



## FIRST DETECTION OF CANINE DISTEMPER VIRUS INFECTION IN VARIEGATED SQUIRRELS (*SCIURUS VARIEGATOIDES*) AND PORCUPINES (*COENDOU MEXICANUS*) IN COSTA RICA

B. LEÓN<sup>1</sup>, C. WIEDERKEHR<sup>2,3</sup>, R. CRUZ<sup>4</sup>, M. CORDERO<sup>5</sup>, A. CHINCHILLA<sup>6</sup>,  
T. SOLORZANO-SCOTT<sup>7</sup>, L. BRENES<sup>8</sup> & F. AGUILAR-VARGAS<sup>9</sup>

<sup>1</sup>LANASEVE, LSE Laboratorio de Virología, SENASA. Heredia, Costa Rica; <sup>2</sup>VISAVET Health Surveillance Centre Universidad Complutense de Madrid, Madrid, Spain; <sup>3</sup>Sol Sanctuary Rescue Center, Guanacaste, Costa Rica; <sup>4</sup>Clínica Nouba Pet, APAMI Wildlife Rescue Center, Guanacaste, Costa Rica; <sup>5</sup>Santuario y Centro de Rescate Las Pumas, Cañas, Guanacaste, Costa Rica; <sup>6</sup>Hospital Veterinario Cavallini, Guanacaste, Costa Rica; <sup>7</sup>Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica; <sup>8</sup>Departamento CUSBSE, Sistema Nacional de Áreas de Conservación, Heredia, Costa Rica; <sup>9</sup>LANASEVE, Laboratorio de Patología Servicio Nacional de Salud Animal, Ministerio de Agricultura y Ganadería, Costa Rica

### Summary

León, B., C. Wiederkehr, R. Cruz, M. Cordero, A. Chinchilla, T. Solorzano-Scott, L. Brenes & F. Aguilar-Vargas, 2026. First detection of canine distemper virus infection in variegated squirrels (*Sciurus variegatoides*) and porcupines (*Coendou mexicanus*) in Costa Rica. *Bulg. J. Vet. Med.* (online first).

Canine distemper virus (CDV) is a multi-host pathogen capable of infecting a wide range of domestic and wild mammals, however, reports of infection in squirrels and porcupines remain scarce. As part of the wildlife rabies surveillance programme in Costa Rica, six wild mammals (four *Sciurus variegatoides* and two *Coendou mexicanus*) with neurological signs were evaluated to rule out rabies. All brain samples tested negative for rabies by direct immunofluorescence and RT-PCR. Rapid distemper antigen tests of ocular and nasal secretions were positive in both porcupines, which were subsequently euthanised. Advanced autolysis prevented histopathological evaluation in five of the animals; however, one squirrel showed severe neutrophilic pneumonia with necrosis and haemorrhage, a moderate lymphoplasmacytic infiltrate in the pulmonary interstitium, intracytoplasmic inclusion bodies in bronchial epithelium, and a focal neutrophilic infiltrate in the midbrain. The findings were consistent with CDV infection accompanied by severe systemic bacterial infection. CDV was confirmed in two of the four squirrels and in the two porcupines by RT-PCR and sequencing. Phylogenetic analysis revealed that the Costa Rican sequences grouped into two distinct clusters: one containing both squirrels and another with both porcupines. All four sequences shared a common ancestor with CDV strains detected in domestic dogs in the United States (2012–2013), and more distantly with a dog strain from Argentina (2005). These cases represent one of the few documented occurrences of CDV in squirrels and porcupines worldwide and the first reported in Costa Rica, underscoring the need for continued molecular surveillance to clarify lineage dynamics and spillover pathways.

**Key words:** canine distemper, Costa Rica, porcupines, variegated squirrel, wildlife

## INTRODUCTION

Canine distemper virus (CDV) is a single-stranded, negative-sense RNA virus belonging to the genus *Morbillivirus* within the family *Paramyxoviridae* (Macías-González *et al.*, 2025). This genus includes agents that cause multisystemic diseases with high morbidity and mortality rates in all terrestrial carnivores (Caro Martínez, 2016) including several mammalian species from the families *Canidae*, *Mustelidae*, *Procyonidae*, *Ursidae*, and *Viverridae*, among others (Macías-González *et al.*, 2025), and livestock (Takeda *et al.*, 2020). CDV is a highly adaptable, multi-host pathogen capable of emerging and re-emerging at the interface between wildlife, and domestic animals (Rios-Usuga *et al.*, 2025). Like other morbilliviruses, it is transmitted through direct contact or aerosol exposure to secretions and excretions from infected animals (Rios-Usuga *et al.*, 2025). Clinical signs include fever, respiratory, gastrointestinal, and/or neurological symptoms, as well as immunosuppression, hyperkeratosis, interstitial pneumonia, and/or encephalopathy (Wipf *et al.*, 2025).

The domestic dog (*Canis familiaris*) is the main reservoir host, and transmission to wildlife has increased due to expanding human activities. This spillover has led to serious outbreaks and poses significant threats to the conservation of vulnerable species (Wipf *et al.*, 2025). The virus's broad host range complicates eradication efforts, as an increasing number of wild species can serve as reservoirs, maintaining active infection cycles. Although dogs were initially considered the primary concern, CDV has now been detected in non-human primates, raising potential concerns for human health, as well as for a wide variety of wild and domestic animals (Loots *et al.*, 2017).

Wild CDV strains are having a significant negative impact on wildlife, contributing to population declines worldwide (Weckworth *et al.*, 2020). For this reason, several studies have attempted to detect CDV in species outside the *Canidae* family to evaluate its potential role in cross-species transmission and its broader impact on wildlife populations. Some of these surveillance efforts have included species such as squirrels, mongooses, marmots, badgers, and porcupines; however, CDV has not been detected or reported in these species (Ricci *et al.*, 2021; Rendon-Marin *et al.*, 2020; Alfano *et al.*, 2025).

In Costa Rica, although there is no official national monitoring programme, CDV is known to circulate widely and remains a disease of major concern in domestic dogs (Conrad *et al.*, 2021). The large number of stray and unvaccinated dogs makes control efforts difficult and poses a risk to wild populations. A recent national passive surveillance pilot study confirmed the presence of CDV in wild species, including gray fox (*Urocyon cinereoargenteus*), coati (*Nasua narica*), raccoon (*Procyon lotor*) (Aguilar-Vargas *et al.*, 2022), and previous reports have documented infections in wild felids (Avendaño *et al.*, 2016) and coatis (Rojas Jiménez *et al.*, 2021). These findings highlight the urgent need for a structured wildlife health monitoring programme (Aguilar-Vargas *et al.*, 2022).

As part of the wildlife rabies surveillance programme conducted under a One Health framework involving the Ministry of Health, the Ministry of Agriculture (SENASA), and the Ministry of Environment (SINAC), in coordination with wildlife management centres, the carcasses of two porcupines and four squirrels showing neurological signs were submitted to

SENASA-LANASEVE Laboratories for rabies testing, in accordance with Article 5 of SENASA Law No. 8495. Although rabies was ruled out, RT-PCR confirmed infection with canine distemper virus (CDV) in the two porcupines and in two of the four squirrels. This represents one of the few documented CDV infections in these species worldwide and the first reported occurrence in Costa Rica.

## MATERIALS AND METHODS

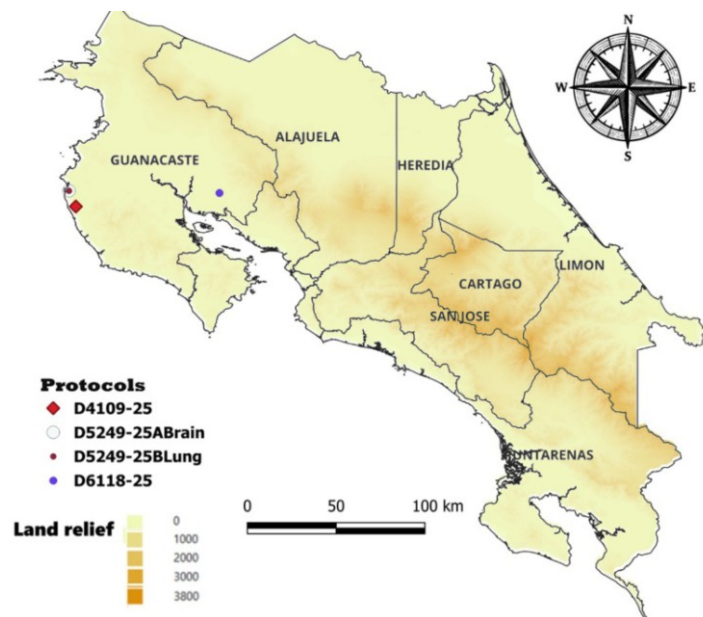
### Cases collected

Three male variegated squirrels (*Sciurus variegatoides*) were collected on May 29, 2025, in Cabo Velas under protocol 4109-25, and a female variegated squirrel was collected on August 12, 2025, in Cañas, protocol 6118-25.

The two female Mexican hairy dwarf porcupines (*Coendou mexicanus*) were collected on July 3, 2025, in Tamarindo under protocol 5249-25. The carcass, blood samples, and swabs (rectal and ocular) were sent frozen with ice on top for histopathological and serological studies of the two individuals. All specimens were obtained in the province of Guanacaste (Fig. 1).

### Rapid immunochromatographic test (RIT)

This kit is designed for the qualitative detection of Canine Distemper Virus (CDV Ag) antigen in conjunctival (ocular), nasal, or salivary secretions, as well as in canine urine or serum samples. Secretions (ocular or nasal) are collected using the provided swab, which is then inserted into the buffer tube and shaken vigorously to release the sample. Using the dropper, the



**Fig. 1.** Distribution of the four wild mammal cases positive for morbillivirus in the province of Guanacaste. The diamond corresponds to the first squirrel found in Cabo Velas, while the small dark dot represents the second squirrel found in Cañas. Both porcupines were found in Tamarindo and are indicated by a circle with a dot inside.

processed sample is drawn from the tube and 3–4 drops are applied to the sample well of the test device. The results were interpreted after 10 minutes. A negative result was indicated by the appearance of the control line (C) only, a positive result by the appearance of both the control line (C) and the test line (T), and the result was invalid when the control line (C) did not appear, in which case the test must be repeated (Silva *et al.*, 2022).

#### *Euthanasia procedure*

Euthanasia of the animals was performed in accordance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. Chemical restraint and anaesthetic induction were achieved via intramuscular injection of tiletamine-zolazepam (Zoletil® 50, Virbac, France) at a dose of 5 mg/kg (0.1 mL/kg). Once a deep plane of anaesthesia was confirmed, as evidenced by the loss of righting, pedal, and corneal reflexes, euthanasia was carried out by intracardiac injection of a solution containing sodium pentobarbital and phenytoin sodium (Eutanyl®, Brouwer, Argentina). Death was clinically confirmed by the permanent cessation of cardiac activity assessed by auscultation and the absence of spontaneous respiratory movements for a minimum of five minutes (Leary *et al.*, 2020). In porcupines, euthanasia was performed using a similar protocol, consisting of anaesthetic induction with Zoletil® 50, followed by administration of sodium pentobarbital (390 mg/mL) and sodium diphenylhydantoin (50 mg/mL) (Euthanex®) to induce death.

#### *Direct fluorescent antibody test (dFAT)*

Direct immunofluorescence was performed following the standard procedure recommended by WOA. For the assay,

FITC-labelled anti-rabies monoclonal globulin (Fujirebio, Tokyo, Japan) was used at a 1:128 dilution (WOAH, 2023).

#### *Histopathology method*

All specimens underwent post-mortem examination according to standard wildlife procedures. Tissue samples were fixed in 10% buffered formalin and processed for histopathological analysis following standard protocols. Additionally, fresh samples were collected for complementary analyses. (McAloose *et al.*, 2018).

#### *RT-PCR diagnostic*

For molecular diagnostics, pooled brain samples from each squirrel (including cerebellum, medulla oblongata, and cerebral cortex) were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

#### *Rabies RT-PCR*

For rabies diagnostic, a conventional reverse transcription polymerase chain reaction (RT-PCR) protocol previously described with modifications was used (Streicker *et al.*, 2019).

#### *CDV RT-PCR*

CDV detection was performed using a modified version of the RT-PCR protocol of Reidarson *et al.* (1998). Reactions were carried out in a final volume of 12.5 µL using the OneStep RT-PCR Kit (Cat. No. 210212, Qiagen, Hilden, Germany). The primers used for amplification of the phosphoprotein gene were DMVC (forward 5'-ATGTTTATGATCACAGCGGT-3') and DMVP2 (reverse 5'-ATTGGGTTGCACCACTTGTC-3'), each added at a final concentration of 0.5 µM. The reaction mixture also contained 0.5 µL of dNTPs (0.4 µM), 2.5 µL of 1× OneStep

buffer, and 0.5 µL of OneStep RT-PCR enzyme mix (1×). Four microliters of extracted RNA were used as template, and nuclease-free water was added to complete the total volume.

PCR products from samples and controls were analysed by electrophoresis on a 1.5% agarose gel. Samples showing bands corresponding to the positive control, 429 bp were confirmed by Sanger sequencing using the same primers as in the RT-PCR. The obtained sequences were submitted to the DDBJ-ENA-GenBank database (Fukuda *et al.*, 2021) under the following accession IDs, LC901454 (4109-25), LC901455 (6118-25), LC901456 (5249-25\_1), and LC901457 (5249-25\_2), corresponding to the squirrel and porcupine sequences.

#### *Phylogenetic analysis*

A total of 100 sequences were downloaded from the GenBank showing identity percentages ranging from 96.42% to 98.33% with the *Coendou mexicanus* sequence. These sequences, together with the four Costa Rican CDV sequences, were aligned using Clustal Omega (Sievers & Higgins, 2014). The measles virus sequence (GenBank accession no. AF266291) was included to root the phylogenetic tree. Identical or duplicate sequences, as well as those lacking information on host species or country of origin, were excluded from the analysis.

The best-fit substitution model for the 79 remaining sequences, according to the Bayesian Information Criterion in MEGA X (Kumar *et al.*, 2018), was K2+G. The phylogenetic tree was constructed using the maximum likelihood method with 1,000 bootstrap replicates.

## RESULTS

The first three variegated squirrels were brought to the rescue center alive but later developed neurological signs and died (protocol 4109-25). The fourth squirrel was found on August 8<sup>th</sup> on a trail in the Las Pumas Sanctuary, walking in circles. She was unable to walk in a straight line and attempted to climb onto a perch but fell, suggesting vertigo rather than simple incoordination. The exhibited neurological signs consistent with vestibular syndrome, including bilateral horizontal nystagmus, head tilt to the right, circling, sensory hyperesthesia, ataxia, and hypothermia. Neither squirrel showed anorexia, haematochezia, or seizures. Horizontal nystagmus was observed, likely related to the circling behaviour. By August 11th, the squirrel appeared depressed and prostrate and was euthanised (protocol 6118-25).

During the same period, two porcupines were admitted to a nearby veterinary hospital. One animal presented ataxia, lethargy, yellowish ocular and nasal discharge, neurological signs consistent with vestibular syndrome, and myiasis on the tail. A rapid distemper test (SensPERT Canine Distemper Ag Test Kit-CDV Ag, Vet All Laboratories, Korea) performed on ocular and nasal secretions yielded a positive result, and the animal was euthanised. The second porcupine presented only ocular discharge, without neurological signs, lethargy, or ataxia. However, the rapid distemper test was also positive, and the animal was subsequently euthanised (protocol 5249-25).

During the necropsy, both porcupines and three of the four squirrels exhibited advanced autolysis and marked freezing artifacts, which precluded histological evaluation. The remaining squirrel showed only mild autolytic changes and, on gross examination, presented abscesses on

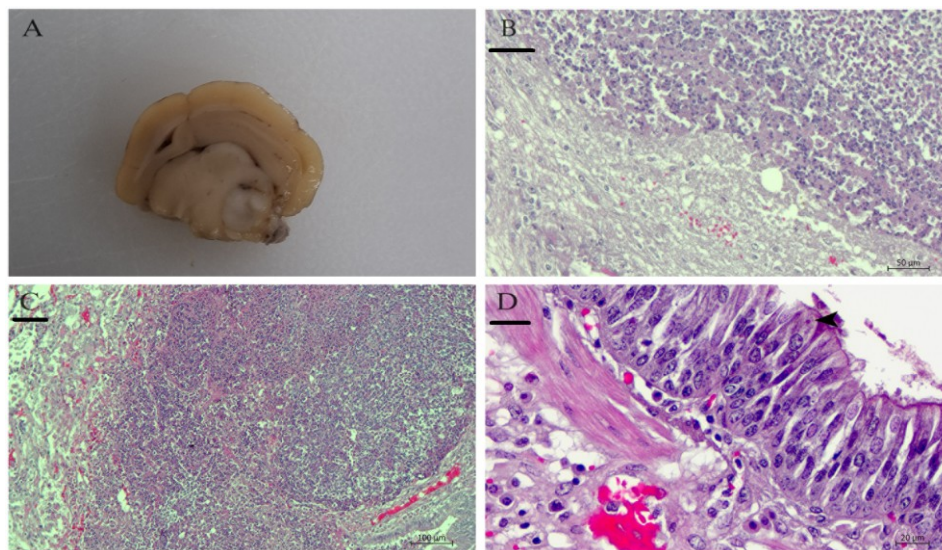
the dorsal thorax, face, and mandible, as well as pulmonary consolidation and abscesses in the right cranial lung lobe and congestion was noted in the brain.

Microscopically, the dermis exhibited a neutrophilic inflammatory infiltrate associated with bacterial-like structures and surrounded by granulation tissue. The lungs showed severe neutrophilic inflammation with extensive necrosis and haemorrhage, accompanied by bacterial-like structures. In the caudal lung lobes, the pulmonary interstitium contained a moderate lymphoplasmacytic infiltrate, with occasional intracytoplasmic inclusion bodies in the bronchial epithelium. A severe focal neutrophilic inflammatory infiltrate was also observed in the midbrain.

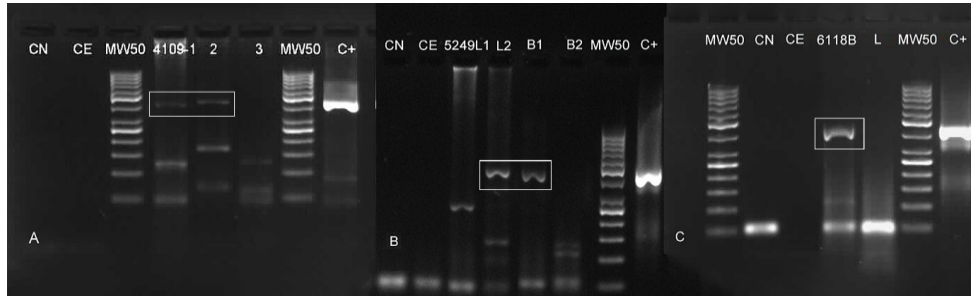
Taken together, these findings supported a diagnosis of lymphoplasmacytic pneumonia consistent with CDV infection, accompanied by severe systemic bacterial

infection that likely contributed to the cause of death (Fig. 2).

All cases tested negative for rabies by both direct immunofluorescence and RT-PCR. Of the three squirrels included under protocol D4109-25, two samples 1 and 2 depicted bands near to the positive control for morbillivirus by RT-PCR, however only the brain tissue of the second squirrel was confirmed positive to CDV (Fig. 3). In case D6118-25, the brain sample of the squirrel tested positive while the lung sample was negative by RT-PCR. The brain of porcupine 1 (protocol D5249-25) tested positive for morbillivirus by RT-PCR, whereas the lung was negative, in contrast to porcupine 2. Four of the five samples highlighted by the white rectangle were confirmed as canine distemper virus (CDV) by sequencing.



**Fig. 2.** Findings and lesions associated with CDV infection in *Sciurus variegatoides*. A) Brain abscess, transverse section at level of thalamus; B) Severe suppurative encephalitis with abundant degenerated neutrophils and cellular debris (bar=50  $\mu$ m); C) Severe suppurative pneumonia (bar=100  $\mu$ m); D) Lymphoplasmacytic inflammatory infiltrate is observed in the pulmonary interstitium; the epithelial cells lining the bronchus present eosinophilic intracytoplasmic structures, suggestive of viral inclusions (arrowhead right superior corner) (bar=20  $\mu$ m).



**Fig. 3.** Agarose gel electrophoresis of RT-PCR amplification products positive for canine distemper virus (CDV). CN corresponds to the negative control, CE to the extraction control, and C+ to the positive control. L indicates lung samples and B brain samples. MW50 represents the 50 bp molecular weight marker; the brighter bands correspond to 250 bp and 500 bp. Amplification products near 429 bp are highlighted with a white rectangle. Panel A shows protocol 4109-25; numbers 1, 2, and 3 correspond to individual squirrels. Panel B shows protocol 5249-25; numbers 1 and 2 correspond to two porcupines, with L indicating lung samples and B brain samples. Panel C shows amplification for protocol 6118-25, in which only the brain sample from another squirrel shows a band corresponding to the positive control, located between the 400 bp and 500 bp marker bands.

Of the 100 CDV sequences downloaded, 21 were duplicates and were discarded. Fig. 4 depicts the phylogenetic tree, with the Costa Rican CDV sequences highlighted by a red rectangle. These sequences shared a common ancestor with four sequences obtained from dogs in the United States between 2012 and 2013, supported by a bootstrap value of 84. Notably, the squirrel sequences shared a common ancestor and cluster separately from the porcupine sequences, which also share a common ancestor among themselves. All these shared a common ancestor with a sequence isolated in a dog from Argentina in 2005.

## DISCUSSION

Canine distemper virus (CDV) was initially described as an infectious disease of domestic dogs but is now recognised as a global multi-host pathogen that infects and causes mass mortalities in a wide range of wild carnivore species. Its impact is particularly severe when it affects endangered species (Loots *et al.*, 2017). Its

ability to infect multiple species has led to mass mortalities among various carnivore families, including wild canids, felids, hyaenids, procyonids, ailurids, ursids, mustelids, and viverrids. Distemper outbreaks have also been reported in marine mammals, such as Baikal seal (*Pusa sibirica*) and Caspian seal (*Pusa caspica*) (Mamaev *et al.*, 1995; Kennedy *et al.*, 2000). Moreover, CDV has been detected in China infecting non-human primates, including rhesus monkeys (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*), where infections were associated with high mortality rates (Sun *et al.*, 2010; Qiu *et al.*, 2011). However, detection of CDV in squirrels or porcupines remains rare and poorly documented. One of the few available reports comes from a study in Mexico, where among 74 wild animals examined, two of six rock squirrels (*Otospermophilus variegatus*) tested positive for CDV, whereas none of the three Mexican gray squirrels (*Sciurus aureogaster*) sampled were positive (Caro Martínez, 2016).



**Fig. 4.** Phylogenetic tree of canine distemper virus (CDV) sequences constructed using the maximum likelihood method in MEGA X with 1,000 bootstrap replicates. The red rectangle highlights the cluster containing the CDV sequences from the squirrels and porcupines reported in this study.

The variegated squirrels exhibited mainly neurological signs, one porcupine presented with ataxia, lethargy, yellowish ocular and nasal discharge, and neurological signs, whereas the other exhibited only ocular discharge. These clinical manifestations are consistent with the neurological and respiratory signs frequently described in CDV-infected carnivores, such as raccoons and foxes, where ataxia, circling, and ocular or nasal discharge are

commonly reported (Pope *et al.*, 2016). Although CDV infection in rodents and hystricomorphs has rarely been documented, the similarity in neurological and mucosal symptoms suggests a comparable disease progression. These findings further support the neurotropic and multi-systemic nature of CDV and highlight its capacity to cause disease across a broad range of mammalian taxa.

Neurological disease typically develops one to three weeks after the onset of initial clinical signs and may present in either acute or chronic forms (Kapil & Yeary, 2011). A further complicating factor is the development of secondary bacterial or protozoal infections resulting from CDV-induced immunosuppression (Martella *et al.*, 2008).

The histopathological findings described here are consistent with those reported in wildlife infected with CDV. The severe systemic bacterial infection CDV induces profound lymphoid depletion through infection of immune cells expressing the signalling lymphocytic activation molecule (SLAM/CD150) and subsequently disseminates to epithelial tissues via nectin-4, increasing susceptibility to opportunistic pathogens and facilitating viral adaptation to new host species (Mares-Guia *et al.*, 2024). Although viral inclusions were scarce in this case, the pulmonary lymphoplasmacytic inflammation, intracytoplasmic inclusion bodies in bronchial epithelium, and central nervous system involvement support the diagnosis. Taken together, these findings suggest that the squirrel experienced systemic CDV infection complicated by severe bacterial sepsis, which likely contributed substantially to the outcome.

Based on the clinical signs and histopathological findings observed, the most appropriate post-mortem tissues for CDV RT-PCR diagnosis were the brain and lung. Interestingly, although both tissues were analysed by RT-PCR, positive results were not consistently obtained from both samples, suggesting variation in viral distribution or stage of infection. Ante-mortem, suitable specimens include ocular and nasal secretion swabs, which are useful not only for rapid antigen testing but also for molecular detection by

RT-PCR not only for CDV and for other viral infections (Alvarado-Fernández *et al.*, 2023). The three squirrels (4109-25) were housed together in the same enclosure and developed similar clinical signs within a short period, supporting the suspicion that all three were affected by CDV. However, the advanced autolysis likely caused substantial nucleic acid degradation, which prevented RT-PCR detection in two of the three individuals.

The sequences most closely related to the cases described here correspond to CDV strains isolated from dogs in the United States between 2012 and 2013, and from Argentina in 2005. Based on our results, there appear to be two distinct CDV strains circulating in Guanacaste: one detected in squirrels and the other in porcupines. Notably, the two squirrel cases were collected 75.11 km apart, suggesting that this strain may be circulating within the squirrel population in the Guanacaste province.

Another possible explanation is spillover from domestic dogs, as the squirrels may have been exposed to dogs infected with the same CDV strain. Guanacaste is one of the provinces that receives the highest number of international tourists in Costa Rica, most of whom come from the United States, followed by Canada and several European countries. In addition, Guanacaste has the highest concentration of retired foreign residents, and a substantial proportion of U.S. and Canadian residents bring their pet dogs with them. This situation could facilitate the introduction and transmission of CDV between domestic dogs and wild animals.

Finally, one of the most important findings in this report was the negative dFAT and RT-PCR results for bat-transmitted rabies virus in the wildlife samples from Costa Rica. This result is

particularly relevant given the public health significance of rabies in the region, where spillover from bat reservoirs to domestic animals and humans remains an ongoing concern. Although rabies virus was not detected in these cases, previous reports of rabies in wildlife, together with the detection of other zoonotic pathogens or diseases causing high mortality in wild species such as CDV in this case highlight the importance of maintaining continuous wildlife health surveillance to support early detection (Leon *et al.*, 2021; Aguilar-Vargas *et al.*, 2022; Estrella-Morales *et al.*, 2025)

In conclusion, this study confirmed the presence of CDV infection in variegated squirrels and Mexican hairy dwarf porcupines in the province of Guanacaste. Notably, no peer-reviewed manuscripts confirming CDV infection in these species were identified in the literature, suggesting that it was either non been previously documented or occurs only very rarely, but nevertheless, the canine distemper virus should be considered an emerging pathogen of concern beyond traditional carnivore hosts.

The clinical, histopathological, and molecular findings demonstrate that CDV can induce neurological and systemic disease in these species, consistent with its neurotropic and immunosuppressive nature observed in other mammals. The phylogenetic relationships and spatial distribution of the detected strains suggest active circulation of at least two CDV lineages in Guanacaste, potentially maintained within wildlife populations and/or introduced through spillover from domestic dogs. Finally, the exclusion of rabies virus alongside the detection of CDV underscores the critical importance of continuous, integrated wildlife health surveillance to enable early detection of emerg-

ing pathogens and to mitigate risks to animal conservation and public health.

## REFERENCES

- Aguilar-Vargas, F., T. Solorzano-Scott, M. Baldi, E. Barquero-Calvo, A. Jiménez-Rocha, C. Jiménez, M. Piche-Ovares, G. Dolz, B. León, E. Corrales-Aguilar, M. Santoro & A. Alfaro-Alarcón, 2022. Passive epidemiological surveillance in wildlife in Costa Rica identifies pathogens of zoonotic and conservation importance. *PLoS One*, **17**, e0262063.
- Alfano, F., M. G. Lucibelli, N. D'Alessio, C. Auremma, S. Rea, G. Sgroi, M. S. Lucente, F. Pellegrini, G. Diakoudi, E. De Carlo, N. Decaro, G. Lanave, V. Martella & G. Fusco, 2025. Detection of canine distemper virus in wildlife in Italy (2022–2024). *Frontiers in Veterinary Science*, **12**, 1527550.
- Alvarado-Fernández, J. C., C. Salas-Rojas, J. Estrella-Morales, R. González, J. M. Cordero-Solórzano, O. Aguilar-Arguedas, Y. Soto-Araya, D. P. Villalobos & B. León, 2023. Evaluation of a rapid immunochromatographic assay for the diagnosis of rabies in regional laboratories of Costa Rica. *Veterinaria México*, **10**, e1106.
- Avendaño, R., F. Barrueta, S. M. Chavarría, O. Monge, G. A. Gutiérrez-Espeleta & A. Chaves, 2016. Canine distemper virus in wild felids of Costa Rica. *Journal of Wildlife Diseases*, **52**, 373–377.
- Caro Martínez, D. M., 2016. Detección de distemper canino en mamíferos silvestres de la Reserva Ecológica del Pedregal de San Ángel (MSc Thesis). UNAM, México.
- Conrad, J., J. Norman, A. Rodríguez, P. M. Dennis, R. Arguedas, C. Jiménez, J. G. Hope, M. J. Yabsley & S. M. Hernandez, 2021. Demographics and pathogens of domestic free-roaming pets and the implications for wild carnivores and human health in the San Luis region of Costa Rica. *Veterinary Sciences*, **8**, 65.

- Estrella-Morales, J., J. C. Alvarado-Fernández, C. Salas, R. González-Barrientos, I. Chacón, O. M. Aguilar, J. M. Cordero-Solórzano & B. León, 2025. First report of gray foxes (*Urocyon cinereoargenteus*) infected with bat-associated rabies virus (Desmodus rotundus rabies virus) in Costa Rica. *Zoonoses*, **5**, 965.
- Fukuda, A., Y. Kodama, J. Mashima, T. Fujisawa & O. Ogasawara, 2021. DDBJ update: Streamlining submission and access of human data. *Nucleic Acids Research*, **49**, D71–D75.
- Kapil, S. & T. J. Yeary, 2011. Canine distemper spillover in domestic dogs from urban wildlife. *Veterinary Clinics of North America: Small Animal Practice*, **41**, 1069–1086.
- Kennedy, S., T. Kuiken, P. D. Jepson, R. Deaville, M. Forsyth, T. Barrett, M. W. Van de Bildt, A. D. Osterhaus, T. Eybatov & C. Duck, 2000. Mass die-off of Caspian seals caused by canine distemper virus. *Emerging Infectious Diseases*, **6**, 637–639.
- Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**, 1547–1549.
- Leary, S., W. Underwood, A. Raymond, S. Cartner & C. Greenacre, 2020. AVMA Guidelines for the Euthanasia of Animals. American Veterinary Medical Association.
- León, B., S. F. González, L. M. Solís, M. Ramírez-Cardoce, A. Moreira-Soto, J. M. Cordero-Solórzano, S.E. Hutter, R. González-Barrientos & C.E. Rupprecht, 2021. Rabies in Costa Rica: Next steps towards controlling bat-borne rabies after its elimination in dogs. *Yale Journal of Biology and Medicine*, **94**, 311–321.
- Loots, A. K., E. Mitchell, D. L. Dalton, A. Kotzé & E. H. Venter, 2017. Advances in canine distemper virus pathogenesis research: A wildlife perspective. *Journal of General Virology*, **98**, 311–321.
- Macías-González, J., R. Granado-Gil, L. Mendoza-González, C. Pedroza-Roldán & R. M. Realpe-Quintero, 2025. Canine distemper virus in Mexico: A risk factor for wildlife. *Viruses*, **17**, 813.
- Mamaev, L.V., N. N. Denikina, S. I. Belikov, V. E. Volchkov, I. K. Visser, M. Fleming, C. Kai, T. C. Harder, B. Liess & A. D. Osterhaus, 1995. Characterisation of morbilliviruses isolated from Lake Baikal seals (*Phoca sibirica*). *Veterinary Microbiology*, **44**, 251–259.
- Mares-Guia, M. A. M., M. C. Furtado, F. L. L. Chalhoub, M. D. Portugal, J. M. C. Oliveira Coelho, A. M. B. Filippis & F. G. Naveca, 2024. Coinfection with canine distemper virus and yellow fever virus in a neotropical primate in Brazil. *Viruses*, **16**, 1670.
- Martella, V. G. & C. Buonavoglia, 2008. Canine distemper virus. *Veterinary Clinics of North America: Small Animal Practice*, **38**, 787–797.
- McAloose, D., K. Colegrove & A. L. Newton, 2018. Wildlife necropsy. In: Pathology of Wildlife and Zoo Animals, eds K. A. Terio, D. McAloose & J. St. Leger, Academic Press, pp. 1–20.
- Pope, J. P., D. L. Miller, M. C. Riley, E. Anis & R. P. Wilkes, 2016. Characterization of a novel canine distemper virus causing disease in wildlife. *Journal of Veterinary Diagnostic Investigation*, **28**, 506–513.
- Qiu, W., Y. Zheng, S. Zhang, Q. Fan, H. Liu, F. Zhang, W. Wang & G. Liao, R. Hu, 2011. Canine distemper outbreak in rhesus monkeys, China. *Emerging Infectious Diseases*, **17**, 1541–1543.
- Reidarson, T. H., J. McBain, C. House, D. P. King, J. L. Stott, A., J. K. Taubenberger, J. Heyning & T. P. Lipscomb, 1998. Morbillivirus infection in stranded common dolphins from the Pacific Ocean. *Journal of Wildlife Diseases*, **34**, 771–776.
- Rendón-Marín, S., M. Martínez-Gutiérrez, J. A. Suárez & J. Ruiz-Sáenz, 2020. Canine distemper virus transit through the Americas: Need to assess the impact on species conservation. *Frontiers in Microbiology*, **11**, 810.

- Ricci, I., Cersini, A., G. Manna, G. A. Marcario, R. Conti, G. Brocherel, G. Grifoni, C. Eleni & M. T. Scicluna, 2021. A canine distemper virus retrospective study conducted from 2011 to 2019 in central Italy. *Viruses*, **13**, 272.
- Ríos-Usuga, C., M. C. Ortiz-Pineda, S. D. Aguirre-Católico, V. H. Quiroz & J. Ruiz-Sáenz, 2025. Concurrent circulation of canine distemper virus at the wild-domestic canid interface in Colombia. *Viruses*, **17**, 649.
- Sievers, F. & D. G. Higgins, 2014. Clustal Omega. In: *Current Protocols in Bioinformatics*, John Wiley & Sons, pp. 3.13.1–3.13.16.
- Silva, P. H. N., B. D. F. Dallo, A. C. Aguiar-Pesenti, J. M. Medeiros, A. Martins & L. Pereira-Machado, 2022. Rapid immunochromatographic test in the diagnosis of canine distemper. *Revista MVZ Córdoba*, **27**, e2046.
- Streicker, D. G., S. L. Fallas-González, G. Luconi, R. G. Barrientos & B. León, 2019. Phylodynamics reveals extinction–recolonization dynamics underpin apparently endemic vampire bat rabies in Costa Rica. *Proceedings of the Royal Society B*, **286**, 20191527.
- Sun, Z., A. Li, H. Ye, Y. Shi, Z. Hu & L. Zeng, 2010. Natural infection with canine distemper virus in hand-feeding rhesus monkeys in China. *Veterinary Microbiology*, **141**, 374–378. h
- Takeda, M., F. Seki, Y. Yamamoto, N. Nao & H. Tokiwa, 2020. Animal morbilliviruses and their cross-species transmission potential. *Current Opinion in Virology*, **41**, 38–45.
- Weckworth, J. K., B. W. Davis, E. Dubovi, N. Fountain-Jones, C. Packer, S. Cleaveland, M. E. Craft, E. Eblate, M. Schwartz, L. S. Mills & M. Roelke-Parker, 2020. Cross-species transmission and evolutionary dynamics of canine distemper virus in African lions. *Molecular Ecology*, **29**, 4308–4321.
- Wipf, A., P. Perez-Cutillas, N. Ortega, A. Huertas-López, C. Martínez-Carrasco & M. G. Candela, 2025. Geographical distribution of carnivore hosts and genotypes of canine distemper virus worldwide. *Transboundary and Emerging Diseases*, **2025**, 6632068.
- WOAH, 2023. Rabies (infection with rabies virus and other lyssaviruses). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 3.1.18.

Paper received 21.11.2025; accepted for publication 15.01.2026

#### Correspondence:

DMV MSc PhD Bernal León,  
LANASEVE, LSE Laboratorio de Virología,  
SENASA. Heredia, Costa Rica  
e-mail: bleonr@senasa.go.cr,  
Bernal\_leon@yahoo.com