SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2026v47n1p201

Molecular detection of dengue virus RNA in dogs (*Canis lupus familiaris*) situated in an important tri-border municipality (Brazil x Argentina x Paraguay)

Detecção molecular de RNA do vírus da dengue em cães (*Canis lupus familiaris*) situados em importante município tricronteiral (Brasil x Argentina x Paraguai)

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Highlights

Dogs may serve as potencial sentinel indicator for dengue fever.
It is of great importance to monitor dogs in the tri-border.
Human-validated qRT-PCR assay showed compatibility with canine samples.

Abstract

Although the role of humans as the main host that maintains the epidemic cycles of some arboviruses

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Received: Jul. 03, 2025 Approved: Abr. 06, 2026

is clarified, there is no consensus that dengue virus (DENV) can be maintained in endemic regions in an enzootic cycle. Therefore, the objective of this study was to investigate the molecular detection of DENV serotypes 1, 2, 3, and 4 RNA in semi-domiciled dogs from urban areas of Foz do Iguaçu, a dengue-endemic municipality located in a tri-border region between Brazil, Paraguay, and Argentina. A total of 100 blood samples were collected from dogs residing in two human *dengue hotspots.* Samples were analyzed by qRT-PCR using a commercial assay originally developed for human diagnostics. The endogenous 18S rRNA internal control amplified in all samples, confirming RNA integrity and absence of detectable PCR inhibition in the canine matrix under the experimental conditions applied. It was possible to identify the presence of DENV-4 RNA signal in six (6%) of the animals, a value considered epidemiologically high. In a region of Triple Frontier, with fluctuating population, great social inequality and different legislative norms between countries, the inclusion of sentinel animals for dengue in epidemiological surveillance programs is in line with PAHO recommendations. The results found provide molecular evidence of DENV RNA detection in dogs from endemic urban areas and raise important questions regarding the potential epidemiological relevance of dogs in dengue-endemic urban environments.

Key words: Arboviruses. Canids. Molecular diagnosis. One health.

Resumo

Embora o papel dos humanos como principal hospedeiro na manutenção dos ciclos epidêmicos de alguns arbovírus esteja bem estabelecido, ainda não há consenso de que o vírus da dengue (DENV) possa ser mantido em regiões endêmicas por meio de um ciclo enzoótico. Assim, o objetivo deste estudo foi investigar a detecção molecular do RNA dos sorotipos 1, 2, 3 e 4 do DENV em cães semidomiciliados de áreas urbanas de Foz do Iguaçu, município endêmico para dengue localizado em uma região de tríplice fronteira entre Brasil, Paraguai e Argentina. Foram coletadas 100 amostras de sangue de cães residentes em dois hotspots de dengue humana. As amostras foram analisadas por qRT-PCR utilizando um ensaio comercial originalmente desenvolvido para diagnóstico em humanos. O controle interno endógeno 18S rRNA amplificou em todas as amostras, confirmando a integridade do RNA e a ausência de inibição detectável da PCR na matriz sanguínea canina sob as condições experimentais aplicadas. Foi possível identificar sinal de RNA do DENV-4 em seis (6%) dos animais, valor considerado epidemiologicamente elevado. Em uma região de Tríplice Fronteira, caracterizada por população flutuante, grande desigualdade social e diferentes normas legislativas entre os países, a inclusão de animais sentinela para dengue em programas de vigilância epidemiológica está alinhada às recomendações da OPAS. Os resultados obtidos fornecem evidência molecular da detecção de RNA do DENV em cães de áreas urbanas endêmicas e levantam importantes questionamentos sobre a possível relevância epidemiológica desses animais em ambientes urbanos endêmicos para dengue.

Palavras-chave: Arbovírus. Canídeos. Diagnóstico molecular. Saúde única.

Introduction

Arboviruses, diseases caused by arboviruses (arthropod–borne viruses), are in almost their entirety, zoonoses kept in wild environments. However, some arboviruses have also emerged in epidemic form in urban centers. These viruses tend to have a restricted geographical and climatic distribution as part of a special ecological subsystem represented by viruses, vectors, "amplifier hosts" and "reservoir hosts" (Abbasi et al., 2025; Côrtes et al., 2023).

All known arboviruses circulate among wild animals and many of them cause diseases in humans, domestic animals and in farm animals. For this reason, all of these can act as accidental hosts for this group of viruses (Almeida et al., 2025).

In the definition of arbovirus by the World Health Organization (WHO), vertebrates can play two roles in transmission, they can be both amplification hosts and also "reservoir hosts". According to Kuno et al. (2017), a more assertive proposal would be to replace the term "reservoir host" with "maintenance host", which is excluded in the WHO definition of arbovirus. According to these authors, for arboviruses in urban transmission cycles, such as dengue virus (DENV) types DENV-1, DENV-2, DENV-3, and DENV-4, a subpopulation of infected humans can be considered as a "transient reservoir". This is based on the fact that these subpopulations have rapid mobility, possibly facilitated mainly by modern transport systems. Since these human subpopulations are usually accompanied by subpopulations of pet animals, such as dogs, and there are suspicions that this animal species may also be acting as a "reservoir host" for the aforementioned arboviruses.

It is interesting to note that some viruses, such as DENV, have lost the need for enzootic amplification and now produce extensive epidemics in urban centers of tropical regions, where they are transmitted to humans. However, although the role of humans as the main host that maintains epidemic cycles is well known, it is still unclear whether DENV can also be maintained in these regions in a similar enzootic cycle (Gwee et al., 2021). The vast majority of studies have focused on the detection of DENV and other arboviruses in human hosts and mosquitoes, however, studies are needed to evaluate the presence of arboviruses in primates and other species of domestic animals that may be reservoirs of these viruses (Thongyuan & Kittayapong, 2017).

Understanding the role played by companion animals, such as dogs, as possible reservoirs amplifiers of arboviruses, can contribute to assess whether their inclusion in epidemiological surveillance programs is necessary. For this reason, to assist and elucidate the role of urban area dogs as possible "reservoir hosts" of arboviruses, the objective of this work is to evaluate the occurrence of infection by arboviruses DENV-1, DENV-2, DENV-3 and DENV-4 in semi-domiciled dogs from an urban area of an endemic municipality for DENV, located in a region between Brazil, Paraguay and Argentina.

Material and Methods

Place of study

Foz do Iguaçu is considered a Cross-Border region (Brazil, Paraguay and Argentina) and according to the Köppen classification, it is located in a region with humid subtropical mesothermal climate, with annual rainfall between 1,900 and 2,200 mm and hot summer. The municipality has an estimated total number of inhabitants of 257,971, distributed in 87,826 households. It has an urbanization rate of more than 99%, and a population density of 414,58 inhabitants per km² (Instituto Brasileiro de Geografia e Estatística [IBGE], 2020). According to the United Nations Development Program [UNDP] (2010), the city has a Human Development Index of 0.751, considered high.

Data analysis and maps

To store the information, the PostgreSQL software - version 14 was used as a database. The maps were produced with geoprocessed information in quantum gis software (QGIS) version 3.10.10. LibreOffice, Power BI version 2.85.985.0, Metabase and Pentaho software were used to generate the reports, all free (Leandro et al., 2021).

Identification of hotspots

Hotspot, a term used to describe the area that presents the highest density by kernel estimation, represents the area where the cases are more crowded with a given disease. Hotspots have been described in different ways as areas of high incidence or prevalence, higher efficiency or risk of transmission or higher probability of disease (Lessler et al., 2017). To identify hotspots for dengue in the municipality of Foz do Iguaçu, we used the number of notifications for dengue in the last five years, taken from the SINAN (Notifiable Diseases Information System) website of the Brazilian Ministry of Health, and were spatially distributed on a heat map. The georeferencing of the collected adult mosquitoes (infected with DENV) was added to the map at hotspot sites between September and November 2018. The collection methodology of *Aedes* sp. was carried out according to Leandro and collaborators (2021). The hotspots were used to delimit two large areas of the municipality, where serum samples were collected from dogs (Figure 1).

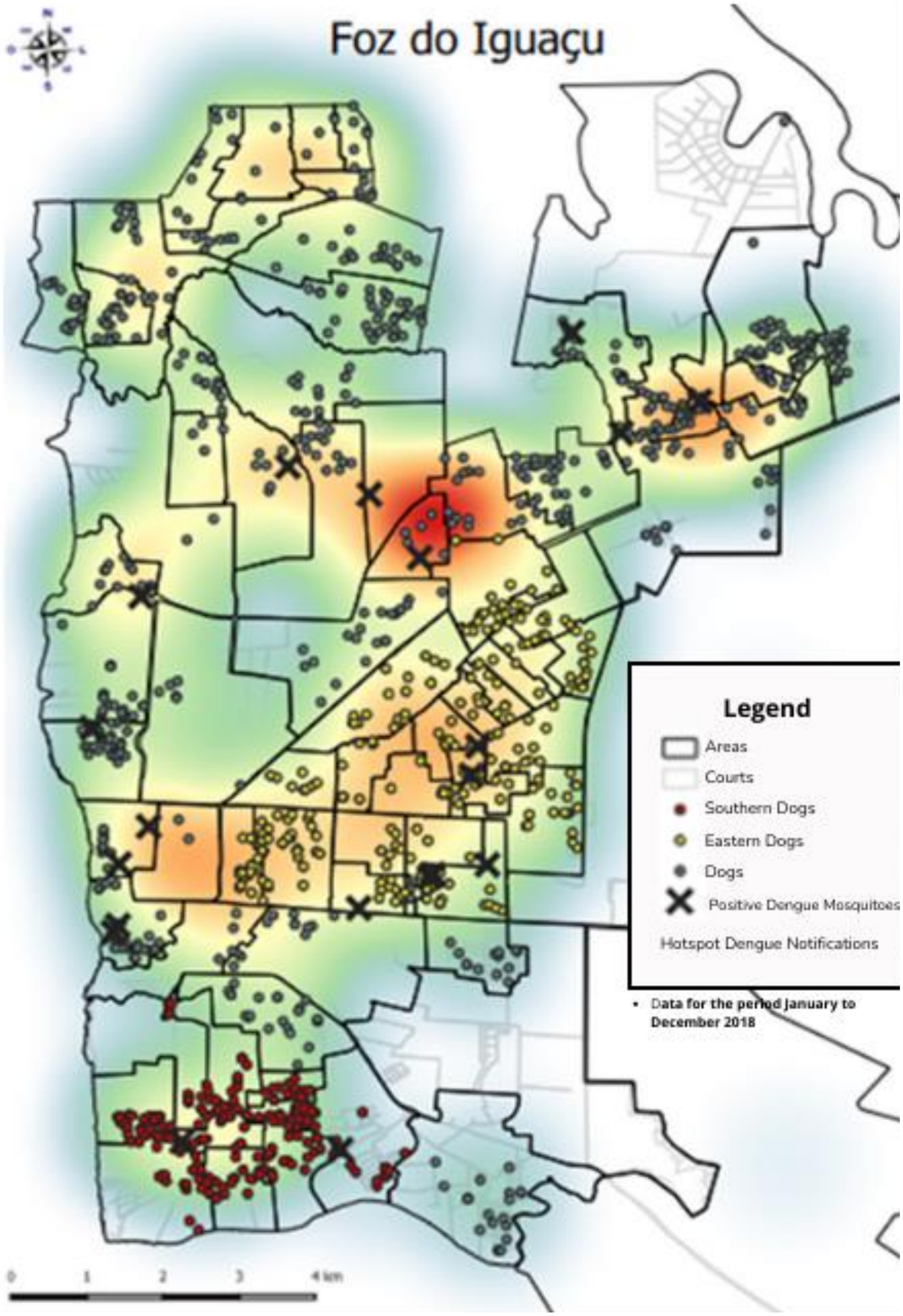

Figure 1. Map of the municipality of Foz do Iguaçu, Paraná, Brazil demonstrating dengue hotspots, the location of the dogs sampled and the points where dengue-positive mosquitoes were collected, 2018.

Blood collection of dogs

The sampling used was provided by the Zoonosis Control Center of Foz do Iguaçu, as part of a routine of work carried out by the center in the identification of pre-existing diseases in these animals. Blood collections of semi-domiciled dogs occurred simultaneously and randomly by qualified field teams, and 5 ml of blood from 1,115 dogs, preferably from the jugular vein, with brachial and saphenous veins as alternatives. These collections were conducted in five sanitary districts of the municipality of Foz do Iguaçu from June to November 2018. At the time of blood collection, physical examinations of each animal were performed to observe the general biological conditions, photographic record for later identification and placement of a collar-seal with the numbering belonging to the animal.

The samples were identified with collar-seal number and date of collection. After the procedure, the material was packed in refrigerated thermal boxes, and sent to the Environmental Laboratory of the Zoonosis Control Center of the respective municipality for proper laboratory procedures and subsequent referral to the Laboratory of the Tropical Medicine Center of the Triple Frontier, where they were stored in Freezer -80° C until the moment of molecular diagnosis.

The spatial location of the capture site of each of the animals on the municipality map is available in Figure 1.

For the arbovirus detection assays evaluated by qRT-PCR, 50 samples of animals captured in each of the regions considered hotspots of DENV in the municipality, south and east zones were randomly selected, totaling 100 samples.

Viral RNA extraction

The extraction of viral RNA from the *blood/serum from Canis lupus familiaris* was performed with the RNA MagMax Viral / Pathogen Nucleic Acid Isolation ™ (Thermo Fisher, USA) kit according to the manufacturer's specifications. The blood samples of the dogs were processed in automated extraction equipment Kingfisher (Thermo Fisher, USA), using the methodology of electromagnetic pearls. After the extraction of the genetic material, the samples were stored in ultrafreezer at -80ºC for subsequent analysis by qRT-PCR.

Real time PCR

For the detection of the viral pathogens studied, the human kit *Biomol* ZDC (Institute of Molecular Biology of Paraná, Brazil) was used to detect DENV. The reactions were conducted in *Quantstudio™ 7 Flex Real-Time PCR System,* with temperature and time protocol: 51° C for 30 min, 95° C for 15 min, 40 cycles of 95° C for 15 seconds and 60° C for 1 min. The results were analyzed using *QuantStudioTM Design & Analysis software* version 1.3.1 and version 1.5.1. The reactions were considered positive when the threshold cycle was CQ and ≤ 36 for the virus analyzed.

Although the commercial qRT-PCR kit employed in this study was originally developed and validated for human clinical samples, an internal analytical verification was performed to assess its technical performance in canine blood samples. The endogenous 18S rRNA gene, used as an internal control, is highly conserved among mammalian species, including humans and dogs. Consistent amplification of the 18S target in 100% of the canine samples confirmed RNA integrity, extraction efficiency, and absence of detectable PCR inhibition in the canine matrix. No formal analytical validation was performed, and therefore the assay should be interpreted as technically compatible with canine samples under the conditions tested.

Results

Of the 100 samples analyzed, six (6%) showed detectable DENV-4 RNA signal according to the assay threshold criteria ($Ct \leq 36$). Four samples (4/50 - 8%) were detected in the eastern area of the municipality, and two (2/50 - 4%) in the southern area. No RNA signal corresponding to DENV serotypes 1, 2, or 3 was detected in the analyzed samples. Although the kit used is intended for use in humans, amplification of the ribosomal 18S gene was observed in all samples, used as internal control. These findings demonstrate adequate RNA extraction and absence of detectable PCR inhibition in canine samples, indicating technical compatibility of the assay with this biological matrix under the experimental conditions applied.

Figure 1 illustrates the municipality of Foz do Iguaçu and the location of dengue infection hotspots in humans. It is also possible to observe the points where the dogs that participated in the experiment were captured and the location of points where positive mosquitoes were collected. It is possible to observe the places where dogs infected by DENV are located, in the east and south, are places where the circulation of DENV occurs both in humans and in mosquitoes.

Discussion

Species *A. aegypti* and *A. albopictus* play an important role as vectors for the transmission of viral and parasitic diseases, especially in countries with tropical and subtropical climates (Álvarez-Rodríguez et al., 2012). For dengue, humans are known to be the main "reservoir hosts" in maintaining urban epidemic cycles (Weaver & Vasilakis, 2009).

According to Weiss (2001), most of these pathogens that affect humans have a zoonotic origin and the transmission process depends on the participation of a reservatory that allows its survival. Embora vertebrate animals are commonly considered "reservoir hosts" for *Flaviviruses* transmitted by Aedes mosquitoes, such as zika virus and yellow fever virus (YFV), there is insufficient evidence suggesting that this occurs for urban transmission of dengue (Chaves et al., 2018).

The results of this study provide molecular evidence of DENV RNA detection in dogs, corroborating findings from Thongyuan and Kittayapong (2017), who detected by qRT-PCR the presence of DENV-2 and 3 in blood samples from dogs in Thailand. These authors found a positivity rate of 0.95% (6/632 samples) while this study obtained a positivity rate of 6% (6/100 samples), a higher positivity rate than that reported in the Thai study. This higher positivity can be explained by the experimental design used. Instead of using samples randomly collected from all over the municipality, we chose to use animals that are in regions where there was a greater number of notifications of dengue diseases over the years (hotspots), which may have contributed to a higher incidence of positive animals.

A limitation of this study is the use of a commercial qRT-PCR assay originally validated for human samples. Although the endogenous 18S rRNA internal control amplified consistently in all canine samples, confirming RNA integrity and absence of detectable PCR inhibition, no formal analytical validation was conducted specifically for canine matrices. Therefore, the findings should be interpreted as molecular detection of DENV RNA within a surveillance framework, rather than validation of a diagnostic protocol for dogs or confirmation of reservoir competence.

The present findings indicate that in urban areas with active dengue transmission, molecular detection of DENV RNA in dogs may occur, particularly in regions with high human case density. These results are consistent with those reported by Thongyuan and Kittayapong (2017) and reinforce the importance of investigating the epidemiological relevance of domestic dogs in dengue-endemic settings, especially given their close ecological and spatial proximity to humans. Such observations raise important questions regarding the potential role of dogs within the broader context of arbovirus circulation in urban environments.

The epidemiological relevance of dogs in dengue-endemic settings depends, among other factors, on their ecological interaction with *Aedes vectors.* Studies investigating mosquito blood meals provide insight into host selection patterns. Estrada-Franco et al. (2020), using next-generation sequencing, analyzed the blood meals of *Aedes aegypti* to determine host feeding sources. Among 145 mosquitoes analyzed, 73.8% had fed on a single vertebrate species and 26.2% on multiple species. Of those with a single-host blood meal, 54.2% had fed on dogs, 28.0% on humans, 16.8% on cats, and 1.0% on turtles. Among mosquitoes with mixed blood meals, 65.9% involved both humans and dogs. These findings highlight the frequent ecological interaction between *Aedes vectors* and domestic dogs in urban environments, supporting the plausibility of shared exposure within the human–animal interface.

Olson et al. (2020) found that only 31% of A's blood *supply. aegypti* were derived from humans, while 50% were from dogs and 19% from other wild and domestic animals. In addition, data from the work Thongyuan and Kittayapong (2017) reported successful isolation of DENV strains from canine blood samples in cell culture, demonstrating the viability of virus samples present in these animals, data that suggest biological plausibility for further investigation of their potential epidemiological role for this disease.

The results of this work also bring to discussion another interesting point and also raised by Thongyuan and Kittayapong (2017), which is the potential use of dogs within epidemiological surveillance frameworks. In the present study, dogs with detectable DENV RNA were identified in areas where viral circulation had been previously documented in humans and mosquitoes. This spatial overlap suggests shared environmental exposure and reinforces the relevance of the human–animal interface in areas with endemic dengue transmission. Although RNA detection does not establish transmission dynamics, these findings support consideration of domestic dogs as potential sentinel indicators within integrated dengue surveillance strategies.

It is interesting to note that for some *flaviviruses such as West Nile Virus and Japanese Encephalitis Virus,* some studies that evaluated the prevalence of positive serology in dogs also recommend its use as an epidemiological surveillance tool (Lan et al., 2011; Kile et al., 2005; Shimoda et al., 2010).

Evaluating the potential epidemiological role of dogs in arbovirus transmission dynamics of arboviruses of interest in public health can help in the construction of a more detailed operational scenario and in the development of tools to control the transmission of viruses. In a region like this one of the Triple Frontier, composed of the municipalities of Foz do Iguaçu - Brazil, Ciudad Del Leste - Paraguay and Puerto Iguazu - Argentina, it is verified that public health is a common problem and the region has faced several dengue epidemics in recent decades. One fact that further aggravates this scenario is that the trinational border presents a floating population and regions characterized by social inequalities, which can influence the incidence rate of dengue. One of the difficulties in articulating public health policies in this region is related to the existence of different laws between the fortifying countries. PAHO/WHO, through the Regional Dengue Program, together with the countries, has implemented since 2003 the Integrated Management Strategy for the control and prevention of dengue in the Americas, known as EGI-dengue. This strategy integrates 6 components: patient care, social communication, environment, integrated vector management, laboratory and epidemiology. Epidemiological surveillance is part of the latter component and is one of the main challenges at regional level.

Based on these findings, a cautious and targeted pilot canine sentinel surveillance approach could be considered in urban dengue hotspots and integrated into existing public health activities. Sampling might be conducted periodically and opportunistically during routine veterinary campaigns to minimize additional logistical burden. However, the use of molecular diagnostics such as qRT-PCR, particularly commercial assays originally developed for human samples, requires substantial investment in reagents, laboratory infrastructure, trained personnel, and sample transport, which may limit scalability in resource-constrained settings. In cross-border regions characterized by population mobility, social inequality, and heterogeneous health regulations, additional coordination challenges must also be considered. Therefore, any operational implementation should be preceded by cost-effectiveness analysis and framed within a One Health perspective, ensuring that it does not divert essential resources from the primary priority of strengthening human dengue surveillance and control.

Significant advances are being made in terms of standardization of indicators, which may allow for more timely interventions. The results allusive to this study help to evaluate the need for the inclusion of dogs in epidemiological surveillance programs, which is in line with the publication of PAHO. The same publication provides structure for the planning and implementation of surveillance, prevention of *A. aegypti,* and control activities, supporting the development of potential operational scenarios at the local level. These operational scenarios serve as a reference for selecting the best vector control tools and using them more efficiently. Thus, future studies of monitoring arboviruses of public health interest in dogs may help to elucidate their function as a sentinel species for dengue and contribute to the elucidation of their role in the dynamics of this infection.

Conclusion

The detection of DENV-4 RNA in domestic dogs from dengue-endemic urban hotspots demonstrates the presence of viral RNA in animals residing in areas of active transmission. Although these findings do not establish viral amplification, reservoir competence, or direct participation of dogs in dengue transmission cycles, the observed positivity rate (6%) was higher than that reported in some previously published studies. This pattern highlights the relevance of the human–animal interface in the Triple Border region and supports the need for continuous, integrated One Health surveillance in complex urban border settings.

Acknowledgment

To the employees of the Zoonosis Surveillance Unit, Itaipu Saúde Working Group, Triple Frontier Tropical Medicine Center, Foz do Iguaçu City Hall, Itaipu Binational, Coordination for the Improvement of Higher Education Personnel [CAPES] / PROSUP) and UNIPAR.

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