

## *Bartonella clarridgeiae* and *Bartonella vinsonii* subsp. *berkhoffii* exposure in captive wild canids in Brazil

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### SUMMARY

Wild canids are potential hosts for numerous species of *Bartonella*, yet little research has been done to quantify their infection rates in South America. We sought to investigate *Bartonella* seroprevalence in captive wild canids from 19 zoos in São Paulo and Mato Grosso states, Brazil. Blood samples were collected from 97 wild canids belonging to four different native species and three European wolves (*Canis lupus*). Indirect immunofluorescent antibody testing was performed to detect the presence of *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, and *B. rochalimae*. Overall, *Bartonella* antibodies were detected in 11 of the canids, including five (12·8%) of 39 crab-eating foxes (*Cerdocyon thous*), three (11·1%) of 27 bush dogs (*Speothos venaticus*), two (8·7%) of 23 maned wolves (*Chrysocyon brachyurus*) and one (12·5%) of eight hoary foxes (*Lycalopex vetulus*), with titres ranging from 1:64 to 1:512. Knowing that many species of canids make excellent reservoir hosts for *Bartonella*, and that there is zoonotic potential for all *Bartonella* spp. tested for, it will be important to conduct further research in non-captive wild canids to gain an accurate understanding of *Bartonella* infection in free-ranging wild canids in South America.

**Key words:** *Bartonella*, bush dog, crab-eating fox, hoary fox, maned wolf, South America, wild canids.

### INTRODUCTION

Carnivores have been shown to be reservoirs of a wide range of *Bartonella* spp. [1]. Worldwide, various wild canids have been identified as natural reservoirs of *Bartonella* spp. In North America, *Bartonella* spp. have been isolated from coyotes (*Canis latrans*), grey

foxes (*Urocyon cinereoargenteus*), island foxes (*Urocyon littoralis*) and raccoons (*Procyon lotor*) and serologically detected in American badgers (*Taxidea taxus*) [2–4]. *Bartonella* have also been detected in river otters (*Lontra canadensis*) [5] and more recently in sea otters (*Enhydra lutris*) [6]. In Europe (France and the Basque Country, northern Spain) and Israel, *B. rochalimae* was isolated or detected from red foxes (*Vulpes vulpes*) and from a wolf (*Canis lupus*) [2, 7]. A species close to *B. clarridgeiae* was detected in badgers (*Meles meles*) from the Basque Country, northern Spain [7]. In Great

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Table 1. Number, species, and zoological park location of sampled wild canids, Brazil

Species	Common name	Location (n)	Total
<i>Speothos venaticus</i>	Bush dog	Am (9), Le (1), Pir (6), SP (8), Cui (3)	27
<i>Cerdocyon thous</i>	Crab-eating fox	Rp (7), Am (3), Ara (6), IS (3), So (2), SC (4), Bau (1), Ita (1), Pir (3), Cat (6), Le (1), Jab (2)	39
<i>Chrysocyon brachyurus</i>	Maned wolf	Rp (2), Am (1), Ara (1), IS (2), So (2), Ita (3), Mg (3), SP (3), Cat (1), NO (3), Bau (2)	23
<i>Lycalopex vetulus</i>	Hoary fox	Rp (1), Am (2), Ara (3), IS (1), So (1),	8
<i>Canis lupus</i>	European wolf	SP (3)	3

AMC, Association Mata Ciliar, Jundiá, SP; Am, Americana Zoo, SP; Ara, Araçatuba Zoo, SP; Bau, Bauru Zoo, SP; Cat, Catanduva Zoo, SP; Cps, Campinas Zoo, SP; Cui, Cuiabá Zoo, Mato Grosso; IS, Ilha Solteira Zoo, SP; Ita, Itatiba Zoo, SP; Jab, Jaboticabal Zoo, SP; Le, Leme Zoo, SP; Mg, Mogi Mirim Zoo, SP; NO, Nova Odessa Zoo, So, Sorocaba; SP; Pir, Piracicaba Zoo, SP; Rp, Ribeirão Preto, SP; SC, São Carlos Zoo, SP; SP, São Paulo Zoo, SP.

Britain, stoats (*Mustela erminea*) were reported to be commonly infected with *Bartonella* [8]. In the Middle East, *Candidatus B. merieuxii* was detected in dogs and jackals from Iraq [9]. In Asia, a new *Bartonella* sp. and one close to *B. washoensis* were isolated respectively from a Japanese badger (*Meles anakuma*) and a Japanese marten (*Martes melampus*) [10]. In Australia, *B. clarridgeiae* was detected in a red fox [11]. *Bartonella* are usually vector-borne, with fleas and possibly ticks being the most likely vectors in canids [12]. Wild carnivores, including wild canids, are hosts or potential hosts for numerous species of *Bartonella*, yet little research has been done to quantify their infection rates in South America. We sought to investigate *Bartonella* seroprevalence in captive wild canids from 17 zoos in São Paulo and Mato Grosso states, Brazil. Blood samples were collected from 97 wild canids belonging to four different native species and three European wolves [13].

## MATERIAL AND METHODS

### Animals

A total of 5 ml of blood was collected from each of 97 Brazilian wild canids belonging to four different species (Table 1), including 27 bush dogs (*Speothos venaticus*), 39 crab-eating foxes (*Cerdocyon thous*), 23 maned wolves (*Chrysocyon brachyurus*), eight hoary foxes (*Lycalopex vetulus*) and three European wolves (*Canis lupus*), maintained in captivity in 17 Brazilian zoos of São Paulo and Mato Grosso states [13]. All samples were collected under IBAMA license numbers S02027.002943/2005 and 15901–1. Animals were immobilized with a mixture of ketamine (Francotar<sup>®</sup>, Virbac, France) (10 mg/kg) and xylazine (Francotar) (1 mg/kg). Physical examination showed

that sampled animals were apparently healthy. No ticks were found on any of the sampled animals.

### Serology

Antibodies against *B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, *B. henselae* and *B. rochalimae* were detected using an indirect immunofluorescent antibody assay (IFA). These four antigens were selected, as *B. henselae*, *B. vinsonii* subsp. *berkhoffii* and *B. rochalimae* have been frequently detected in domestic dogs as well as *B. clarridgeiae*, which can also be a good substitute for detection of *B. rochalimae* [14, 15]. The IFA procedure was similar to a procedure described previously [16], with the following modifications. A 90% confluent tissue culture flask (containing MDCK cells) was inoculated with a 4-day-old culture of *B. vinsonii* subsp. *berkhoffii* (ATCC 51672) resuspended in 0.5 ml saline. Similarly, flasks containing Vero tissue cultures were inoculated with *B. clarridgeiae* (ATCC 51734), *B. rochalimae* (ATCC BAA-1498) or a mixture of *B. henselae* (ATCC 49882) and *B. henselae* U4 (University of California, Davis, strain). Drops (40 µl) of the infected tissue culture were spotted onto 12-well glass slides (Cel-Line<sup>®</sup>, Thermo Scientific, USA), the tissue culture allowed to adhere overnight, and the slides were then washed in PBS and fixed with acetone for 20 min. Serum samples added to the test wells were screened at 1:64 dilution in PBS with 5% milk. Slides were incubated at 37 °C for 30 min, followed by three washes in PBS. Fluorescein-conjugated goat anti-dog immunoglobulin G (IgG; ICN Biomedicals Inc., USA) was diluted in PBS (1:1400 for *B. vinsonii* subsp. *berkhoffii*, 1:3600 for *B. clarridgeiae* and *B. rochalimae* and 1:2800 for *B. henselae*) with 5%

Table 2. *Bartonella* antibody titres for seropositive captive wild canids, Brazil

Common name	Zoological park	B.v.b.	B.c.	B.h.	B.r.
Crab-eating fox	Ilha Solteira	Neg.	1:64	1:64	Neg.
Crab-eating fox	Ilha Solteira	Neg.	Neg.	1:64	Neg.
Crab-eating fox	Ilha Solteira	Neg.	1:64	Neg.	Neg.
Crab-eating fox	Araçatuba	Neg.	1:64	Neg.	Neg.
Crab-eating fox	Araçatuba	Neg.	1:64	Neg.	Neg.
Bush dog	São Paulo	Neg.	1:128	1:64	Neg.
Bush dog	São Paulo	1:512	1:64	1:256	1:128
Bush dog	Leme	Neg.	1:256	Neg.	Neg.
Maned wolf	Sorocaba	1:128	Neg.	Neg.	Neg.
Maned wolf	Nova Odessa	Neg.	1:64	Neg.	Neg.
Hoary fox	Ribeirão Preto	Neg.	1:128	Neg.	Neg.

B.v.b., *Bartonella vinsonii* subsp. *berkhoffii*; B.c., *Bartonella clarridgeiae*; B.h., *Bartonella henselae*; B.r., *B. rochalimae*.

milk containing 0.001% Evans Blue, and 20  $\mu$ l of the dilution was applied to each well. The slides were incubated at 37 °C for 30 min and again washed in PBS three times. The intensity of bacillus-specific fluorescence was scored subjectively from 1 to 4. Samples with a fluorescence score of  $\geq 2$  at a dilution of 1:64 were reported as positive and final titration was performed (last dilution with a score  $\geq 2$ ). The same two readers performed a double-blind reading of each slide. Negative and positive control samples were included on each slide.

## RESULTS

Overall, *Bartonella* antibodies were detected in 11 (11%) of the canids, including five (12.8%) of the 39 crab-eating foxes, three (11.1%) of 27 bush dogs, two (8.7%) of 23 maned wolves and one (12.5%) of eight hoary foxes, with titres ranging from 1:64 to 1:512 (Table 2). Antibodies against *B. clarridgeiae* were most frequently detected (82%, 9/11), whereas only four animals were seropositive for *B. henselae* (36.4%), two animals for *B. v. berkhoffii* (18.2%) and one for *B. rochalimae* (9.1%).

Two of the three seropositive bush dogs were from São Paulo Zoo (25%, 2/8) and the third one from Leme Zoo. The positive hoary fox was from Ribeirão Preto. The two seropositive maned wolves were from Nova Odessa and Sorocaba zoos, respectively, and three of the five crab-eating foxes were from Ilha Solteira (100%, 3/3); the two others being from Araçatuba (33%, 2/6). None of the three European wolves from São Paulo Zoo tested positive for *Bartonella* spp. One of the three seropositive bush dogs was seropositive for all four antigens, with the

highest titre for *B. v. berkhoffii*. All three dogs were seropositive for *B. clarridgeiae* and only one of the three dogs was seropositive for *B. clarridgeiae* only (titre 1:256), which was also the case of the only seropositive hoary fox, one of the two seropositive maned wolves, but four of the five crab-eating foxes. The bush dog seropositive for two antigens was positive for *B. clarridgeiae* but also for *B. henselae*. The other maned wolf was only seropositive for *B. v. berkhoffii*. Two crab-eating foxes were seropositive for *B. henselae*, one of the two being also *B. clarridgeiae* positive.

## DISCUSSION

We report the first evidence of presumptive *Bartonella* exposure in captive wild South American canids. Antibodies were detected in all four species, with an overall prevalence of 11% (range 8.7–12.8%, according to the canid species tested). Prevalence also varied depending of the location where these animals were in captivity from 0% to 100%. All animals tested were clinically healthy. Unfortunately, no specific information on age and sex of these animals was available regarding whether these animals were born in captivity or trapped in the wild. *Bartonella* are usually vector-borne, with fleas and possibly ticks being the most likely vectors in canids [12]. It was recorded that none of the animals had ticks attached at time of examination, but no information on fleas were given. However, it is likely that if massive infestation had occurred, it would have been recorded.

In contrast to Northern America, limited information on infection of domestic dogs from South and Central America with various *Bartonella* spp.

has been reported. The main data come from Brazil, Peru and Colombia, where dogs were found to be positive and/or seropositive by polymerase chain reaction (PCR) for *B. v. berkhoffii*, *B. rochalimae* and *B. clarridgeiae*. In Brazil, less than 3% of sick dogs from São Paulo region presenting at a veterinary teaching hospital were seropositive for *B. v. berkhoffii* or *B. henselae* and both species were detected by PCR in these dogs [17, 18]. In stray dogs from São Paulo, seroprevalence was more than double (7.6%, 9/118) that reported in sick pet dogs presenting at a veterinary teaching hospital and 10.7% of 258 stray dogs from Bogota, Colombia [19]. Both *B. v. berkhoffii* and *B. rochalimae* were detected by PCR in two of the seropositive dogs from Bogota. In Peru, a much higher seroprevalence was detected in free-roaming dogs from various Andean locations [20], as seropositivity for *B. rochalimae* was detected in 67 dogs (62%), and for *B. v. berkhoffii* in 43 (40%) of the 108 dogs for which serum samples were available. Reciprocal titres  $\geq 1:256$  for *B. rochalimae* were detected in 19% of dogs, and for *B. v. berkhoffii* in 6.5% of dogs. *Bartonella* DNA was detected in 21 (10%) of the 205 dogs for which DNA was extracted. Fifteen dogs were infected with *B. rochalimae*, while six dogs were infected with *B. v. berkhoffii* genotype III. Out of 95 free-roaming dogs from Isabella Island in the Galapagos archipelago, Ecuador, 13 (13.7%) dogs were PCR positive, including eight dogs PCR positive for *B. clarridgeiae*, four dogs positive for *B. elizabethae* and one dog positive for *B. henselae* [21]. In the Caribbean islands, six (8%) of 73 dogs brought to the veterinary teaching hospital in St George, Grenada were seropositive for *B. v. berkhoffii* and *Bartonella* DNA was detected by PCR from one (1.4%) of these 73 dogs [22].

Our data widen the spectrum of *Bartonella* infection to four species of wild canids native of South America, as none of the three imported European wolves were seropositive. On the contrary, *B. rochalimae* was detected by PCR from a free-ranging wolf from northern Spain [7]. The captive South American wild canids were mainly seropositive to *Bartonella* spp. also endemic in domestic dogs in South and Central America. It is particularly interesting to note that most of our seropositive captive wild canids were positive (positive only for that antigen or with the highest titre) to *B. clarridgeiae*, which was also the species most commonly detected by PCR in dogs from Isabella Island in the Galapagos and the antigen for which dogs from Colombia had the highest prevalence

and highest titres [19, 21]. Cross-reactivity between antigens is still possible; however, it was observed for only 3/11 seropositive animals and as it was a cross-sectional study, it is also possible that a given animal had been exposed to different *Bartonella* spp. In experimental infection of specific pathogen-free (SPF) dogs, cross-reactivity was not observed [23, 24]. Therefore, *B. clarridgeiae*, either of feline origin or closely related strains specific to canids could be highly prevalent in domestic dogs and wild canids in South America. Further investigation is warranted in free-ranging wild canids from this part of the world in order to better determine the *Bartonella* spp. for which they are natural or accidental hosts. Furthermore, it will be important to determine if these *Bartonella* spp. can cause endocarditis in domestic dogs and captive wild canids, as shown for domestic dogs in North America for *B. v. berkhoffii*, *B. clarridgeiae*, *B. rochalimae* and *B. henselae* [25–28].

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#### DECLARATION OF INTEREST

None.

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