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Comparison of infection by *Brucella* spp. in free-ranging and captive wild animals from São Paulo State, Brazil

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Abstract: The aim of the current study was to evaluate the infection rate by *Brucella* spp. in wild and in captive animals. Serum samples from 121 animals (94 free-ranging and 27 captive) of different mammal species were evaluated. Sera were submitted to rose Bengal test (RBT) for screening and serum agglutination tests (SAT) and 2-mercaptoethanol test (2-ME) for confirmatory results. Nine animals (five free-ranging and four captive) tested positive in RBT, but negative in the confirmatory tests. Several domestic animal diseases that have control programs are not focused on wild reservoirs, such as brucellosis in Brazil. The study of new reservoirs in wildlife is essential to prevent emerging diseases.

Key words: brucellosis, wildlife, zoonosis.

The key for controlling several zoonoses – including brucellosis – is to focus on animal reservoirs (1). Wild animals as reservoirs of classical and emerging zoonoses persist in numerous countries and substantially hinder the efforts for controlling these infections (2). The fast-growing demand for milk and meat in urban centers has provoked the intensification of livestock production in periurban areas which, in turn, has increased the risk for zoonosis transmission (3).

The bacteria of the genus *Brucella* spp. are generalists when it comes to hosts, including wild animals (4). Brucellosis has been reported in marsupials, peccary, swine, camelids, cattle, pinnipeds and cetaceans (5-10). It is suggested that *B. abortus* infection occurs by eating carcasses, placental or fetal remnants of diseased animals (11). Abortions, orchitis, epididymitis and infertility are the main clinical manifestations in wild mammals (12). Infectious pathogens affect not only public health but also the economy, and wildlife conservation. Wild animals that are treated as exotic pets pose a risk to public health from exposure to zoonosis (13). Wildlife can become a new source of infection and re-contaminate domestic animals, as in the case of domestic pigs infected with brucellosis by wild boars in Europe (14). This study aimed to detect the infection rate by *Brucella* spp. in serum samples of free-ranging and captive wild animals.

Serum samples of 121 animals attended at the Center of Medicine and Research of Wild Animals, CEMPAS, UNESP, Botucatu, Brazil, were collected. The animals were both captive (from zoos or rehabilitation centers) and free-ranging, found in localities adjacent to the municipality of Botucatu (22°53'S 48°26'W). Of the 121 samples collected from 2006 to 2009, 94 belonged to free-ranging animals and 27 to captive ones (Table 1). The samples were serologically analyzed for *Brucella* spp. infection according to

the National Program for Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) of the Brazilian Ministry of Agriculture Livestock and Food Supply (MAPA) (15).

The serum samples were first evaluated by the screening test (RBT) and if they were considered reagent, they were then submitted to the confirmatory tests (tube agglutination test – TAT, and 2-mercaptoethanol test – 2-ME). Analysis of the data included calculation of statistical rates. The hypothesis was that free-ranging animals could have a higher frequency of infection than captive animals. Statistical analyses were performed in 2 x 2 small table [origin: (free-

Table 1. Serological results for *Brucella* spp. infection in free-ranging and captive wild animals from São Paulo state, Brazil

Species	Free-ranging (FR)*	Captivity (C)	RB	SAT	2-ME
Lion (*Panthera leo*)	0	2	R (1C)	N	N
Jaguarundi (*Puma yagouaroundi*)	1	0	NR	N	N
Tiger (*Panthera tigris*)	0	1	NR	N	N
Porcupine (*Sphiggurus* spp.)	2	0	NR	N	N
Coati (*Nasua nasua*)	3	2	NR	N	N
Patas monkey (*Erythrocebus pata*)	0	2	NR	N	N
Maned wolf (*Chrysocyon brachyurus*)	3	3	R (1C)	N	N
Tufted Capuchin (*Cebus apella*)	0	2	NR	N	N
Mandrill (*Mandrillus sphinx*)	0	1	NR	N	N
Capybara (*Hydrochoerus hidrochaeris*)	1	0	NR	N	N
Lowland paca *(Cuniculus paca)*	2	6	R (1FR and 1C)	N	N
Crab-eating fox (*Cerdocyon thous)*	3	1	NR	N	N
Hoary fox (*Lycalopex vetulus*)	1	1	R (1C)	N	N
Brown howler monkey *(Alouatta guariba)*	0	3	NR	N	N
Jaguar (*Panthera onca*)	0	1	NR	N	N
White-eared opossum *(D. albiventris)*	50	0	R (3FR)	N	N
Gray brocket (*Mazama gouazoubira*)	3	0	NR	N	N
European hare (*Lepus europaeus*)	2	0	NR	N	N
Chimpanzee (*Pan troglodytes)*	0	1	NR	N	N
Lesser anteater (*Tamandua tetradactyla*)	1	0	R (1FR)	N	N
South African porcupine (*Hystrix africaeaustralis*)	0	1	NR	N	N
Nutria (*Myocastor coypus*)	1	0	NR	N	N
Greater naked tailed armadillo (*Cabassous tatouay*)	1	0	NR	N	N
Nine-banded armadillo (*Dasypus novemcinctus*)	17	0	NR	N	N
Six-banded armadillo (*Euphractus sexcinctus*)	3	0	NR	N	N
Total	**94**	**27**			

RB: rose Bengal; SAT: serum agglutination test; 2-ME: 2 mercaptoethanol test; R: reagent; NR: not reagent; P: positive; N: negative.

* OR: 3.23; 95%CI: 0.72-13.80; $p > 0.05$.

ranging animals or captive animals) and RBT (positive or negative)], and submitted to chi-square analysis, and Fisher's exact test ($p \leq 0.05$).

As displayed in Table 1, nine animals (five free-ranging and four captive) were positive for RBT, but none remained positive in the confirmatory tests. In the current study, no significant association between origin and RBT was observed (OR: 3.23; 95%CI: 0.72-13.80; $p > 0.05$). Fisher's exact test suggested that the origin of the animals is not significant for the source of infection.

The serological method employed in the present work is recommended by MAPA and is also utilized in serological surveys in wild animals (15-17). Serology is a standard technique for the epidemiological surveillance of brucellosis; however, cross-reactions between *Brucella* species and other gram-negative bacteria are a major problem of serological assays (18). The source of antigenic cross-reactions is the O-chain of the smooth lipopolysaccharide (S-LPS) present on the surface of the bacterial cell, which shows great similarity in smooth *Brucella* spp. False-positive serological results due only to *Yersinia enterocolitica* O:9 affect up to 15% of the cattle herds in regions free from brucellosis. However, there are no reports of *Y. enterocolitica* infection on wildlife species analyzed in this study. False-negative results have also been observed in serological diagnosis of brucellosis (19). They occur mostly due to the fact that the antibody response depends on the stage of infection during sample collection. Detectable amounts of antibodies are not recorded in the first 12 to 16 days after artificial inoculation of goats with *Brucella abortus* (20). On the other hand, when the disease becomes chronic, the antibody titer can drop to undetectable levels, which is the case of intracellular organisms such as *Brucella* spp. (20).

The results of serology in wild animals are controversial. Absence of positivity in experimental infection of opossums (*D. virginiana*) was previously reported (11). In the present study, the majority of the samples (52%) were from white-eared opossums (*Didelphis albiventris*), resulting in an underestimated prevalence of brucellosis in this animals. However, in opossums, we suggest a direct investigation of brucellosis in reproductive organs. In Austria, European hares (*Lepus europaeus*) are considered a source of human brucellosis while in Switzerland the disease is responsible for the declining population of hares (21, 22). Although this hare species is exotic, there are no reports of brucellosis infections in hares in Brazil, and the number of such animals captured in this study was small. There are studies on *Brucella* spp. microbiological isolation in buffalo (*Bubalus bubalis*), fox (*Dusicyon gymnocercus antiquus*), gray weasel (*Didelphis marsupialis*), capybara (*Hydrochoerus hydroachaeris*) and ferret (*Galictis furax huranox*) (23). In other species analyzed in the present study, there were no observed cases of brucellosis.

The present work reports for the first time serological positive results of *Brucella* spp. infection by RBT test in lions (*Panthera leo*), maned wolves (*Chrysocyon brachyurus*), lowland pacas (*Cuniculus paca*), hoary foxes (*Lycalopex vetulus*), white-eared opossums (*Didelphis albiventris*), and lesser anteaters (*Tamandua tetradactyla*). From the 121 animals tested, 7.43% were positive by RBT. According to Lage *et al*. (15), brucellosis in Brazil ranged from 4 to 5% in bovines, and exploratory surveys are currently being carried out in most Brazilian states. According to MAPA, there are no specific tests recommended for wildlife. However, the methods employed in the present study were previously utilized in studies involving wild animals (17-20, 23). Consequently, there are no data available about the sensitivity and specificity of these tests for wildlife, and the possibility of cross-reactions with non-*Brucella* strains cannot be dismissed. The rose Bengal test and the tube agglutination test are considered by World Organization for Animal Health (OIE) standardized tests for diagnosing *Brucella suis* and *Brucella abortus* infection in wild animals (24). Unfortunately, there are no studies on the pathogenesis of brucellosis in wild animals and their immune response. Moreover, it is not clear how the immunoglobulins of these animals behave in such tests.

Brucellosis is considered an important infectious disease that affects public health (1). The devastation of forests, aggressive development of agriculture, increased meat production and even the tourism industry are associated with zoonosis transmission (13). Consequently, the development of surveillance programs for emerging diseases in wild animals is extremely important to avoid new cases of such infections (1, 13). Several diseases that affect domestic animals that have control programs are not focused on wild reservoirs, as

the case of brucellosis in Brazil (15). Therefore, monitoring the presence of the pathogen in wildlife, and analyzing other infectious agents are necessary. Medicine conservation, sustainable agriculture, education of tourists, public health education, and risks of acquiring an exotic pet are also critical for preventing emerging diseases and new reservoirs (13).

Research on brucellosis in wild animals is scarce, especially in Brazil. Positivity by RBT must be carefully evaluated and monitored since this may represent a source of infection. The consequences are the impact on wild animal species and their role as reservoir to other animal species. Further studies involving a larger number of animals should be conducted in order to discover the impact of brucellosis in wild animals and the importance of the transmission of this disease to humans and livestock.

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CONFLICTS OF INTEREST
There is no conflict.

ETHICS COMMITTEE APPROVAL
The present study was approved by the Institutional Ethics and Animal Welfare Commission of the School of Veterinary Medicine and Animal Husbandry, UNESP, Botucatu Campus (ethics committee protocol n. 82/2009-CEUA) and the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA n. 16900-1).

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