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REVIEW

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Rabies in Costa Rica – Next Steps Towards Controlling Bat-Borne Rabies After its Elimination in Dogs

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Rabies is an acute, progressive encephalitis caused by a lyssavirus, with the highest case fatality of any conventional infectious disease. More than 17 different lyssaviruses have been described, but rabies virus is the most widely distributed and important member of the genus. Globally, tens of thousands of human fatalities still occur each year. Although all mammals are susceptible, most human fatalities are caused by the bites of rabid dogs, within lesser developed countries. A global plan envisions the elimination of human rabies cases caused via dogs by the year 2030. The combination of prophylaxis of exposed humans and mass vaccination of dogs is an essential strategy for such success. Regionally, the Americas are well on the way to meet this goal. As one example of achievement, Costa Rica, a small country within Central America, reported the last autochthonous case of human rabies transmitted by a dog at the end of the 1970s. Today, rabies virus transmitted by the common vampire bat, *Desmodus rotundus*, as well as other wildlife, remains a major concern for humans, livestock, and other animals throughout the region. This review summarizes the historical occurrence of dog rabies and its elimination in Costa Rica, describes the current occurrence of the disease with a particular focus upon affected livestock, discusses the ecology of the vampire bat as the primary reservoir relevant to management, details the clinical characteristics of recent human rabies cases, and provides suggestions for resolution of global challenges posed by this zoonosis within a One Health context.

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Abbreviations: PEP, postexposure prophylaxis; PAHO, Pan American Health Organization; ENSO, El Niño Southern Oscillation; mAbs, monoclonal antibodies; anti-RNP, anti-ribonucleoprotein mAbs; N, nucleocapsid; G, glycoprotein; MRCA, most recent common ancestor.

Keywords: Costa Rica, lyssavirus, One Health, prophylaxis, rabies, vampire bat, vaccination, zoonosis

INTRODUCTION

"If you know the enemy and know yourself, you need not fear the result of a hundred battles. If you know yourself but not the enemy, for every victory gained, you will also suffer a defeat. If you know neither the enemy nor yourself, you will succumb in every battle [1]." As described in *The Art of War: Sun Zi's Military Methods*, to master any enemy you must achieve considerable internal and external familiarity, a classical adage relevant to warfare and comparable to disease management, particularly zoonoses [2]. As a classical zoonosis, rabies is an acute, progressive encephalitis caused by a lyssavirus, with the highest case fatality of any infectious disease [3]. Transmission is direct, after animal bites and transdermal inoculation of virus-laden saliva [4]. Tens of thousands of human fatalities still occur in the world each year, with the majority caused by the bites of rabid dogs in developing countries [5]. Unlike zoonoses that involve only a few definitive hosts, all mammals are susceptible to the rabies virus [6]. Despite being a well-known pathogen for which sensitive and specific diagnostics and effective vaccines are available, rabies is not a candidate for eradication [7]. Besides prevention in humans by postexposure prophylaxis (PEP), canine rabies can be eliminated by mass immunization and wildlife rabies can be controlled by oral vaccination [8]. International organizations (FAO, OIE, and WHO) support the concept of the worldwide elimination of human rabies caused via dogs by the year 2030 [9]. Within the Americas, the Pan American Health Organization (PAHO) has been instrumental in working with member countries to achieve this goal [10,11]. In Costa Rica, a small tropical country in Central America (ie, approximately 51,000 km^2; nearly 5 million inhabitants, bordered by Nicaragua to the north, Panama to the south, the Pacific Ocean to the west, and the Caribbean Sea to the east (Figure 1), no indigenous human rabies cases caused by dogs have been reported since the 1970s, achieving dog rabies elimination goals decades in advance. However, elimination of canine rabies does not remove all risk, because of the perpetuation of the disease among bats. Bats rabies control is still an unresolved puzzle, and to resolve it, we need to understand how the virus strains are spread in the country. The objectives of this review are to describe the history of rabies in Costa Rica, the currents role of bats as rabies hosts, the vampire bat as the primary reservoir host, detail extant viral variants, reports on recent human cases, and discuss future challenges in rabies management.

HISTORY OF RABIES

A Brief History of Canine Rabies

Rabies was well recognized and widespread throughout Europe at the height of global "discovery" during the 15th through 17th centuries. During the 18th century, rabies was reported in the Americas from anecdotal colonial reports. Using phylogenetic-dating analyses, it was estimated that cosmopolitan canine rabies was introduced to the Americas between 1642-1782 [12-14]. The only available reports of dog rabies outbreaks in Costa Rica were recorded during 1714, 1721, and 1763 [15]. In 1763, acting Governor Joseph Antonio de Oreamuno pointed out the need to repress dogs, especially from the towns, ordering that they be hanged. Sentiments were reflected in statements by the Governor, who stated: "… for how much time has been experienced in this Province for more than two years, from my command, general Contagion of the Ravia of the Dogs, and that in time they have bitten some people who have died raging pitifully, without finding any remedy to free their lives, and that every day with the multitude of Dogs like this in this city... [16]."

Preceding the use of vaccination, measures focused upon the killing of stray dogs. No further cases of canine rabies were reported for 189 years. Geographic features of this rather narrow country, hemmed in by oceans on two sides and marked by volcanic mountain ranges, may have assisted health control at the borders and limited contact among stray dog populations. A factor for eventual rabies reappearance might have been the opening of the Inter-American highway in 1956 [15,17]. By comparison, African studies reveal fine connectivity between disease events by roads from source to sink localities, illustrating a role for human movement in disease dispersal [18,19]. After reintroduction in 1956, two children died, one from Liberia, in the north part next to the border with Nicaragua, and another from Cañas, 51 km away in the Guanacaste province – both bitten by rabid dogs. In 1958, cases of canine rabies were reported in two dogs, a cat, and five cattle. By 1959, canine rabies was again thought to be eliminated. However, in 1960, cases reappeared in La Cruz (near the Nicaraguan border). This outbreak was controlled only after substantial measures by the Ministry of Public Health, including dog vaccination and elimination of stray dogs. Five years later, a new epizootic began in Liberia, with 24 cases. Over the next two years, rabies presented as a serious outbreak, encompassing the entire northern and central area of Guanacaste, the most densely populated area of the province [17]. In 1966, the first case was reported in Puntarenas (a neighboring province to Guanacaste), and 34 animals died. In 1967, rabies reached the capital city, San José, and continued to Turrialba and San Isidro de El General in the southern part of San José province, where 329 animals succumbed [15]. Concomitantly, a dog extermination campaign was conducted, and more than 4,000 persons were vaccinated [15]. While vaccine campaigns were established for outbreak control, from January to July 1968, 399 animals

Figure 1. **Primitive historical rendering of a map of Costa Rica, relative to other Central American countries, during the Spanish colonial period.** Cassini, GM. *Nuovo atlante geografico universale delineate suelle ultime osservazioni*. Rome, 1798 edition.

died and from November 1967 to September 1970, six children succumbed in the National Children's Hospital [15,17]. The vaccine used was probably an avian embryo vaccine. This vaccine was applied in outbreaks since 1957, producing high antibody levels and fewer neurological complications in human beings, in comparison with nervous tissue vaccine (today, all vaccines contain inactivated virus and are produced in Vero cells) [17]. In response, the Ministry of Public Health, through its Department of Zoonosis, carried out intensive campaigns, which consisted of three primary points: free vaccination of domestic dogs; the killing of stray dogs; and vaccination of exposed people. Other measures included the closing of the transit of small animals at the border between Panama and Costa Rica and the establishment of an agreement between Costa Rica and Nicaragua to control the movement of dogs in the border area [19,20].

In 1987, after 17 years without an autochthonous case, two rabid dogs were imported in Salinas, La Cruz, Guanacaste, at a place near the Nicaraguan border, and 68 dogs were killed as part of contingency actions. The conclusion that the case was imported was based on the epidemiological nexus, considering the proximity to the border with Nicaragua, the absence of cases of rabies in dogs in Costa Rica for several years, and that the infected dogs were not known to the inhabitants of the area. With concern over risks posed by importation and translocation, the Ministry of Health vaccinated 20,000 dogs along the Nicaraguan border [21]. Thereafter, no additional cases of canine rabies were reported in the country to date

Table 1. Animals submitted in Costa Rica for rabies diagnosis to the SENASA laboratory, 1986 to 2020.

Animal	Positive	Negative	Total samples	Percentage Positive
Cattle	193	2,083	2,276	8%
Dogs	4	169	173	2%
Bats	3	79	82	4%
Horses	4	63	67	6%
Non-human primates	0	39	39	0%
Rodents	0	36	36	0%
Cats (including wild felids)	0	29	29	0%
Small ruminants	0	18	18	0%
Raccoons (*Procyon lotor*)	0	15	15	0%
Coatis (*Nasua narica*)	0	13	13	0%
Swine	1	3	4	25%
Sloths (*Choloepus hoffmani, Bradypus variegatus*)	0	7	7	0%
Opossums	1	1	2	50%
Other wildlife	0	10	10	0%
Not specified	6	13	19	32%
Total	**212**	**2,578**	**2,790**	**8%**

(Table 1).

A SHIFT IN THE EPIDEMIOLOGICAL PARADIGM FROM CANINE RABIES TO BAT RABIES CONTROL

A Short History of Rabies Transmitted by D. rotundus

Significantly, as human rabies cases caused by dogs over the last two decades decreased throughout Latin America, an apparent transition occurred as fatalities became associated with wildlife, especially due to rabies virus transmitted by the hematophagous vampire bat, *Desmodus rotundus*, distributed broadly from Mexico to Argentina [22]. A similar appreciation occurred in Costa Rica during the 1980s, with large outbreaks among livestock, after the disruption of canine rabies the decade before [23]. Regardless of the temporal 20th-century recognition and later apparent epidemiological trend throughout the 21st century, bat rabies is not a recent phenomenon. Rather, the rabies virus was likely affecting bats long before colonization. Anthropogenic changes exacerbated transmission dynamics drastically [24-27].

If "bat rabies" preceded the Columbian exchange, historical cases should support its existence. Multiple records allude to this: in 1514, Spanish colonists reported that soldiers died after bat bites in Panama; animals and troops were bitten in 1527 in the Yucatan; and bitten livestock succumbed in 1576 in Guatemala, 1745 in Ecuador, and 1858 in Trinidad [28]. There are several reasons for the extremely limited information on this pathogen in the Americas before European arrival and subsequent introduction of canine rabies. Parts of Mesoamerica were sparsely populated and only oral histories were the norm. The Maya was the only pre-Columbian civilization to develop independently a sophisticated writing system. Mayans wrote numerous folding books (ie, "codices"), but most were destroyed during attempts to convert the inhabitants to Christianity. Whether the few surviving fragments actually describe such a disease associated with bats remains uncertain [29]. Additionally, before Spanish arrival to Central America, there was no common large-bodied prey. Vampire bat populations were smaller, as would have been rabies incidence. Only after colonization and livestock introduction would a veritably unlimited food supply occur, as forests were cleared for grazing, disrupting ecosystems, and providing exponential growth in bat populations [28,29]. Finally, livestock deaths on range by vampire bats during the colonial period were probably few compared to alternate mortality sources, such as other diseases, trauma, predation, etc., and not necessarily linked to rabies by these European neo-agricultural settlers, perhaps due in part to different clinical manifestations of rabies virus variants associated with vampire bats (ie, usually ascending paralysis) in contrast to dog rabies virus infection (ie, more aggressive/violent), post-18th century [28]. The relationship between vampire bat bites and rabies was not established firmly

until the first quarter of the 20th century, in Brazil [28]. Most Latin American countries corroborated this widely held suspicion over ensuing decades, such as notification of bovine paralytic rabies in Costa Rica in 1952.

Bats as Lyssavirus Reservoirs

Currently, more than 17 recognized or putative lyssavirus species are described [30]. Bats are the known or suspected reservoir for most of these lyssavirus species [4-8,31]. Curiously, rabies virus perpetuates among bats only in the Americas (in contrast to distinct bat lyssaviruses in Africa, Australia, and Eurasia), whereas other specific rabies viruses are maintained among carnivores [14,25].

Throughout the Americas, rabies occurs in several bat genera [32]. Within North America, this includes varied insect-eating bat genera including: *Eptesicus, Lasiurus, Lasionycteris, Myotis, Parastrellus,* and *Tadarida* [33]. In Latin America, six of nine families of Neotropical bats contain representatives that have been diagnosed with rabies virus, implying additional bat species are potential reservoirs. Nevertheless, current reports of rabies in taxa other than *D. rotundus* are relatively few [34]. In Costa Rica, as elsewhere throughout Central America, *D. rotundus* is considered the main rabies virus reservoir, even though other taxa of non-hematophagous bats have been recorded [35,36]. Unlike in North America, documentation of wild carnivores as important reservoirs has not been described in Costa Rica. Occasionally, incidents of spillover infection do surface, such as a human case transmitted apparently by a rabid raccoon in El Salvador in 2001, but many are only isolated records with high recall bias [22,36]. This is a conundrum, as tropical countries such as Costa Rica are considered "megadiverse," with 114 bat species and over 260 carnivore species [37,38]. While canine rabies, considered as any virus rabies circulating in dogs, but also any dog-derived rabies variant that has spilled over and establishes within any wild-carnivore or mammal species [5], has been eliminated in most countries, according to PAHO, circulation continues in Bolivia, Guatemala, Haiti, and the Dominican Republic, while Argentina, Brazil, Cuba, Peru, and Venezuela report isolated cases that make up 10% of the total [39-41]. Thus, more widespread surveillance (not only in livestock but also more systematic surveillance in wild mammals) and greater characterization of rabies viruses is fundamental to zoonosis control, given the major impact that canine rabies has upon public health, veterinary medicine, and conservation biology. The high likelihood of unrecognized wildlife reservoirs and the opportunities for potential host shifts with subsequent emergence in a high biodiversity country such as Costa Rica is to be expected and warrants further study [42].

The Influence of Climate on Vampire Bats and Disease Occurrence

As in other zoonoses, rabies virus dynamics are closely related to reservoir ecology. Host distribution, foraging activities, and social interactions are central facets to viral transmission and disease dispersal. As with other heterotherms, climate plays a substantial role in the behavior of *D. rotundus*. For example, in southern Brazil, vampire bats live in smaller roosts without a buffered climate during the warmer, rainy season, but concentrate into larger caves with a more stable climate in the drier, cooler season [43]. Since bats migrate to warmer areas during cooler periods, the temperature is one critical climatic factor in seasonal behavior. Physiologically for vampire bats, the 10°C minimal winter isotherm mirrors their distribution [44].

Vampire bats are also influenced by humidity, losing mass "…due to high rates of evaporative water loss, which may explain why vampires normally frequent humid roosts… [45]." In Costa Rica, vampire bats had a longer daily foraging period during the dry season and more bats foraged per day compared to the wet season [46]. The time of attachment to prey was also longer during the dry season (17 minutes versus 9 minutes), and more bats were attached to a single prey animal than in the rainy season. A study on the influence of the El Niño Southern Oscillation (ENSO) in Costa Rica on rabies cases in cattle showed that outbreak occurrence and size were not directly associated. However, both ENSO phases and rabies outbreaks showed oscillations of a similar 5-year period. Outbreak numbers decreased with rainfall, but increased with temperature, as did cattle mortality. Further efforts are needed to explain mechanisms underlying this relationship between weather alterations and cattle rabies outbreaks, such as an influence on vampire bat abundance [47]. Disagreement persists on the seasonality of *D. rotundus* births. Earlier publications cite an increase in pregnancies during the rainy season in Costa Rica [48,49]. Others suggest vampire bats breed throughout the year in Costa Rica, which seems to be the case for Central America in general [37,50,51].

Diversity of mammalian hosts suggests a high variation and evolution rate among viral pathogens. A study done with hundreds of rabies virus isolates collected from bats throughout the Americas revealed that viral evolutionary rates were labile following historical jumps between bat species and nearly four times faster in tropical and subtropical bats compared to temperate species. The association between geography and viral evolution could not be explained by host metabolism, phylogeny, or variable selection pressures, and instead appeared to be a consequence of reduced seasonality in bat activity and virus transmission associated with climate [52].

Antigenic and Molecular Characterization of Rabies Viruses

For agents once thought to be indistinguishable, hybridoma technology and generation of monoclonal antibodies (mAbs) allowed comparative antigenic differentiation of viral variants during the late 1970s [53]. Panels of mAbs were used to differentiate laboratory strains from field isolates obtained from bats, dogs, foxes, raccoons, and other species worldwide, including Latin America [54-60]. Commonly used panels employed anti-ribonucleoprotein (anti-RNP) mAbs, specific to viral nucleocapsid (N) epitopes.

From 1987 to 1992, the Centers for Disease Control and Prevention (CDC) and the Pan American Zoonoses Center (CEPANZO-OPS) analyzed isolates from throughout the Americas, establishing a panel of eight anti-RNP mAbs for regional characterization of common rabies viruses [61]. This panel distinguished 11 major variants: dog/mongoose (V1); dog (V2); *D. rotundus* (V3, V5 & V11); *T. brasilensis* (V4 & V9); *Lasiurus cinereus* (V6); Arizona grey fox (V7); and skunk (V8 & V11) [49-51].

Over the past 20 years, the use of mAb panels in most Latin American laboratories allowed delineation of the geographical distribution of antigenic variants associated with different reservoirs, the discovery of new variants, and identification of the likely source of infection in human and domestic animal cases when the history of exposure was not clear or was absent [61-69]. However, as regional introspection increased, panels of more than 20 mAbs were necessary to distinguish "atypical" variants, equating with an increased level of laboratory scrutiny and a higher likelihood for error [70]. Gradually accumulating data demonstrated the need for additional molecular tools, considering that mAbs did not provide the same type of information nor variant resolution compared to nucleotide sequencing. Although mAb typing gave a rapid but coarse-grained insight to potential identity, nucleotide sequencing provided a much better resolution for antigenic variants [64,71-74]. The use of such molecular techniques identified synonymous and non-synonymous mutations, as well as detailed information about evolutionary relationships, temporal and spatial dynamics, and genetic similarities among isolates [75]. For these reasons, instead of implementing antigenic typing with mAb, Costa Rica opted to implement the classification of variants using molecular techniques as of 2013, allowing opportunities to determine how rabies virus is spread by *D. rotundus*.

Either the inner viral N or the outer glycoprotein (G) gene sequences are used routinely in molecular studies of rabies viruses because they render consistent and informative data [75-77]. Given conserved regions, the N gene is widely used for diagnostics in RT-PCR, molecular epidemiological, and phylogenetic studies [65,69,78-89]. Such work translates into a considerable amount of available partial and complete N gene sequences available for diverse comparative studies. For example, phylogenetic investigations reveal not only how bats rabies virus spreads into countries, but also by sequencing nuclear and mitochondrial DNA of *D. rotundus*, provide insight on the viral spread from colony to colony, with relevant implications for control [90].

Vampire Bats and the Perpetuation of Rabies Virus

Throughout Mexico to Argentina, Chile, and Uruguay, from arid to humid parts of the tropics and subtropics, the common vampire bat*, D. rotundus* (grouped taxonomically within the *Phyllostomidae*, a highly diverse family that includes neotropical taxa that feed upon arthropods, small vertebrates, fruit, nectar, and pollen and includes two other species of blood-feeding bats), has a major economic impact upon the livestock industry [91]. Some of this burden is attributed to deaths from rabies virus transmission during obligate feeding and generating enormous production losses. Preferred prey is livestock (ie, cattle, small ruminants, horses, pigs, etc.) but they will feed on other animals (including birds and reptiles) and humans [91,92-100]. Vampire bats will die of starvation if unsuccessful in procuring a blood meal for approximately three consecutive nights, which keeps bats actively searching for prey to meet costly energetic needs associated with flight and reproduction [44]. In cattle-raising areas, vampire bat populations are almost twice the expected number in an untouched natural ecosystem [101].

Vampire bats are restricted to warm climates, found from 0 to 2400 meters of altitude [91]. There are no recent reports of *D. rotundus* on Baja California (Mexico) or in the Caribbean, except on three islands located close to the mainland: Trinidad and Tobago and Margarita [89,102,103]. These bats do not hibernate or estivate and live in social groups from 20 to 100 individuals, although larger colonies have been reported. Typical roosts include caves and hollow trees. In addition to their natural diurnal roost, they can be found in fabricated structures such as bridges, tunnels, or wells. Besides regular intraspecific transmission through bites or even the blood-sharing process, on which bats share their meal with others, opportunities for spillover infection to other bat species may occur, as they are known to roost with approximately 45 other bat species [91]. In Costa Rica, *D. rotundus* may share roosts with bats belonging to several genera of insect, nectar, and fruit-eating bats, including *Micronycteris, Glossophaga, Carollia, Sturnira, Saccopteryx,* and *Artibeus* [49].

Contrasting to the period of canine rabies before the 1980s, nowadays most rabies cases are diagnosed among livestock, perpetrated by infected vampire bats [23]. To

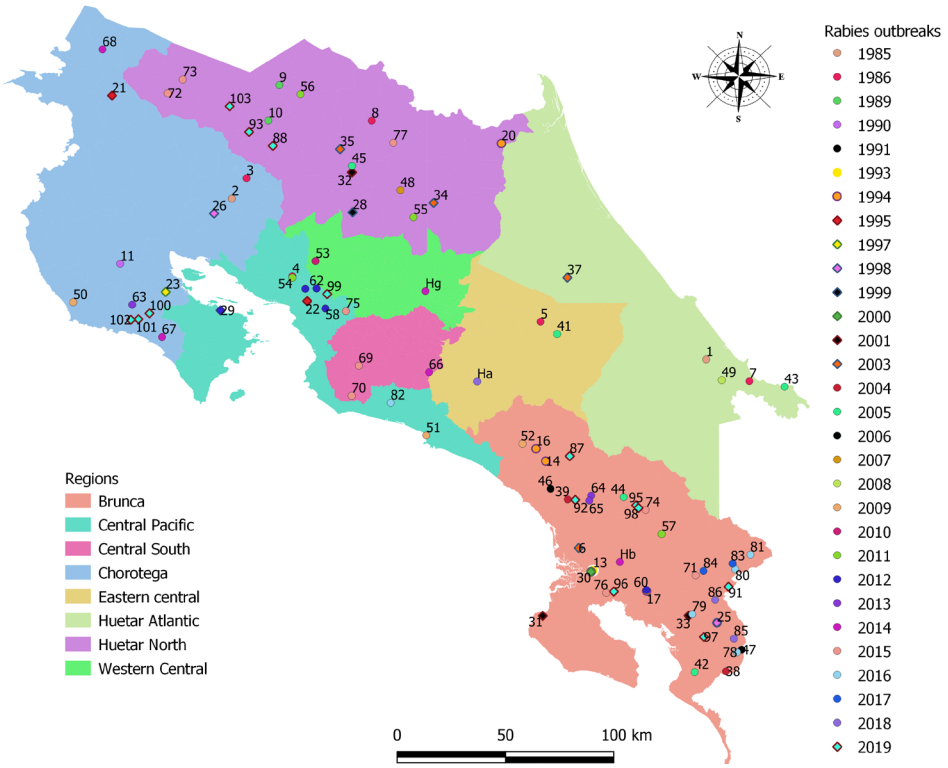

Figure 2. Animal rabies outbreaks in Costa Rica. The number of animal rabies outbreaks registered throughout Costa Rica, from 1985 to October 2020. Ha=Human adult, Hb=human boy, and Hg=human girl.

control rabies transmitted by bats, there is a need for molecular characterization of bat-associated viruses, to assess which other bat species could be important hosts.

Since 1985, more than 80 outbreaks have been registered, with more than 800 head of rabid livestock (Figure 2). Rabies is enzootic, as shown through the distribution by regional outbreaks, from 1985 to 2019 (Figure 3). Cattle outbreaks are defined operationally as: "A laboratory-confirmed event of bovine rabies, in which one or more cattle were infected and died within a 5 km radius and/or within 6 months from the result date of the first laboratory-confirmed case [23]." The foraging behavior of *D. rotundus* depends on food availability, normally restricted to a radius of approximately 5-8 km around their diurnal roost [104]. In support of this observation, a study conducted in Costa Rica in 1968 found that vampire bats can locate their original roost from as far away as 16km, but not 29 km [46]. Based on these data, we hypothesized that all cases in Costa Rica from the same region should be related genetically. Using the available surveillance data, comprising 31 rabies virus N gene sequences from 2004 until 2015, we constructed a phylogenetic analysis (Figure 4). Of the 31 sequences, 30 correspond to the *D. rotundus* variant, 29 were isolated in cattle, one was from a child (LSE0304-14), and one was associated with *T. brasiliensis* (LSE0582-14), detected in a bovine host [23]. The phylogenetic tree can be divided into three clusters (Figure 4). The time of the most recent common ancestor (TMRCA) of cluster 1 was 1994. This set was composed of two related viral sequences, one from the Brunca region collected in February 2006, and the other from the Huetar Norte Region 1 year later. These outbreaks were about 151.5 km apart. The same observation pertained to other clusters (Figure 4). The location diffusion rate of the rabies viruses associated with these sequences was approximately 11.7 km/year, in concordance with the vampire bat movement data. The 95% Highest Posterior Density (HPD) Interval (5.1-18.6 km/year) also corresponded with the expected range of the bat from the roost to the food source. Additional information on the methodology can be found in Box 1.

How are two similar viral sequences, phylogenetically linked, obtained from outbreaks a year apart, but separated by more than 150 km, if an infected host flies on average only less than 29 km? A study done in Brazil demonstrated that *D. rotundus* establishes a complex society formed by a harem (mostly by females and pups), bachelors (young males), and overnight roosts (used as a temporary resting stop during foraging and digestion). The harem and overnight roosts were more associated with rabies virus outbreaks in livestock [105]. Vampire bats are sexually mature at 9 months and ges-

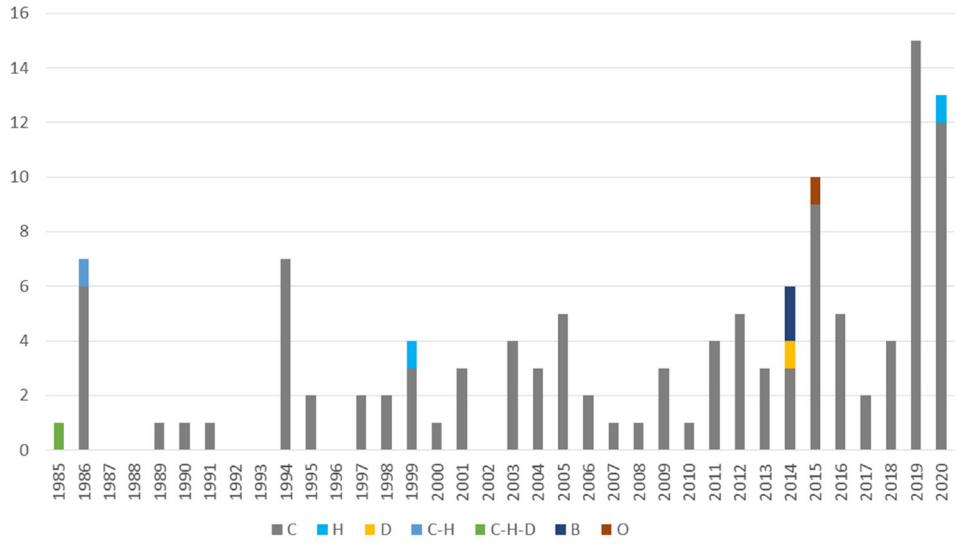

Figure 3. **Cases of rabies in cattle**. The distribution of rabies virus outbreaks in livestock, from 1985 to 2019, is displayed by Costa Rican regions.

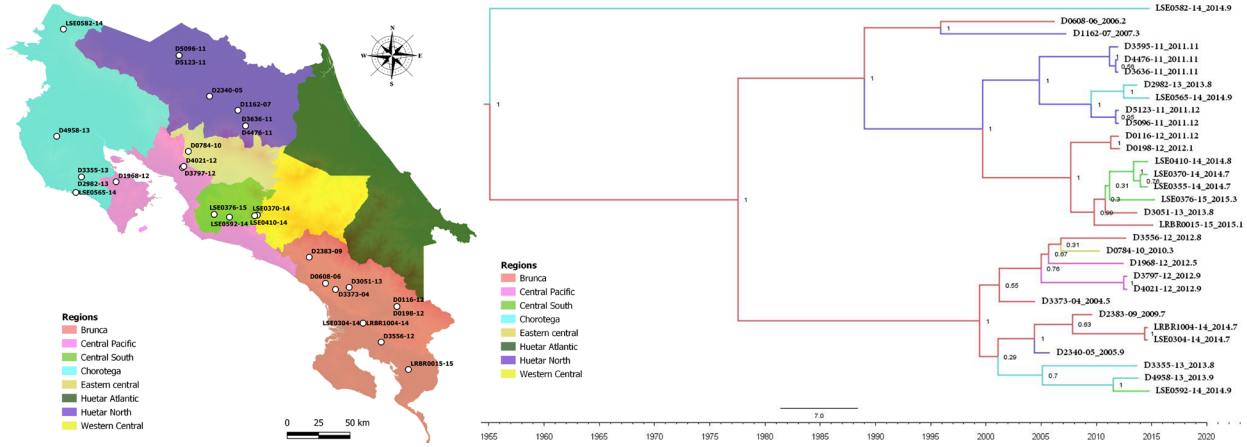

Figure 4. **Phylogeography of rabies viruses in Costa Rica**. A complete N gene sequence tree of 31 isolates, with 100 million generations, sampled every 10,000 steps with a burn-in of 10% generations, was built using BEAST, (shown on the right and the regional distribution of the samples used in the phylogeographic tree is presented on the left). In the tree, posterior values are indicated on the nodes, and the terminal branches are colored according to the sample collection region, while the color of the internal branches represents the inferred origin (ie, region) of their parental nodes. The tips show the sequence name, including the year and month when the sample was collected.

tation lasts approximately 205-214 days. One new bat generation occurs around every 16 months. Upon sexual maturity, individuals leave natal colonies, forming new settlements approximately 20 km away. After a decade and approximately seven generations, one bat with a progenitor virus could be responsible figuratively for infection to a conspecific over an expanse of 150 km. Besides the spatio-temporal influence of reproduction and demographics, differential transmission by sex is also operative, with male bats responsible primarily for spread to females [90], and females to the offspring. Since juveniles rarely groom adults, they are less affected by culling by anticoagulant paste [105]. These juveniles set up new colonies, spreading rabies virus from one colony to a new one. Phylogenetically, Clade 1 is represented by two sequences, with the oldest MRCA (Figure 4). No

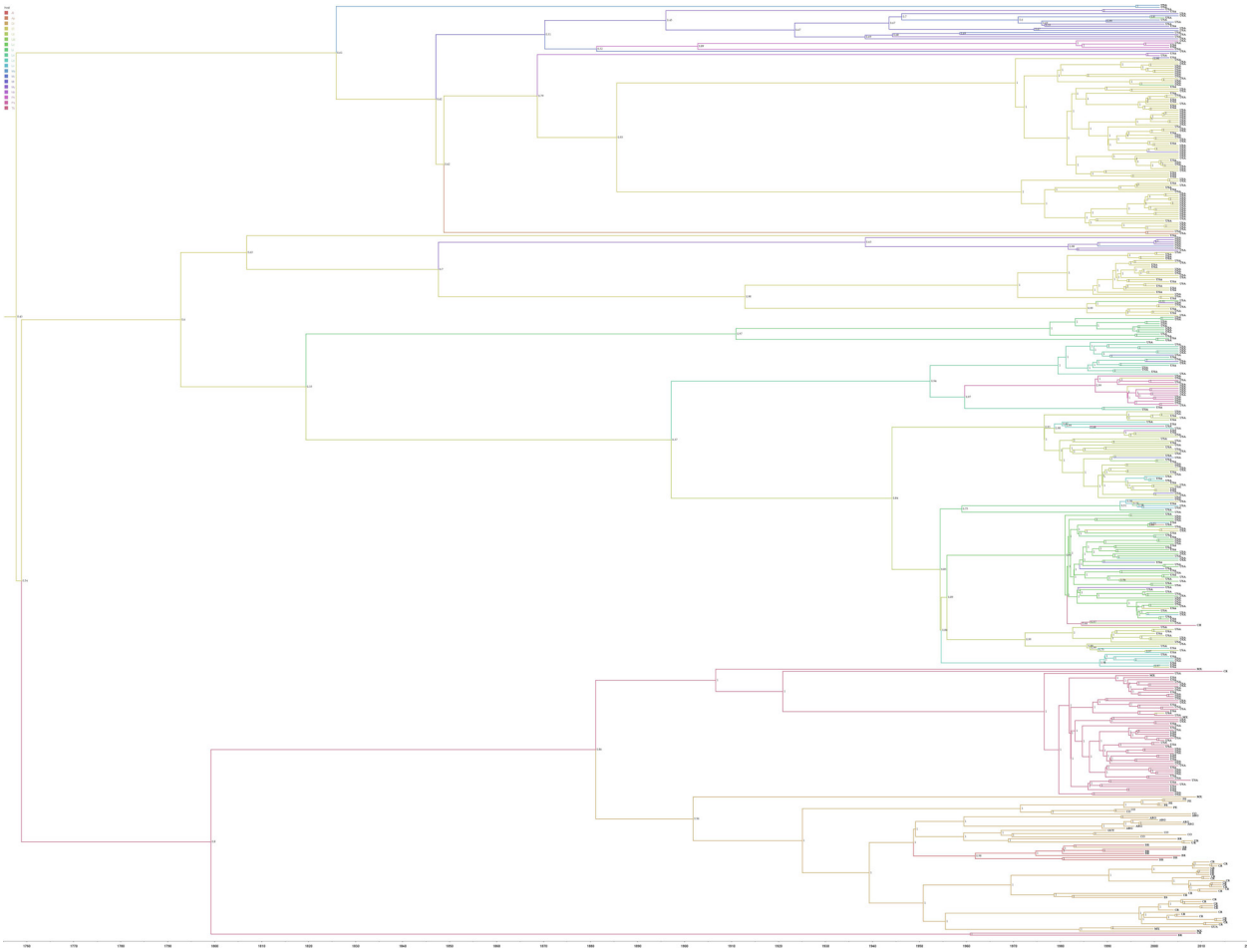

Figure 5. **The most recent common ancestor (MRCA) of rabies virus isolates from Costa Rica**. Thirty-one isolates from Costa Rica identify as CR, were compared to 414 nucleotide sequences of bat rabies viruses throughout the Americas. The tips show the rabies country source abbreviations (Argentina, ARG; Brazil, BR; Chile, CH; Colombia, CO; Costa Rica, CR; El Salvador, ES; Guatemala, GUA; Guyana, GUY; Mexico, Mx; Peru, PE, Uruguay, UR; United States, USA) while the abbreviations in the legend and the branch color represent the virus variants, (*Artibeus lituratus* (Al), *Antrozous pallidus* (Ap), *Desmodus rotundus* (Dr), *Eptesicus fuscus* (Ef), *Lasiurus borealis* (Lb), *Lasiurus blossevillii* (Lbl), *Lasiurus cinereus* (Lc), *Lasiurus intermedius* (Li), *Lasionycteris noctivagans* (Ln), *Lasiurus seminolus* (Ls), *Lasiurus xanthinus* (Lx), *Myotis austroriparious* (Ma), *Myotis californicus* (Mc), *Myotis evotis* (Ml), *Myotis yumanensis* (My), *Nycticeius humeralis* (Nh), *Parastrellus hesperus* (Ph), *Perimyotis subflavus* (Ps), and *Tadarida brasiliensis* (Tb), the node number depicts the probability of the MRCA host, the time scale is shown.

similar sequences are in this cluster (at least not recently), despite the lack of geographical restriction (Figure 4). Notwithstanding surveillance bias, this begs the question if the individual represented by the case 1162-07 died before viral transmission to a new generation, triggering a putative local viral extinction explaining the absence of rabies cases since 2008. Unfortunately, not only do viral lineages go extinct, but reintroduction occurs routinely within Costa Rica, and probably elsewhere in Central America [106]. This flow of new viral variants must be considered to effectively reduce the number of rabies cases (or even to think about eliminating rabies virus in the Central American region).

To infer the MRCA of 31 Costa Rican isolates, 414 rabies virus sequences in GenBank from 19 bat species in northern and southern countries were introduced to the analyses. The MRCA of the *T. brasiliensis*, Costa Rica sequence was 101 years (ie, 1914), with a 100% probability that the ancestor originated from Mexico (Figure 5). This observation suggested that not only *D. rotundus*

rabies virus reintroductions arise from other countries, but also from other bat species such as *T. brasiliensis* which unlike *D. rotundus* is a migratory species. A previous study detailed that the MRCA for all bat rabies virus lineages harkens back to approximately 1585 (95% HPD: 1493–1663) [107]. The MRCA of the *D. rotundus* Costa Rica sequences was 1930. The likely country source of such an ancestor was unknown. The MRCA between the *D. rotundus* source sequences and the *T. brasiliensis*, bat source sequences was 1888, with a probability of 86% that this ancestor was a *T. brasiliensis* bat. In another study, the date of the potential viral shift between these two bat species was 1837 (range 1656–1892), in agreement with Costa Rican estimations [108]. However, based upon other comparative data, adaptations of rabies viruses associated with nascent infections of species such as *T. brasiliensis* and *D. rotundus* was not a recent evolutionary event, but a relatively lengthy, pre-colonial process [109], suggesting more sequences are required to reach further phylogenetic conclusions.

Challenges Posed by Recent Human Rabies Cases

With canine rabies controlled, human cases are now uncommon in Costa Rica [110]. Nevertheless, any cases are considered public health failures and illustrate challenges posed in regard to surveillance, detection, characterization, and response. Bites from bats are more subtle than from dogs. Often, people may not realize they were bitten by bats while sleeping. In other cases, people believe that only *D. rotundus* can transmit rabies virus and may overlook the minor lesions caused by other bats. As an example, below are the histories of the last three cases of human rabies in Costa Rica. The common denominator is ignorance about rabies in bats.

In May 2014, a child was evaluated at a local medical facility in Ciudad Neily and transferred to a regional hospital with a history of fever, malaise, myalgia, and vomiting. The differential diagnoses were Dengue and Chikungunya. Cerebrospinal fluid (CSF) analysis ruled out meningitis. Regardless, antibiotics were begun but symptoms persisted. The child was referred to the National Children's Hospital when symptoms became more severe, including mental status fluctuations with alternating excitement and depression, aggressive behavior, tachycardia, and hypertension. Symptoms progressed to neurological impairment and apnea that required intubation. Second CSF analysis showed a slight increase in leucocytes but was negative for Enterovirus and Herpes Virus by PCR. The child declined to dysautonomia, characterized by tachycardia, bradycardia, hyper/hypotension, and sialorrhea. A CT and MRI were performed, without specific findings, and an EEG showed no relevant alterations. Upon questioning, the mother recalled that the child and his dog were bitten by a squirrel (ie, anecdotal data without actual species data corroboration) 3 months earlier. Other exposures were not reported. With suspicion of rabies, urine, saliva, CSF, and a skin biopsy were sent to the SENASA LANASEVE laboratory. Only the saliva tested positive for rabies virus nucleic acid by RT-PCR. The patient died 2 weeks after hospitalization. Brain samples were submitted to the LANASEVE laboratory. The case (ie, Case 1) was confirmed as rabies by an immunochromatographic test strip (ICTS), direct immunofluorescence test (DFT), RT-PCR, and the mouse inoculation test (MIT). The variant was characterized as associated with rabies viruses maintained in vampire bats (96.2% of identity with sample AB201819.1).

A second human case (ie, Case 2) was diagnosed at the National Children's Hospital in August 2014. This involved an 11-year-old Nicaraguan girl who entered the country in January 2014. At the time of hospitalization, her mother reported that after minor trauma sustained while playing, the girl showed paresis and hypoesthesia of her lower left limb, later affecting the other limb, impairing the ability to walk. In less than a week, she could not move her lower limbs and complained of fever. She was evaluated in the emergency department, where she was described as having paralysis from the neck down, with diminished sensitivity. She was admitted to the intensive care unit (ICU), where she was conscious and stable. Two days after admittance, she deteriorated rapidly, with loss of consciousness and sialorrhea, requiring intubation. After an epidemiological investigation, the mother reported that the patient was bitten by an opossum (ie, anecdotal data without actual species data corroboration) on her foot in August 2013, while living in Nicaragua. No other exposures were recalled. Under suspicion of rabies, urine, saliva, CSF, and skin biopsy samples were sent to the LANASEVE laboratory. Only the CSF sample tested positive for rabies virus nucleic acid by RT-PCR. The patient died 2 days after the onset of symptoms. Brain samples were submitted to the LANASEVE laboratory, and all samples were positive by ICTS, DFT, RT-PCR, and MIT. The variant was characterized as associated with a canine rabies virus origin, by nucleotide sequencing (98.2% of identity with sample HQ450386).

The most recent case (ie, Case 3) was a 43-year-old male biologist and high-school teacher who was bitten by an insectivorous bat on August 15th, 2018 but did not receive PEP. Two months later, on October 14th, he showed pain, hyperesthesia, and paresis of his upper left arm and hand. A week later, the patient complained of fever, malaise, loss of appetite, nausea, and vomiting, and fluctuations in mental status with paresis affecting the lower limbs, impairing his ability to walk. He was evaluated in a hospital emergency department, where he was described as anxious, irritable, and confused, with

mild sialorrhea, urine retention, and paralysis from the neck down, with diminished sensitivity. He showed hemodynamic instability and neurological impairment requiring intubation and was admitted to the ICU. The differential diagnoses were Guillain-Barre Syndrome and acute meningitis. With a suspicion of rabies, samples of urine, saliva, CSF, and a neck skin biopsy were sent to the LANASEVE laboratory. The CSF analysis ruled out meningitis and samples were negative for pathogens included in the FilmArray® Meningitis/Encephalitis Panel. A CT was performed without specific findings. Only the skin biopsy samples tested positive for rabies virus nucleic acid by RT-PCR. The patient died on November 14th, a month after the illness. Postmortem, the positive RT-PCR product was sequenced, with 93.5% identity to variants associated with the big brown bat, *Eptesicus fuscus* (compared to sample AY170404 from *E. fuscus*).

These cases represent challenges posed by a rarely diagnosed human zoonosis within the realm of the enzootic perpetuation of rabies viruses. For Case 1, the exposure was reported to be by a "squirrel," but the variant was associated with vampire bats. Whether this was a misidentification or an instance of spillover infection is unknown, as rabies is not commonly diagnosed among rodents and human cases after rodent exposures have not been documented [111]. Case 2 illustrates the omnipresent dangers of canine rabies and until global elimination has been verified, translocation opportunities remain, even in highly developed countries, such as the US [112]. As with Case 1, whether the history of "opossum" exposure was the actual source of the infection (from spillover by a rabid dog, cat, etc.) or an unrecognized canine exposure could not be ascertained, given that no human cases have ever been attributed to a marsupial, further complicated by recall bias and incubation periods that may vary from months to years [113]. Case 3 exemplifies the threat posed by bat rabies, particularly in regions where vampire rabies predominates and the risk by even relatively minor exposures to non-hematophagous species may be grossly underappreciated [114]. Moreover, every suspect human rabies case requires a thorough public health investigation, but epidemiological implications of isolated transmission from a bat are quite different from the identification of canine rabies if indicative of an otherwise "silent" community event and potentially broader local circulation.

FUTURE RECOMMENDATIONS

Rabies Management

While human PEP, mass parenteral immunization of dogs, and oral wildlife vaccination are highly effective for zoonosis control, a diversity of hosts perpetuates rabies throughout the world [8]. In contrast, within Costa Rica, most detected cases occur in livestock. Likely, cases in other species may go undetected, as found in other parts of Latin America [115]. Reasons are many, including low levels of disease awareness and non-specific clinical manifestations [107]. Enhanced and passive surveillance data coupled with viral characterization are valuable sources of information for risk analyses, targeted education, and focused public health activities [116,117].

In general, animal rabies detection, prevention, and control techniques have improved over the past 20 years, particularly related to modern diagnostics and biologics [8]. Unfortunately, most of this progress is targeted to human and domestic animal health, rather than wildlife, such as bats. Although bats should be humanely excluded from human dwellings rather than killed, unfortunately culling and non-specific destruction of roosting sites of bats after an outbreak in domestic animals is still widely practiced in Latin America. Even the use of anti-coagulants specifically applied to vampire bats has downstream concerns [47,91]. Increasing evidence indicates that this practice is counterproductive and should be discontinued [107,118]. Non-specific destruction of roosting sites is a highly disruptive approach that impacts other non-hematophagous taxa of bats that roost together. These species act as seed dispensers, insect predators, and forest pollinators, performing critical ecological services [119].

Independent of rabies, economic losses generated by vampire bats, such as anemia, reduced milk production and myiasis (ie, "fly strike") in bite lesions must be considered before abandoning all vampire bat control [47,91]. Otherwise, farmers will not support alternatives [118]. One novel suggestion takes advantage of the grooming behavior of vampire bats, substituting anticoagulant paste by application of an oral vaccine [120-124]. Future research may lead to the development of these, and dual contraceptive or transmissible biologics for vampire bat control [125,126]. No such vaccines are available commercially at this moment.

Despite such useful public health and veterinary measures, animal cases continue to escalate – attributable to both ecological and social changes. In response, all domestic animals at risk, especially dogs and cats, should be vaccinated. The lack of awareness of the public towards rabies continues, in regard to avoiding exposures, thorough risk assessments, and timely PEP. Proper professional and public education, training, and preexposure vaccination of persons at risk should be improved [8].

In retrospect, several significant events have occurred in the successful history of rabies management in Costa Rica (Table 2). Building on this progress, over the next 4 years (2020-2024) a regional study will occur among the International Regional Organization of Agricultural Health (OIRSA) countries in cooperation with The University of Glasgow (Glasgow, Scotland), to characterize

Table 2. Highlights of rabies occurrence, prevention, and control in Costa Rica.

PERIOD	EVENT
Pre-colonial	Cultural, historical, and genetic evidence of bat rabies existence
Colonial period	Introduction of domestic animals as reservoirs and hosts, including dogs and livestock
18th century	First report of rabies in dogs
1952	Notification of bovine paralytic rabies
1956-1970	Periodic canine rabies outbreaks
1983	Initiation of Seller's staining for the detection of Negri bodies in CNS tissues
1985	Establishment of a national animal rabies surveillance and control program, following a large outbreak with 139 cattle deaths
1987	Last imported rabies cases in dogs
1993	Use of the direct fluorescent antibody test for rabies diagnosis
1994	Massive dog vaccination along Costa Rican and Nicaraguan border
2001	Transmission of vampire bat rabies virus via a cat to a 62-year-old caregiver and a 9-year-old child
2002	Large outbreak with at least 194 confirmed cattle deaths
2008	Apparent decade-long vampire bat rabies virus lineage disappearance along the entire Caribbean coast, reflective of colonization and extinction events
2013	Addition of molecular techniques to laboratory diagnosis and typing
2014	Case of spillover infection of a vampire bat rabies virus variant to a dog and a 9-year-old child
2014	Imported human case from Nicaragua with a canine rabies virus variant
2014	First confirmation of a bovine with a *Tadarida brasiliensis* rabies virus variant in Costa Rica
2018	Human rabies infection by an insectivorous bat variant, *E. fuscus*

rabies virus variants, delineate enzootic cycles, and establish potential dispersal patterns among countries. With such information, we anticipate identifying new variants, predict foci of future outbreaks in livestock and humans, identify localities where rabies vaccination programs could be more effective, and eventually conduct novel interventions against *D. rotundus*, such as the use of oral vaccination.

CONCLUSIONS – WHAT DO WE SUGGEST?

As exemplified by Costa Rica and several other countries in the Americas, canine rabies can be prevented, controlled, and selectively eliminated by mass immunization [127]. Yet, threats for re-introduction remain [128-130]. To avoid canine rabies virus reintroduction, several measures are proposed:

Sterilization and vaccination campaigns in stray dogs and with owners: Modify the URBAN FAUNA CONTROL AND ZOONOSIS PREVENTION LAW draft. File No. 19,837. This project stated that: it would be the responsibility of the municipalities to conduct a survey to determine the possession of pets per household (dogs and cats) that would be done when the different taxes or municipal procedures are paid. This information will allow an estimation of the number of pets that require sterilization, which will be free and annual, systematic in all districts of each municipality for dogs and cats between 5 and 6 months of age. In addition, rabies vaccination will be done at cost and subsidized for those families that cannot afford the vaccine. Also, there will be a follow-up of the rabies vaccination in these animals. Fines will be charged to those citizens who do not comply with the provision.

Enhanced surveillance of wildlife: SENASA, in coordination with National Parks, rescue centers, and wildlife refuges should receive samples of animals with suspect clinical signs for rabies testing. In the case of positive samples, oral vaccination of free-ranging carnivores may be a consideration in disease management [131-133]. A domestic animal control program close to national parks is envisioned. A rabies control program should focus on limiting the contact of domestic animal species with wildlife. Within this program, stray animals will be brought to shelters, to help maintain the canine rabies-free status. The elimination of canine rabies saves human and animal lives and brings significant health economic advantages and the epidemiological luxury for laboratory-based surveillance to detect wildlife rabies [8,41,134,135].

Bats and Wildlife Measures

The vaccination of livestock in rural areas with frequent rabies outbreaks would reduce economic losses to farmers, and could reduce human exposure, which occurs through the manipulation of infected animals.

As such, to achieve a meaningful reduction of bovine rabies, all herds within endemic or high-risk regions should be vaccinated, requiring an enormous economic effort by stakeholders. Nevertheless, such a measure does not guarantee that spillover to wildlife does not occur. Management should be directed to *D. rotundus* populations while avoiding adverse events in other bat species. Recent studies with bioluminescent paste (ie, to mimic a vaccine) are an attempt to determine the potential utility of transfer among the *D. rotundus* colony members, as well as between other non-hematophagous bats, when sharing the same roost. The dual need to reduce populations of *D. rotundus* and to vaccinate this reservoir to decrease the spread of rabies is a complicated need. In addition, controlling the excess abundance of food sources to the vampire bats, which is driving the disruption of the population, might in the long run, lower vampire bat population numbers. This might be attained by costly measures such as stabled livestock and requires again enormous effort by public stakeholders. However, such measures should be taken in all endemic countries for a comprehensive approach. Moreover, besides vampire bat rabies, non-hematophagous taxa, such as the migratory insectivorous bat *T. brasiliensis,* can be also infected and require further investigation. Beyond intra-specific perpetuation, bat rabies virus spill-over infection can occur to carnivores, such as coatis, coyotes, foxes, and skunks, as has been reported in Mexico. Such lack of information on rabies in carnivores requires attention by the authorities to assess if other control measures, such as oral vaccination, should be considered in the country [136,137].

Prevention of Human Cases

At a minimum, public education should be implemented to avoid exposures, seek pertinent PEP, and apply appropriate domestic animal vaccination, which will remain relevant throughout this hemisphere and beyond [8].

Also, people with occupational risk of exposure to animals (eg, veterinarians, biologists, etc.) should be vaccinated against rabies. Considering that not only *D. rotundus* transmit rabies virus in Costa Rica, but also insectivorous bats, any bat bite should receive PEP. Wildlife rabies constitutes an enormous problem for public awareness, case detection, and control in Costa Rica, specifically considering permissive hosts and disease emergence, for which limited information exists in the country [138,139].

Figure 6. **Brain sample collected from a rabies-suspect horse with compatible clinical signs**. In large-bodied animals, such as livestock, the collection of the appropriate parts of the brain is critical for laboratory-based surveillance for a variety of conditions, including rabies, equine encephalitis viruses, and transmissible spongiform encephalopathy in the diagnosis of bovine cases.

Collaboration Among Stakeholders and Decentralization of Diagnostic Testing

Given progress over the past decade, the current outlook for improved laboratory-based surveillance and management of rabies in Costa Rica is promising. Measures are underway to generate additional information about viral variants, using improved diagnostic tools, together with a more rapid local response and improved data collection and sharing, to compare with trends in other countries of the region and update preparedness plans [23]. In this context, while most of the epidemiological focus is upon outbreaks among livestock due to vampire bat rabies, confirmation of suspect cases via the routine collection of appropriate tissues for diagnosis under field conditions will remain a challenge (Figure 6).

Currently, SENASA is comparing linear flow antigen detection test kits, as other countries have done [140-142]. Nevertheless, conflicting results by other research groups suggest caution [143]. The need is to implement surveillance near the border of neighboring countries to be used in livestock, dogs, and wild carnivores, aiming to detect putative re-introductions. This expansion of diagnostics to regional laboratories, with careful confirmation in the central SENASA laboratories, allows a more rapid public health response to wild or domestic animal rabies cases. Working under the umbrella of the One Health concept, the coordination in Costa Rica between the Health Ministry and the Agriculture Ministry through SENASA is

vital, as outbreaks should be monitored in exposed populations of both domestic animals and humans [144]. This facilitates a more comprehensive investigation of human exposures, ensuring appropriate PEP, as well as associated animal control, including notification, vaccination, quarantine, and euthanasia. Additionally, implementing relevant management practices for the control of vampire bats and expanding vaccine coverage in livestock and dogs with owners and strays are urgent tasks [145].

CONCLUDING REMARKS

Despite being considered a canine "rabies-free" country, official validation of the status by international health authorities is still necessary. Moreover, a high risk of canine rabies resurgence exists if translocation occurs, due in part to a large population of free-ranging dogs, suboptimal levels of vaccination of companion animals (suggested to be less than 20%), and the presence of rabies in other countries of the region [146].

This review constitutes an effort to gather pertinent information from the multiple institutions involved in rabies control and to begin a national conversation on the difficulties represented by this ancient zoonosis. Health authorities should recognize that prevention, control, and selective elimination of rabies requires a modern transdisciplinary approach, with the engagement of diverse professionals in the country, region, and also international cooperation. Over the past 300 years, Costa Rica has grappled with this "enemy" and came to realize its internal needs, wants, and expectations. Sharing such lessons learned and prioritizing collaborative research endeavors towards the development of future tools will help pave the final stretch on the global road towards 2030, as well as the reality of post-elimination aftermath [8,147]. In conclusion, rabies has maintained its status as a neglected tropical disease for decades, demonstrating a critical need regarding data gaps for understanding epidemiological alterations, ecological patterns, and host adaptations of such a complex disease of nature, particularly in light of the "Zero by Thirty" ideal and the ongoing COVID-19 pandemic [147-149].

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Box 1. A starting phylogenetic tree with 31 rabies virus sequences, employing IQtree with HKY + G4 as a substitution and 10000 bootstrap replicates [150], was used to generate a molecular clock model. Analyses using the TempEst program [151] were conducted, and the clock rate was estimated. We constructed a tree with BEAST [152], under a strict clock model, with an evolution rate of 0.00061, based on the TempEST estimate, and with a prior tree with a constant coalescence size [153,154]. The ESS for all parameters was greater than 1000, indicating that a stationary phase was reached. The Maximum Clade Credibility (MCC) tree was created using TreeAnnotator v1.8.2, (Figure 4). To calculate the relative speed of rabies virus spread (location. diffusion Rate), latitude and longitude were added to the model and the diffusion rate was estimated in km/yr, under a lognormal relaxed molecular clock (Uncorrelated) [155], with the initial value of the Rate set to 0.00061, with the prior tree selected as coalescent exponential growth. Using the Tracer software, the evolution rate of the whole tree was 4.37 E-4, 95% HPD Interval (1.81E-4- 7.3 E-4). The IQtree calculated this rate as 6.1E-4, while the evolution rate estimated by TreeAnnotator and displayed by FigTree was 4.36 E-4. However, these variations were included in the 95% HPD established by the Tracer software. Beast analysis was performed in Cipres portal [156].

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