



SHORT COMMUNICATION

First Report of Gray Foxes (*Urocyon cinereoargenteus*) Infected with Bat-Associated rabies Virus (*Desmodus rotundus* rabies virus) in Costa Rica

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Abstract

Objective: Rabies is a lethal zoonotic viral disease that affects mammals. Although Costa Rica has not reported any cases of canine rabies since 1987, cases of bat-transmitted rabies virus in domestic animals are recorded annually. Cases in wild carnivores remain sporadic, and surveillance of these species has intensified during the past 7 years. Herein, we present three cases of gray foxes (*Urocyon cinereoargenteus*) infected with bat-associated rabies virus (*Desmodus rotundus* rabies virus) in residential neighborhoods of Costa Rica.

Methods: Brain tissue samples from the foxes were analyzed with histological and molecular methods, including RT-PCR and Sanger sequencing.

Results: Test findings confirmed the presence of vampire bat rabies virus in wild mammals (gray fox) inhabiting peri-urban areas near humans and domestic animals.

Conclusions: This study underscores the urgent need to review current rabies protocols to ensure timely provision of prophylactic medical care for people and pets with suspected exposure. Furthermore, educating the public regarding the risks associated with rabies transmission in wildlife is essential to prevent incidents with potential to endanger public health.

Key words: Rabies, gray fox, *Desmodus rotundus*, zoonotic, wildlife surveillance, necropsy, One Health, Costa Rica

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INTRODUCTION

Rabies is a fatal zoonotic disease caused by a neurotropic virus belonging to the genus *Lyssavirus*, family *Rhabdoviridae*. Rabies virus (RABV) affects the central nervous system in mammals, and is transmitted through bites or scratches from infected animals [1].

Rabies has a worldwide distribution, and Asia and Africa are the most

affected continents. In the Americas, the incidence of RABV transmitted by domestic dogs is declining, whereas RABV transmitted by bats is becoming more prevalent [2].

In Costa Rica, although the last reported case of rabies transmitted by dogs occurred in 1987, rabies transmitted by bats remains a major public health concern [3]. The main vector is the common vampire bat (*Desmodus rotundus*),

which, when infected, can transmit the virus and infect cattle or, less frequently, horses, pigs, sheep, dogs, humans, and wild mammals [4]. Rabies transmitted by insectivorous bats, such as *Tadarida brasiliensis* and *Eptesicus fuscus*, has also been detected in the country [3].

Costa Rica has a national animal rabies surveillance and control program, which was established by the National Animal Health Service (SENASA) in the 1980s. This program is based on passive syndromic surveillance of domestic, farm, and wild animals showing neurological signs of rabies [4].

When a positive case of rabies is confirmed, disease mitigation measures are applied, as established in SENASA protocols for susceptible animals present in the area where the outbreak was detected, as well as those established by the Costa Rican Ministry of Health when a human is affected [4].

However, little is known regarding the effects and dynamics of RABV in wild mammals. Consequently, epidemiological surveillance in wildlife is crucial for detecting infected animals and decreasing rabies cases in domestic animals and humans, in alignment with the “One Health” approach.

The objective of this work was to report three cases of gray foxes (*Urocyon cinereoargenteus*) infected with a bat-associated RABV (*D. rotundus* RABV) found in residential areas of Costa Rica’s metropolitan region. These cases were identified as part of the wildlife surveillance program established by SENASA, in coordination with private wildlife rescue centers across the country.

METHODS

Case histories

First case

On July 23, 2021, a disoriented gray fox was found in Curridabat, an urban area of San José (Fig 1A). The animal was allowed to shelter from the rain overnight in a private garden (Fig 1B).

On the following day, the fox appeared listless and was taken to the Rescue Center Costa Rica, located in La Guácima, Alajuela. On arrival, the fox was hypothermic and showed diminished vital signs. The animal was warmed and stabilized with fluid therapy. However, after the fox regained consciousness, neurological signs became apparent, including paralysis of both hindlimbs and forelimbs, incoordination, abnormal neck movements, aggression, confusion, howling, and atypical vocalizations.

These signs persisted, and the animal died on the following day. The case was reported to the SENASA, and the body was transferred for necropsy and subsequent histopathological analysis.

Second case

The second case was reported on November 11, 2022, when a gray fox was found in an urban area in Tres Ríos, Colinas de Montealegre, Cartago. The animal was transported to the Toucan Rescue Ranch in San Isidro, Heredia. It exhibited hind limb paresis, tremors, apathy, unresponsiveness to stimuli, and stupor.

Third Case

The third case was also reported in Tres Ríos and was collected on December 23, 2024. The gray fox was transferred to the Toucan Rescue Ranch in San Isidro, Heredia. The animal was found in a prostrate state and exhibited hindlimb paresis and circular movements.

Necropsy

The animals were sent to the SENASA pathology laboratory for rabies diagnosis. From the first case, the complete carcass of a gray fox was provided, and underwent necropsy including physical inspection, dissection, examination, and organ removal [5]. Samples were collected for subsequent diagnostic tests.

From the second and third foxes, only the head was received; therefore, the brain was extracted for analysis [5].



FIGURE 1 | (A) Map showing the three locations where gray foxes have been reported. These sites are separated by 4.53 km, as indicated by the white line. (B) Shows the gray fox from the first case, found in the backyard of a house in Cipreses, Curridabat.

The fox brains were dissected sagittally. The left hemisphere was used to collect fresh tissue for diagnostic tests, whereas the right hemisphere was fixed in 10% formalin for histopathological examination with routine hematoxylin & eosin (H&E) staining.

Diagnostic testing

The brain samples were analyzed for evidence of RABV with a rapid immunochromatographic test (Antigen Rapid Rabies Ag Test Kit, BioNote Inc., South Korea) [6], direct immunofluorescence (FITC anti-rabies monoclonal globulin, Fulirebio Diagnostics, Inc.) [6], and reverse transcription polymerase chain reaction (RT-PCR) targeting a partial fragment of the nucleoprotein gene [7]. Samples were extracted with columns from a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. One-step RT-PCR was performed with a Qiagen OneStep RT-PCR Kit (Qiagen, Hilden, Germany). The primers were RAB20, 5'-ACGCTTAACAACAARATCARAG-3'; RAB30 4, 5'-TTGACGAAGATCTTGCTCAT-3'; and 1242F, 5'-TATACTCGAATCATGATGAATGGAGGTCGACT-3', targeting the complete nucleoprotein (N) gene of RABV [7].

RT-PCR was performed under the following cycling conditions: 45°C for 30 minutes (reverse transcription); 95°C for 15 minutes (initial denaturation); and 40 cycles of 94°C for 10 seconds, 53°C for 45 seconds, and 68°C for 1 minute 30 seconds. A final extension was performed at 68°C for 10 minutes. The resulting 1,534 base pair amplicons were visualized under UV light after electrophoresis on a 1% agarose gel stained with GelRed Nucleic Acid Stain (Biotium). A slice of the gel containing the amplicon was excised and purified with a QIAquick Gel Extraction Kit (Qiagen) [7].

Sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) with the following thermal profile: 30 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. The products were purified with a BigDye XTerminator Purification Kit and sequenced on a SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Massachusetts, USA) [7].

Additionally, samples were tested for morbillivirus with RT-PCR [8].

The RT-PCR positive samples were subjected to Sanger sequencing of the full nucleoprotein gene [9,10].

Phylogenetic analysis

A total of 43 nucleoprotein gene sequences were downloaded from the GenBank database (S1 Table). After alignment of the sequences, a phylogenetic tree was built in MEGA X [10], with the maximum likelihood method with 1000 bootstrap replicates and the Tamura 3-parameter model with gamma distribution and invariant sites (T92+G+I) as the best-fit substitution model [10].

RESULTS

Necropsy findings

During necropsy of the first fox, bilateral ulcerations measuring 0.5 cm in diameter were observed on the oral mucosa of the cheeks at the level of the molar and pre-molar teeth. An evident dislocation of the left elbow was also noted. In the subcutaneous area of the left costal arch, a reddened area measuring 3 cm in diameter containing a blood clot was observed. Similar injuries were present on the chest wall between the 8th and 11th ribs. Blood was found in both the abdominal and thoracic cavities, and partially digested food was present in the esophagus. The surface of the cerebral cortex appeared reddened with engorged blood vessels. On the basis of these macroscopic findings, rabies, canine distemper, or physical trauma were considered possible causes of death. Brain samples were collected for molecular and histopathology analyses.

Histopathological examination

Histopathological examination of the central nervous system in the foxes, including the brainstem, cerebellum, hippocampus, cortex, and basal ganglia, revealed severe freeze-thaw artifacts and moderate postmortem autolysis, thus hindering the evaluation of subtle morphological changes, including the presence of viral inclusion bodies. However, in multiple areas, the Virchow-Robin spaces around small neuroparenchyma capillaries were infiltrated by small numbers of lymphocytes, plasma cells, and macrophages (Fig 2A). Mild gliosis, occasional glial nodules, and neuronal satellitosis were observed. Neurons with adequate cell preservation exhibited multiple intracytoplasmic, round, eosinophilic inclusions approximately 5 µm in diameter, which were consistent with Negri bodies (Fig 2B).

Molecular and serological testing

Molecular tests for morbillivirus were negative in all three cases. In addition, brain samples from all three foxes tested positive for RABV according to rapid immunochromatographic tests, direct immunofluorescence, and RT-PCR [6,7]. Sanger sequencing confirmed that the strains belonged to the *D. rotundus* RABV lineage, with nucleoprotein gene fragment sizes of 1,353 bp for the first case; 1,369 bp for the second case; and only 1,056 bp, with a 310 bp region missing in the middle, for the third case. Despite multiple attempts, a longer fragment was unable to be obtained for the third case, possibly because of a low viral load in the sample or degradation of the viral genome.

Phylogenetic analysis

The sequence obtained from the first case showed 99.63% identity with the *D. rotundus* MK766721 variant, which was isolated from a bovine in Puntarenas, Costa Rica, in March 2010. The amplified nucleoprotein fragment

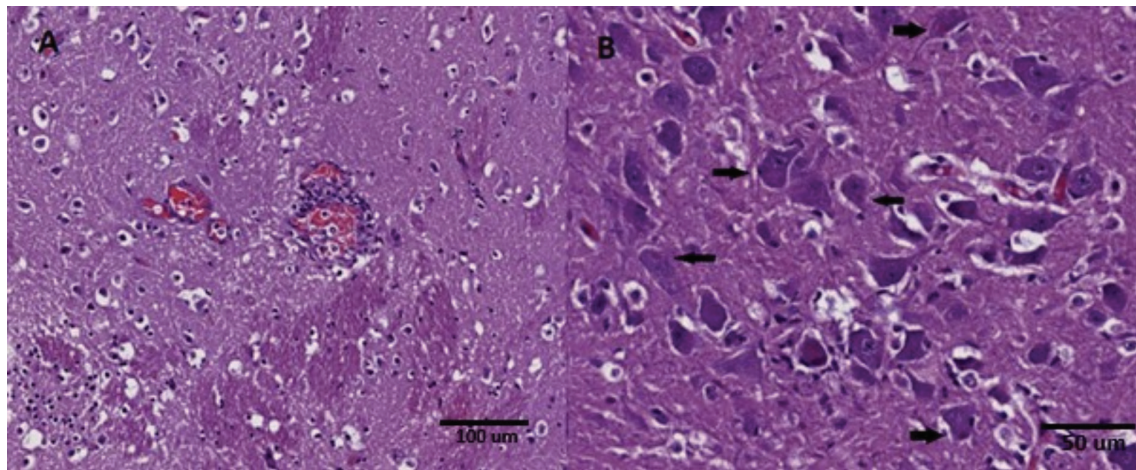


FIGURE 2 | Histopathological images of brain tissue, H&E staining. A) Perivascular lymphocytic infiltration surrounding neuroparenchymal vessels (100X). B) Neurons showing multiple intracytoplasmic eosinophilic inclusion bodies (Negri bodies), indicated by black arrows (200X).

from the second case, although only 105 bp in length, was confirmed as a *D. rotundus* RABV strain. This sequence demonstrated 100% identity with sequence KU550097 (LRBR1004-14), which was isolated on July 15, 2014, from a 9-year-old boy and his dog, believed to have been infected by a squirrel (genus *Sciurus*) in Osa, Palmar, Puntarenas, Costa Rica [3]. Furthermore, the sequence obtained from the third fox also shares 99.04% identity with the sequence from the aforementioned 9-year-old boy and his dog (Fig 3).

Figure 3 illustrates the topology of the RABV phylogenetic tree, which is divided into two main clusters. The sequences related to *T. brasiliensis* (Tb), shown in blue, serve as an outgroup to the *D. rotundus* virus-associated sequences. The vampire bat RABV sequences are further divided into the following groups: (1) sequences isolated exclusively in South America (orange) and indicated as lineage 3 SA, and (2) sequences isolated in Colombia, Central America, and North America, which were further separated into two clusters: lineage 1, including sequences from Central and South America CSA (green), and lineage 2, consisting of sequences from Central and North America CNA (red).

The sequences isolated from the gray foxes clustered within lineage 2, containing sequences isolated between 2004 and 2014, and shared a most recent common ancestor with RABV strains from Mexico (Fig 3), thus further supporting the southward spread of a North American strain [7].

DISCUSSION

The clinical signs, necropsy findings, histopathological evidence, and molecular analyses of three gray foxes were consistent with those of rabies cases reported in various mammals [11]. This study describes the first diagnosed and documented instances of rabies in gray foxes in Costa Rica.

Immediately after the first case tested positive, the rescue center was informed. A foreign volunteer was then

reported to have been bitten by the fox while handling the animal on the day before it died. The volunteer had visited a private clinic and received a tetanus shot but had been incorrectly informed that vaccination against rabies was unnecessary. She returned to her home country the following day. The rescue center personnel contacted her, and she was fortunately able to receive post-exposure prophylaxis, including immunoglobulin against RABV and a full vaccination regimen, in her country.

The implications of this case were considered according to the One Health framework [12], and viral samples were sent to the Pan American Center for Foot-and-Mouth Disease and Veterinary Public Health (PANAFTOSA/HPV), a World Health Organization (WHO) reference laboratory in Brazil. The reference laboratory's results corroborated our findings.

Genetic studies have demonstrated how RABV circulating in bats can spread to terrestrial mammals. Enzootic cycles of RABV found in bats have been established through host-switching events, particularly in raccoons (*Procyon lotor*) and skunks (*Mephitis mephitis*) [12].

In recent decades, wildlife rabies cases in the Americas have increased, and bats and terrestrial carnivores (raccoons, foxes, and skunks) are the main carriers of the virus [12]. The gray fox, widely distributed from southern Canada to northern South America, has been reported to play an important role in the epidemiological dynamics of rabies in the United States [13], Mexico [14], and Colombia [15].

In the United States, circulation of RABV in gray foxes is particularly common [13]. For example, New Mexico has more than three enzootic RABV cycles, involving skunks (south-central skunk variant), gray foxes (Arizona gray fox variant), and several non-hematophagous bat-associated variants [16].

Mexican studies conducted between 1990 and 1995 indicated that rabies cases were associated primarily with dogs and *D. rotundus*. However, two cases in bobcats were associated with the Arizona gray fox strain [14]. In

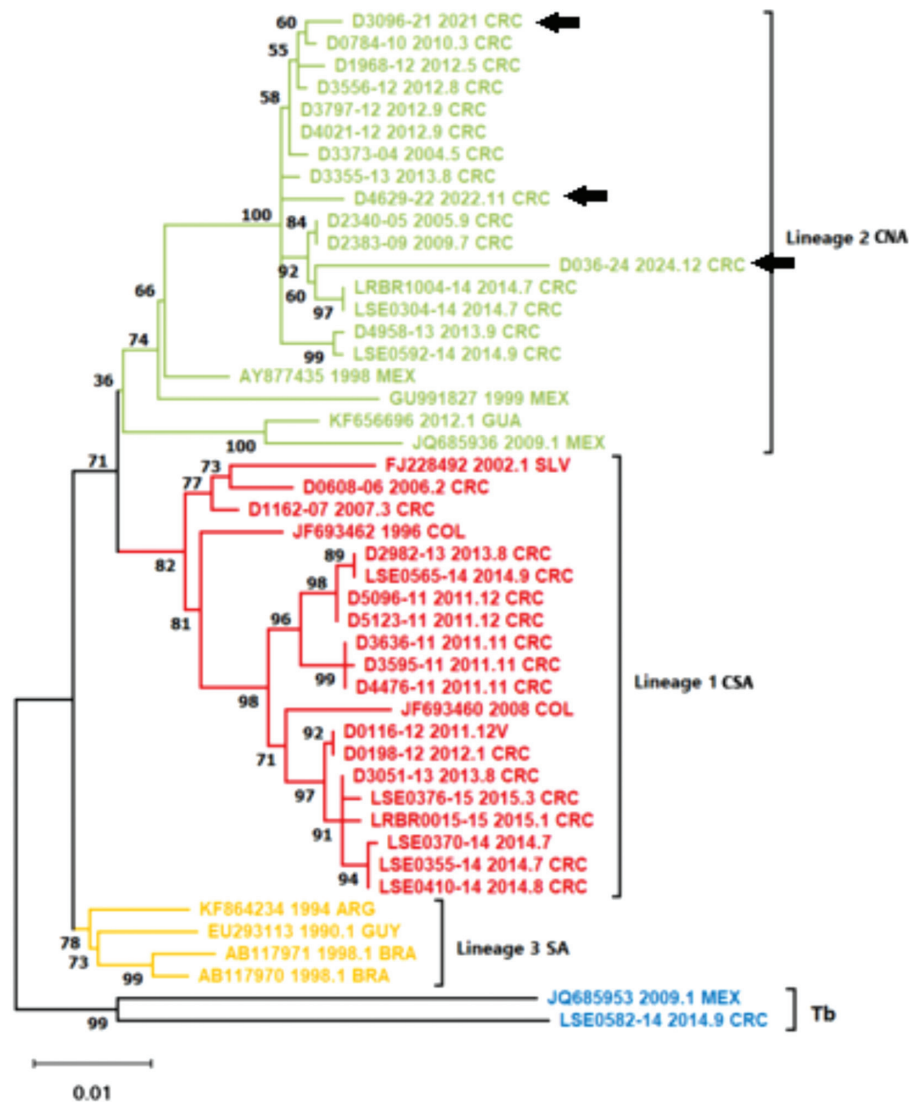


FIGURE 3 | The phylogenetic tree shows lineage 2 sequences in green and lineage 1 sequences in red. The arrows indicate the sequences obtained from foxes in Costa Rica.

Colombia, rabies cases in gray foxes have been linked to the transmission of canine RABV [15].

This study reports the first cases of rabies in gray foxes in Costa Rica, in which a *D. rotundus* virus strain was detected. This strain was previously documented in gray foxes in other countries in the Americas. Such rabies cases are believed to be associated with the predatory behavior of gray foxes, given that they might consume infected bats and contract the virus through bites during predation [17].

The occurrence of rabies in gray foxes in Costa Rica is notable for two reasons. First, it highlights that wild mammals inhabiting peri-urban environments can become infected with the virus, thereby posing a risk to public health through potential contact with humans or domestic animals (e.g., dogs and cats). Second, this finding demonstrates that RABV transmitted by vampire bats continues to occur in areas without prior disease outbreaks, particularly in livestock. This finding presents a major public

health concern, because the 2015 Anti-Rabies Protocol stipulates that in regions with no rabies outbreaks, vaccination treatment is not required for humans bitten by domestic or wild animals, thus placing people's health at risk [18].

In the past decade, fatal cases of human rabies in Costa Rica have been linked to bites from wild animals [3]. Importantly, several species, such as bats, skunks, foxes, raccoons, and coyotes, can transmit RABV [19,20]. However, in Costa Rica, knowledge regarding the presence, occurrence, and dynamics of rabies in wild species other than bats is limited.

Therefore, clear and assertive communication regarding the presence of RABV in wild mammals, and its association with *D. rotundus*, is critical for the general public and people working directly with wildlife, such as students and professionals in the biological sciences, veterinary and human medicine; wildlife rescue center personnel; and

public and animal health authorities. This goal is a primary objective of the global strategic plan for rabies elimination “Zero by 2030 and One Health” [21].

Increased rabies awareness might potentially lead to improved management of incidents involving contact between wild mammals and domestic animals or people, particularly in accident cases, thereby enabling provision of adequate prophylaxis, potentially preventing the tragic loss of human or domestic animal life, and averting potential outbreaks [21].

Unfortunately, in Costa Rica, the current protocol for managing bites or scratches from wild or domestic animals does not include post-exposure prophylaxis unless the incident occurs in an area with a previously reported rabies case or outbreak [18].

Finally, public education is crucial to discourage contact with wild animals, particularly those appearing sick, unusually tame, or disoriented, to prevent incidents of a preventable disease with potential to jeopardize the health and lives of people or pets.

CONCLUSION

We confirmed the presence of a vampire bat strain of RABV in gray foxes residing in an urban area near humans and domestic animals. This finding highlights the urgent need for adequate communication between Costa Rican animal health and human health authorities regarding the presence of this virus in atypical hosts, and to improve surveillance and ensure timely prophylactic medical care for people and pets when necessary. In addition, educating the general population about these risks is crucial to prevent incidents with potential to compromise public health.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

No ethics approval was required, in accordance with the criteria of the Bioethics and Animal Welfare Commission of the Faculty of Veterinary Medicine of the National University of Costa Rica. The Commission conducted a thorough review of the study and concluded that, because it involved collaboration with SENASA, an entity legally authorized to conduct research in animal health, no further ethics approval procedures were necessary. However, the commission did not identify any ethical concerns regarding the conduct or publication of the study, and fully supports the research protocol and methods used.

AUTHOR CONTRIBUTIONS

Josimar Estrella-Morales and Bernal León contributed to the development and initial drafting of the manuscript. Josimar Estrella-Morales, Juan C. Alvarado-Fernández, and Carolina Salas were responsible for sample processing and diagnostic testing. Rocío González-Barrientos collaborated in the histopathological interpretation. Idania Chacón, Olga Aguilar, Juan M. Cordero-Solórzano, and Bernal León collaborated in the molecular and phylogenetic analysis of the samples. All authors contributed to the editing and review of the manuscript. Furthermore, all authors approved the final version for submission.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://zoones-journal.org/wp-content/uploads/2025/09/zoones20250001_Suppl.pdf.

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