (11.2) 1/19 (5.3) 0.31 (0.04-2.43) 0.212 Hunting experience - - - - - <1 year	No	84/170 (49.4)	15/84 (17 Q)	(rof)	0.100
Yes 169/170 (99.4) 24/169 (14.2) 0.859 No 1/170 (0.6) 0 (ref) Deworming - - - Yes 170/170 (100.0) 24/170 (14.1) - - No 0 (ref) - - Dog mobility - - - - Unlimited 151/170 (88.8) 23/151 (15.2) (ref) - Unlimited 19/170 (11.2) 1/19 (5.3) 0.31 (0.04-2.43) 0.212 Hunting experience - - - - <1 year	Vaccination	04/1/0 (49.4)	15/04 (17.5)	(101)	
No 1/170 (0.6) 0 (ref) Deworming 7 0 0 (ref) Deworming 0 (ref) - - No 0 (ref) - - Dog mobility 151/170 (88.8) 23/151 (15.2) (ref) - Unlimited 19/170 (11.2) 1/19 (5.3) 0.31 (0.04-2.43) 0.212 Hunting experience - - - - - <1 year	Yes	169/170 (99.4)	24/169 (14.2)		0.859
Deworming Yes 170/170 (100.0) 24/170 (14.1) - - - - - 0 (ref) Dog mobility - - - 0 0 (ref) Dolation 0 1100000000000000000000000000000000000	No	1/170 (0.6)	0	(ref)	
Yes $170/170 (100.0)$ $24/170 (14.1)$ No0(ref)Dog mobility151/170 (88.8) $23/151 (15.2)$ (ref)Limited19/170 (11.2) $1/19 (5.3)$ $0.31 (0.04-2.43)$ 0.212 Hunting experience<1 year	Deworming				
No0(ref)Dog mobility Limited151/170 (88.8)23/151 (15.2)(ref)Unlimited19/170 (11.2)1/19 (5.3)0.31 (0.04-2.43)0.212Hunting experience </td <td>Yes</td> <td>170/170 (100.0)</td> <td>24/170 (14.1)</td> <td>-</td> <td>-</td>	Yes	170/170 (100.0)	24/170 (14.1)	-	-
Dog mobility Limited $151/170 (88.8)$ $23/151 (15.2)$ (ref)Unlimited $19/170 (11.2)$ $1/19 (5.3)$ $0.31 (0.04-2.43)$ 0.212 Hunting experience $(19/170 (25.3))$ $7/43 (16.3)$ (ref)>1-<3 years	No	0		(ref)	
Limited $151/170 (88.8)$ $23/151 (15.2)$ (ref)Unlimited $19/170 (11.2)$ $1/19 (5.3)$ $0.31 (0.04-2.43)$ 0.212 Hunting experience (11.2) $(1/19 (5.3)$ $0.31 (0.04-2.43)$ 0.212 $< 1 year$ $43/170 (25.3)$ $7/43 (16.3)$ (ref) $> 1-<3 years$ $87/170 (51.2)$ $12/87 (13.8)$ $0.73 (0.21-2.53)$ 0.626 $> 3 years$ $40/170 (23.5)$ $5/40 (12.5)$ $0.89 (0.29-2.73)$ 0.842 Hunting frequency (per month) $0nce$ $29/170 (17.1)$ $3/29 (10.3)$ (ref)Once $63/170 (37.1)$ $8/63 (12.7)$ $2.48 (0.34-17.83)$ 0.368 4 times $69/170 (40.6)$ $11/69 (15.9)$ $1.96 (0.35-11.16)$ 0.446 8 times $9/170 (5.3)$ $2/9 (22.2)$ $1.51 (0.28-8.23)$ 0.663 Tick collection Yes $7/170 (4.1)$ $1/7 (14.3)$ $1.01 (0.12-8.82)$ 0.663 No $163/170 (95.8)$ $23/163 (14.1)$ (ref)Biome $Z_{31/70 (13.5)}$ $3/23 (13.0)$ (ref) Hunter variables Sex $Female$ $7/49 (14.3)$ $0/7 (0.0)$ 0.314	Dog mobility				
Unlimited 19/1/0 (11.2) 1/19 (5.3) 0.31 (0.04-2.43) 0.212 Hunting experience 43/170 (25.3) 7/43 (16.3) (ref) >1-<3 years	Limited	151/170 (88.8)	23/151 (15.2)	(ref)	
Hunting experience <1 year	Unlimited	19/170 (11.2)	1/19 (5.3)	0.31 (0.04-2.43)	0.212
< 1 year	Hunting experience		7/42 (10 2)	(106)	
>3 years 37/170 (31.2) 12/37 (13.8) 0.73 (0.21-2.33) 0.020 >3 years 40/170 (23.5) 5/40 (12.5) 0.89 (0.29-2.73) 0.842 Hunting frequency (per month) 29/170 (17.1) 3/29 (10.3) (ref) Twice 63/170 (37.1) 8/63 (12.7) 2.48 (0.34-17.83) 0.368 4 times 69/170 (40.6) 11/69 (15.9) 1.96 (0.35-11.16) 0.446 8 times 9/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 Tick collection 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) Biome 41antic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) 0.586 Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)		43/170 (25.3)	//43 (10.3)	(rer) 0 72 (0 21 2 52)	0 626
Hunting frequency (per month) 29/170 (17.1) 3/29 (10.3) (ref) Twice 63/170 (37.1) 8/63 (12.7) 2.48 (0.34-17.83) 0.368 4 times 69/170 (40.6) 11/69 (15.9) 1.96 (0.35-11.16) 0.446 8 times 9/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 Tick collection 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) 0.663 Biome 4tlantic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) 0.586 Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	>1-<5 years	40/170 (31.2) 40/170 (23.5)	5/40 (12.5)	0.73 (0.21-2.53)	0.626
Once 29/170 (17.1) 3/29 (10.3) (ref) Twice 63/170 (37.1) 8/63 (12.7) 2.48 (0.34-17.83) 0.368 4 times 69/170 (40.6) 11/69 (15.9) 1.96 (0.35-11.16) 0.446 8 times 9/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 Tick collection 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) 0.663 Biome 4tlantic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) 0.586 Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Hunting frequency (per month)	40/1/0 (25.5)	5/40 (12.5)	0.05 (0.25 2.75)	0.042
Twice 63/170 (37.1) 8/63 (12.7) 2.48 (0.34-17.83) 0.368 4 times 69/170 (40.6) 11/69 (15.9) 1.96 (0.35-11.16) 0.446 8 times 9/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 Tick collection 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) Biome 4tlantic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) 0.586 Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Once	29/170 (17.1)	3/29 (10.3)	(ref)	
4 times 69/170 (40.6) 11/69 (15.9) 1.96 (0.35-11.16) 0.446 8 times 9/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 Tick collection 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) Biome 7 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables 23/170 (13.5) 3/23 (13.0) (ref) Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Twice	63/170 (37.1)	8/63 (12.7)	2.48 (0.34-17.83)	0.368
8 times 9/170 (5.3) 2/9 (22.2) 1.51 (0.28-8.23) 0.636 Tick collection 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) 0 Biome 7/170 (4.1) 1/7 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables Sex 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	4 times	69/170 (40.6)	11/69 (15.9)	1.96 (0.35-11.16)	0.446
Tick collection Yes 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) Biome 4tlantic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables Sex 5ex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	8 times	9/170 (5.3)	2/9 (22.2)	1.51 (0.28-8.23)	0.636
Yes 7/170 (4.1) 1/7 (14.3) 1.01 (0.12-8.82) 0.663 No 163/170 (95.8) 23/163 (14.1) (ref) Biome 4tlantic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables Sex 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Tick collection				
No 163/170 (95.8) 23/163 (14.1) (ref) Biome Atlantic Forest 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables Sex 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Yes	7/170 (4.1)	1/7 (14.3)	1.01 (0.12-8.82)	0.663
Biome 147/170 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	No	163/170 (95.8)	23/163 (14.1)	(ref)	
Atlantic Forest 14//1/0 (86.5) 21/147 (14.3) 1.11 (0.30-4.07) 0.586 Cerrado 23/170 (13.5) 3/23 (13.0) (ref) Hunter variables Sex Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Biome				0
Cerrado 23/1/0 (13.5) 3/23 (13.0) (ref) Hunter variables 7/49 (14.3) 0/7 (0.0) 0.314 Sex 7/49 (14.3) 0/7 (0.6) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)	Atlantic Forest	14//170 (86.5)	21/147 (14.3)	1.11 (0.30-4.07)	0.586
Function Figure 1 Figure 2	Cerrado	23/1/0 (13.5)	3/23 (13.0)	(ref)	
Female 7/49 (14.3) 0/7 (0.0) 0.314 Male 42/49 (85.7) 7/42 (16.6) (ref)					
Male 42/49 (85.7) 7/42 (16.6) (ref)	Female	7/49 (14 3)	0/7 (0 0)		0 314
	Male	42/49 (85.7)	7/42 (16.6)	(ref)	0.017

(Contd...)

Table-1: (Continued).						
Risk factors of <i>Rickettsia</i> spp.	Total Yes/total (%)	Positive Yes/total (%)	OR (95% CI)	p-value		
Occupation						
Retired and student	8/49 (16.3)	0/8 (0.0)	(ref)			
Private work	32/49 (65.3)	5/32 (15.6)		0.644		
Public work	9/49 (18.4)	2/9 (22.2)		0.118		
Number of minimum wages						
Up to three	28/49 (57.1)	4/28 (14.3)	(ref)			
Four to eight	16/49 (32.6)	2/16 (12.5)	0.7 (0.1-7.6)	0.744		
Up to eight	5/49 (10.2)	1/5 (20.0)	0.6 (0.1-8.0)	0.678		
Basic education	5/49 (10.2)	0/5 (0.0)				
School level						
High education	12/49 (24.5)	2/12 (16.7)	(ref)	0.43		
Graduate	22/49 (44.9)	2/22 (9.1)		0.151		
Postgraduate	10/49 (20.4)	3/10 (30.0)		0.220		
Rural household location						
Yes	18/49 (36,7)	3/18 (16.7)	1.3 (0.3-6.8)	0.512		
No	31/49 (63.3)	4/31 (12.9)	(ref)			
Registered hunter	- / - (/					
Yes	32/49 (65.3)	6/32 (18.8)	3.7 (0.4-33.5)	0.219		
No	17/49 (34.7)	1/17 (5.9)	(ref)			
Forest nearby the household		_/ _: (=:=)	(1)			
Yes	23/49 (46.9)	4/23 (17.4)	1.6 (0.3-8.1)	0.429		
No	26/49 (53.1)	3/26 (11.5)	(ref)			
Dog owner	20, 10 (0012)	0,20 (22.0)	(101)			
Yes	5/49 (10.2)	5/38 (13.2)	0.7 (0.1-4.1)	0.500		
No	11/49 (22.4)	2/11 (18.2)	(ref)	01000		
Dogs location		_, (,	(1 2 1)			
Without dogs	12/49	2/12 (16.7)	(ref)			
Peridomicile	22/49	3/22 (13.6)	0.8(0.1-5.5)	0.812		
Peridomicile and domiciled	2/49	2/14 (14.3)	0.8(0.1-7.0)	0.867		
Domiciled	1/49	0/1		01007		
Contact with dogs of other hunters	_,	0, 1				
Yes	39/49	6/39 (15.4)	1.6 (0.2-15.4)	0.996		
No	10/49 (20.4)	1/10(100)	(ref)	0 559		
Presence of capybaras in hunting areas	10, 10 (2017)	1, 10 (1010)	(101)	0.000		
Yes	24/49 (49 0)	4/24 (16 7)	15(03-74)	0 476		
No	25/49 (51.0)	3/25 (12.0)	(ref)	0.170		
Presence of opossum in hunting areas	20, 10 (01:0)	5/25 (12.0)				
Yes	25/49 (51 0)	3/25 (12.0)	07(01-34)	0.641		
No	23/49 (31.0) 24/49 (49 N)	4/24 (16 7)	(ref)	0.041		
	27/79 (79.0)	7/27 (10.7)	(161)			

seasons, 13/30 (43.3%) hunters reported exposure during summer, 1/30 (3.3%) hunter reported exposure during spring, and 1/30 (3.3%) hunter reported exposure during autumn and winter. A tick check inspection after hunting was reported by 30/49 (59.2%) hunters, and the use of a repellent was reported by only 1/49 (0.2%) hunter. After hunting, 14/49 (28.6%) hunters mentioned taking a shower, and 35/49 (71.4%) hunters reported slaughtering wild boars (Table-1).

Discussion

In this study, no associated risk factor was statistically significant for wild boars, hunting dogs, or hunters with seropositivity to *Rickettsia* spp., except for wild boar sex, with females being more likely positive (p=0.034). This finding may be due to the high exposure of in-park wild boars, mostly females, because previous research has shown a "forest border effect" risk for dogs and human beings [5].

Hence, the statistically significant differences in seropositivity between 17/29 (58.6%) wild boar males

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and 41/51 (80.4%) females could be related to the most prevalent wild boar sex and tick species sampled in the natural areas. Not surprisingly, 14/21 (66.6%) wild boar females were slaughtered in-park, all of which were sero-positive to *Rickettsia* spp. and had *A. brasiliense* ticks [1].

Although Brazilian hunters may equally hunt male and female wild boars, hunting has been prohibited in state parks, which may serve as a nursery to females [3]. Moreover, male wild boars have demonstrated a higher variation of roaming distance [6], indicating that they are more likely to cross park limits toward surrounding agricultural areas.

The frequency of dog contact with forests has been associated with the occurrence of *Rickettsia* spp. and *Amblyomma* spp. transmission [7]. In the present study, the absence of statistical differences in sex, age, body size, hygiene, vaccination, mobility, hunting experience, and frequency, tick collection, the group size of dogs, and biomes may indicate that hunting dogs are exposed to *Rickettsia* spp., irrespective of the associated risk factors. Although no study has focused on hunter exposure, people infected with BSF were found to be primarily white males aged between 20 and 64 years from the rural areas of South and Southeast Brazil, visiting natural environments (66.7%), exposed to ticks (72.7%), and contacting capybaras (15.6%), dogs and cats (42.4%), cattle (17.2%), and horses (17.4%) [8]. Therefore, the absence of statistical differences in the present study may indicate that hunters are exposed to *Rickettsia* spp., irrespective of their sex, age, occupation, income, education, household location, owning a dog, contact with hunting dogs, presence of capybaras or opossums, number of years lived in rural areas, hunting experience, and number of people and dogs in the hunter's household.

Finally, all ticks collected in these areas were negative for the presence of *Rickettsia* spp. as they have rarely infected BSF-non-endemic areas [1]. In Southeast Brazil, high capybara population has been associated with a high tick infestation rate in BSF-endemic areas [9], with wild boar occurrence reportedly overlapping such areas [10].

Conclusion

The entry of wild boars, hunting dogs, and hunters into tick habitats may lead to bites and consequent infection with *Rickettsia* spp. The only statistically significant association with seropositivity found in this study was the increased risk due to hunting female wild boars, which is probably associated with in-park exposure to *A. brasiliense* and *Rickettsia* spp. Furthermore, the absence of statistical differences in the associated risk factors for hunting dogs and hunters may indicate a random exposure to *Rickettsia* spp.

Authors' Contributions

LBK, APS, and AWB: Conceptualization. LBK, TFM, RVWB, CMM, IRB, LCL, GMF, APS, and AWB: Data collection and data analysis. LBK and AWB: Drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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