

Ranavirus and Its Impact on the Amphibian Population

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Abstract: Mass mortality of amphibians has occurred globally since at least the early 1990s from viral pathogens that are members of the genus Ranavirus, family Iridoviridae. This disease has a wide host range and with the movement of ranavirus-infected animals in commerce, ranaviruses have become an increasing threat to captive and wild populations, especially amphibians. The clinical signs of amphibians include haemorrhaging, lethargy, swelling, and ulcerative skin lesions. The virus is highly contagious, spreading through direct contact with infected individuals or contaminated environments, such as water bodies. Environmental factors, including temperature and pollution, can influence the severity and spread of ranavirus infections. The ecological impact of ranavirus is profound, as it threatens biodiversity and disrupts ecosystems. Consequently, understanding the epidemiology, pathology, and control measures for ranavirus is critical for conserving amphibian populations. Current research efforts are focused on developing effective strategies for monitoring, managing, and mitigating the impacts of this pathogen on amphibian communities. This review presents distribution, hosts, and epidemiology to better understand this disease.

Keywords: Amphibian, Virus, Ranavirus, Mortality, Iridoviridae.

REVIEW PAPER

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How to cite this paper:

Andreia Garcês & Isabel Pires
(2024). Ranavirus and Its Impact
on the Amphibian Population.
Middle East Res J Biological Sci,
4(4): 127-138.

Article History:

| Submit: 14.07.2024 |
| Accepted: 13.08.2024 |
| Published: 14.08.2024 |

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1. INTRODUCTION

Ranaviruses are a group of viruses in the family Iridoviridae that infect a wide range of ectothermic vertebrates, including fish, amphibians, and reptiles (Brenes *et al.*, 2014). They are known to cause significant disease and mortality in these populations, and their distribution is global. The virus was first detected in northern leopard frogs (*Rana pipiens*) in 1965 (Granoff, Came, e Rafferty Jr. 1965). Mass mortality of amphibians associated with this agent occurred globally around 1990 (Gray, Miller, e Hoverman 2009). It has a high mortality rate of 90%-100%, with the potential to eliminate entire species if not controlled (Brenes *et al.*, 2014). The commerce of amphibian species, whether for the pet trade, research, or food, plays a significant role in the spread of Ranavirus. The global trade in amphibians often involves the movement of individuals across international borders, which can inadvertently introduce Ranavirus into new regions and populations. This translocation increases the risk of outbreaks in wild populations, as infected individuals can transmit the virus to native species. Furthermore, the conditions under which amphibians are often kept and transported—crowded and stressed environments—can

exacerbate the spread and severity of infections (Picco e Collins 2008).

Ranavirus in amphibians is now listed as notifiable by the World Organization for Animal Health (OIE) Aquatic Animal Health Code. World Organization for Animal Health (OIE) developed the World Animal Health Information System (WAHIS) where the OIE member states are required to regularly update their national and regional statutes concerning listed diseases such as ranavirus («WOAH», s.d.). This reporting database facilitates risk analysis related to international trade in animal products and sanitary practices in the global food trade, including amphibians, thereby helping to prevent pathogen pollution and reduce the risk of new disease outbreaks (Black, Meredith, e Price 2017)

The ecology of this virus is complex, with the interaction of reservoir hosts, transmission routes, environmental persistence, host immunity, and stressors (Gray, Miller, e Hoverman 2009). This paper aims to present a brief review of ranavirus and its impact on the amphibian population, particularly endangered species.

2. Aetiological Agent

Ranaviruses belong to the genus *Ranavirus* of the Family Iridoviridae. Iridoviruses have an icosahedral capsid with a diameter ranging from 120 to 300 nanometers. They possess a complex protein shell surrounding a lipid bilayer and the DNA core. The genome comprises double-stranded DNA, typically ranging from 150 to 300 kilobase pairs. It contains several open-reading frames that code for proteins involved in virus replication, host interaction, and immune evasion (King *et al.*, 2012). At electron microscopic images is possible to observe the distinctive icosahedral shape in the cytoplasm of infected cells (Daszak *et al.*, 1999).

Although 6 species of Ranavirus were identified, the most common to infect amphibians: Frog

Virus 3 (FV3), Bohle iridovirus (BIV) and *Ambystoma tigrinum* virus (Gray e Chinchar 2015a) (Table 1). Research on well-characterized isolates shows that FV3-like ranavirus strains can cause mortality in diverse and geographically separated hosts, such as the *Rana temporaria* in the UK or the *Lithobates sylvaticus* near the Arctic Circle in Canada. Another ranavirus, *Ambystoma tigrinum* virus (ATV), has been found only in *Ambystoma tigrinum* in western North America (Lesbarrères *et al.*, 2011). Phylogenetic evidence suggests that several testudinal reptile ranaviruses are closely related to amphibian ranaviruses, indicating interclass host shifts and that ancestral ranaviruses likely infected fish before jumping to amphibians and reptiles (Black, Meredith, e Price 2017; Lesbarrères *et al.*, 2011).

Table 1: Ranaviruses reported from amphibians. Adapted from (Gray e Chinchar 2015a; Lesbarrères *et al.*, 2011)

Location	Virus	Amphibian species	References
China	Tiger frog virus (TFV)	<i>Rana tigrina</i>	(He <i>et al.</i> , 2002)
	Rana grylio virus (RGV)	<i>Rana grylio</i>	(Zhang <i>et al.</i> , 2001)
Thailand	Rana tigrina virus (RTV)	<i>Rana tigrina</i>	(Essbauer e Ahne 2001)
Australia	Bohle Iridovirus (BIV)	<i>Limnodynastes ornatus</i> , <i>Litoria caerulea</i> , <i>Rhinella marina</i>	(Speare 2001)
	Mahaffey Road ranavirus (MHRV)	<i>Litoria splendida</i>	(Weir <i>et al.</i> , 2012)
Canada	Regina ranavirus (RRV)	<i>Ambystoma tigrinum diaboli</i>	(Bollinger <i>et al.</i> , 1999)
USA	Tadpole edema virus (TEV)	<i>Lithobates catesbeianus</i>	(Wolf <i>et al.</i> , 1968)
	Frog virus 3 (FV3), (FV1, 2, 9-23), LT1-LT4	<i>Rana pipiens</i>	(Hyatt <i>et al.</i> 2000)
	<i>Ambystoma tigrinum</i> virus (ATV)	<i>Ambystoma tigrinum</i>	(J. K. Jancovich <i>et al.</i> , 2001)
	T6-20	<i>Diemictylus viridescens</i>	(Essbauer e Ahne 2001)
	NVT	<i>Notophthalmus viridescens</i>	(Essbauer e Ahne 2001)
	FV1-3, FV9-23	<i>Rana pipiens</i>	(Essbauer e Ahne 2001)
	Xenopus virus (XV)	<i>Xenopus laevis</i>	(Essbauer e Ahne 2001)
	Rana catesbeiana virus Z (RCV-Z)	<i>Lithobates catesbeianus</i>	(Majji <i>et al.</i> , 2006)
	Redwood Creek virus	<i>Rana aurora</i>	(Mao <i>et al.</i> , 1999)
Venezuela	Zoo rana virus (Bohle-like iridovirus)	<i>Anaxyrus boreas boreas</i>	(Majji <i>et al.</i> , 2006)
Venezuela	Guatopo virus (GV)	<i>Rhinella marina</i>	(Hyatt <i>et al.</i> , 2000)
Croatia	Rana esculenta iridovirus	<i>Rana esculenta</i>	(Fijan <i>et al.</i> , 1991)
UK	Rana UK virus	<i>Rana temporaria</i>	(Drury, Gough, e Cunningham 1995)
	Bufo UK virus	<i>Bufo bufo</i>	(Essbauer e Ahne 2001)
	Rana esculenta iridovirus (REIR)	<i>Rana esculenta</i>	(Essbauer e Ahne 2001)

2. Hosts, Transmission Mechanisms and Detection

Ranaviruses infect amphibians, reptiles, and fish. In the case of amphibians, the orders Anura and Caudata are affected. It has been detected in 72 different species of amphibians (14 families), with most cases being reported in the family Ranidae (Gray, Miller, e Hoverman 2009). Some amphibian species seem to be more susceptible to the virus, which may be related to their co-evolutionary history with the pathogen (Gray, Miller, e Hoverman 2009).

Some of the most susceptible species are the *Lithobates sylvaticus*, *L. capito* and *Scaphiopus holbrookii*. Rare amphibian species affected by ranavirus-induced mortality include the *Rana muscosa*, *R. draytonii*, *Anaxyrus boreas boreas*, and *Andrias davidianus* (Miller, Gray, e Storfer 2011; Ruggeri *et al.*, 2024). Table 2 represents some of the species where ranavirus was reported.

Table 2: Distribution of ranavirus in wild and captive amphibians. Adapted from (Miller, Gray, e Storfer 2011)

Country	Familiy	Specie	Wild (W), Captivity (C)
Cameroon	Pipidae	<i>Xenopus longipes</i>	W
China	Ranidae	<i>Rana dybowskii</i>	C
		<i>Rana grylio</i>	W
		<i>Hoplobatrachus tigerinus</i>	C
	Hynobiidae	<i>Hynobius nebulosus</i>	C
	Ranidae	<i>Lithobates catesbeianus</i>	W
Japan	Hynobiidae	<i>Hynobius nebulosus</i>	C
	Ranidae	<i>Lithobates catesbeianus</i>	W
Thailand	Ranidae	<i>Hoplobatrachus tigerinus</i>	C
Australia	Hylidae	<i>Litoria caerulea</i>	W, C
		<i>Litoria splendida</i>	C
	Myobatrachidae	<i>Limnodynastes ornatus</i>	C
		<i>Pseudophryne coriacea</i>	C
Belgium	Bufonidae	<i>Bufo bufo</i>	W
	Ranidae	<i>Lithobates catesbeianus</i>	W
	Salamandridae	<i>Tylotriton kweichowensis</i>	C
Croatia	Ranidae	<i>Pelophylax esculenta</i>	W
Denmark	Ranidae	<i>Pelophylax esculenta</i>	W
France	Ranidae	<i>Rana temporaria</i>	W
Germany	Ranidae	<i>Pelophylax esculenta</i>	C
	Salamandridae	<i>Neurergus crocatus</i>	C, W
Israel	Bufonidae	<i>Pseudepidalea viridis</i>	W
Italy	Ranidae	<i>Pelophylax esculenta</i>	-
Netherlands	Dendrobatidae	<i>Dendrobates auratus</i>	C
		<i>Phyllobates bicolor</i>	C
		<i>Phyllobates vittatus</i>	C
	Ranidae	<i>Pelophylax spp.</i>	W
	Salamandridae	<i>Lissotriton vulgaris</i>	C
Portugal	Alytidae	<i>Alytes obstetricans</i>	W
	Salamandridae	<i>Lissotriton boscai</i>	W
		<i>Triturus marmoratus</i>	W
		<i>Triturus boscai</i>	W
Spain	Alytidae	<i>Alytes obstetricans</i>	W
	Salamandridae	<i>Ichthyosaura alpestris</i>	W
Switzerland	Ranidae	<i>Pelophylax ridibunda</i>	C
UK	Alytidae	<i>Alytes obstetricans</i>	W
	Bufonidae	<i>Bufo bufo</i>	W
	Ranidae	<i>Rana temporaria</i>	W
	Salamandridae	<i>Lissotriton vulgaris</i>	W
Canada	Ambystomatidae	<i>Ambystoma mavortium</i>	W
		<i>Ambystoma spp.</i>	W
	Hylidae	<i>Hyla versicolor</i>	W
		<i>Pseudacris crucifer</i>	W
		<i>Pseudacris crucifer</i>	W
	Ranidae	<i>Lithobates clamitans</i>	W
		<i>Lithobates pipiens</i>	W, C
		<i>Lithobates sylvaticus</i>	W
		<i>Rana pretiosa</i>	W
Costa Rica	Salamandridae	<i>Notophthalmus viridescens</i>	W
	Bufonidae	<i>Rhaebo haematiticus</i>	W
		<i>Rhinella marina/Bufo marinus</i>	W, C
	Hylidae	<i>Scinax elaeochroa</i>	W, C
		<i>Smilisca baudinii</i>	W
	Centrolenidae	<i>Teratohyla spinosa</i>	W
	Craugastoridae	<i>Craugastor bransfordii</i>	W
<i>Craugastor fitzingeri</i>		W	

Country	Familiy	Specie	Wild (W), Captivity (C)	
		<i>Craugastor megacephalus</i>	W	
	Dendrobatidae	<i>Oophaga pumilio</i>	W	
Nicaragua	Hylidae	<i>Agalychnis callidryas</i>	W	
	Ranidae	<i>Lithobates forreri</i>	W	
USA	Ambystomatidae	<i>Ambystoma jeffersonianum</i>	W	
		<i>Ambystoma macrodactylum</i>	W	
		<i>Ambystoma maculatum</i>	W	
		<i>Ambystoma mavortium</i>	W	
		<i>Ambystoma opacum</i>	W	
		<i>Ambystoma talpoideum</i>	W	
		<i>Ambystoma tigrinum</i>	W, C	
	Bufonidae	<i>Anaxyrus americanus</i>	W	
		<i>Anaxyrus boreas boreas</i>	C	
		<i>Anaxyrus fowleri</i>	W	
		<i>Melanophryniscus stelzneri</i>	C	
	Cryptobranchidae	<i>Cryptobranchus alleganiensis</i>	C	
	Dendrobatidae	<i>Dendrobates auratus</i>	C	
		<i>Phyllobates terribilis</i>	C	
	Hylidae	<i>Acris crepitans</i>	W	
		<i>Hyla chrysoscelis</i>	W,C	
		<i>Hyla chrysoscelis/Hyla versicolor Complex</i>	W	
		<i>Hyla cinerea</i>	W	
		<i>Pseudacris clarkii</i>	W	
		<i>Pseudacris crucifer</i>	W	
		<i>Pseudacris feriarum</i>	W	
		<i>Pseudacris regilla</i>	W	
		<i>Pseudacris sierra</i>	W	
		Megophryidae	<i>Megophrys nasuta</i>	C
	Plethodontidae	<i>Desmognathus conanti</i>	C	
		<i>Desmognathus folkertsi</i>	C	
		<i>Desmognathus fuscus</i>	W	
		<i>Desmognathus imitator</i>	W	
		<i>Desmognathus marmoratus</i>	W	
		<i>Desmognathus monticola</i>	W	
		<i>Desmognathus ocoee</i>	W	
		<i>Desmognathus orestes</i>	W	
		<i>Desmognathus organi</i>	W	
		<i>Desmognathus quadramaculatus</i>	W	
		<i>Desmognathus santeetlah</i>	W	
		<i>Desmognathus wrighti</i>	W	
		<i>Eurycea cirrigera</i>	W	
		<i>Eurycea longicauda</i>	W	
		<i>Eurycea lucifuga</i>	W	
		<i>Eurycea wilderae</i>	W	
		<i>Gyrinophilus porphyriticus</i>	W	
		<i>Plethodon glutinosus complex</i>	W	
		<i>Plethodon jordani</i>	W	
		<i>Plethodon montanus</i>	W	
		<i>Plethodon welleri</i>	W	
		Ranidae	<i>Lithobates blairi</i>	W
			<i>Lithobates catesbeianus</i>	W,C
<i>Lithobates clamitans</i>	W			
<i>Lithobates palustris</i>	W			
<i>Lithobates pipiens</i>	W			
<i>Lithobates septentrionalis</i>	W			
<i>Lithobates sphenoccephalus</i>	W			
<i>Lithobates sylvaticus</i>	W			

Country	Family	Specie	Wild (W), Captivity (C)
		<i>Pyxicephalus adspersus</i>	C
		<i>Rana aurora</i>	W
		<i>Rana draytonii</i>	W
		<i>Rana heckscheri</i>	W
		<i>Rana luteiventris</i>	W
		<i>Rana mucosa</i>	W
	Rhacophoridae	<i>Rhacophorus dennysi</i>	C
	Salamandridae	<i>Notophthalmus viridescens</i>	W
	Scaphiopodidae	<i>Scaphiopus holbrookii</i>	W
Argentina	Leptodactylidae	<i>Atelognathus patagonicus</i>	w
Brazil	Ranidae	<i>Lithobates catesbeianus</i>	C
Uruguay	Ranidae	<i>Lithobates catesbeianus</i>	C
Venezuela	Bufonidae	<i>Rhinella marina/Bufo marinus</i>	W
	Leptodactylidae	<i>Leptodactylus</i> spp.	W

The pathogenesis of this disease is still under investigation and is not understood, but recent findings support viral entry through epithelial surfaces, with subsequent dissemination through the body of the animals. Transmission occurs by indirect and direct routes. It can occur due to direct contact with infected animals, ingestion of viral particles, or contact with contaminated surfaces (bodies of water, soil particles) (Gray, Miller, e Hoverman 2009; Gray e Chinchar 2015a). Vertical transmission is thought to occur but remains unproven. Figure 1 represents a conceptual model of amphibian ranavirus ecology in amphibians adapted from Gray *et al.*, 2009 (Gray, Miller, e Hoverman 2009). Ranavirus can affect animals at all life stages. Animals that contract the virus during a juvenile or larval stage (hatchlings and metamorphs) have higher mortality rates (Gray, Miller, e Hoverman 2009).

Ranavirus infection can be diagnosed through primary isolation and cell culture of the virus, molecular identification (PCR and sequencing), and light or electron microscopy.

Genetic variability within populations is correlated with susceptibility, with inbred individuals, like *Xenopus laevis*, being more susceptible than outbred ones. In the wild, this variation may be due to the adaptation of host immune genes to local ranavirus strains. Factors such as faster larval development, restricted distributions, and inhabiting semi-permanent breeding sites increase susceptibility in North American amphibian species (Robert *et al.*, 2005). Geographic factors, such as higher elevation watersheds, and environmental conditions like ambient temperature or pesticides influence susceptibility (Miller, Gray, e Storfer 2011). The developmental stage seems to affect susceptibility significantly. Adults are generally less susceptible due to more competent immune systems, but adult die-offs have been reported in Europe and in captivity (Rollins-Smith 2001). Metamorphosis is the

most vulnerable stage for several North American species, especially those in the Ranidae family. The *Scaphiopus holbrookii* is most vulnerable during the hatchling stage, likely due to delayed immune development from rapid growth. Conversely, eggs are the least susceptible stage, possibly protected by a mucopolysaccharide/mucoprotein capsule or vitelline membrane (Miller, Gray, e Storfer 2011).

Ranaviruses frequently cause epizootics and sometimes lead to population declines, making them a strong selective pressure on their hosts. This pressure often results in host-pathogen coevolution, as evidenced in several ranavirus-host systems. For instance, common frogs exhibit higher frequencies of specific MHC Class I alleles in previously infected individuals compared to uninfected ones. In the *Ambystoma tigrinum* and ATV system, multiple studies support coevolution (Storfer *et al.*, 2007). One line of evidence is the negative correlation between disease prevalence and cannibalism rates in salamander populations in Arizona, USA. Although cannibalistic salamanders gain a performance advantage, they face a higher risk of ATV infection, which leads to natural selection favouring reduced cannibalism in areas with high ATV prevalence (Collins, Zerba, e Sredl 1993). Common garden experiments indicate that these differences have a genetic basis, as salamanders did not alter their cannibalistic behaviour based on the presence of ATV, and field patterns were replicated in the lab. Additionally, phylogenetic analyses suggest coevolution between salamanders and ATV. Apart from three host switches due to the use of infected salamanders as fishing bait, the phylogenetic trees of salamanders and the virus show complete concordance, indicating a coevolutionary history. Moreover, spatial variations in the amino acid sequences of ATV's immune evasion genes among host populations further support the coevolutionary relationship (Teacher, Garner, e Nichols 2009).

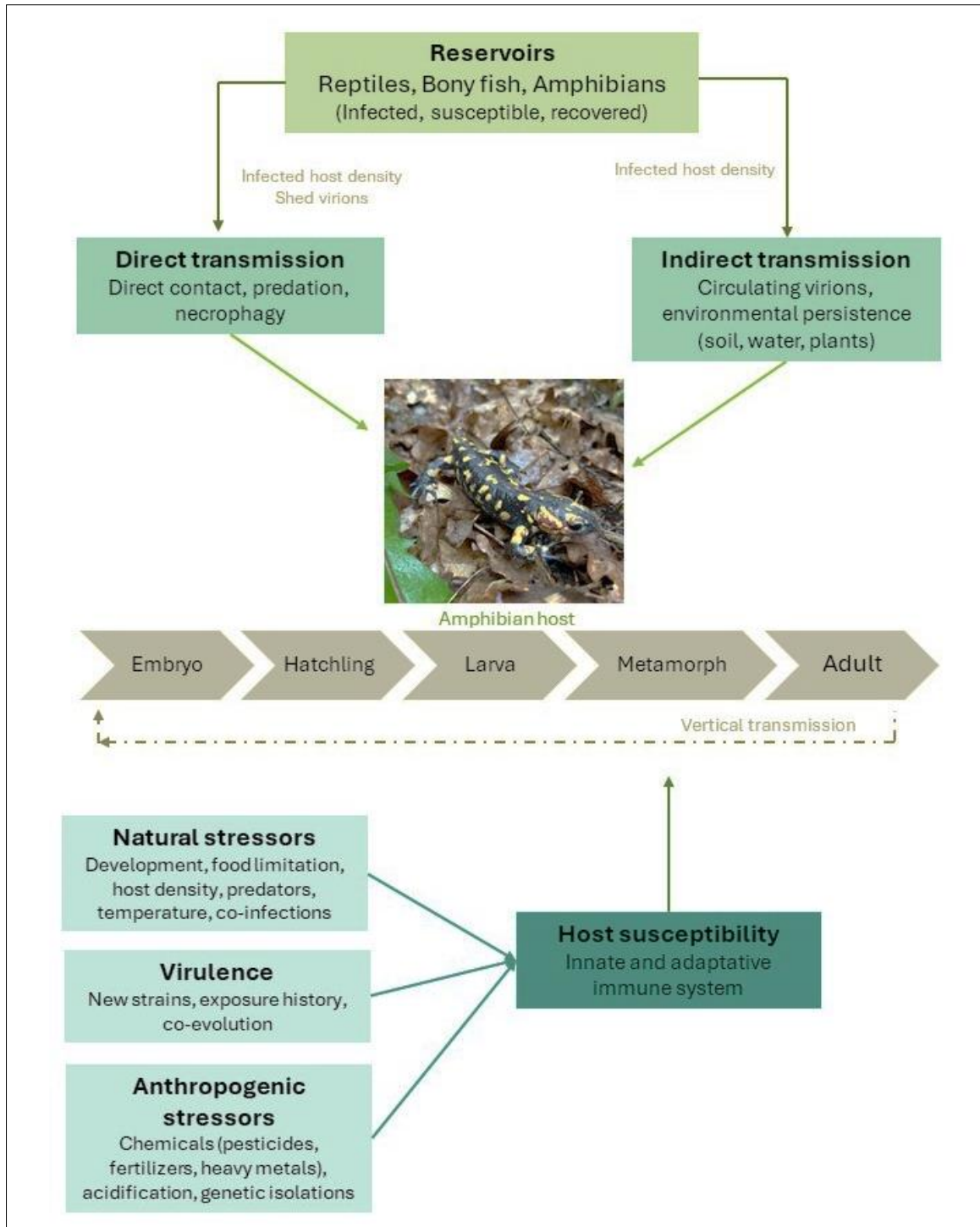


Figure 1: Conceptual model of amphibian ranavirus ecology. Adapted from (Gray e Chinchar 2015a)

2. DISTRIBUTION

Ranavirus is widely distributed across the globe. Figure 3 shows the distribution of ranavirus worldwide in 2024, based on the information provided by World Animal Health in both wild and domestic animals («World Animal Health Information System WAHIS», s.d.). It is not distributed uniformly across the landscape, with North and South America, Asia and Europe being the most affected (Campbell, Pawlik, e Harrison 2020; Une *et al.*, 2009; Roh *et al.*, 2022).

Some factors have been influencing the distribution of this agent, such as the international trade of amphibians, reptiles, and fish. Changes in land use and habitat destruction can stress populations and make them more susceptible to infections. Alterations in temperature and precipitation patterns can affect the prevalence and severity of ranavirus outbreaks (Brenes *et al.*, 2014; Duffus *et al.*, 2015).

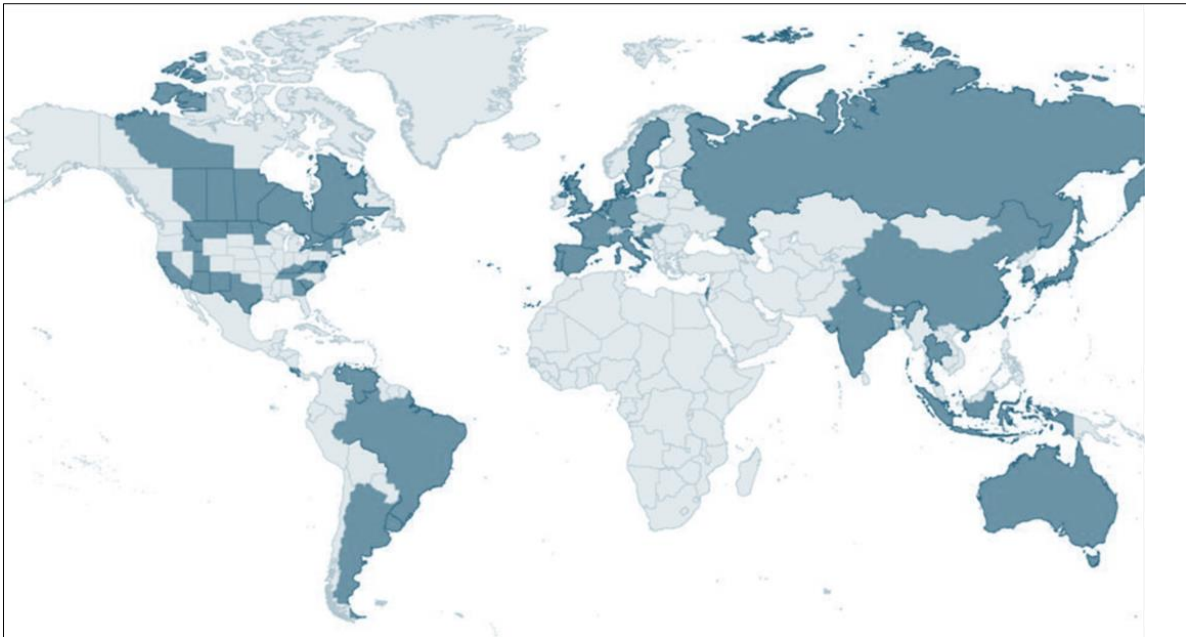


Figure 3: Ranavirus distribution worldwide. Dark blue represents the countries where the virus has been detected. Adapted from (Duffus *et al.*, 2015; Gray e Chinchar 2015b).

4. Clinical Signs and Post-Mortem Examination

Clinical signs observed in amphibians include lordosis, weak or erratic swimming, lethargy, mild to severe haemorrhages in the skin (especially near the base of the hind limbs and the vent opening), buoyancy problems, gasping for air, and mild to severe fluid

accumulation under the skin of the abdomen or hind legs, loss of pigmentation (Figure 4). Clinical signs and gross lesions are usually apparent in sublethal infections, but the severity depends on the extent of the disease (Gray, Miller, e Hoverman 2009; Miller, Gray, e Storfer 2011).

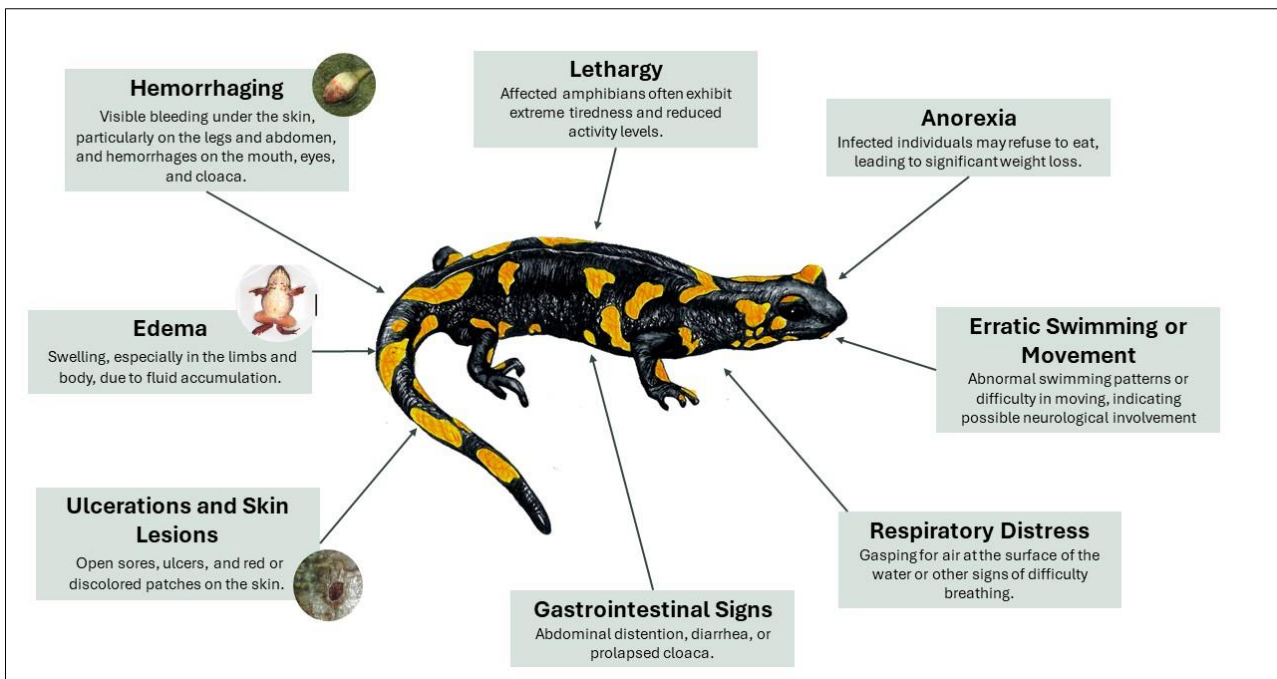


Figure 4: Clinical signs of ranavirus in amphibians (Author: Andreia Garcês)

Postmortem lesions can vary depending on the life cycle in which the animals are infected. In larvae were observed erythema at the base of the gills, ventrum, and legs, and swelling of the legs, body, and gular region. Histological lesions can be non-specific as

lymphocytolysis, lymphoid depletion, vacuolation of hepatocytes and renal tube epithelium, and extensive organ necrosis (liver, spleen, kidney, and intestines the most affected) (Miller, Gray, e Storfer 2011).

Ranaviral inclusion bodies are typically seen as variably-sized basophilic inclusions, although they can also appear eosinophilic, depending on the disease stage or host. Identifying these inclusions in necrotic areas is challenging due to their similarity with cellular debris. Jancovich *et al.*, (J. K. Jancovich *et al.*, 2001) observed intranuclear inclusion bodies in late-stage ranaviral disease in tiger salamanders, though viral particles were only found in the cytoplasm and intercellular spaces. While rare, intranuclear inclusions have been noted in other species, and their composition is still unclear. Recently, ranavirus presence in the nuclei of infected lizards was documented in Europe, which may help clarify these intranuclear inclusions (Gray e Chinchar 2015b).

Secondary pathogens often complicate ranavirus disease diagnosis (Gray e Chinchar 2015b). For example, hemorrhagic gastroenteritis, systemic haemorrhages, ulcerations, and bacterial infections have been observed in *Rana temporaria* (Cunningham *et al.*, 1996). Similar bacterial contaminations occur in captive anurans, where lesions are primarily linked to ranavirus but exacerbated by bacterial infections. Subclinically infected individuals rarely show secondary infections (Miller, Gray, e Storfer 2011).

5. Impact on Amphibian Populations

Ranaviruses have had a devastating impact on amphibian populations globally, contributing significantly to the decline of many species, including those already endangered (Gray, Miller, e Hoverman 2009; Gray e Chinchar 2015b). Table 3 shows some of those mass mortalities. These pathogens cause severe disease outbreaks characterized by high mortality rates, particularly affecting larval and juvenile amphibians. Some studies in Europe showed higher rates of adult mortality, as the study by von Essen *et al.*, (2020) in Spain (Essen *et al.*, 2020).

The rapid spread and high virulence of ranaviruses have led to mass die-offs, severely disrupting local populations and ecosystems (Gray e Chinchar 2015b; Black, Meredith, e Price 2017). Ranavirus poses a particularly severe threat to endangered species of amphibians, many of which already face significant pressures from habitat loss, climate change, and pollution. For these vulnerable species, an outbreak of Ranavirus can result in catastrophic population declines, potentially pushing them closer to extinction (Campbell, Pawlik, e Harrison 2020; Wheelwright *et al.*, 2014).

Table 3: Some examples of mass deaths caused by Ranavirus in wild populations of amphibians

Country	Year	Specie affected	Reference
UK	1991-1999	<i>Rana temporaria</i>	(Drury, Gough, e Cunningham 1995)
Australia	1992	<i>Limnodynastes ornatus</i>	(Speare e Smith 1992)
Canada (Saskatchewan)	1997	<i>Ambystoma tigrinum diaboli</i>	(Bollinger <i>et al.</i> , 1999)
USA (Arizona)	1998	<i>Ambystoma tigrinum stebbinsi</i>	(James K. Jancovich <i>et al.</i> , 1997)
USA (Dakota)	1998	<i>Ambystoma tigrinum</i>	(Docherty <i>et al.</i> , 2003)
USA (Maine)	1998	<i>Ambystoma maculatum</i>	(Docherty <i>et al.</i> , 2003)
USA (Utah)	1998	<i>Ambystoma tigrinum</i>	(Docherty <i>et al.</i> , 2003)
Canada (Ontario)	2002	<i>Rana sylvatica</i> , <i>Rana pipiens</i>	(Greer, Berrill, e Wilson 2005)
Spain	2008	<i>Mesotriton alpestris cyreni</i> , <i>Alytes obstetricans</i>	(Balseiro <i>et al.</i> , 2010)
Netherlands	2010	<i>Pelophylax spp.</i> , <i>Lissotriton vulgaris</i>	(Kik <i>et al.</i> , 2011)
USA (Maine)	2013	<i>Lithobates sylvaticus</i>	(Wheelwright <i>et al.</i> , 2014)

For endangered amphibian species, which often have limited distributions and small population sizes, ranavirus infections can push them closer to extinction. The loss of these species not only reduces biodiversity but also affects ecosystem functions, as amphibians play crucial roles in their habitats as both predators and prey (Earl *et al.*, 2016).

A significant concern is the anthropogenic movement and introduction of invasive, nonnative amphibian, fish, and reptile species that can act as ranavirus carriers, a phenomenon known as pathogen pollution (James K. Jancovich *et al.*, 2010). *Lithobates catesbeianus*, often infected with FV3 and FV3-like ranaviruses, are believed to spread these viruses through commercial trade (Miller, Gray, e Storfer 2011). Colonies of these species harbor highly virulent ranavirus strains, supporting the theory that high

pathogen virulence can evolve in densely populated captive environments. It was reported that over 28 million amphibians were imported into the United States from 2000 to 2005, with 8.5% infected with ranavirus (Schloegel *et al.*, 2009). Likewise, ATV has been spread by infected salamanders used as fishing bait. Salamanders from bait shops often test positive for ATV, and their introduction by anglers has spread the virus. Experiments indicate that bait ATV strains are more virulent than native strains, raising concerns about introducing highly virulent strains into areas with naïve hosts or those adapted to other ranavirus strains (Miller, Gray, e Storfer 2011; Mao *et al.*, 1996). Captivity-induced stress likely increases susceptibility to ranavirus infections in ranaculture and aquaculture operations, which typically focus on single-species production. Ranaviruses that infect fish can also cause disease in amphibians, suggesting host switches have occurred.

Thus, introducing fish species to new areas without proper precautions can pose serious disease threats to native amphibians (Williams, Barbosa-Solomieu, e Chinchar 2005).

6. Prevention and Future

To date, there is no treatment or vaccine for Ranavirus. Management is focused on biosecurity, quarantine, and decontamination to prevent the spread (Duffus *et al.*, 2015; Black, Meredith, e Price 2017) of the virus to other animals and new environments. Ranaviruses can survive outside their hosts for extended periods, facilitating their spread via sampling equipment, recreational gear, and fomites. Any infected animals should be quarantined to prevent infection. Wildlife biologists, veterinarians, rehabilitators, and anyone involved in amphibian or reptilian fieldwork should employ strict biosecurity protocols and clean and disinfect all equipment and clothes before and after working at a field site (Gray, Miller, e Hoverman 2009; Campbell, Pawlik, e Harrison 2020).

Following the guidelines of the World Organization for Animal Health (OIE), regulations should mandate testing amphibians reared in captivity for commercial purposes, especially those that could be released or escape into wild populations. For amphibians traded as bait regulations should either prohibit their use entirely or restrict their use to the watershed where they were captured (Miller, Gray, e Storfer 2011).

Managing ranavirus disease in captivity is simpler than in the wild. Key steps include isolating infected individuals, disinfecting enclosures, reducing stressors, and following strict biosafety protocols to prevent cross-contamination. Warm, frequently filtered water and low host densities can help prevent ranavirus outbreaks in captive settings (Gray e Chinchar 2015b; Balseiro *et al.*, 2010).

The development of a Ranavirus vaccine shows promise. Prior infection with a ranavirus has been found to enhance immunity against future exposure. While a vaccine may have limited use in the wild, it could be beneficial for captive populations. Researchers in Japan have successfully developed a vaccine for the red sea bream Iridovirus, which may serve as a basis for creating a Ranavirus vaccine. An oral formulation of this vaccine is currently being tested for use in fish, indicating potential future applications (Rojas *et al.*, 2005).

6. CONCLUSIONS

Ranavirus, a highly infectious pathogen, poses a significant threat to amphibian populations worldwide. It can cause rapid and severe outbreaks, leading to high mortality rates among affected species. The impact of Ranavirus on amphibians includes not only immediate population declines but also long-term ecological consequences. The reduction in amphibian numbers can disrupt local ecosystems, as amphibians play crucial

roles in pest control, nutrient cycling, and as prey for other wildlife. Furthermore, the loss of amphibian biodiversity can weaken ecosystem resilience, making habitats more vulnerable to other environmental stresses. Ranavirus research has extensively covered several key areas, including epidemiology and host range, documenting infections across various amphibians, reptiles, and fish, and revealing interspecies transmission potential. Pathogenesis studies have focused on how ranaviruses invade host cells, replicate, evade the immune response, and cause symptoms like haemorrhaging and organ necrosis. Genomics and molecular biology research have provided insights into the genetic diversity and evolution of ranaviruses and the functions of viral proteins. The environmental and ecological impact of ranavirus outbreaks on amphibian populations and biodiversity has been explored, along with factors like water temperature and habitat disturbance that facilitate virus transmission. Immune response studies have investigated how different species and life stages respond to infection, providing insights into innate and adaptive immune mechanisms. Conservation and management efforts have developed strategies to mitigate outbreaks, including exploring vaccination and other prophylactic measures to protect vulnerable species.

Topics such as the American and European FV3 strain's origin and processes of global spreading of the ranavirus are still not very understood, and more research is needed in the future. Several key areas of ranavirus research remain under-explored, including the long-term effects on population dynamics and ecosystem health, which require multi-year post-outbreak studies. The impact of climate change on ranavirus transmission and virulence is also emerging, necessitating research into how changing environmental conditions affect host susceptibility and virus ecology. Understanding virus evolution and adaptation, particularly the genetic mutations influencing virulence and host range, is crucial. The genetic basis for host susceptibility or resistance remains unclear, and identifying specific genetic markers could aid conservation. Additionally, interactions between ranavirus and other pathogens in co-infected hosts need investigation to inform disease management. Finally, while basic immune responses are known, the detailed molecular mechanisms of host immunity and viral evasion strategies require further study to develop effective therapeutic approaches. Future advances in the genomics of Ranavirus can be used to develop vaccines and control transmission in captive facilities, and at the same time protect wild populations.

Ethics Statement: Not applicable.

Informed Consent Statement: Not applicable.

Funding

This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020, funded

by the Portuguese Foundation for Science and Technology (FCT) (Pro-ject UIDB/CVT/0772/2020).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

- Balseiro, A., Dalton, K. P., Del Cerro, A., Márquez, I., Parra, F., Prieto, J. M., & Casais, R. (2010). Outbreak of common midwife toad virus in alpine newts (*Mesotriton alpestris cyreni*) and common midwife toads (*Alytes obstetricans*) in Northern Spain: A comparative pathological study of an emerging ranavirus. *The Veterinary Journal*, 186(2), 256-258.
- Black, Y., Meredith, A., & Price, S. J. (2017). Detection and reporting of ranavirus in amphibians: evaluation of the roles of the world organisation for animal health and the published literature. *Journal of wildlife diseases*, 53(3), 509-520.
- Bollinger, T. K., Mao, J., Schock, D., Brigham, R. M., & Chinchar, V. G. (1999). Pathology, isolation, and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. *Journal of Wildlife Diseases*, 35(3), 413-429.
- Brenes, R., Gray, M. J., Waltzek, T. B., Wilkes, R. P., & Miller, D. L. (2014). Transmission of ranavirus between ectothermic vertebrate hosts. *PloS one*, 9(3), e92476.
- Campbell, L. J., Pawlik, A. H., & Harrison, X. A. (2020). Amphibian ranaviruses in Europe: important directions for future research. *Facets*, 5(1), 598-614.
- Collins, J. P., Zerba, K. E., & Sredl, M. J. (1993). Shaping intraspecific variation: development, ecology and the evolution of morphology and life history variation in tiger salamanders. *Genetica*, 89, 167-183.
- Cunningham, A. A., Langton, T. S., Bennett, P. M., Lewin, J. F., Drury, S. E. N., Gough, R. E., & Macgregor, S. K. (1996). Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 351(1347), 1539-1557.
- Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E., & Speare, R. (1999). Emerging infectious diseases and amphibian population declines. *Emerging infectious diseases*, 5(6), 735.
- Docherty, D. E., Meteyer, C. U., Wang, J., Mao, J., Case, S. T., & Chinchar, V. G. (2003). Diagnostic and molecular evaluation of three iridovirus-associated salamander mortality events. *Journal of Wildlife Diseases*, 39(3), 556-566.
- Drury, S. E., Gough, R. E., & Cunningham, A. A. (1995). Isolation of an Iridovirus-like Agent from Common Frogs (*Rana Temporaria*). *The Veterinary Record*, 137(3), 72-73. <https://doi.org/10.1136/vr.137.3.72>.
- Duffus, A. L., Waltzek, T. B., Stöhr, A. C., Allender, M. C., Gotesman, M., Whittington, R. J., ... & Marschang, R. E. (2015). Distribution and host range of ranaviruses. *Ranaviruses: Lethal pathogens of ectothermic vertebrates*, 9-57.
- Earl, J. E., Chaney, J. C., Sutton, W. B., Lillard, C. E., Kouba, A. J., Langhorne, C., ... & Gray, M. J. (2016). Ranavirus could facilitate local extinction of rare amphibian species. *Oecologia*, 182, 611-623.
- Essbauer, S., & Ahne, W. (2001). Viruses of lower vertebrates. *Journal of Veterinary Medicine, Series B*, 48(6), 403-475.
- Fijan, N., Matasin, M., Perinec, Z., Valpotic, I., Zwillenberg, e LO. (1991). Isolation of an iridovirus-like agent from the green frog *Rana esculenta* L. *Veterinaski Archiv Zagred*, 61, 151-58.
- Granoff, A., Came, P. E., & Rafferty Jr, K. A. (1965). The isolation and properties of viruses from *Rana pipiens*: their possible relationship to the renal adenocarcinoma of the leopard frog. *Annals of the New York Academy of Sciences*, 126(1), 237-255.
- Gray, M. J., Brunner, J. L., Earl, J. E., & Ariel, E. (2015). Design and analysis of ranavirus studies: surveillance and assessing risk. *Ranaviruses*, 209.
- Gray, M. J., Miller, D. L., & Hoverman, J. T. (2009). Ecology and pathology of amphibian ranaviruses. *Diseases of aquatic organisms*, 87(3), 243-266.
- Gray, Matthew J., e V. Gregory Chinchar, eds. 2015b. *Ranaviruses*. Cham: Springer International Publishing. <https://doi.org/10.1007/978-3-319-13755-1>.
- Greer, A. L., Berrill, M., & Wilson, P. J. (2005). Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. *Diseases of aquatic organisms*, 67(1-2), 9-14.
- He, J. G., Lü, L., Deng, M., He, H. H., Weng, S. P., Wang, X. H., ... & Chan, S. M. (2002). Sequence analysis of the complete genome of an iridovirus isolated from the tiger frog. *Virology*, 292(2), 185-197.
- Hyatt, A. D., Gould, A. R., Zupanovic, Z., Cunningham, A. A., Hengstberger, S., Whittington, R. J., ... & Coupar, B. E. H. (2000). Comparative studies of piscine and amphibian iridoviruses. *Archives of virology*, 145, 301-331.
- Jancovich, J. K., Bremont, M., Touchman, J. W., & Jacobs, B. L. (2010). Evidence for multiple recent host species shifts among the ranaviruses (family Iridoviridae). *Journal of virology*, 84(6), 2636-2647.
- Jancovich, J. K., Davidson, E. W., Morado, J. F., Jacobs, B. L., & Collins, J. P. (1997). Isolation of a

- lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. *Diseases of Aquatic Organisms*, 31(3), 161-167.
- Jancovich, J. K., Davidson, E. W., Seiler, A., Jacobs, B. L., & Collins, J. P. (2001). Transmission of the *Ambystoma tigrinum* virus to alternative hosts. *Diseases of aquatic organisms*, 46(3), 159-163.
 - Kik, M., Martel, A., Spitzen-van der Sluijs, A., Pasmans, F., Wohlsein, P., Gröne, A., & Rijks, J. M. (2011). Ranavirus-associated mass mortality in wild amphibians, The Netherlands, 2010: A first report. *The Veterinary Journal*, 190(2), 284-286.
 - King, Andrew M. Q., Michael, J., Adams, E. B., Carstens, E., Elliot, J., & Lefkowitz, (2012). Family - Iridoviridae. Em *Virus Taxonomy*, 193–210. San Diego: Elsevier. <https://doi.org/10.1016/B978-0-12-384684-6.00019-7>.
 - Lesbarrères, D., Balseiro, A., Brunner, J., Chinchar, V. G., Duffus, A., Kerby, J., & Miller, D. L. (2011). Ranavirus: past, present and future. *Biology Letters*, 8(4), 481–83. <https://doi.org/10.1098/rsbl.2011.0951>.
 - Majji, S., LaPatra, S., Long, S. M., Sample, R., Bryan, L., Sinning, A., & Chinchar, V. G. (2006). *Rana catesbeiana* virus Z (RCV-Z): a novel pathogenic ranavirus. *Diseases of Aquatic Organisms*, 73(1), 1-11.
 - Mao, J., Green, D. E., Fellers, G., & Chinchar, V. G. (1999). Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. *Virus research*, 63(1-2), 45-52.
 - Mao, J., Tham, T. N., Gentry, G. A., Aubertin, A., & Chinchar, V. G. (1996). Cloning, sequence analysis, and expression of the major capsid protein of the iridovirus frog virus 3. *Virology*, 216(2), 431-436.
 - Miller, D., Gray, M., & Storfer, A. (2011). Ecopathology of ranaviruses infecting amphibians. *Viruses*, 3(11).
 - Picco, A. M., & Collins, J. P. (2008). Amphibian commerce as a likely source of pathogen pollution. *Conservation Biology*, 22(6), 1582-1589.
 - Robert, J., Morales, H., Buck, W., Cohen, N., Marr, S., & Gantress, J. (2005). Adaptive immunity and histopathology in frog virus 3-infected *Xenopus*. *Virology*, 332(2), 667-675.
 - Roh, N., Park, J., Kim, J., Kwon, H., & Park, D. (2022). Prevalence of Ranavirus infection in three anuran species across South Korea. *Viruses*, 14(5), 1073.
 - Rojas, S., Richards, K., Jancovich, J. K., & Davidson, E. W. (2005). Influence of temperature on Ranavirus infection in larval salamanders *Ambystoma tigrinum*. *Diseases of aquatic organisms*, 63(2-3), 95-100.
 - Rollins-Smith, L. A. (2001). Neuroendocrine-immune system interactions in amphibians: implications for understanding global amphibian declines. *Immunologic research*, 23, 273-280.
 - Ruggeri, J., Pontes, M. R., Ribeiro, L. P., Gendreau, K. L., Sousa, R. L. M., & Toledo, L. F. (2024). Predominant Prevalence of Ranavirus in Southern Brazil, a Region with Widespread Occurrence of the Amphibian Chytrid. *Animal Conservation*, 27(3), 338–49. <https://doi.org/10.1111/acv.12911>.
 - Schloegel, L. M., Picco, A. M., Kilpatrick, A. M., Davies, A. J., Hyatt, A. D., & Daszak, P. (2009). Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation*, 142(7), 1420-1426.
 - Speare, R. (2001). «REPORT TO ENVIRONMENT AUSTRALIA».) Developing management strategies to control amphibian diseases: decreasing the risks due to communicable disease.
 - Speare, R., & Smith, J. R. (1992). An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. *Diseases of Aquatic Organisms*, 14, 51-51.
 - Storfer, A., Alfaro, M. E., Ridenhour, B. J., Jancovich, J. K., Mech, S. G., Parris, M. J., & Collins, J. P. (2007). Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic. *Ecology Letters*, 10(11), 1075-1083.
 - Teacher, A. G., Garner, T. W., & Nichols, R. A. (2009). Evidence for directional selection at a novel major histocompatibility class I marker in wild common frogs (*Rana temporaria*) exposed to a viral pathogen (Ranavirus). *PloS one*, 4(2), e4616.
 - Une, Y., Nakajima, K., Taharaguchi, S., Ogihara, K., & Murakami, M. (2009). Ranavirus infection outbreak in the salamander (*Hynobius nebulosus*) in Japan. *Journal of Comparative Pathology*, 141(4), 310.
 - von Essen, M., Leung, W. T., Bosch, J., Pooley, S., Ayres, C., & Price, S. J. (2020). High pathogen prevalence in an amphibian and reptile assemblage at a site with risk factors for dispersal in Galicia, Spain. *PloS one*, 15(7), e0236803.
 - Weir, R. P., Moody, N. J. G., Hyatt, A. D., Crameri, S., Voysey, R., Pallister, J., & Jerrett, I. V. (2012). Isolation and characterisation of a novel Bohle-like virus from two frog species in the Darwin rural area, Australia. *Diseases of Aquatic Organisms*, 99(3), 169-177.
 - Wheelwright, Nathaniel, Matthew Gray, Rachel Hill, e Debra Miller. 2014. «Sudden Mass Die-off of a Large Population of Wood Frog (*Lithobates sylvaticus*) Tadpoles in Maine, USA, Likely Due to Ranavirus». *Herpetological Review* 45 (2).
 - Williams, T., Barbosa-Solomieu, V., & Chinchar, V. G. (2005). A decade of advances in iridovirus research. *Advances in virus research*, 65, 173-248.

- Wolf, K., Bullock, G. L., Dunbar, C. E., & Quimby, M. C. (1968). Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. *The Journal of infectious diseases*, 253-262.
- «WOAH». s.d. WOAH - World Organisation for Animal Health. Acedido a 22 de junho de 2024. <https://www.woah.org/en/home/>.
- «World Animal Health Information System WAHIS». s.d. WOAH - World Organisation for Animal Health. Acedido a 5 de junho de 2024. <https://www.woah.org/en/what-we-do/animal-health-and-welfare/disease-data-collection/world-animal-health-information-system/>.
- Zhang, Q. Y., Xiao, F., Li, Z. Q., Gui, J. F., Mao, J., & Chinchar, V. G. (2001). Characterization of an iridovirus from the cultured pig frog *Rana grylio* with lethal syndrome. *Diseases of Aquatic Organisms*, 48(1), 27-36.