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Ranaviruses in Wild Reptiles in the USA

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Introduction:

Ranaviruses (family *Iridoviridae*, genus *Ranavirus*) are double-stranded DNA viruses that replicate at temperatures of 12-32 °C. Ranaviruses are highly persistent and can survive outside of a host in aquatic environments for months and at temperatures < 40 °C (Daszak et al. 1999; Chinchar et al. 2017; La Fauce et al 2012; Nazir et al. 2012). They infect and can cause mass mortality events in reptiles, amphibians and fishes (Duffus et al. 2015). Studies on ranavirus pathogenesis and disease ecology have focused largely on amphibians and fishes, and have demonstrated that susceptibility and severity of infection vary with age and species of host, virus strain, and presence of environmental stressors (Brunner et al. 2005; Brunner et al. 2015; Forson & Storfer 2006; Schock et al. 2009; Hoverman et al. 2010; Whittington et al. 2010). The impact of ranaviruses on reptilian population dynamics and factors contributing to pathogenicity and host susceptibility have been largely unexplored.

In the USA, ranaviral infections have been confirmed in eight wild reptile species including seven chelonians and one lizard (Table 1). While ranaviruses have been found in snakes in other countries (Duffus et al. 2008), in the USA, infection has only been documented for a closely related iridoviridus (*Thamnophis sauritus* erythrocytic virus (TsEV); Wellehan et al. 2008).

Clinical Signs of Ranaviral Disease:

Among reptiles, ranavirus infection has been studied most in **chelonians**, in which signs include lethargy; anorexia; ulcerative stomatitis and yellow-white plaques on the tongue; nasal and ocular discharge; aural abscesses; palpebral and cervical edema; and necrosis and ulceration of esophagus, stomach, and spleen (Allender et al. 2006; Johnson et al. 2008; Marschang 2011; Miller et al. 2015). In **squamates**, where study of ranavirus infection is more limited, signs include ulceration of the nasal mucosa, ulcerative-necrotizing glossitis, granulomatous lesions in the tongue and liver, enlargement of liver, and glomerulosclerosis (Drury et al. 2002; Marschang et al. 2005; Marschang 2011; Miller et al. 2015). Microscopic examination may also reveal the presence of intracytoplasmic inclusions, or viral aggregates in the cytoplasm, in certain cell types (e.g., red blood cells; Miller et al. 2015).

Importantly, turtles can test positive for ranavirus and shed the virus without showing any signs of disease (Goodman et al. 2013; Johnson 2006). Also, many signs described above are not specific to ranavirus

disease. Therefore, the aforementioned signs cannot be considered pathognomonic for ranavirus infection, and infection does not necessarily indicate that an animal will appear diseased.

Surveillance and Diagnostic Testing:

To date, rigorous surveys for ranavirus in wild populations of reptiles have only been conducted in chelonians. Additionally, the absence of prevalence data for ranavirus in snakes may be related to issues with PCR tests that target portions of the major capsid protein gene. They appear to amplify part of the host genome creating background noise, although this hypothesis has not been formally tested (R. Goodman, personal observation; A. Stöhr, personal communication). In infected hosts, ranavirus can be isolated from various organs, including liver, kidney, and spleen, as well as any lesions (e.g., of the oral mucosa or intestines). Samples of these organs and lesions are routinely used to sample for ranavirus presence (Johnson et al. 2007; Gray et al. 2012). However, many tissues can only be obtained via necropsy, and large sample sizes are often necessary to detect ranavirus as the prevalence of infection varies widely in different populations (<5 to >90%; Gray et al. 2009; Goodman et al. 2013; O'Bryan et al. 2012). Non-lethal sampling methods are preferred for surveillance of wildlife populations, and two methods (oral-cloacal swabbing and skin tissue sampling) have been used and compared in the past decade. While these samples involve less invasive methods, there is a risk of a false-positive result if virions are physically present in the saliva or feces or on the skin surface, but have not actually infected the animal. Gray et al. (2012) conducted a controlled infection study with American bullfrog (*Lithobates catesbeianus*) tadpoles and found false-negative and -positive rates of 20% and 6% for tail samples, and 22% and 12% for external body swabs, when compared to liver as the standard tissue to indicate infection. This suggests a similar rate of false-negative results for tissue and swab samples in amphibians; however, a field comparison of sampling methods in reptiles suggested a higher rate of false-negative results with swabs (though a different swabbing technique was used) relative to tissue sampling. Goodman et al. (2013) detected ranavirus in 11 out of 63 Eastern painted turtles (*Chrysemys picta picta*; pooled over three sites) using tail clips (distal portion, 5mm in length), but failed to detect ranavirus in any of 50 turtles using oral-cloacal swabs. Clearly, more research is needed to select the best method for field surveillance of ranavirus in reptiles. Additionally, researchers should expect some false negatives to result from non-lethal sampling. Both tissue sampling and oral-cloacal swabs are recommended, until a direct comparison of their effectiveness is made for lizards and snakes.

To prevent potential spread of ranavirus between sampling sites, the following disinfectants must be applied to all equipment, footwear, and other surfaces for at least 1 min at these concentrations or higher: Nolvasan, 0.75%; bleach, 3.0%; or Virkon S, 1.0% (Bryan et al. 2009). Any mud or debris should be scrubbed from surfaces before disinfection.

Tissue samples collected for PCR detection of ranavirus should be either kept frozen without liquid or, in the case of field conditions, stored at room temperature in 70% ethanol. Methods for PCR detection are described by Gray et al. (2012). Recent studies in reptiles have detected ranavirus using sections of liver, kidney, spleen, and small intestine (Goodman, unpub. data; Gray & Miller, unpub. data). Whole animals that are suspected to have succumbed to ranavirus (deceased or humanely euthanized) should preferably be kept cold, but not frozen, on ice and shipped overnight to a laboratory that can perform wildlife necropsies. If this is not possible, carcasses should be stored in 10% neutral buffered formalin (after removing tissue samples for PCR testing). In order to ensure penetration of formalin into tissues, use at least a 10:1 volume of formalin to tissue/carcass, and make an incision in the body wall before placing the carcass in formalin.

Table 1: Reptile species from the USA with known ranavirus infections in wild populations. (From Bartlett et al. In Prep).

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

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