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Evidence of Natural Zika Virus Infection in Captive Cervid Species in Brazil

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ABSTRACT: As part of an epidemiologic study of the Zika virus (ZIKV) in deer (*Cervidae*), samples from 56 captive deer in south and southeastern Brazil were tested for evidence of ZIKV. Three samples were positive using reverse-transcription quantitative PCR, although no samples were positive by virus isolation.

In 2015, circulation of Zika virus (ZIKV) in northeastern Brazil was confirmed (Zanluca et al. 2015). After being associated with neurologic disorders in humans in 2016, it was declared a global health emergency. Conservation and environmental professionals are also concerned about this pathogen in wildlife, as many viruses are known to cause population declines that may be of particular concern for threatened and endangered species (Althouse et al. 2016; Bueno et al. 2016). Although ZIKV is of zoonotic origin, information on transmission and exposure of nonhuman vertebrates to ZIKV is lacking (Pauvolid-Corrêa et al. 2019).

In Brazil, nine species of deer (*Cervidae*) have been identified (Duarte and Jorge 2003); they may be affected by various diseases and can serve as reservoirs of arthropod-borne pathogens. Thus, our objective was to evaluate the participation of the deer in the epidemiology of ZIKV.

We analyzed 56 blood samples from cervids, selected from the sample bank of the Bovid Virus Laboratory (BVL) of the Biological Institute in the state of São Paulo, Brazil, where viral diagnostics of domestic and wild ruminants are conducted. The laboratory maintains a partnership with the Deer Research and Conservation Center (NUPECCE), which collaborates with national parks. The random samples had been sourced from deer in conservation breeding herds of NUPECCE in the Brazilian municipalities of Jaboticabal, São Paulo, and Blumenau, Santa Catarina, covering the period from 2018 to 2019. The species analyzed were marsh deer (*Blastocerus dichotomus* (Illiger, 1815)), red brocket (*Mazama americana* (Erxleben, 1777)), small red brocket deer (*Mazama jucunda* (Thomas, 1913)), brown brocket deer (*Subulo gouazoubira* (Fischer, 1814)), Brazilian dwarf brocket (*Mazama nana* (Hensel, 1872)), Amazonian brown brocket deer (*Passalites nemorivagus* (Cuvier, 1817)), white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)), and pampas deer (*Ozotoceros bezoarticus* (Linnaeus, 1758)).

For the detection of ZIKV RNA, samples were subjected to nucleic acid extraction using the commercial kit cador® Pathogen 96 in the automated system QIAcube® HT (Qiagen, Hilden, Germany). The extracted RNA was then analyzed by reverse-transcription quantitative PCR (RT-qPCR) using the commercial Bio Gene Zika Virus PCR kit (Quibasa, Belo Horizonte, Minas Gerais, Brazil).

Virus isolation was performed in Vero cells lineage CCL-8, grown at 37 C and 5% CO_2, in minimum essential medium. The Vero cells were seeded at a density 2×10^5 cells/mL 24 h before inoculation. Each blood sample (200 µL) was inoculated onto the Vero cell monolayer. After 1-h incubation at 37 C, the inoculum was removed and replaced by 1 mL of maintenance medium (minimum essential medium, 2% fetal bovine serum, and 1% antimicrobial solution). As a negative control for each experiment, Vero cells seeded in one culture flask were mock inoculated with culture medium. The presence of infectious viral

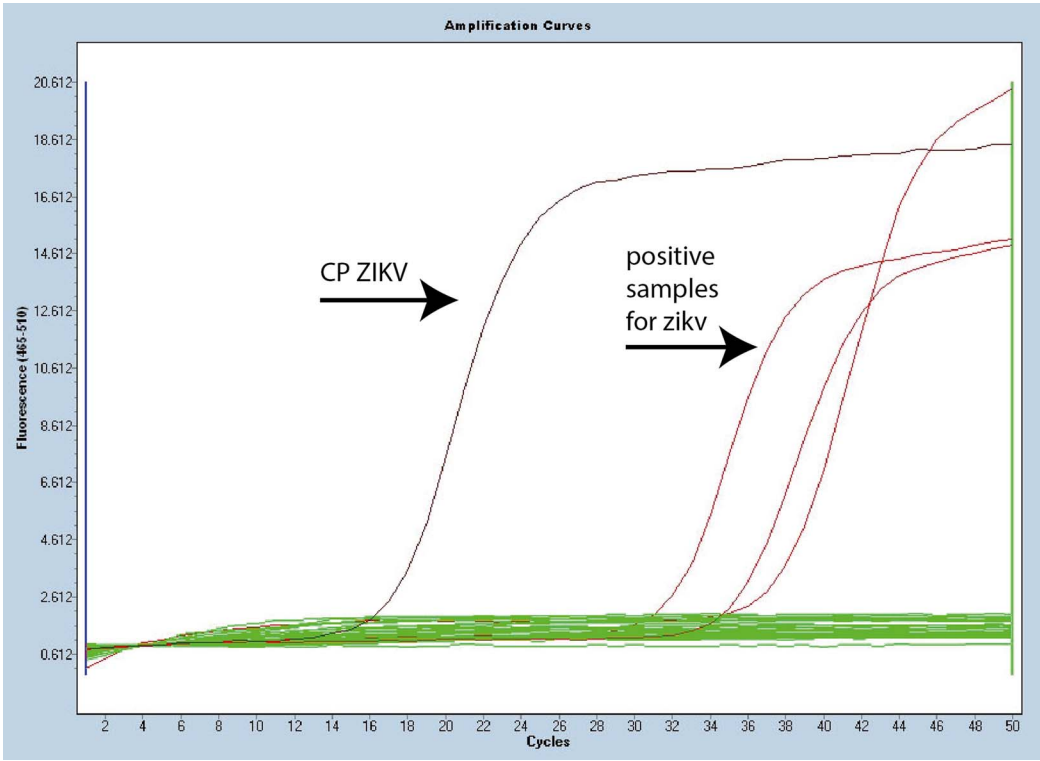

FIGURE 1. Result of reverse-transcription quantitative PCR for Zika virus in deer in Brazil, showing the three deer with cycle threshold (Ct) values ranging from 31 to 35. Positive control (CP) Ct 15.

particles was controlled by observation of cytopathic effects.

In the RT-qPCR, 3 of 56 (5%) cervid samples tested positive (Fig. 1): one Brazilian dwarf brocket and two marsh deer, all originating from Jaboticabal, São Paulo, and submitted to BVL for analysis in 2018. Both species are classified as vulnerable (Duarte et al. 2015, 2016). For verification, the positive samples were replicated in triplicate, with a cycle threshold (Ct) ranging from 31 to 35 and subsequently subjected to virus isolation in Vero cells. Viral isolation was unsuccessful in all samples. During 2018–19, the years covered by our study, nine deaths and 18,676 probable cases of Zika in humans were confirmed in Brazil (Coordenação-Geral de Vigilância das Arboviroses do Departamento de Imunização e Doenças Transmissíveis 2020), indicating viral circulation.

With the occurrence of ZIKV, there was concern about the possibility of spillback from an urban cycle to a sylvatic cycle in the Americas (Althouse et al. 2016). Our results suggest that deer have been exposed to ZIKV and are susceptible to infection. Therefore, monitoring these species becomes important for understanding the dynamics of the ZIKV in wild environments, as cervids may serve as viral reservoirs and contribute to the maintenance of the virus in nature (Drolet et al. 2013).

The results of our study differ from a study published earlier in which all deer samples tested were negative for ZIKV (Reuter and Nelson 2018). At least 79 animal species have been identified as naturally infected or experimentally susceptible to ZIKV infection. Mammals, especially primates, are the most represented taxonomic group, although birds, reptiles, and amphibians have also been identified (Gutiérrez-Bugallo et al. 2019).

According to Catenacci and Alcântara (2019), there is little information about the clinical signs of ZIKV infection in animals, as its occurrence

was found mainly accidentally during serologic research in search of other pathogens. In our study, one of the positive animals for ZIKV (Ct 31) showed inappetence, which was initially associated with a dental problem; however, after the RT-qPCR for ZIKV, the clinical sign might be associated with ZIKV. After the detection of ZIKV in cervids, NUPECCE reported the occurrence of stillbirths of deers. Bueno et al. (2016) suggested that wild mammals infected with ZIKV show few clinical signs. In a sentinel study in Uganda in 1947, a rhesus macaque had mild pyrexia (Dick et al. 1952). Experimental studies with rhesus macaques showed mild to moderate inappetence, which resulted in weight loss in four animals, and two animals developed a mild rash around the inoculation site 1 d after infection (DPI) that persisted for 4–5 d. No other abnormal clinical signs were observed (Dudley et al. 2016). Two other studies with rhesus macaque showed that within 8–10 DPI, the animals developed fever (axillary temperature 38.9 C), with maximum temperatures of 40.1 C (Dudley et al. 2016) and 39.5 C (Osuna et al. 2016).

A limitation of our study was the small number of deer samples available for testing. The species studied can exhibit elusive behavior and avoid human contact, thereby making samples difficult to obtain. (da Silva et al. 2020). However, one of the strengths of our study is the use of a highly sensitive and specific molecular method for ZIKV detection. According to Hayes (2009), molecular tests for ZIKV detection, including RT-qPCR, are the best option to detect ZIKV RNA. Nonetheless, virus isolation is considered the gold standard for ZIKV detection. Hayes (2009) mentions that the use of Vero or C636 cells is efficient in the isolation of ZIKV; however, in our study there was no viral isolation. The Ct values obtained in RT-qPCR show low viral load, which is probably the reason we were unable to isolate virus.

Despite the positive results obtained in this study, more work is needed to thoroughly evaluate the role, if any, deer play in ZIKV transmission cycles. As such, field and experimental studies should be carried out to aid the interpretation of the data obtained in these investigations, as they may be useful in defining effective measures to control this arbovirus.

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