UNIVERSIDAD TÉCNICA DE MANABÍ
Fundada en 1952

LA TÉCNICA
Revista de las Agrociencias

Regional spread and phylogenetic perspectives on rabies virus transmission in cattle in southern Ecuador

Propagación regional y perspectivas filogenéticas sobre la transmisión del virus de la rabia en el ganado bovino del sur de Ecuador

Authors

[1]*Jorge Rodrigo Espinoza Samaniego*
joespinozasa@uide.edu.ec

[1]*Edilberto Chacón Marcheco*
edilberto.chacon@utc.edu.ec

[2]*Luis Alfredo Mena Miño*
luismenamino@hotmail.com

[3]*Rubén Alexander Maldonado Orbe*
ruben.maldonado@uisek.edu.ec

[1]Universidad Técnica de Cotopaxi, Maestría en Ciencias Veterinarias, Unidad Académica de Posgrado, Latacunga, Cotopaxi, Ecuador.
[2]Agencia de Regulación y Control Fito y Zoosanitario (AGROCALIDAD), Sanidad Animal, Quito, Pichincha, Ecuador.
[3]Universidad Internacional SEK-UISEK, Facultad de Ciencias de la Salud, Enfermedades Desatendidas, Emergentes, Ecoepidemiología y Biodiversidad (GIEED), Biomedicina Experimental y Aplicada (GIBEA), Quito, Pichincha, Ecuador.

Suggested citation: Espinoza Samaniego, J. R., Chacón Marcheco, E., Mena Miño, L. A., Maldonado Orbe, R. A. (2025). Regional spread and phylogenetic perspectives on rabies virus transmission in cattle in southern Ecuador. *La Técnica, 15*(2), 103-111. DOI:https://doi.org/10.33936/latecnica.v15i2.7321

Received: February 26th, 2025
Accepted: October 10th, 2025
Published: November 01st, 2025

Abstract

Rabies is a lethal zoonosis that impacts both humans and animals. To understand the evolution and epidemiology of the disease, its molecular characterization is essential. Therefore, the objective of this study was to perform the molecular characterization of virus isolates obtained from bovine brain tissue in Zone 7 of Ecuador, with the aim of providing useful information that contributes to strengthening national surveillance and control programs. This study included cases in the provinces of Zamora Chinchipe, Loja, and El Oro during the period 2015-2020. Twenty-six positive samples were analyzed by direct immunofluorescence and culture in BHK-21 cells, confirming the presence of the rabies virus. Phylogenetic analysis revealed that isolates from Ecuador are distributed in specific clades, with evident links between historical variants and those isolated in the provinces of Loja and Zamora Chinchipe. On the other hand, the isolates from El Oro showed less genetic diversity, which could indicate that local transmission models are more limited. Loja and Zamora Chinchipe play a fundamental role in the dissemination of the virus, likely due to ecological conditions that benefit blood-sucking bats. Furthermore, the observed genetic diversity highlights the importance of genomic surveillance, which allows for the detection of genetic mutations that could affect the effectiveness of existing vaccines and control methods. This study highlights the importance of establishing health policies that align with local distribution patterns of the virus and emphasizes the substantial use of molecular and phylogenetic tools for epidemiological surveillance. The information generated will contribute to the strengthening of more effective control programs, aimed at reducing the impact of this disease in the region by improving surveillance and control programs.

Keywords: rabies, molecular characterization, phylogenetic analysis, Ecuador, epidemiological surveillance.

Resumen

La enfermedad de la rabia es una zoonosis letal que tiene un impacto en humanos como en animales. Para entender la evolución y la epidemiología de la enfermedad, es fundamental su caracterización a nivel molecular. Por esta razón, el objetivo de este estudio fue realizar la caracterización molecular de los aislamientos del virus obtenidos a partir de tejido cerebral bovino en la Zona 7 de Ecuador, con el propósito de brindar información útil que contribuya al fortalecimiento de los programas nacionales de vigilancia y control. El estudio incluyó en las provincias de Zamora Chinchipe, Loja y El Oro, durante el período 2015-2020, en el cual fueron analizadas 26 muestras positivas mediante inmunofluorescencia directa y cultivo en células BHK-21, confirmándose la presencia del virus de la rabia. El análisis filogenético reveló que los aislados de Ecuador se distribuyeron en clados específicos, con vínculos evidentes entre las variantes históricas y las aisladas en las provincias de Loja y Zamora Chinchipe. Por otra parte, los aislados de El Oro mostraron una menor diversidad genética, lo que podría indicar que los modelos de transmisión a nivel local fueron más limitados. Loja y Zamora Chinchipe tuvieron un rol fundamental en la diseminación del virus, probablemente debido a las condiciones ecológicas que benefician a los murciélagos hematófagos. Asimismo, la diversidad genética que se ha observado resalta lo crucial de la vigilancia genómica que permita detectar mutaciones genéticas que podrían afectar la efectividad de las vacunas y los métodos de control existentes. Este estudio destaca la relevancia de establecer políticas sanitarias que se alineen con los patrones de distribución local del virus y enfatiza el empleo sustancial de herramientas moleculares y filogenéticas para la vigilancia epidemiológica. La información generada contribuirá al fortalecimiento de programas de control más efectivos, orientados a reducir el impacto de esta enfermedad en la región mejorando los programas de vigilancia y control.

Palabras clave: rabia, caracterización molecular, análisis filogenético, Ecuador, vigilancia epidemiológica.

Introduction

The rabies virus (RABV), a member of the genus *Lyssavirus* within the family Rhabdoviridae, is a zoonotic pathogen responsible for rabies, a fatal neurological disease that affects mammals, including humans. With a length of approximately 200 nm and a width of about 80 nm, the virus has a bullet-shaped morphology. Its genome consists of negative-sense single-stranded RNA and encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (L) (Holmes and Holmes, 2023; Parija, 2023). The nucleoprotein, among others, plays an essential role in transcription and replication and has previously been used in molecular typing studies and phylogenetic analyses to clarify the evolutionary dynamics of RABV (Omodo et al., 2020; Nadal et al., 2022; Schreiber and Fachinetto, 2022).

Rabies is a viral zoonosis that represents a serious public health problem and has a strong economic impact worldwide (World Organization for Animal Health (WOAH), 2023). Each year, more than 55,000 people die from this disease, of whom 40% are children under 15 years of age, frequently bitten by animals suspected of being infected (Khairullah et al., 2023; Wallace and Muller, 2024). Wildlife rabies can generate outbreaks of urban rabies that directly affect people, since contact with infected animals occurs in the environment, which contributes to the spread of this disease (Ortiz et al., 2017). Once clinical signs appear, treatment options are very limited and lethality approaches 100%. Although effective vaccines exist, access to them remains a challenge for many people in high-risk conditions (Scheffer et al., 2014).

At the global level, organizations such as the World Health Organization (WHO) and the Pan American Health Organization (PAHO) have implemented strategies that have made it possible to control and, in some cases, eradicate rabies in certain countries. This is because, as a zoonosis, its control has a direct impact on human health (Ortiz et al., 2017). In Ecuador, the monitoring of this disease is carried out by two main institutions: the Phyto- and Zoosanitary Regulation and Control Agency–Agrocalidad, attached to the Ministry of Agriculture, which leads the official program for the prevention and control of bovine paralytic rabies in the agricultural sector; and the Ministry of Public Health, in collaboration with the National Institute of Public Health Research (INSPI), which manages zoonosis control under guidelines such as NOM-011-SSA2-1993. As a Regulation and Control Agency, Agrocalidad has conducted diagnoses of bovine paralytic rabies using techniques such as direct immunofluorescence, viral isolation, and molecular tests since 2014. However, even in the face of these efforts, the high prevalence of wildlife rabies in the country has hindered the acquisition of specific data on the viral variants circulating in the regions with the highest incidence, such as the Amazon region. Molecular characterization of the virus and analysis of the relationships among outbreaks would make it possible to identify the variants present and establish more effective strategies within the national program, with the aim of controlling the disease and, in the long term, eradicating it. This is important, since this disease has caused significant economic losses in the livestock sector (Ortiz et al., 2017).

Zone 7 of Ecuador includes the provinces of Zamora Chinchipe, Loja, and El Oro, where a high incidence of wildlife rabies has been reported, and where demographic and geographic characteristics constitute a favorable environment for the hematophagous bat, which is the main vector of the disease (Ortiz et al., 2017). Between 2015 and 2020, 263 positive cases of wildlife rabies were recorded in the country, which highlights the need to detect the reservoirs involved in the spread of this disease and to implement actions to prevent subsequent re-emergence (Vizcaíno et al., 2016; Ortiz et al., 2017). Despite the efforts made, it is important to understand the genetic relationship among the outbreaks that have been recorded. Furthermore, the lack of molecular data that would allow characterization of the virus in this region limits epidemiological surveillance and outbreak control capacities.

Therefore, the objective of this research was to molecularly characterize virus isolates obtained from bovine brain tissue of positive cases from Zone 7 of Ecuador, in order to establish phylogenetic relationships in this region, providing valuable information that will help strengthen national surveillance and control programs.

Materials and methodology

The research was conducted in Zone 7 of Ecuador, comprising the provinces of Zamora Chinchipe, Loja, and El Oro, using rabies virus isolates detected during the 2015-2020 period.

Study design and data collection

abies is a mandatory notifiable disease in Ecuador, which requires the reporting of suspected cases and outbreaks in production animals. According to national guidelines, cases presenting neurological symptoms, such as sudden behavioral changes or progressive paralysis, are investigated. In this context, field technicians from the Phyto- and Zoosanitary Regulation and Control Agency–Agrocalidad collected bovine brain samples from affected farms, as part of the guidelines established for routine and emergency surveillance. In order to ensure an accurate diagnosis of this disease, brainstem and cerebellum samples were prioritized due to their high diagnostic value.

Sample selection

The direct immunofluorescence (DIF) test was used for the diagnosis of rabies samples during the 2015-2020 period, following the guidelines of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (WOAH, 2023). The samples were evaluated according to criteria that included visual inspection of the tissue in order to rule out autolysis, confirmation of the anatomical integrity of the brain (presence of the medulla oblongata or hippocampus), control of storage temperature, and absence of external contamination.

During this period, 263 brain tissue samples from Zone 7 of Ecuador were recorded as positive and reported in the Ecuadorian Zoosanitary Information System (SIZSE). From these samples, 136 were initially evaluated as they met the criteria for viral isolation and molecular testing. From this group, 46 samples were selected, taking into account technical aspects such as preservation quality, anatomical characteristics, detection of viral antigen, and epidemiological importance.

Rabies virus isolation

The neonatal hamster kidney cell line (BHK-21) was used to isolate the rabies virus. Prior to viral isolation, the samples were confirmed by direct immunofluorescence (DIF), a technique that made it possible to demonstrate the presence of viral proteins characteristic of the rabies virus. Subsequently, the virus was isolated by means of cell culture, which was demonstrated through fluorescent viral particles observed in the cultured cells (figure 1).

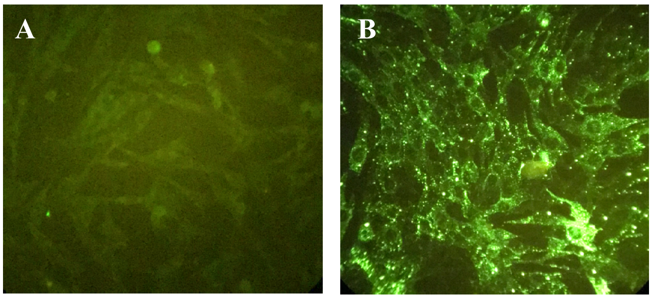

Figure 1. isolation of the rabies virus in BHK-21 cells. BHK-21 cells were infected with viral isolates obtained from bovine brain tissue and analyzed by the direct fluorescent antibody test (DIF). A. Negative control: uninfected cells with no evidence of fluorescence, indicating the absence of rabies virus proteins. B. Positive samples: infected cells showing bright green fluorescence foci, characteristic of the presence of rabies virus–specific viral proteins.

The material obtained from the cell cultures was stored at -80 °C to preserve its viability during the development of subsequent tests. This approach ensured the quality and stability of the isolated virus, which was crucial for the molecular study and for the construction of virus banks intended for future research.

The effectiveness of culture in BHK-21 cells and its analysis by DIF has been supported by previous studies, which have demonstrated the usefulness of this cell line for the isolation and diagnosis of the rabies virus, also employing it in the analysis of human brain sections to confirm viral infection (Qin et al., 2019; Wardhani et al., 2021; Harsha et al., 2022; Markbordee et al., 2024). The results of the present study reinforce this evidence, highlighting the sensitivity and specificity of BHK-21 cells as an in vitro model for virus isolation.

RT-PCR, electrophoresis, and sequencing

The SuperScript IV First-Strand cDNA Synthesis Kit was used to synthesize complementary DNA (cDNA), and the TRIzol reagent (Thermo Fisher Scientific™) was used to extract total RNA from infected cells. The nucleoprotein (N) gene was amplified using primers JW12 (5′-ACGCTTAACAACAARATCARAG-3′) and P784 (5′-CCTCAAAGTTCTTGTGGAAGA-3′), reported for the rabies virus (Fernandes et al., 2020; Dettinger et al., 2022; Claassen et al., 2023). PCR conditions included 30 cycles of denaturation, annealing, and elongation, and the amplicons were visualized on 2% agarose gels stained with SafeView. In order to ensure data quality, the samples were sequenced in both directions using the Sanger technique (Macrogen, South Korea).

Phylogenetic analysis

A dataset of nucleoprotein gene sequences was compiled, which included both Ecuadorian isolates and sequences from neighboring countries available in GenBank. These countries were: Brazil (MN103483), Colombia (JF693463), Ecuador (MF467498, MF467497, HM368179, MF467501, MF467504), and Peru (KU938717), with the aim of performing a comparison among the sequences. The rabies virus genome was used as the reference sequence (NC_001542), while the European bat lyssavirus (EBLVF, U22845), a lyssavirus not related to rabies, was used as the outgroup.

SMetadata, such as the year and location of isolation, were included when available. The sequences were aligned using MAFFT (v7.407_1) (Katoh and Standley, 2013), and conserved regions with divergence or poorly aligned sites were removed using the Gblocks tool (Castresana, 2000; Talavera and Castresana, 2007). For the phylogenetic analysis, the statistical method of maximum likelihood (ML) was used, while the Tamura–Nei model with gamma distribution (TN+F+G2) was identified by the ModelFinder tool (Kalyaanamoorthy et al., 2017) as the best model to estimate genetic distances. Clade support was constructed using the IQ-TREE software (v1.6.12) (Nguyen et al., 2015) with 10,000 bootstrap replicates (Hoang et al., 2018). FigTree (v1.4.4) and iTOL (Letunic and Bork, 2024) were used for tree visualization and annotation.

Results and discussion

Cases of bovine rabies in southern Ecuador between 2015 and 2020

During the 2015–2020 period, a total of 263 cases of bovine rabies were confirmed by Agrocalidad in the provinces of El Oro, Loja, and Zamora Chinchipe. The distribution of bovine cases from 2015 to 2020 among the different provinces is presented in figure 1. The highest number of confirmed cases occurred in Zamora Chinchipe (189 cases; 71.86%), followed by Loja (70 cases; 26.62%) and El Oro (four cases; 1.52%). These results are consistent with previous studies. Cárdenas (2022) reported that Zamora Chinchipe was one of the provinces with the highest incidence of bovine rabies cases due to its environmental conditions favorable for virus proliferation. Similarly, between 2010 and 2018, 67 positive cases of bovine rabies from 127 analyzed samples were reported in Loja (Briceño-Loaiza and Alegría-Morán, 2019), underscoring the epidemiological importance of this province. On the other hand, the results of this study confirmed that the province of El Oro showed a lower incidence, in agreement with the historical record of only four positive cases reported during the same period (Briceño-Loaiza and Alegría-Morán, 2019; Cárdenas, 2022).

Detection of viral antigens, amplification of viral RNA, and virus isolation

To characterize the rabies virus, brain samples were collected from cattle in the high-incidence area of Ecuador, which is Zone 7. Table 1 summarizes the results of viral antigen detection, virus isolation, viral RNA amplification, and sample sequencing.

Viral antigen was detected in the 46 selected samples, of which 26 exhibited a cytopathic effect in cell culture, indicating the presence of viable virus. In addition, in these same samples the presence of viral RNA was confirmed by RT-PCR, thereby confirming the detection of the rabies virus. The results are presented in the following table 1.

Isolates rabies virus distribution

Table 2 summarizes the geographical distribution of the 26 rabies virus isolates obtained in the provinces of Zamora Chinchipe, Loja, and El Oro between 2015 and 2020. The results showed Zamora Chinchipe as the province with the highest number of cases, including 16 isolates from samples collected during the study period. Zamora Chinchipe presented geographic and climatic conditions that favored the development of hematophagous bats, such as *Desmodus rotundus*. This may be a factor contributing to the presence of the disease (Fornace et al., 2013; Meske et al., 2021). On the other hand, eight isolates were obtained in the province of Loja, which showed a notable increase in cases in 2018. This increase could be related to the strengthening of agricultural and livestock activities in rural areas, which could represent a factor of exposure of cattle to the virus vectors (Soler-Tovar and Escobar, 2025). In contrast, El Oro reported only two isolated cases in 2017 and one case each in 2018 and 2019, indicating a lower incidence.

Table 1. Positive bovine rabies samples in Zone 7 of Ecuador.

Year	Province	Positive IFD samples*	Ideal samples	Viral isolation	Positives RT-PCR	Sequenced samples
2015	El Oro	0	0	0	0	0
	Loja	1	1	1	1	1
	Zamora Chinchipe	10	7	4	4	4
2016	El Oro	0	0	0	0	0
	Loja	3	3	2	1	1
	Zamora Chinchipe	18	11	5	2	2
2017	El Oro	2	2	2	2	2
	Loja	9	6	1	1	1
	Zamora Chinchipe	45	24	6	3	3
2018	El Oro	1	0	0	0	0
	Loja	35	15	4	2	2
	Zamora Chinchipe	88	37	10	2	2
2019	El Oro	1	0	0	0	0
	Loja	13	8	3	2	2
	Zamora Chinchipe	26	13	4	2	2
2020	El Oro	0	0	0	0	0
	Loja	4	3	1	1	1
	Zamora Chinchipe	7	6	3	3	3
	Total	263	136	46	26	26

*Source: Agrocalidad disease notification base .

Geographically, the latitudinal and longitudinal coordinates revealed a clustering of cases in rural areas, where interactions between livestock and virus vectors were more frequent due to the absence of physical barriers, traditional livestock management practices, and proximity to natural habitats (Nahata et al., 2021). These findings underscored the need to implement control strategies focused on these specific regions and periods of higher incidence, especially in Zamora Chinchipe, in order to mitigate the impact of rabies in endemic areas.

Molecular identification

The molecular analysis of the 26 bovine brain tissue samples from the provinces of Zamora Chinchipe, Loja, and El Oro corresponded to the nucleoprotein of the rabies virus. The BLAST results showed 98.48% similarity with the reference sequence of the rabies virus nucleoprotein gene, which confirmed the identification of the infectious agent. These results were consistent with previous studies, in which 98.75% similarity was observed between rabies virus variants associated with European bats and detected in cases of canine rabies (Mantari et al., 2019). This highlighted the need to study the evolution of the virus in different hosts and geographic areas.

Table 2. Bovine rabies virus isolates from Zone 7 of Ecuador (2015–2020) with their corresponding geographic coordinates.

Isolate	Year	Month	Province	Latitude	Longitude
Isolate 1	2015	July	Zamora Chinchipe	-4.55	-79.13
Isolate 2	2015	March	Zamora Chinchipe	-3.89	-78.81
Isolate 3	2015	September	Zamora Chinchipe	-3.71	-78.73
Isolate 4	2015	July	Loja	-4.36	-79.39
Isolate 5	2015	June	Zamora Chinchipe	-4.03	-78.89
Isolate 6	2016	February	Loja	-4.40	-79.51
Isolate 7	2016	October	Zamora Chinchipe	-3.54	-78.66
Isolate 8	2016	April	Zamora Chinchipe	-4.02	-78.65
Isolate 9	2017	September	Zamora Chinchipe	-4.76	-79.05
Isolate 10	2017	April	Zamora Chinchipe	-3.80	-78.76
Isolate 11	2017	October	Loja	-3.66	-79.38
Isolate 12	2017	April	Zamora Chinchipe	-3.90	-78.85
Isolate 13	2017	March	El Oro	-3.65	-79.71
Isolate 14	2017	September	El Oro	-3.49	-79.77
Isolate 15	2018	January	Loja	-3.85	-79.19
Isolate 16	2018	November	Loja	-3.93	-79.21
Isolate 17	2018	October	Zamora Chinchipe	-3.98	-79.02
Isolate 18	2018	March	Zamora Chinchipe	-3.60	-78.93
Isolate 19	2019	January	Loja	-3.92	-79.23
Isolate 20	2019	May	Loja	-4.41	-79.45
Isolate 21	2019	April	Zamora Chinchipe	-3.72	-78.80
Isolate 22	2019	May	Zamora Chinchipe	-3.90	-78.83
Isolate 23	2020	March	Zamora Chinchipe	-3.70	-78.71
Isolate 24	2020	February	Zamora Chinchipe	-4.02	-78.82
Isolate 25	2020	July	Zamora Chinchipe	-3.60	-78.90
Isolate 26	2020	August	Loja	-3.73	-79.26

Nota: The isolates are listed by year, month, and location, providing a comprehensive overview of the viral samples collected. Information on the place and date of sampling, corresponding to the death of the animal, was extracted from the Agrocalidad rabies disease notification database.

The remarkable similarity among the sequences suggested that the virus exhibited a high level of genetic conservation in the analyzed area, which could help to establish diagnostic and control strategies. In addition, Mantari et al. (2019) reported that the genetic variability of the virus had the potential to affect vaccine efficacy, as well as transmission and pathogenesis, which represents a relevant factor to consider.

In addition, molecular characterization made it possible to infer specific transmission routes and virus dispersion patterns. In this context, the slight genetic divergences found could be associated with local adaptations of the virus to its main host or to the ecological conditions of the area (Cai et al., 2022; Li et al., 2023; Durrant et al., 2024). This reinforced the need to carry out continuous monitoring of the viral genome in order to identify changes that could compromise vaccination and control efforts in the region. Likewise, the results obtained highlighted the importance of using molecular tools not only to confirm diagnoses, but also to advance the understanding of the epidemiology and evolution of the rabies virus in endemic areas (Manjunatha et al., 2023; Islam et al., 2025), in line with international recommendations for the control of this zoonosis.

Phylogenetic Analysis

Through phylogenetic analysis, it was possible to observe the evolutionary connections that existed between the international reference sequences and the local isolates, as well as the reference genome of the rabies virus. The phylogenetic tree (figure 2) revealed a clear clustering of the Ecuadorian isolates into multiple well-defined clades, which is supported by the bootstrap values (bootstrap > 70 in the main clades), ensuring the robustness of the phylogenetic relationships.

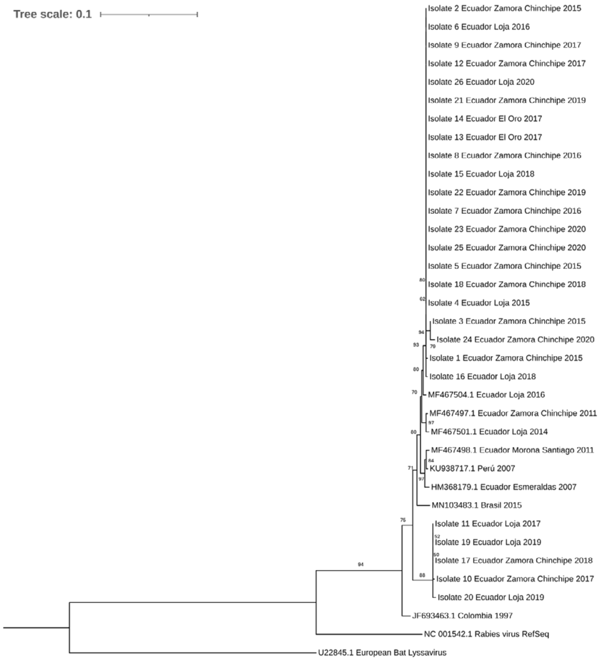

Figure 2. Phylogenetic tree of the rabies virus based on the nucleoprotein gene. The analysis included 26 isolates from bovine brain tissue from Zone 7 of Ecuador (provinces of Zamora Chinchipe, Loja, and El Oro) collected between 2015 and 2020, together with reference sequences from Ecuador, neighboring countries (Peru, Colombia, and Brazil), and the reference genome of the rabies virus. The sequence of the European bat lyssavirus (European Bat Lyssavirus, U22845.1) was used as the outgroup.

The isolates from El Oro, Loja, and Zamora Chinchipe showed a strong relationship with other isolates in Ecuador that have been previously characterized, such as those from Loja (MF467501.1 and MF467504.1) and Morona Santiago (MF467498.1). In addition, a clear differentiation was established with respect to the international reference sequences, such as those from Brazil (MN103483.1) and Colombia (JF693463.1). This indicated that the lineages circulating in Ecuador have undergone local evolution, with a possibly restricted genetic exchange among neighboring countries.

The isolates from Loja and Zamora Chinchipe clustered with historical sequences from Loja and Morona Santiago, which indicated continuity in the circulation of specific lineages in these regions. On the other hand, in other areas of Latin America it has been reported that the genetic diversity of the rabies virus has been influenced by local adaptations and by interactions among host species (Mantari et al., 2019; Meganck and Baric, 2021; Rupprecht et al., 2023). According to Escobar et al. (2023), bat-related variants exhibited greater genetic stability, which could explain the clustering of Ecuadorian isolates into well-defined clades.

The clustering of isolates from Zamora Chinchipe and Loja into specific clades suggested that these provinces were key points for the circulation and perpetuation of the virus in Zone 7. This may be associated with ecological factors, such as the high activity of hematophagous bats (*D. rotundus*), and the proximity among farms in these regions (Castelo-Branco et al., 2023; Briceño-Loaiza et al., 2024). In Ecuador, a prevalence of *D. rotundus* attacks of 69% has been reported in farms, while a prevalence of 24% has been reported in attacks on cattle. In addition, it has been reported that, of the total number of captured bats, 93% have been identified as *D. rotundus*, and no rabies virus infections were found in 30% of the analyzed specimens (Orlando et al., 2019). This information is considered useful for improving surveillance and control of rabies transmission in the region.

On the other hand, the lower diversity observed in the isolates from El Oro could be the result of reduced contact between livestock populations and virus vectors, as occurs in areas with a lower density of hematophagous bats or less interaction with wild ecosystems (Bárcenas-Reyes et al., 2019). Although the Ecuadorian isolates were very similar, the genetic differences among clades may have an impact on the effectiveness of vaccination strategies that are based on particular strains. These results emphasized the importance of strengthening molecular surveillance programs for the detection of genetic variations that have the potential to affect the epidemiology of the virus or to jeopardize current control programs.

Conclusion

The present analysis provides a comprehensive perspective on the molecular and phylogenetic characterization of the rabies virus in cattle located in Ecuador's Zone 7, which has a significant impact in terms of public health and the control of this zoonosis. According to the findings, a higher prevalence and greater evolutionary complexity were observed in the provinces of Zamora Chinchipe and Loja, underscoring the genetic diversity of the virus across the three provinces: El Oro, Zamora Chinchipe, and Loja.

The phylogenetic analysis shows that the variants circulating in Zone 7 are closely related to the historical variants from Ecuador, which indicates that the virus continues to circulate locally, making it important to implement measures to control and monitor hematophagous bats as the main carriers of the disease. Likewise, the genetic similarity of the isolates to the global reference sequence of the rabies virus, together with the discrepancies observed among clades, emphasizes the importance of maintaining continuous molecular monitoring to detect possible mutations that could have an impact on the effectiveness of current vaccines and surveillance and control strategies.

Zamora Chinchipe and Loja emerge as key areas for virus spread, whereas El Oro shows a lower incidence, possibly due to ecological factors and reduced contact with hematophagous bats. These geographic distribution patterns highlight the importance of intensifying epidemiological monitoring in Zamora Chinchipe and Loja, as well as determining the specific factors that limit incidence in El Oro. Likewise, the data obtained help to better understand the epidemiology of rabies in the area, providing essential information for the design of targeted control strategies.

The use of cell cultures and molecular tests are tools that contribute to a better understanding of the epidemiology of the virus in endemic regions. The data generated not only strengthen national efforts to control rabies in livestock, but also provide valuable information for the development of effective vaccination programs focused on hotspots.

Conflict of interest

The authors declare not having any conflict of interest in the present publication at any of its stages.

References

Bárcenas-Reyes, I., Nieves-Martínez, D. P., Cuador-Gil, J. Q., Loza-Rubio, E., González-Ruiz, S., Cantó-Alarcón, G. J. and Milián-Suazo, F. (2019). Spatiotemporal analysis of rabies in cattle in central Mexico. *Geospatial Health*, *14*(2). https://doi.org/10.4081/gh.2019.805

Briceño-Loaiza, C. y Alegría-Morán, R. (2019). Distribución espacio-temporal de la rabia animal durante el periodo 2010 al 2018, en la provincia de Loja, Ecuador. *Revista Científica y de Opinión CRIALZH*, *2*(1), 153-163.

Briceño-Loaiza, C., Fernández-Sanhueza, B., Benavides-Silva, C., Jimenez, J. Y., Rubio, A. V., Ábalos, P. and Alegría-Morán, R. A. (2024). Spatial clusters, temporal behavior, and risk factors analysis of rabies in livestock in Ecuador. *Preventive Veterinary Medicine*, *226*, 106188. https://doi.org/10.1016/j.prevetmed.2024.106188

Cai, M., Liu, H., Jiang, F., Sun, Y., Wang, W., An, Y., Zhang, M., Li, X., Liu, D., Li, Y., Yu, Y., Huang, W. and Wang, Y. (2022). Analysis of the evolution, infectivity and antigenicity of circulating rabies virus strains. *Emerging Microbes & Infections*, *11*(1), 1474-1487. https://doi.org/10.1080/22221751.2022.2078742

Cárdenas, V. M. (2022). *Áreas de riesgo futuro de dispersión del virus de la rabia con el uso de modelos de distribución de especies de murciélagos vectores*. Quito: UCE. http://www.dspace.uce.edu.ec/handle/25000/28503

Castelo-Branco, D. S. C. M., Nobre, J. A., Souza, P. R. H., Diógenes, E. M., Guedes, G. M. M., Mesquita, F. P., Souza, P. F. N., Rocha, M. F. G., Sidrim, J. J. C., Cordeiro, R. A. and Montenegro, R. C. (2023). Role of Brazilian bats in the epidemiological cycle of potentially zoonotic pathogens. *Microbial Pathogenesis*, *177*, 106032. https://doi.org/10.1016/j. micpath.2023.106032

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*(4), 540-552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Centro Nacional de Programas Preventivos y Control de Enfermedades. (2011). *NOM-011-SSA2-2011 Para la prevención y control de la rabia humana y en los perros y gatos.*

Claassen, D. D., Odendaal, L., Sabeta, C. T., Fosgate, G. T., Mohale, D. K., Williams, J. H. and Clift, S. J. (2023). Diagnostic sensitivity and specificity of immunohistochemistry for the detection of rabies virus in domestic and wild animals in South Africa. *Journal of Veterinary Diagnostic Investigation*, *35*(3), 236-245. https://doi.org/10.1177/10406387231154537

Dettinger, L., Gigante, C. M., Sellard, M., Seiders, M., Patel, P., Orciari, L. A., Yager, P., Lute, J., Regec, A., Li, Y. and Xia, D. (2022). Detection of apparent early rabies infection by LN34 Pan-Lyssavirus Real-Time RT-PCR assay in Pennsylvania. *Viruses*, *14*(9), 1845. https://doi.org/10.3390/v14091845

Durrant, R., Cobbold, C. A., Brunker, K., Campbell, K., Dushoff, J., Ferguson, E. A., Jaswant, G., Lugelo, A., Lushasi, K., Sikana, L. and Hampson, K. (2024). Examining the molecular clock hypothesis for the contemporary evolution of the rabies virus. *PLOS Pathogens*, *20*(11), e1012740. https://doi.org/10.1371/journal.ppat.1012740

Escobar, L. E., Velasco-Villa, A., Satheshkumar, P. S., Nakazawa, Y. and Van de Vuurst, P. (2023). Revealing the complexity of vampire bat rabies ”spillover transmission”. *Infectious Diseases of Poverty*, *12*(01), 102-110.

Fernandes, M. E. S., Carnieli, P., Gregório, A. N. F., Kawai, J. G. C., Oliveira, R. N., Almeida, L. L., Rosa, J. C. A., Ferreira, J. C., Traverso, S. D., Roehe, P. M. and Batista, H. B. C. R. (2020). Phylogenetic analysis of rabies viruses isolated from cattle in southern Brazil. *Virus Genes*, *56*(2), 209-216. https://doi.org/10.1007/s11262-020-01730-y

Fornace, K., Liverani, M., Rushton, J. and Coker, R. (2013). Effects of land-use changes and agricultural practices on the emergence and reemergence of human viral diseases. *Viral infections and global change*, 133-149. https://doi.org/10.1002/9781118297469.ch8

Harsha, P. K., Ranganayaki, S., Yale, G., Dey, G., Mangalaparthi, K. K., Yarlagadda, A., Chandrasekhar Sagar, B. K., Mahadevan, A., Srinivas Bharath, M. M. and Mani, R. S. (2022). Mitochondrial dysfunction in rabies virus-infected human and canine brains. *Neurochemical Research*, *47*(6), 1610-1636. https://doi.org/10.1007/s11064-022-03556-6

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., and Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*(2), 518-522. https://doi.org/10.1093/molbev/msx281

Holmes, E. C. and Harvey, E. H. (2023). *The diversity, evolution and emergence of rabies virus in the Americas. In history of rabies in the Americas:* From the pre-columbian to the present, Vol. Insights to specific cross-cutting aspects of the disease in the Americas (pp. 43-59). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-25052-1_3

Islam, M. M., Naeem, A., Mshelbwala, P. P., Dutta, P., Hassan, M. M., K. Elfadl, A., Kodama, C., Zughaier, S. M., Farag, E. and Bansal, D. (2025). Epidemiology, transmission dynamics, risk factors, and future directions of rabies in the Arabian Peninsula using one health approach: a review. *European Journal of Public Health*, *35*(Supplement_1), i14–i22. https://doi.org/10.1093/eurpub/ckae164

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. and Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*(6), 587-589. https://doi.org/10.1038/nmeth.4285

Katoh, K. and Standley, D. M. (2013). MAFFT Multiple sequence alignment software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772-780. https://doi.org/10.1093/molbev/mst010

Khairullah, A., Kurniawan, S., Hasib, A., Silaen, O., Widodo, A., Effendi, M., Ramandinianto, S., Moses, I., Riwu, K. and Yanestria, S. (2023). Tracking lethal threat: In-depth review of rabies. *Open Veterinary Journal*, *13*(11), 1385. https://doi.org/10.5455/OVJ.2023.v13.i11.1

Letunic, I. and Bork, P. (2024). Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research*, *52*(W1), W78-W82. https://doi.org/10.1093/nar/gkae268

Li, G., Chen, X., Li, X., Liang, Y., Li, X., Liang, W., Yan, Z., Wang, Y., Wang, Y., Luo, J., Guo, X.-F. and Zhu, X.-T. (2023). Analyzing the evolution and host adaptation of the rabies virus from the perspective of codon usage Bias. *Transboundary and Emerging Diseases*, 1-17. https://doi.org/10.1155/2023/4667253

Manjunatha, K. G., Chandrahasa, C., Akshay, S. D., Sannejal, A. D., Vittal, R., Kavitha, G., Goh, K. W., Isloor, S and Devegowda, D. (2023). Comprehensive update on rabies: A neglected zoonotic disease of public health concern. *Progress In Microbes & Molecular Biology*, *6*(1). https://doi.org/10.36877/pmmb.a0000385

Mantari Torpoco, C. R., Berrocal Huallpa, A. M., Espinoza-Culupú, A. O. and López-Ingunza, R. L. (2019). Molecular characterization of the rabies virus' nucleoprotein in dogs from Arequipa, Peru. *Revista Peruana de Medicina Experimental y Salud Publica*, *36*(1), 46-53. https://doi.org/10.17843/rpmesp.2019.361.3938

Markbordee, B., Cabic, A. G. B., Iamohbhars, N., Shiwa-Sudo, N., Kimitsuki, K., Espino, M. J. M., Nacion, L. B., Manalo, D. L., Inoue, S. and Park, C. H. (2024). Histopathological and immunohistochemical examination of the brains of rabid dogs in the Philippines. *Journal of Veterinary Medical Science*, *86*(12), 0224-0249. https://doi.org/10.1292/jvms.24-0249

Meganck, R. M. and Baric, R. S. (2021). Developing therapeutic approaches for twenty-first-century emerging infectious viral diseases. *Nature Medicine*, *27*(3), 401-410. https://doi.org/10.1038/s41591-021-01282-0

Meske, M., Fanelli, A., Rocha, F., Awada, L., Soto, P. C., Mapitse, N. and Tizzani, P. (2021). Evolution of rabies in South America and inter-species dynamics (2009-2018). *Tropical Medicine and Infectious Disease*, *6*(2), 98. https://doi.org/10.3390/tropicalmed6020098

Nadal, D., Hampson, K., Lembo, T., Rodrigues, R., Vanak, A. T. and Cleaveland, S. (2022). Where rabies is not a disease. Bridging healthworlds to improve mutual understanding and prevention of rabies. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets. 2022.867266

Nahata, K. D., Bollen, N., Gill, M. S., Layan, M., Bourhy, H., Dellicour, S. and Baele, G. (2021). On the use of phylogeographic inference to infer the dispersal history of rabies virus: a review study. *Viruses*, *13*(8), 1628. https://doi.org/10.3390/v13081628

Nguyen, L. T., Schmidt, H. A., von Haeseler, A. and Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268-274. https://doi.org/10.1093/molbev/msu300

Omodo, M., Ar Gouilh, M., Mwiine, F. N., Okurut, A. R. A., Nantima, N., Namatovu, A., Nakanjako, M. F., Isingoma, E., Arinaitwe, E., Esau, M., Kyazze, S., Bahati, M., Mayanja, F., Bagonza, P., Urri, R. A., Lovincer, M. N., Nabatta, E., Kidega, E., Ayebazibwe, C., Nakanjako, G., Sserugga, J., Ndumu, D. B., Mwebw, R., Mugabi, K., Gonzalez, J.-P. and Sekamatte, M. (2020). Rabies in Uganda: rabies knowledge, attitude and practice and molecular characterization of circulating virus strains. *BMC Infectious Diseases*, *20*(1), 200. https://doi.org/10.1186/s12879-020-4934-y

Orlando, S. A., Panchana, V. F., Calderón, J. L., Muñoz, O. S., Campos, D. N., Torres-Lasso, P. R., Arcos, F. J. and Quentin, E. (2019). Risk factors associated with attacks of hematophagous bats (*Desmodus rotundus*) on cattle in Ecuador. *Vector-Borne and Zoonotic Diseases*, *19*(6), 407-413. https://doi.org/10.1089/vbz.2017.2247

Ortiz, I., Burbano, A., Villarreal, V., Santiana, I., Vargas, J. y Vizcaíno Cabezas, D. (2017). Manual de procedimientos para la prevención y control de rabia bovina en Ecuador -Programa Nacional Sanitario de Prevención y Control de Rabia Bovina.

Parija, S. C. (2023). Introduction to viruses. In: Textbook of microbiology and immunology (pp. 687-713). *Springer Nature Singapore*. https://doi.org/10.1007/978-981-19-3315-8_48

Qin, S., Volokhov, D., Rodionova, E., Wirblich, C., Schnell, M. J., Chizhikov, V. and Dabrazhynetskaya, A. (2019). A new recombinant rabies virus expressing a green fluorescent protein: A novel and fast approach to quantify virus neutralizing antibodies. *Biologicals*, *59*, 56-61. https://doi.org/10.1016/j.biologicals.2019.03.002

Rupprecht, C. E., Mshelbwala, P. P., Reeves, R. G. and Kuzmin, I. V. (2023). Rabies in a postpandemic world: resilient reservoirs, redoubtable riposte, recurrent roadblocks, and resolute recidivism. *Animal Diseases*, *3*(1), 15. https://doi.org/10.1186/s44149-023-00078-8

Scheffer, K. C., Iamamoto, K., Asano, K. M., Mori, E., Estevez Garcia, A. I., Achkar, S. M. y Fahl, W. O. (2014). Murciélagos hematófagos como reservorios de la rabia. *Revista Peruana de Medicina Experimental y Salud P*ública, *31*, 302-309.

Schreiber, M. D. S. and Fachinetto, J. M. (2022). Phylogenetic relationship of rabies virus (*Rabies lyssavirus*) in two different host species. Research Square, https://doi.org/10.21203/rs.3.rs-2207887/v1

Soler-Tovar, D. and Escobar, L. E. (2025). Rabies transmitted from vampires to cattle: An overview. *PloS one*, *20*(1), e0317214. https://doi.org/10.1371/journal.pone.0317214

Talavera, G. and Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*(4), 564-577. https://doi.org/10.1080/10635150701472164

Vizcaíno Cabezas, D., Vargas Estrella, J., Yánez Ortiz, I., Burbano Enríquez, A., Villarreal Benavides, V., Santiana Jara, I. y Espinosa Salme, C. (2016). *Manual de procedimientos para la prevención y control de rabia bovina en el Ecuador* (Issue 593).

Wallace, R. M. and Muller, T. (2024). Challenges and opportunities for the next miles in global rabies control. *Revue Scientifique et Technique de l'OIE*, Special Edition, 74-82. https://doi.org/10.20506/rst.SE.3561

Wardhani, S. W., Wongsakul, B., Kasantikul, T., Piewbang, C. and Techangamsuwan, S. (2021). Molecular and pathological investigations of selected viral neuropathogens in rabies-negative brains of cats and dogs revealed neurotropism of carnivore Protoparvovirus-1. *Frontiers in Veterinary Science*, *8*. https://doi.org/10.3389/fvets.2021.710701

World Organization for Animal Health (WOAH). (2023). *Chapter 3.1.18 – Rabies (infection with rabies virus and other lyssaviruses).* https://talk.ictvonline.org/ictv-reports/ictv_online_report/

Declaration of authorship according to CRediT

Jorge Rodrigo Espinoza Samaniego: conceptualization, methodology, investigation, formal analysis, writing–original draft, writing–review and editing. **Edilberto Chacón Marcheco:** conceptualization, methodology, formal analysis, writing–original draft, writing–review and editing. **Luis Alfredo Mena Miño:** methodology, formal analysis, writing–original draft, writing–review and editing. **Rubén Alexander Maldonado Orbe:** methodology, formal analysis, writing–original draft, writing–review and editing.