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Natural Infection of the South American Tapir (*Tapirus terrestris*) by *Theileria equi*

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ABSTRACT: *Theileria equi* is a tick-borne piroplasm considered endemic in equines in Brazil. The cohabitation of domestic and wild animals in areas of extensive cattle breeding favors the close contact between different species and the sharing of vectors and, consequently, pathogens. We report the natural infection of a young South American tapir (*Tapirus terrestris*) by *T. equi* in Mato Grosso do Sul, Brazil. Although it was not possible to associate the clinical and hematologic status of the animal with the infection by the protozoan parasite, our report represents an alert on the sharing of pathogens between domestic and wild animals.

The South American tapir (*Tapirus terrestris*) is an ungulate belonging to the Family Tapiridae and Order Perissodactyla. In South America, these animals are found from northern Argentina to the far north of the continent (Venezuela, Colombia, and Guyana). In Brazil, tapirs can be found in all biomes (Pantanal, Atlantic forest, and Amazon), with the exception of the Caatinga (Medici et al. 2012).

Deforestation and the conversion to agricultural and pasture land for livestock have had important impacts on the conservation status of this species. A recent study addressing the conservation of tapirs was conducted in accordance with the criteria of the International Union for Conservation of Nature, classifying the status of this animal in Brazil as vulnerable (Medici et al. 2012). In the Pantanal biome, the species was considered near-threatened (Medici et al. 2012). The expansion of agriculture threatens native species due to the reduction of their natural habitat and close contact between wild and

domestic animals. This proximity facilitates the transmission of pathogens which may represent a risk to the animals involved (wild and domestic) and to the human population (zoonosis; Williams et al. 2002).

Evidence of natural infection of tapirs by piroplasms has been described in both captive (Vroege and Zwart 1972) and free-living animals (Armando and Castellanos 2013); however, there are no reports of *Theileria equi* confirmed by molecular tools. Therefore, we aimed to report the natural infection of a tapir by *T. equi* in Brazil as confirmed by PCR and DNA sequencing.

A female tapir, aged approximately 3 mo, was rescued on a beef cattle farm in the municipality of Campo Grande (20°26'34''S, 54°38'47''W) and sent to a rehabilitation center for wild animals (Centro de Reabilitação de Animais Silvestre, Mato Grosso do Sul, Brazil). The animal had a compound fracture of the left pelvic leg and myiasis on exposed tissues. The clinical and hematologic parameters of the animal were assessed in the Veterinary Hospital of the Universidade Federal de Mato Grosso do Sul (Campo Grande, Mato Grosso do Sul, Brazil). The animal was hyperthermic (38.9 C), apathetic, and anorexic. Blood cell counts indicated a normochromic microcytic anemia, leukocytosis to neutrophilia without a left shift, and lymphocytosis according to the reference values for the species (Teare 2006). We observed anisocytosis and suggestive erythrocytic inclusions resembling piroplasms in 1% of cells on the blood smear. Due to the severity of the fracture and the degeneration

of clinical and hematologic parameters, the animal was euthanized in accordance with current legislation.

Genomic DNA was extracted from a blood sample as described by Araújo et al. (2009). Subsequently, the DNA was subjected to PCR using universal *Babesia*-*Theileria* primers (BT1F 5'GGTTGATCCTGCCAGTAGT3' and BT1R 5'GCCTGCTGCCTTCCTTA3'), which amplified a fragment of the 18S ribosomal RNA gene (Criado-Fornelio et al. 2003). Amplified fragments were purified using a QIAEX II kit (Qiagen, Hilden, Germany) and sequenced in both directions using the Sanger method. The chromatograms were analyzed and edited using BioEdit v.7.2.5 software (Hall 1999). Consensus sequences were submitted to a BLASTn search (Altschul et al. 1990) to determine the sequence identity based on comparisons with orthologous sequences available in the GenBank database.

We observed a 98% identity among the DNA sequences (414 base pairs) obtained in the present study and sequences of *T. equi* available in GenBank (accession numbers Z15105, CP001669, AY150062, and AY150063). The sequence obtained in the present study was deposited in GenBank under accession number KX364475.

Vroege and Zwart (1972) microscopically diagnosed a case of natural infection by *T. equi* in a Malayan tapir (*Tapirus indicus*) raised in captivity in Europe. The animal died a few days after being transferred from the zoo, but the authors did not attribute the cause of death to the presence of the parasite. The clinical characterization of *T. equi* infection in *Tapirus* species has not been described so far.

In affected horses with the acute form of *T. equi* infection, severe regenerative hemolytic anemia and jaundice occur. In chronic cases, clinical signs are mild or often absent, keeping the animal as an asymptomatic carrier (Wise et al. 2013). In the present report, it was not possible to attribute the clinical and laboratory abnormalities to the presence of the parasite due to the osteomyelitis resulting from the exposed fracture in the left pelvic limb. In

addition, due to the scarcity of infection reports, there are no hematologic or clinical parameters that characterize the action of the protozoan in tapirs.

In the region where the present case occurred, the extensive breeding of beef cattle predominates. It is common for there to be cohabitation of domestic animals (cattle, horses) with wild animals such as tapirs, cervids, and capybaras, among others. In addition piroplasms, such as *T. equi*, are endemic in the region and prevalences as high as 91% have been found among horses in this region (Heim et al. 2007; Barros et al. 2015). Furthermore, there is molecular evidence of the occurrence of *Theileria* sp. in free-living and captive cervids (*Mazama gouazoubira* and *Blastocerus dichotomus*; Silveira et al. 2011).

The transmission of *T. equi* occurs from the bite of infected ticks, especially of the genus *Amblyomma* (Wise et al. 2013). We observed these ticks parasitizing our case animal, indicating the possible source of the infection. *Amblyomma cajennense* was the most prevalent tick species in nonurban areas of the region, parasitizing both domestic and wild animals (Garcia et al. 2013). Therefore, in this habitat the exchange of vectors is common and, consequently, so too is the pathogens that they carry.

The natural infection of *Tapirus terrestris* by *T. equi* in South America has not been previously reported. Despite the fact that the case in question was an accidental finding, because the protozoan infection cannot be correlated with the clinical condition of the animal it raises an alert about the exchange of pathogens between domestic and wild animals.

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