

Tracking ranavirus infections: an integrative review of epidemiological research on pathogen dynamics in anurans

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Abstract. Emerging infectious diseases have contributed to the decline in amphibian species around the world. Among them, ranavirus infections have major importance, as they significantly impact biodiversity and frog rearing. This study aims to conduct an integrative review of the recent literature, including epidemiological data on the distribution, prevalence, and circulating species and strains, to identify gaps that may still exist and suggest potential directions for future research. We synthesized data collected from 68 articles found in two scientific databases (Scopus and PubMed), and we provide a situational update on ranavirus infections in anurans. We found relevant reports confirming the high prevalence of this pathogen and the risk it poses, including to endangered species. However, there is still information to be clarified regarding its distribution in different territories and the identification of the species involved in infections.

1 Introduction

Amphibians are a diverse and cosmopolitan class of vertebrates, comprising over 8000 species (Frost, 2024). Despite this diversity, amphibians are at risk of extinction. According to the Red List of Threatened Species of the International Union for the Conservation of Nature (IUCN), 41 % of amphibian species are at risk of disappearing (IUCN, 2023). Several factors are behind this decline, including climate change, deforestation, habitat fragmentation (Santi and Corrêa, 2018), and the introduction of alien species (Kraus, 2015). Moreover, the occurrence of infectious diseases significantly impacts the numbers of many of these species (Daszak et al., 2003). Ranaviruses, viruses belonging to the Iridoviridae family (Chinchar et al., 2017) that infect fish, amphibians, and reptiles (Brenes et al., 2014), are among the most important emerging pathogens.

Ranaviruses are large viruses (150 to 200 nm in diameter), with a genome consisting of a double strand of DNA (dsDNA); are covered by an inner membrane, which in turn is covered by a capsid of icosahedral symmetry; and can be enveloped by an outer envelope (in the case of species that bud from the host cell's plasma membrane) or not. These morphological characteristics are common to all members of this taxon (Chinchar et al., 2017). Seven species are currently recognized: *Ambystoma tigrinum virus* (ATV), *Common midwife toad virus* (CMTV), *Epizootic haematopoietic necrosis virus* (EHNV), Santee-Cooper virus (*Largemouth bass virus*, LMBV), *Singapore grouper iridovirus* (SGIV), *European North Atlantic ranavirus* (LfRV), and *Frog virus 3* (FV3) (Jancovich et al., 2012). The main species that affect amphibians are CMTV, ATV and FV3, and studies have shown that anurans and urodeles are more susceptible to ATV and FV3, respectively (Schock et al., 2008).

Ranaviruses are considerably resistant in natural environments: for example, a laboratory study showed that FV3 persisted outside a host at low temperatures $(4^{\circ}C)$ for up to 102 d in filtered river water and 57 d in unfiltered water (Nazir et al., 2012). In addition, these viruses can also remain in sediments, where they are able to conserve their infecting capacity, under the same temperature range (Munro et al., 2016). Virions are inactivated by acidic $(< 3.0$) and alkaline (> 11.0) pH, exposure to UV irradiation, and high temperature ($> 55^{\circ}$ C for 30 min). In addition, ranaviruses are sensitive to the action of disinfectants such as chlorhexidine, sodium hypochlorite, and potassium compounds (Bryan et al., 2009).

The first description of ranavirus infection occurred in the 1960s; since then, several die-off events have been recorded. These viruses have been identified as causing epizootic outbreaks worldwide (Gray and Miller, 2013; Maclachlan; Dubovi, 2016), with cases reported in several countries (Duffus et al., 2015). The disease has a high mortality rate and can reach 100 % death rates in infected populations (World Organisation for Animal Health, 2021), thus bringing significant losses to aquaculture and biodiversity: there are scientific reports that reveal the decline in amphibian species in the wild due to infections by ranavirus in Europe (Price et al., 2014), as well as mass mortalities of animals in the wild (Miaud et al., 2016) and captivity (Une et al., 2014). For these reasons, ranavirus infection was listed as a notifiable disease by the World Organisation for Animal Health (WOAH) in 2009, although it is not a zoonotic disease (Black et al., 2017).

The ecology of ranaviruses involves a complex chain of interaction between host species, transmission routes, environmental persistence, stressors, and the immunity of the host individual (Gray et al., 2012). The virus is transmitted horizontally. A high population density can facilitate transmission between individuals, through direct contact between healthy and sick animals. In addition, natural behaviors among amphibians, such as predation (cannibalism and necrophagy), are risk factors for direct transmission of the virus (Harp and Petranka, 2006; Latney and Klaphake, 2013). The virus can also be dispersed through water, soil, and sediment and infect other individuals indirectly (Nazir et al., 2012).

Affected animals can show symptoms such as altered buoyancy, erratic swimming, lethargy, edema, erythema, and skin ulcers that can develop into skin necrosis with loss of extremities (Forzán et al., 2015; Miller et al., 2007; Stöhr et al., 2013). Despite the variety of clinical signs, some animals may harbor a subclinical but transmissible infection of the virus (Brunner et al., 2019); these carriers contribute to the dispersal of the pathogen, especially when considering the wild-animal trade, which some authors point to as a "source of pathogen pollution" (Picco and Collins, 2008).

In the last few years, there has been a significant increase in research involving ranaviruses, evidenced by the growth in the number of articles published on the subject (Wirth et al., 2021). Anurans are the main models used to evaluate important aspects of the infection, such as immunity, pathogenesis (Forzán et al., 2017; Morales et al., 2010), diagnostic techniques (Ford et al., 2022), transmission potential (Cunningham et al., 2007), and environmental drivers (Hall et al., 2020). Furthermore, since ranaviruses can accelerate the process of extinction of threatened species, the risk of disappearance of anurans is concerning from a conservation perspective (Earl et al., 2016). Given the amount of available information on ranavirus infection in anurans, this study aims at conducting an integrative literature review to collect and synthesize data published between 2013 and 2022. We intend to provide a situational update on the epidemiology of ranavirus infections in anurans worldwide and identify possible gaps in knowledge that could be the subject of future research.

2 Materials and methods

This research consisted of an integrative review to analyze data on the prevalence, geographical distribution, and diversity of ranavirus species infecting anurans worldwide. The search criteria included studies published within a 10 year period (2013–2022). The search for articles was conducted in two bibliographic databases: Scopus and PubMed. The search terms were applied using Boolean operators: ranavirus AND infection AND (anurans OR amphibian) AND (epidemiology OR prevalence OR occurrence). We predefined the inclusion and exclusion criteria for the articles (Table 1) and applied them for screening and subsequent selection.

The search in the two databases yielded 921 articles. After retrieval, the titles and abstracts were read. A total of 746 articles did not meet the established criteria and were excluded. The articles that passed the screening were read in full; the inclusion and exclusion criteria were also considered. Those that did not provide the full text for reading were rejected (3 articles). In the end, 68 articles were selected (Fig. 1).

Importantly, when the study did not provide the prevalence of *Ranavirus*, we calculated it if access to the data needed to do so for the species of interest were available. In articles where the prevalence was defined for other orders of anurans (e.g., Caudata), a prevalence ratio was conducted considering only anurans. Studies wherein this information was impossible to retrieve were discarded. To calculate prevalence, the equation comprising the ratio between the total number of animals detected as positive and the total number of animals tested was applied. This calculation follows the prevalence principle described by Lima et al. (2018), in which it is measured by dividing the number of known cases of a given disease by the number of individuals in the population at a

Table 1. Predefined inclusion and exclusion criteria for the selection of articles.

| Inclusion criteria | Exclusion criteria |
|--|--|
| Observational prevalence/occurrence studies Studies on ranavirus infection in anurans Articles published between 2013 and 2022 Research containing data to calculate the prevalence Research conducted in any country/language | Review articles and book chapters Clinical trials (experimental infection) Studies/reports with species other than anurans Studies that do not focus on the prevalence/occurrence of the disease/infection Studies that do not explain the observed prevalence Duplicate articles |

Figure 1. Flowchart showing the sequence of article selection for the integrative review.

defined point in time (day, month, or year) (Eq. 1, calculation for measuring prevalence):

prevalence ratio =
$$
\frac{\text{(number of known cases of disease)}}{\text{population}} \times 100. \quad (1)
$$

3 Results

We selected 68 articles published between 2013 and 2022 that reported cases of ranavirus infections in anurans, confirmed by laboratory techniques. The reports came from countries in all continents, except Antarctica; of these, most (39.7 %) described occurrences in the United States. Consequently, the number of cases recorded in North America was higher than in other continents (33 records or 48.5 % of

publications). In contrast to North America, only a few cases have been recorded in continents such as Oceania (1 record) (Wynne, 2020) and Africa (2 records) (Box et al., 2021; Kolby et al., 2015), highlighting a gap in the knowledge of ranaviruses on these continents.

Several species of anurans have been affected by the infection: 16 families were recorded in the reports, including species threatened with extinction (Table 2). Assessed animals came from various sources: free-living animals, animals bred in captivity (commercial farms and aquariums), and zoological collections. It is worth noting that some articles reported the first case of detection of the pathogen in amphibians, either in a natural environment or in captivity (Park et al., 2021; Vörös et al., 2020; Kwon et al., 2017; Ruggeri et al., 2019) in order to create a collaborative, informationexchanging network, demonstrating the increased efforts to identify the cause behind the outbreaks of this emerging disease.

Moreover, some of the studies reported the occurrence of ranavirus infection in species included in the IUCN Red List categories. The concerned species are *Craugastor ranoides* (Critically Endangered), *C. taurus* (Endangered), *Agalychnis lemur* (Critically Endangered), *Duellmanohyla legleri* (Endangered), *Litoria lorica* (Critically Endangered), *Rana muscosa* (Endangered), and *R. sierrae* (Vulnerable). The assessment considers various factors to define the conservation status of species of different taxa (IUCN, 2023).

Of the 68 articles analyzed, 27 (39.7 %) identified the viral species involved, with *Frog virus 3* (FV3) being reported in most cases (13 records). The spatial distribution of ranavirus species in different countries, according to the studies we analyzed, is shown in Fig. 2. Ranaviruses are widespread globally, aided by the international animal trade, whether for the pet market or for food. Studies showed the presence of exported animals with the infection (Kolby et al., 2014), which facilitate the spread of the virus across borders.

We present the prevalence values found in the articles evaluated, along with each country in which the studies were performed (Table 3). In some cases, it was necessary to calculate the prevalence, as this parameter was not indicated. A new calculation was performed considering only anuran amphibians in articles that presented data for other orders of amphibians. There is a wide variation in prevalence among species

| Order | Family | Species |
|-------|----------------------|---|
| Anura | Alytidae | Alytes obstetricans |
| | Bombinatoridae | Bombina bombina, Bombina orientalis, Bombina variegata |
| | Bufonidae | Bufo bufo, Bufo spinosus, Anaxyrus (Bufo) hemiophrys, Anaxyrus americanus, Anaxyrus boreas boreas, Duttaphrynus dhufarensis, Epidalea calamita, Rhinella manu, Rhaebo haematiticus, Rhinella marina, Rhinella horribilis |
| | Calyptocephalellidae | Calyptocephalella gayi |
| | Centronelidae | Teratohyla spinosa |
| | Craugastoridae | Craugastor ranoides*, Craugastor bransfordii, Craugastor fitzingeri, Craugastor megacephalus, Craugastor mimus, Craugastor crassidigitus, Craugastor podiciferus, Craugastor stejnegerianus, Craugastor taurus* |
| | Hylidae | Agalychnis callidryas, Agalychnis lemur*, Agalychnis spurrelli, Acris gryllus, Acris blanchardi, Duellmanohyla legleri*, Dendropsophus phlebodes, Hyla cinerea, Hyla molleri, Hyla squirella, Hyla chrysoscelis, Hyla versicolor, Hyliola regilla, Hyloscirtus palmeri, Hypsiboas gladiator, Dendropsophus phlebodes, Dendropsophus microcephalus, Pseudacris crucifer, Pseudacris fouquettei, Pseudacris maculata, Pseudacris ornata, Osteopilus septentrionalis, Dryophytes japonicus, Scinax elaeochroa, Smilisca baudinii, Teratohyla spinosa |
| | Microhylidae | Gastrophryne carolinensis, Gastrophryne olivacea |
| | Mantellidae | Mantidactylus cowanii, Mantidactylus mocquardi |
| | Pelobatidae | Pelobates cultripes |
| | Pelodryadidae | Litoria nannotis, Litoria lorica* |
| | Pipidae | Xenopus laevis |
| | Pyxicephalidae | Pyxicephalus spp. |
| | Ranidae | Rana dybowskii, Rana draytonii, Rana sylvatica, Rana iberica, Rana pyrenaica, Rana temporaria, Rana catesbeiana, Rana clamitans, Rana huanrensis, Rana sphenocephala, Rana pipiens, Rana palustris, Rana grylio, Rana heckscheri, Rana okaloosae, Rana virgatipes, Rana muscosa*, Rana sierrae*, Lithobates capito, Lithobates forreri, Lithobates vibicarius, Lithobates warszewitschii, Lithobates clamitans melanota, Hoplobatrachus occipitalis, Hylarana spp., Ptychadena spp., Pelophylax esculentus, Pelophylax ridibundus, Pelophylax lessonae |
| | Scaphiopodidae | Scaphiopus holbrookii |
| | Strabomantidae | Pristimantis lindae, Pristimantis pharangobates, Pristimantis platydactylus, Pristimantis toftae |
| | Telmatobiidae | Telmatobius marmoratus |

Table 2. List of anurans grouped by families and species found in the 68 records selected.

[∗] Species classified as Vulnerable (VU), Endangered (EN), or Critically Endangered (CR) according to the IUCN Red List.

and sites, with values reaching 100 % in cases wherein outbreaks with high mortality have been reported (Wheelwright et al., 2014; Miaud et al., 2016; Kwon et al., 2017; Hartmann et al., 2022).

4 Discussion

The field of ranavirus studies has seen significant expansion over the past few years. The articles in our study maintain a relative consistency of publications per year (Fig. 3); however, it must be noted that these data represent just a fraction of the extensive volume of publications on this topic. The Global Ranavirus Reporting System is a database created by well-known researchers in the study of ranavirus, and the existing dataset reveals an exponential rise in publications from 2010 onwards (Brunner et al., 2021). On the other hand, the count of scientific reports is not equal to the number of notifications transmitted to WOAH. Black et al. (2017) made such a remark regarding this underreporting, which is reflected in the disparity between articles and official reports. This underscores the need not only for ongoing research but also for informing animal health authorities of cases of ranavirosis.

The decline in endangered species is a matter of significant concern. It is estimated that, since the 1960s, around 200

Figure 2. Geographical distribution of the ranavirus species and isolates identified in the records. Publisher's remark: please note that the above figure contains disputed territories.

Figure 3. Box plot showing the number of articles selected according to year of publication.

species of frog have become extinct and that, in the next century, hundreds more may follow a similar path (Alroy, 2015). Many of them are located in biodiversity hotspots, biomes that contain great biological diversity and face a high degree of threat (Mittermeier et al., 2004). Thus, the preservation of ecosystems is of crucial importance for the maintenance of endemic species, both animal and plant.

In addition, invasive species intensify this process: as well as competing for food resources and preying on native species, they have a role as pathogen carriers (Blackburn et al., 2014), serving as a source of infection and acting as conduits in the dynamics of agent transmission. Some articles cited invasive species, such as *Eleutherodactylus planirostris* (Rivera et al., 2019) and *Lithobates catesbeianus* (Ruggeri et al., 2019), currently named *Aquarana* *catesbeiana* (Jorgewich-Cohen et al., 2022). In one of the articles reviewed, *E. planirostris* had a higher prevalence and intensity of infection than the other native species evaluated, highlighting the amplifying role that invaders play in disseminating ranaviruses as reservoir hosts (Rivera et al., 2019).

The American bullfrog (*A. catesbeiana*) is a species widely raised in production for human consumption. This anuran has been introduced into several countries, as various farming sites have been built to breed them on a commercial scale (Ribeiro and Toledo, 2022). However, reports of animals escaping or being abandoned in the wild after the closure of facilities are not uncommon, and the species, which is highly adaptable, has managed to thrive outdoors. In several countries, such as Argentina (Akmentins and Cardozo, 2010), Uruguay (Laufer et al., 2008), and Brazil (Both et al., 2011), feral populations of the bullfrog have been recorded. American bullfrogs have demonstrated resistance to *Ranavirus* infection (Hoverman et al., 2011) and can carry subclinical levels of the virus (Brunner et al., 2019); this suggests they could impact community transmission dynamics, also acting as reservoirs for the virus.

In addition to intra-class interaction, ranaviruses can be transmitted through inter-class contact since the pathogen can infect different host classes. Studies have reported the occurrence of ranavirus infection in sympatric amphibians and chelonians (Currylow et al., 2014), as well as in anurans and urodeles that share the same territory in a prey–predator relationship (Rothermel et al., 2016). This dynamic favors the transmission of the agent, increasing the likelihood of infection. When considering an environment with great biodiversity, other mobile taxa, such as birds and reptiles, can carry

Table 3. Continued.

the virus as carriers or reservoirs, contributing to spreading the pathogen in the environment (Tornabene et al., 2018).

Some of the studies we evaluated also highlighted the increased impact of co-infection with other emerging pathogens, including the chytrid fungus *Batrachochytrium dendrobatidis* (Longcore et al., 1999); this zoospore-forming fungus can infiltrate the host's epidermis, causing a disturbance in its osmoregulatory function, which can result in death (Sewell et al., 2021). One hypothesis to be confirmed is that infection by one of these pathogens may favor subsequent infection by the other (Warne et al., 2016). Seven studies reported animals co-infected with the two pathogens (Whitfield et al., 2013; Reshetnikov et al., 2014; Warne et al., 2016; Olori et al., 2018; Talbott et al., 2018; Watters et al., 2018; Julian et al., 2019).

Co-infection with these pathogens, combined with factors such as climate change, exacerbates the impacts caused by these agents, increasing disease severity and triggering new outbreaks (Thumsová et al., 2022). Hall et al. (2018) observed a relationship between higher ranavirus mortality and higher water temperatures. Temperature is a major factor in the behavior, reproduction, metabolism, and immune function of amphibians (Blaustein et al., 2010), so high temperatures interfere with the full functioning of their bodies, making them susceptible to infection.

The environment is also a key factor in the occurrence of ranavirus infections. Studies showed that certain environmental aspects can increase the predisposition of animals to infection. Elements such as the season or environment-linked aspects (e.g., distance from the nearest lake with a ranavirus infection) can be used to predict infections (Tornabene et al., 2018). The deleterious consequences of human action for the environment also impact amphibians, making them susceptible to infection; for example, ammonia from livestock farming and fertilizers used in agriculture is extremely toxic to aquatic animals and impairs their development and interferes with their immune function, which renders them more vulnerable to the virus (Talbott et al., 2018). A higher severity of infections was also associated with environments contaminated with wastewater that led to an increase in salinity in wetlands (Hall et al., 2020).

Regarding diagnosis, all studies used polymerase chain reaction (PCR) as the primary test to confirm infection; however, other techniques, such as viral isolation (Miaud et al., 2016; Julian et al., 2019), histopathology (Landsberg et al., 2013; Rothermel et al., 2016; Sluijs et al., 2016; Eustace et al., 2018; Hsieh et al., 2021), immunohistochemistry (Forzán et al., 2019), in situ hybridization, and electron microscopy (Hsieh et al., 2021), were also used. Of all the methods used, PCR is the technique recommended by the World Organisation for Animal Health (WOAH) due to its availability, utility, and diagnostic sensitivity and specificity (World Organisation for Animal Health, 2021). The use of samples from the environment to recover environmental DNA (eDNA) is a growing trend, and it was used in three articles retrieved in our search. This method not only eliminates the need to handle and/or euthanize animals but also provides quantitative data on viral elimination in the environment during outbreaks, and it has provided satisfactory results (Hall et al., 2018; Miaud et al., 2019; Tornabene et al., 2021).

Despite the numerous studies reporting ranavirus infections, there is limited understanding of the specific species and strains involved. Among the studies evaluated, the most prevalent species was FV3, followed by CMTV. One study only identified the *Bohle iridovirus* (BIV) in animals housed in an aquarium with anuran species from various regions, which makes it difficult to identify where the virus originated (Cheng et al., 2014). BIV is a ranavirus frequently found in Australia (Jerrett et al., 2015), and its ability to infect is not limited to amphibians, as cases of epizootic outbreaks have already been reported in bony fish and reptiles (Chinchar et al., 2017). A notable study documented the diagnosis and molecular characterization through phylogenetic analysis of a ranavirus strain in Brazil (Oliveira et al., 2020). This strain exhibited a distinct phylogenetic profile compared to other strains identified globally. We emphasize that studies providing molecular data of detected *Ranavirus* species and strains (i.e., sequencing) are crucial for uncovering the evolutionary history of the virus. Such data can also shed light on genes that may influence the replication cycle and viral pathogenesis.

We would also highlight the lack of studies on ranavirus infections in continents such as Africa and Oceania; although the presence of the virus is known, there is a scarcity of information on the distribution of the pathogen in the territories and on which species are prevalent, as well as on which animal species are susceptible to the virus. We therefore suggest that more studies on ranavirus infection should be carried out

in order to increase knowledge of the virus distribution and prevent outbreaks.

In epidemiology, coefficients are essential for measuring events related to the health–disease process. The prevalence coefficient, point prevalence (or simply prevalence), is one of the most commonly used measures of disease frequency. It quantifies the proportion of individuals in a population affected by a disease at a specific time. Wagner (1998) likens this indicator to a photograph, capturing a static moment in time (even if data collection spans days, months, or years). Thus, it is vital to measure the probability of morbid events (Merchán-Hamann et al., 2000) and evaluate the frequency of disease risk factors (Lima et al., 2018). Prevalence studies are helpful when investigating wildlife mortality events; however, assessing the death rate is a difficult task, as it is not always possible to record all the dead animals. For this reason, for diseases of relevance to wildlife, measuring the point prevalence is recommended; this is done by sampling specimens from the target population, whether these animals are alive or dead (Mörner et al., 2002).

Regarding the prevalence data of our study, it is noteworthy that lower values do not necessarily imply a lower occurrence of infection compared to other cases. For instance, Miaud et al. (2016) described a ranavirus outbreak where hundreds of dead animals were observed in three lakes in the southeastern Alps, France. In that study, 21 animals were collected for the diagnostic test, and all tested positive. In contrast, Price et al. (2017) conducted a 25-year retrospective study evaluating the occurrence of infections in individuals from an archive. Although the number of positive animals detected was higher (87), the prevalence was low due to the large number of tested individuals.

Equal importance must be given to studies with negative data. Ten of the studies evaluated showed negative results, in which the pathogen investigated was not detected (Titus and Green, 2013; Richter et al., 2013; Sainsbury et al., 2017; Eustace et al., 2018; Hall et al., 2018; Goodman et al., 2019; Jacinto-Maldonado et al., 2020; Tornabene et al., 2021; Kostanjšek et al., 2021; Strachinis et al., 2022). Negative data are sometimes neglected and rarely published; however, data such as these are valuable for a better understanding of the epidemiology of the disease and of its distribution and infection patterns according to different species and time periods (Stallknecht, 2007). Besides, due to the damage caused by this disease, null results should be valued from a conservation perspective.

Eradicating a disease that circulates among wild animals is quite difficult; therefore, prevention and risk analysis are necessary to avoid the spread of ranaviruses. Sainsbury et al. (2017) described the application of a program for the reintroduction of *Pelophylax lessonae* for the conservation of this species. In this sense, quarantine and testing of animals to be reintroduced into the wild are essential to ensure that these animals do not introduce pathogens into the receiving environment. If an outbreak is already in progress, the goals are the early detection of the pathogen and preventing its dissemination. One management action aiming to restrict the spread of the disease is the removal of host species, as reported by Martel et al. (2020) in relation to a *Batrachochytrium salamandrivorans* outbreak in Spain; however, this measure has been highly questioned and should not be carried out when the species in question is at risk of extinction (Bozzuto et al., 2020). For captive animals, control and prevention of the disease are based on preventive management measures (quarantine, sanitization of the facilities), as well as on the elimination of infected animals (Batista et al., 2021). There are currently no vaccines available, although there is ongoing research into the development of immunizers against ranavirus (Chen et al., 2018; Zhang et al., 2012).

5 Conclusions

The global rise in *Ranavirus* infections among amphibians, particularly anurans, highlights the urgent need for coordinated efforts in disease surveillance and management. Despite the increasing number of reports, several knowledge gaps remain, especially in regions where infections have not been thoroughly studied. To mitigate the impact on vulnerable species and ecosystems, a multi-faceted approach to surveillance, early detection, control, and post-outbreak analysis is essential. The following key points outline the critical actions required to advance our understanding of ranavirosis:

- *Exploratory research in new locations.* While reports of *Ranavirus* are becoming more frequent, there is a pressing need for comprehensive epidemiological studies in regions where the infection status remains unknown.
- *Molecular characterization.* Accurate identification of *Ranavirus* species through molecular characterization is crucial for understanding viral diversity, guiding control measures, and predicting potential future outbreaks.
- *Surveillance for conservation.* Implementing targeted surveillance programs is vital, particularly for endangered species, to monitor emerging infectious diseases and ensure timely reporting to both national and international animal health authorities.
- *Post-outbreak monitoring.* Post-outbreak strategies, such as serological testing, are critical to determine the extent of infection and evaluate the health status of surviving populations.

Data availability. The authors are open to sharing any information and data about the research upon contact and request.

Author contributions. JWPJ and DdSR: conceptualization; JWPJ: methodology; RdCCM, GJBdM, and RLMdS: validation; DdSR: formal analysis, investigation, data curation, and writing (original draft preparation); JWPJ, RdCCM, GJBdM, and RLMdS: writing (review and editing); JWPJ, RdCCM, GJBdM, and RLMdS: visualization; JWPJ and RdCCM: supervision. All authors have read and agreed to the published version of the article.

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