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RANAVIRUSES IN NORTH AMERICA: A BRIEF REVIEW IN WILD HERPETOFAUNA

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ABSTRACT: Ranaviruses are globally emerging pathogens of poikilothermic vertebrates. They affect over 40 species of amphibians in the USA and at least nine species in Canada. Additionally, ranaviruses have been found in at least eight species of reptiles in the USA and two species in Canada. Several of the species that are known to be affected are listed by state or federal agencies as they are of conservation concern (e.g., *Cryptobranchus alleganiensis alleganiensis* and *Terrapene carolina carolina*). In this brief review, we discuss the diversity and distribution, the species affected, the clinical signs of ranavirus disease (ranavirosis) that one would see in the field, morbidity and mortality events and their likely triggers, and conservation implications from the emergence of ranaviruses in populations of North American herpetofauna. We also would like to stress that ranaviruses are reportable infections in amphibians (according to the OIE, World Health Organization for Animals) and if an infection is suspected, the incident should be reported to the proper authorities. Additionally, biosecurity measures should be taken to avoid the spread of ranaviruses between individuals and between field sites.

Key Words: infection; Ranavirus; amphibians; reptiles; wild; North America

INTRODUCTION

The family of viruses known as *Iridoviridae* are double-stranded DNA-containing viruses that affect poikilothermic animals. *Ranavirus*, a genus of *Iridoviridae*, specifically targets species of reptiles, amphibians, and bony fish (Chinchar et al., 2017). The first *Ranavirus* to be described, *Frog virus 3* (FV3) was isolated from *Lithobates pipiens* in the United States in the mid-1960s (Granoff et al., 1965; Clark et al., 1968). More than 30 years later, a second type of ranavirus known as *Ambystoma tigrinum virus* (ATV) was isolated in Arizona, and then again in Saskatchewan, and the connection was made between several mass die-off events of amphibians, which had previously been attributed to a possible bacterial infection, and this viral infection (Jancovich et al., 1997; Bollinger et al., 1999). Since then, ranaviruses have been found in more than 30 countries around the world, with more than 145 species of amphibians and at least 29 species of reptiles shown to be affected (Chinchar et al., 2021; Duffus et al., 2015). There are currently three recognized species of ranaviruses found in the wild in North America (Chinchar et al., 2017), as well as several chimeric strains which appear to be more fatal (Claytor et al., 2017; Peace et al., 2019; Vilaça et al., 2019).

In this brief review, we will examine the salient points of ranavirus biology in wild North American herpetofauna. While amphibian ranavirus infections have been more extensively studied than reptiles, the number of ranavirus infections in North American reptiles is also increasing. We will first explore the diversity and distributions of ranaviruses, the clinical field signs of infection associated with ranavirus infections, the suspected triggers of morbidity and mortality events, conservation implications and some brief conclusions.

DIVERSITY AND DISTRIBUTION

Ranaviruses are widely distributed in North America (see Figures 1 and 2). In fact, nearly everywhere researchers have looked for ranaviruses, they have found them in wild herpetofaunal species. A notable exception in North America has been that no ranavirus infections were found in wild invasive amphibian species in Hawai'i (Goodman et al., 2019). In the USA, there are currently at least eight species of Ambystomatids, five species of

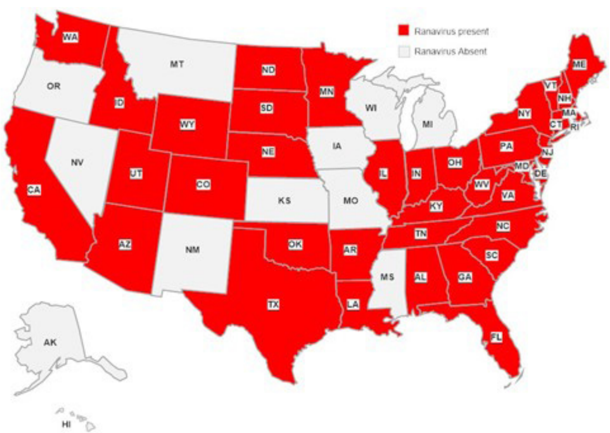

Figure 1. Known distribution of *Ranavirus* in wild herpetofauna of the USA.

Figure 2. Known distribution of *Ranavirus* in Canadian herpetofauna.

Table 1. Family and species of amphibians known to be infected with *Ranavirus* in the USA. (Adapted from Chinchar et al., 2021). Bolded species are those that are of conservation concern at the state, federal, or international level.

Family	**Species Name**	**First report**
Ambystomatidae	***Ambystoma californiense***	Picco et al., 2008
	Ambystoma jeffersonianum	Green & Ip (Unpublished) In Miller et al., 2011
	Ambystoma macrodactylum	Green & Ip (Unpublished) In Miller et al., 2011
	Ambystoma maculatum	Green et al., 2002
	Ambystoma opacum	Todd-Thompson, 2010
	Ambystoma talpoideum	O'Bryan et al., 2012
	Ambystoma tigrinum	Green et al., 2002
	Ambystoma tigrinum stebbinsi	Jancovich et al., 1997
Bufonidae	*Anaxyrus americanus*	Hoverman et al., 2012a
	Anaxyrus boreas	Patla et al., 2016
	Anaxyrus fowleri	Monson-Collar et al., 2013
	Anaxyrus terrestris	Love et al., 2016
	Anaxyrus woodhousii	Davis and Kerby, 2016
Cryptobranchidae	***Cryptobranchus alleganiensis alleganiensis***	Souza et al., 2012
Eleutherodactylidae	*Eleutherodactylus planirostris*	Rivera et al., 2019
Hylidae	***Acris blanchardi***	Davis and Kerby, 2016
	Acris crepitans	Hoverman et al., 2012b
	Hyla chrysoscelis/Hyla versicolor Complex	O’Bryan et al., 2012
	Hyla cinerea	Converse and Green, 2005
	Hyla squirella	Rivera et al., 2019
	Osteopilus septentrionalis	Galt et al., 2021
	Pseudacris clarkii	Torrence et al., 2010
	Pseudacris crucifer	Green et al., 2002
	Pseudacris feriarum	Todd-Thompson, 2010
	Pseudacris fouquettei	Davis et al., 2019
	Pseudacris maculata	Davis and Kerby, 2016
	Pseudacris regilla	Green & Ip (Unpublished) In Miller et al., 2011

Table 1, continued

	Pseudacris sierra	Russell et al., 2011
	Pseudacris streckeri	Davis et al., 2019
Microhylidae	*Gastrophryne carolinensis*	Davis et al., 2019
	Gastrophryne olivacea	Davis et al., 2019
Plethodontidae	***Aneides aeneus***	Blackburn et al., 2015
	Desmognathus conanti	Gray et al., 2009
	Desmognathus folkertsi	Rothermel et al., 2013
	Desmognathus fuscus	Davidson and Chambers, 2011
	Desmognathus imitator	Gray et al., 2009
	Desmognathus marmoratus	Rothermel et al., 2013
	Desmognathus monticola	Gray et al., 2009
	Desmognathus ocoee	Gray et al., 2009
	Desmognathus orestes	Hamed et al., 2013
	Desmognathus organi	Hamed et al., 2013
	Desmognathus quadramaculatus	Gray et al., 2009
	Desmognathus santeetlah	Gray et al., 2009
	Desmognathus wrighti	Gray et al., 2009
	Eurycea cirrigera	Davidson and Chambers, 2011
	Eurycea longicauda	Davidson and Chambers, 2011
	Eurycea lucifuga	Davidson and Chambers, 2011
	Eurycea wilderae	Gray et al., 2009
	Gyrinophilus porphyriticus	Gray et al., 2009
	Plethodon chlorobryonis	Love et al., 2016
	Plethodon glutinosus	Davidson and Chambers, 2011
	Plethodon jordani	Gray et al., 2009
	Plethodon montanus	Hamed et al., 2013
	Plethodon welleri	Hamed et al., 2013
Ranidae	*Lithobates blairi*	Green & Ip (Unpublished) In Miller et al., 2011
	Lithobates catesbeianus	Wolf et al., 1968
	Lithobates clamitans	Green et al., 2002
	Lithobates palustris	Green et al., 2002
	Lithobates pipiens	Clark et al., 1968
	Lithobates septentrionalis	Green et al., 2002
	Lithobates sphenocephalus	Hoverman et al., 2012b
	Lithobates sylvaticus	Green et al., 2002
	Rana aurora	Mao et al., 1999
	Rana chiricahuensis	Isidoro-Ayza et al., 2017
	Rana draytonii	Green & Ip (Unpublished) In Miller et al., 2011
	Rana heckscheri	Green & Ip (Unpublished) In Miller et al., 2011
	Rana luteiventris	Converse & Green, 2005; Green & Converse, 2005
	Rana muscosa	Converse and Green, 2005
	Rana pretiosa	Isidoro-Ayza et al., 2017
Salamandridae	*Notophthalmus viridescens*	Granoff et al., 1965
Scaphiopodidae	*Spea bombifrons*	Davis and Kerby, 2016

Table 2. The families and species names of reptiles known to be affected by *Ranavirus* in the USA. Bolded species are those that are of conservation concern at the state, federal or international level.

Family	Species Name	First Report
Emydidae	*Chrysemys picta picta*	Goodman et al., 2013
	Graptemys pseudogeographica	Butterfield, 2019
	Terrapene carolina bauri	Johnson et al., 2008
	Terrapene carolina carolina	Mao et al., 1997
Kinosternidae	*Kinosternon subrubrum*	Winzeler et al., 2015
Phrynosomatidae	*Sceloporus undulatus*	Goodman et al., 2018
Testudinidae	***Gopherus polyphemus***	Westhouse et al., 1996

Table 3. The families and species names of amphibians known to be infected with *Ranavirus* in Canada. Bolded species are those that are of conservation concern at the provincial/territorial, federal or international level.

Family	Species Name	First Report
Ambystomatidae	*Ambystoma tigrinum diaboli*	Bollinger et al., 1999
Bufonidae	*Anaxyrus hemiophrys*	Grant et al., 2019
Hylidae	*Hyla versicolor*	Duffus et al., 2008
	Pseudacris maculata	Forzan et al., 2019
	Pseudacris spp.	Duffus et al., 2008
Ranidae	*Lithobates clamitans*	St. Amour and Lesbarrères, 2007
	Lithobates pipiens	Greer et al., 2005
	Lithobates sylvaticus	Greer et al., 2005
Salamandridae	*Notophthalmus viridescens*	Duffus et al., 2008

Table 4. The reptilian species in Canada that are known to be infected by *Ranavirus*.

Family	Species Name	First Report
Chelydridae	*Chelydra serpentina*	McKenzie et al., 2019
Emydidae	*Chrysemys picta*	Carstairs et al., 2019

Bufonids, one species of Cryptobranchid, one species of Eleutherodactylid, 14 species of Hylids, two species of Microhylids, 23 species of Plethodontids, 15 species of Ranids, one species of Salamandrid, and one Scaphiodid that are known to be affected by ranavirus infections in the wild (see Table 1). In reptiles, there are at least four species from the Emydidae, one Kinosternid, one Phrynostomatid, and one Testudinid that are known to be affected by ranavirus infection in the wild. Affected herpetofaunal species are known to occur in every state, yet not every state reports infection in these animals (See Figure 1), most likely because of a lack of surveillance and/or publication in the literature.

In Canada, there are at least nine species of amphibians (see Table 2) and two species of testudinids (see Table 3) that are known to be affected with ranavirus in the wild. Again, the absence of known ranavirus infections in several provinces and territories is likely due to a lack of surveillance and/or publication in the academic literature.

There are two main species of ranaviruses that are present in North America. *Frog virus 3* (FV3) was originally isolated from *Lithobates pipiens* sourced from Wisconsin or Minnesota in the 1960s (Granoff et al., 1965). This is the dominant species of ranavirus in the eastern portion of North America. *Ambystoma tigrinum virus* (ATV) was originally isolated from a mortality event involving *Ambystoma tigrinum stebbinsi* from the San Rafael Valley in Arizona (Jancovich et al., 1997). This is the dominant species of ranavirus in the western portion of North America.

In addition to FV3 and ATV, there are also chimeric ranaviruses. In 2017, Claytor et al. reported a chimeric ranavirus in the southeastern portion of the USA. This virus is a combination of the *Common midwife toad virus* (CMTV) and FV3 (Claytor et al., 2017). Until Claytor et al. (2017), CMTV had not been described in North America, having only been found in Europe and Asia. In 2019, Vilaça et al. reported chimeric CMTV-FV3 ranaviruses in central and Northwestern Canada. The presence of these chimeric ranaviruses should be of concern because they tend to be more deadly than non-chimeric viruses (Earl and Gray, 2014; Claytor et al., 2017; Peace et al., 2019; Vilaça et al., 2019). Additionally, we do not know the full extent of the ranges of these chimeric viruses, nor do we know how widespread CMTV is in North America.

CLINICAL FIELD SIGNS OF DISEASE

Unfortunately, many of the clinical field signs associated with ranaviral disease (also called ranavirosis) are non-specific and can be caused by other infectious agents. (See Table 5 for a clinical field signs associated with ranavirosis in herpetofauna). Therefore, it is important to include multiple methods of diagnosis, such as pathology, histopathology, molecular methods (e.g. polymerase chain reaction, immunohistochemistry), and

Table 5. Examples of some of the clinical field signs associated with ranavirosis in herpetofauna. Table adapted from Miller et al. (2015).

Class	Abnormality/Lesion
Amphibian Larvae	Loss of buoyancy; erratic swimming; swelling of the body, head, and legs; external hemorrhages, especially near the vent, eyes, gular region, abdomen, and legs; anorexia.
Adult Anurans	Lethargy; anorexia; in aquatic species, loss of buoyancy and/or erratic swimming; swelling of the legs, feet and body; skin ulcerations; hemorrhages in the skin and in the mouth.
Adult Caudates	Lethargy; anorexia; in aquatic species, loss of buoyancy and/or erratic swimming; swelling; hemorrhages, especially on the tail and bottoms of the feet; skin ulcerations.
Chelonians	Difficulty respiring; anorexia; necrotic plaques in the mouth; swelling of the head, eyes, neck, and legs; skin ulcerations; external hemorrhages.
Lizards	Lethargy; anorexia; necrotic plaques in the mouth; skin ulcerations; swelling.

if possible, virus isolation and sequencing for diagnosing ranaviral disease and/or associated mortality. For an excellent review of all the potential diagnostic methods for ranavirus infections, please see Miller et al. (2015).

MORBIDITY AND MORTALITY EVENTS

There are many factors which may encourage ranaviral infections in wild populations. One such factor may be seasonal temperatures; mortality rates seem to increase as the weather warms up, peaking in mid-to-late summer in late-stage tadpoles and those that have recently gone through metamorphosis (Green et al., 2002), with the majority of die-off events for amphibians occurring June through August in North America (Brunner et al., 2015).

While the role of humans in the spread of ranavirus is still being studied, there does seem to be a correlation between human activity and the prevalence of ranaviral infections in the wild, with proximity to homes and industry increasing the chances of infected frogs (St. Amour et al., 2008). Humans also exacerbate the spread by altering the natural landscape and changing the amount of vegetation in ponds, such as in cattle fields (Greer and Collins, 2008; Hoverman et al., 2012a). Widespread use of pesticides and herbicides and the runoff of those substances from fields into waterways, as well as parasites, also contribute to increases in the mortality rates by decreasing overall animal health (Kerby and Storfer, 2009; Marcogliese and Pietrock, 2011, Goodman et al. 2021).

If you happen upon a morbidity and/or mortality event that involves amphibians or reptiles, it is important to report them. Ranaviruses in amphibians are reportable infections according to the OIE (OIE 2019). An easy way to report them in North America is by using the Partners in Amphibian and Reptile Conservation (PARC) Herpetofaunal Disease Alert System (HDAS). To report the event, you will need to include your name, email address, the date and time of the event that you saw, a description of what you saw and where it was, the types of herpetofauna that were involved, what ages they were (eggs, larvae, juveniles, adults), if the event is ongoing (i.e. are there sick animals that are still in the area or are there only dead/rotting animals), photographs if possible and any other observations that you made at the time (e.g. strange smells, sheens on the water, recent weather events). The email to report this information to the PARC HDAS is herp_disease_alert@parcplace.org and more information can be found at https://parcplace.org/resources/parc-disease-task-team/. However, even if you report the morbidity and/or mortality event through PARC's HDAS system, it is advisable that you also report them to your local and state authorities separately to ensure that they receive the report.

CONSERVATION IMPLICATIONS

There are several implications of the wide distribution of ranaviruses in North America. While declines in North American species have not been explicitly linked to ranavirus emergence, there are other places where they have been. In Europe, the emergence of novel ranaviruses has resulted in the decline of amphibian populations in the UK and in Spain (Teacher et al., 2010; Bosch et al., 2021). However, in North America we do not appear to have the long-term data sets that permit us to assess population level declines and attribute them to ranaviruses. If they do exist, they have not yet been published in the academic literature. The fact that the emergence of ranaviruses can cause population declines is concerning because there are several amphibian and reptile species of conservation concern that are known to be affected by these viruses in the wild (e.g. hellbenders, *Cryptobranchus alleganiensis alleganiensis*, and box turtles, *Terrapene carolina* spp.; see Tables 1 and 2). Additionally, models predict that the emergence of the more virulent chimeric viruses in common species (*Lithobates sylvaticus*) can cause localized extirpations of tadpoles (Earl and Gray, 2014; Peace et al., 2019). Little is known about the effects of these viruses on adult individuals, as most studies are performed on tadpoles.

During field studies, it is imperative to follow biosecurity protocols to reduce the possibility of transmission of ranaviruses (and other pathogens) between sites. There are a number of resources available that detail proper decontamination procedures and appropriate disinfectants for the infectious agents that can cause diseases in herpetofauna (e.g. Gray et al., 2017). There are also protocols available on the PARC Disease Task Team's website (see above) and the Southeast Partners in Amphibian and Reptile Conservation (SEPARC) Disease Task Team website (http://separc.org/diseases-herpetofauna). Following biosecurity protocols between sites is effective at reducing transmission between field sites and more information about this can be found in Olson et al. (2021).

CONCLUSION

Ranaviruses pose an underestimated threat to wild North American herpetofauna. They are widely distributed and persistent pathogens not only at the population level, but at the community level as well. While they have been documented in North America since the 1960s (Granoff et al., 1965), the number of species experiencing negative impacts and mortality events since the late

1990s has increased (e.g. Green et al., 2002; Duffus et al., 2015; Chinchar et al., 2021). The taxonomic and geographic distributions of ranaviruses in North America are unknown, and both are likely to be underestimates. Importantly, they are known to affect species that are of conservation concern and therefore require more attention ecologically than they currently receive. We need to understand what is 'normal' with ranaviruses across North America. To do this, we need to increase surveillance in wild populations of herpetofauna and other affected species (i.e., fish). We also need to understand the different species, strains, and chimeric viruses that are present in these populations and assess the threats that they pose to other susceptible species. In addition to this, we need to further understand community transmission of ranaviruses. To understand all these different facets of ranavirus biology in North America, interdisciplinary teams will be necessary, and a collaborative effort will yield the best results.

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