Brief Communication

Molecular detection of *Brucella abortus* in wild and captive felids

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**ABSTRACT**

**Purpose:** Brucellosis is a zoonotic disease of great public health importance. In wild animals, *Brucella abortus* is one of the most diagnosed species, mainly in enzootic environments where domestic animals share the same environment. *B. abortus* is common in environments shared by cattle, wild, and domestic animals. This study aimed to detect the presence of *B. abortus* DNA in free-ranging and captive felids at Mato Grosso State, Brazil.

**Method:** Polymerase chain reaction, based on the genetic element IS711, was performed in blood samples collected from 23 free-ranging and captive felids. The species represented include *Leopardus colocolo*, *Leopardus pardalis*, *Leopardus wiedii*, *Panthera onca*, *Puma concolor*, and *Puma yagouaroundi*.

**Results:** DNA amplification of *B. abortus* was observed in only one captive *P. concolor* (4.34%).

**Conclusion:** The detection of this pathogen in captive animals using molecular tools demonstrates the importance of monitoring, as it raises concerns about the possibility of transmission between humans and wild and domestic animals, especially in regions of vast biodiversity, such as in the State of Mato Grosso, Brazil.

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Zoonotic diseases contribute to 60% of the emerging infectious diseases and out of these 71.8% originate from wildlife. Among pathogens, *Brucella* spp. have great zoonotic potential, with more than 500,000 new cases emerging each year. In wild animals, *B. abortus* is one of the most diagnosed species, especially in enzootic regions where domestic animals share the same environment. The presence of infectious pathogens in wild populations contributes to the spread of diseases, decline in species population, and persistence in reservoir hosts. In recent years, control, eradication, and prevention of brucellosis at the wildlife, livestock, and human interface have been addressed, considering the complex eco-epidemiological aspects of this zoonosis and the importance of wild felids in the maintenance of the functional ecosystem. This study aimed to detect the presence of *B. abortus* DNA in blood samples from free-ranging and captive wild felids at Mato Grosso State, Brazil.

Whole blood samples (1 mL) were collected from 23 wild felids between August 2014 and August 2018 in the state of Mato Grosso, Brazil. Of these, 10 from captivity and 13 wilds were rescued by environmental government agencies and admitted for rehabilitation at the Medical Clinic of Wild Animals, Veterinary Hospital, Federal University of Mato Grosso.
Animal handling and sample collection were carried out in accordance with the national "Sistema de Autorização e Informação em Biodiversidade" (SISBIO) nº 40617-1 e 42303.

Genomic DNA extraction from the samples was performed using 250 μL of whole blood plus 1 μL of lysis buffer (100 mM NaCl, 25 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.5% SDS, and 0.1 mg proteinase K), which was incubated at 56°C overnight, and subsequently treated with phenol-chloroform. The DNA was resuspended in 50 μL ultrapure water and stored at -20°C until use.

Polymerase Chain Reaction (PCR) was performed based on the IS711 genetic element from Brucella abortus using the following primers: forward 5'-GAC GAA CGG AAT TTT TCC AAT CCC-3' and reverse 5'-TGC CGA TCA CTG TAG GGC CTG ATT TGC CAG-3', which amplified a fragment of 500 bp. Each reaction consisted of 10 ng of DNA, 0.4 pmol of each primer (forward and reverse), 0.2 nM of dNTPs, 3 mM of MgCl₂, 1 × PCR buffer, 1 U of Taq DNA polymerase (Invitrogen), and ultrapure water for obtaining a final volume of 25 μL. The amplification protocol was as follows: initial denaturation for 5 min at 94°C, 35 cycles of denaturation for 1 min at 94°C, hybridization for 45s at 60°C, and extension for 30s at 72°C, followed by a final extension cycle at 72°C for 5 min. PCR products were stained with GelRed (Biotium), separated by electrophoresis on 1.5% agarose gel (10 V/cm), and visualized on a transilluminator.

Out of the total sample tested, one (4.34%) captive P. concolor was positive for B. abortus (Table 1). A likely source of infection in felines raised in captivity is their diet, which is mostly composed of carcasses and fetuses from bovine slaughterhouses.

Little is known about the prevalence of Brucella spp. in wild cats. Reports of Brucella spp. in populations of felids detected using PCR are rare. However, B. abortus by PCR have been detected in P. onca and L. pardalis, and B. canis in P. concolor captive animals.

DNA or antibodies against Brucella abortus were detected in some species, such as Panthera leo from Tanzania, P. onca, P. concolor and L. pardalis from the Cerrado-biome in Brazil, and Lynx rufus from the United States. Antibodies against B. canis were detected in L. rufus from the United States, and in P. concolor from Brazil.

In the wild, B. abortus infections in wild cats are generally associated with predation of infected cattle. Brucellosis occurs when contact is made between the agent and the respiratory tract, skin lesions, and/or gastrointestinal tract.

Bovine brucellosis in Mato Grosso is associated with beef cattle and is the most frequent infection in animals that share the same habitat with cattle, domestic, and wild animals. In captivity, a possible source of infection in zoo animals may be associated with the ingestion of contaminated meat and water.

Another risk factor for transmission could be close contact with domestic animals like stray cats, once these animals can access captive animal enclosures and infect them as well as the environment.

The five specimens studied were at extremely high risk of becoming extinct in the wild: L. colocolo, L. wiedii, P. onca, P. concolor, and P. yagouaroundi. It is noteworthy that P. concolor is listed as threatened with extinction in Brazil and are considered vulnerable species. The impact of wildlife Brucella infections on the emergence of brucellosis in animals and humans is difficult to assess, as bacterial transmission is rarely described and poorly understood.

The present study showed that B. abortus circulates in wild felines in the state of Mato Grosso. These animals can play an important role in ecological function, as carriers of emerging infectious pathogens and indicators of environmental health, even without the development of clinical disease. The detection of this pathogen in captive animals using molecular tools highlights the importance of monitoring, as it raises concerns about the possibility of transmission between humans and wild and domestic animals, especially in regions of vast biodiversity and ecological interest, such as the state of Mato Grosso.

Table 1 – Molecular detection by Polymerase Chain Reaction (PCR) of Brucella abortus in the blood of wild free-living and captive felids from the state of Mato Grosso, Brazil, during 2014–2018.

| Species (n)   | Habitat     | Municipality            | Brucella abortus |
|--------------|-------------|-------------------------|------------------|
| Leopardus colocolo | Free-living | Várzea Grande | 0               |
| Leopardus pardalis | Captive    | Cuiabá            | 0               |
| Leopardus pardalis | Captive    | Cuiabá            | 0               |
| Leopardus pardalis | Captive    | Cuiabá            | 0               |
| Leopardus pardalis | Free-living | Várzea Grande | 0               |
| Leopardus pardalis | Free-living | Barra do Bugres | 0               |
| Leopardus pardalis | Free-living | Barra do Bugres | 0               |
| Leopardus pardalis | Free-living | Várzea Grande | 0               |
| Leopardus wiedii   | Captive    | Cuiabá            | 0               |
| Panthera onca     | Free-living | Marcellândia | 0               |
| Panthera onca     | Free-living | NF                 | 0               |
| Panthera onca     | Captive    | Cuiabá            | 0               |
| Puma concolor     | Free-living | Acorizal         | 0               |
| Puma concolor     | Free-living | Tangará da Serra | 0               |
| Puma concolor     | Free-living | Cáceres           | 0               |
| Puma concolor     | Free-living | Pontes e Lacerda | 0               |
| Puma concolor     | Captive    | Cuiabá            | 1 (4.34%)       |
| Puma concolor     | Captive    | Cuiabá            | 0               |
| Puma concolor     | Captive    | Cuiabá            | 0               |
| Puma concolor     | Captive    | Cuiabá            | 0               |
| Puma yagouaroundi | Free-living | NA                | 0               |

NA, Not informed.

Disclaimers

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451:990–3.

2. Wareth G, Melzer F, El-Diasty M, Schmoock G, Elbauomy E, Abdel-Hamid N, et al. Isolation of Brucella abortus from a dog and a cat confirms their biological role in re-emergence and dissemination of Bovine Brucellosis on dairy farms. Transbound Emerg Dis. 2017;64:e27–30.

3. Yon L, Duff JP, Agren EO, Erdélyi K, Ferroglio E, Godfroid J, et al. Recent changes in infectious diseases in European wildlife. J Wildl Dis. 2019;55:3–43.

4. Godfroid J. Brucellosis in livestock and wildlife: zoonotic diseases without pandemic potential in need of innovative health approaches. Arch Public Health. 2017;75:34.

5. Sambrook J, Russel DW. Molecular Cloning: a Laboratory Manual. New York, NY: Cold Spring Harbor Laboratory Press; 2004.

6. Bricker BJ, Halling SM. Differentiation of Brucella abortus bv. 1, 2, and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. J Clin Microbiol. 1994;32:2660–6.

7. Almeida ABPF, Silva CPA, Pitchenin LC, Arabia M, Silva GCP, Souza VRF, et al. Brucella abortus and Brucella canis in captive wild felids in Brazil. Int Zoo Yearbook. 2013;47:204–7.

8. Sachs R, Staak C, Groocock CM. Serological investigation of brucellosis in game animals in Tanzania. Bull Epizoot Dis Afr. 1968;16:93–100.

9. Hoq A. A serologic survey of Brucella agglutinins in wildlife and sheep. Calif Vet. 1978;32:15–7.

10. Furtado MM, Gennari SM, Ikuta CY, Jácomo ATA, Morais ZM, Pena HFJ, et al. Serosurvey of Smooth Brucella, Leptospira spp. and Toxoplasma gondii in Free-Ranging Jaguars (Panthera onca) and domestic animals from Brazil. PloS One. 2015;10:e0145816.

11. Barddal JHI, Quixbeira-Santos JC, Lopes IF, Ferreira Neto JS, Ferreira F, Amaku M, et al. Effect of vaccination in lowering the prevalence of bovine brucellosis in the state of Mato Grosso. Brazil Semina. 2016;37:3479–92.

12. Amjadi O, Rafiei A, Mardani M, Zafari P, Zarifian A. A review of the immunopathogenesis of Brucellosis. Infect Dis. 2019;51:321–33.

13. Oliveira A, Macedo GC, Rosinha G, Melgarejo JL, Alves AGL, Barreto W TG, et al. Detection of Brucella spp. in dogs at Pantanal wetlands. Braz J Microbiol. 2019;50:307–12.

14. Oliveira-Filho EF, Junior Pinheiro JW, Souza MMA, Santana VLA, Silva JCR, Mota RA, et al. Serologic survey of brucellosis in captive neotropical wild carnivores in northeast Brazil. J Zoo Wildl Med. 2012;43:384–7.

15. Instituto Chico Mendes de Conservação da Biodiversidade. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. ICMBio; 2018. p. 4162.

16. Dadar M, Shahali Y, Fakhri Y, Godfroid J. The global epidemiology of Brucella infections in terrestrial wildlife: a meta-analysis. Transbound Emerg Dis. 2021;68:715–29.