Ranaviruses and reptiles

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Ranaviruses can infect a number of vertebrate classes including fish, amphibians and reptiles, but for the most part, research has been focused on amphibians and these viruses have therefore been called amphibian specialist. More recently, reports of ranaviral infections of reptiles are increasing with over 12 families of reptiles currently known to be susceptible to ranaviruses, most of which are turtles and lizards. Reptiles are infected by ranavirus that are genetically similar to, or the same as the viruses that infect amphibians and fish, however, physiological and ecological differences results in differences in effective study designs. Although disease in these animals is often influenced by host species, viral strain and environmental differences, general trends in pathogenesis are emerging. More experimental studies using a variety of reptile species, life stages and routes of transmission are required to start to unravel the complexity of wild ranavirus transmission. Our understanding of the reptilian immune system is still lacking, especially in reference to ranaviral infection, although the considerable amount of work conducted in amphibians, will serve as a useful guide. In this review we summarise the latest findings of reptilian ranavirus research, identify major gaps in the field of knowledge and include recommendations for future research directions.

1 Ranaviruses and Reptiles

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9 **Abstract**
10 Ranavirus can infect a number of vertebrate classes including fish, amphibians and reptiles, but
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12 been called amphibian specialist. More recently, reports of ranaviral infections of reptiles are
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19 reptile species, life stages and routes of transmission are required to start to unravel the
20 complexity of wild ranavirus transmission. Our understanding of the reptilian immune system is
21 still lacking, especially in reference to ranaviral infection, although the considerable amount of
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23 latest findings of reptilian ranavirus research, identify major gaps in the field of knowledge and
24 include recommendations for future research directions.

25

26

Introduction

Ranaviruses (family Iridovirdae) are emerging lethal pathogens of ectothermic vertebrates. First discovered in 1965 (Granoff et al., 1965), ranaviruses were initially studied for their interesting molecular biology but rose to reportable pathogen status as more epizootics were discovered (Schloegel et al., 2010; Gray & Chinchar, 2015). The vast majority of research on the genus *Ranavirus* has been conducted in amphibians (*Rana* being the Latin for frog), but despite their name, ranaviruses do not only occur in amphibians (Chinchar and Waltzek, 2014). This group of viruses infects over 175 species of ectothermic vertebrates; including reptile species from at least 12 different families (Duffus et al., 2015).

Reptiles are a diverse group that evolved over 300 million years ago and currently inhabit every continent except Antarctica. They include turtles, crocodilians, snakes, lizards, tuatara, and their extinct cousins, comprising over 10,000 species, and contributing more to biodiversity than amphibians (The Reptile Database http://www.reptile-database.org, 2017). However, with the increase in ranaviral disease around the globe comes an increased threat to reptile species (Marschang et al., 2016).

Since the initial report of ranaviruses in reptiles in the early 1980s (Heldstab & Bestetti, 1982) many infections have been noticed in wild and captive reptiles and the literature continues to grow. In this review we summarise the latest findings in all areas of reptilian ranavirus research. We identify major gaps in the field of knowledge and include recommendations for future research directions.

Survey Methodology

To ensure this review included as many publications focusing on ranaviruses and reptiles as possible, an extensive search of multiple databases using broad search queries was conducted. Databases used in the search strategy included; Web of Science, PubMed, and Google Scholar. The search strategy included keywords such as 'ranavirus' and 'reptiles' and their conjugations as well as more specific terms such as 'turtle', 'lizard', and 'snake'. To broaden the search further references of articles found in the initial database search were then assessed for content relating to ranaviruses and reptiles. As a baseline for general ranavirus literature, relevant references were extracted from the 2015 *Ranavirus* book (Gray and Chinchar, 2015).

Taxonomy

Ranaviruses are large (~150 nm), nucleocytoplasmic viruses with icosahedral virions and double-stranded DNA genomes that contain approximately 100 genes (Jancovich et al., 2015). *Ranavirus* is a genus in the family Iridoviridae: a group of five related viral genera. Of the five Iridoviridae genera, only ranaviruses cause significant disease in wild reptiles.

The taxonomy of the genus *Ranavirus* is changing; as more viruses are isolated and sequenced a clearer picture of the phylogenetic distribution of this group is developing. The current phylogeny of the Ranavirus genus can be subdivide into three major groups: Epizootic haematopoietic necrosis virus-like (EHNV-like), the Common midwife toad virus-like (CMTV-like), and the Frog virus 3-like (FV3-like) (Stohr et al., 2015; Claytor et al., 2015; Price et al., 2017). All of the viruses that infect reptiles belong to the CMTV-like and FV3-like groups (Duffus

72   et al., 2015). The factors that control the host specificity of these viruses remain unknown.
73   Phylogenetic analyses of sequences from different reptilian and amphibian viruses has revealed
74   that reptilian ranaviruses are often more closely related to amphibian ranaviruses from the same
75   geographical region than to each other (Stohr et al., 2015). This provides support for the recent
76   emergence of these viruses in new hosts (Jancovich et al., 2010, Stohr et al., 2015).
77

78   **Bibliometrics**
79   Despite ranaviruses' lack of host specificity, the vast majority of ranaviral literature is on
80   amphibians. As of February 2018, 449 references were returned when the Web of Science ™
81   database was queried for the topic 'ranavirus'. Of these, over 200 used the term 'amphibian' in
82   their title or abstract while fewer than 60 used the term 'reptile'. However, plotting the usage of
83   these terms over the last 10 years shows a steady increase in the ratio of 'reptile' to 'amphibian',
84   possibly reflecting an increase in reptilian ranavirus research or an increased awareness of the
85   role of reptiles in this disease (Figure 1). Many advances have been made since the discovery
86   of ranaviruses, however, for the most part, this research is specific to amphibians. Reptiles and
87   amphibians are very different physiologically and although they sometimes share habitats, their
88   ecology is divergent. Some results from one host group can translate to the other; however,
89   there is no substitute for host specific research. As ranavirus research continues, it is important
90   to focus efforts on all hosts, including reptiles.
91

92   **Diagnostics and surveillance**
93   Because of the variability in ranaviral disease signs and severity within and among reptile
94   species (see pathology section), suspected cases of ranaviral disease must be confirmed with
95   laboratory diagnostic techniques.
96

97   The World Organization for Animal Health (OIE) provides guidelines for diagnostic methods in
98   their Diagnostic Manual for Aquatic Animal Health (OIE, 2012), and Miller et al. (2015) have
99   summarised the diagnostic techniques used in ranaviral research. The most commonly used
100   methods to confirm the presence of a ranavirus in host samples have included electron
101   microscopy, antigen-capture enzyme-linked immunosorbent assays, viral isolation,
102   immunohistochemistry (IHC), amplification using polymerase chain reaction (PCR) and more
103   recently next generation sequencing.
104

105   All of these techniques have been used at some stage in the study of ranaviruses from reptiles;
106   however, the selection of diagnostic technique is highly dependent on the resident expertise in
107   the laboratory, the required results, and the type of study (Miller et al., 2015). Before their
108   application, all diagnostic techniques should be thoroughly tested and optimised with
109   appropriate controls for use in new hosts or against new pathogens (e.g. different species of
110   reptiles or ranavirus or both) (Wobeser, 2007).
111

112   Most ranaviruses can be grown using commercially available fish cell lines (Miller et al., 2015).
113   Reptile cell lines such as Russell's viper heart cells, gecko lung cells, and turtle heart cells have
114   also been used successfully to isolate ranaviruses from reptiles (Hyatt et al., 2002, Johnson et
115   al., 2008).

116
117 Serological surveys, employing various ELISAs, have been used to assess reptiles for anti-
118 ranaviral antibodies (Johnson et al., 2010; Ariel et al., 2017). Several attempts at developing
119 ranaviral ELISAs to detect antibodies have, however, failed (Meddings, 2011; Allender, 2012).
120 Anti-ranaviral monoclonal antibodies have been used in a double antibody sandwich enzyme-
121 linked immunosorbent assay (ELISA) to detect viral particles in soft-shelled turtles (Zhang et al.,
122 2010). In this case, the virion was detected with 98% specificity when compared with
123 conventional PCR as the gold standard.
124
125 Most serological tests measure the host's response to the pathogen, not the agent of the
126 disease itself (Wobeser, 2007) and therefore a lack of *Ranavirus*-specific antibodies does not
127 definitively indicate a lack of past ranaviral exposure. It could be that antibodies are no longer
128 present or that the animal has not produced anti-ranaviral antibodies due to immunosuppression
129 or early stage infection. It is also important to note that reptile antibody titres vary seasonally
130 (more antibodies being produced in the warmer months), which must be taken into account
131 when determining sensitivity and specificity cut-off values (Wobeser, 2007; Zimmerman et al.,
132 2010; Meddings, 2011). Using total IgY levels as an internal control may minimise diagnostic
133 errors resulting from seasonality.
134
135 Polymerase chain reaction (PCR) based assays have been used conventionally and in
136 quantitative real-time assays to detect reptilian ranaviruses in a number of sample types
137 including blood, oral and cloacal swabs, and fresh and fixed tissues (Pallister et al., 2007;
138 Allender et al., 2013a; Goodman et al., 2013a, Butkus et al., 2017; Leung et al., 2017; Macline
139 et al., 2018). Molecular surveys of turtle populations for ranavirus have revealed that swabs and
140 blood samples are not equally valid targets for ranavirus detection (Allender et al., 2013a).
141 Goodman et al., (2013) also found that swabs were not as effective for ranavirus detection when
142 compared with tissue samples. Given possible differences in sample type sensitivity, it would
143 seem advisable to collect multiple sample types when conducting a molecular survey for
144 reptilian ranaviruses. It is also possible to use bone marrow as a source of DNA for ranavirus
145 detection from reptile carcases in which other viable tissue samples may have decayed (Butkus
146 et al., 2017). The preferred target of ranaviral PCR assays is the major capsid protein (MCP)
147 gene as it is highly conserved throughout the ranaviral lineage (Miller et al., 2015). As the cost
148 of sequencing decreases, it is becoming increasingly popular to use sequencing to more
149 accurately identify and characterise viral isolates (Stöhr et al., 2015; Hick et al., 2016;
150 Subramaniam et al., 2016).
151
152 Environmental DNA (eDNA) -based detection may be an effective method for assessing the
153 presence of ranaviral DNA in populations of aquatic reptiles. These methods of eDNA PCR
154 have been used to detect outbreaks of ranaviruses in aquatic frog populations (Hall et al.,
155 2016). Aquatic reptiles with ranaviral infections shed virions into their surroundings, indicating
156 that eDNA detection may be possible, although no publication has yet confirmed this *in situ*
157 (Brenes et al., 2014).
158
159 Immunohistochemistry (IHC) and other *in situ* labelling methods have been successfully used in
160 reptilian ranaviral studies to visualise the location of the viral protein in tissue samples (Hyatt et
161 al., 1991; Ariel et al., 2015). Ranaviral IHC assay results combined with histopathology can be

162   used to correlate pathology with the location of viral antigens (Becker et al. 2013; Ariel et al.,
163   2015; Forzán et al., 2015).
164
165   Using a combination of these diagnostic methods would improve the rigour of epidemiological
166   studies of ranavirus in reptile populations. For example: eDNA methods could be used to
167   cheaply and broadly screen aquatic environments for the presence of ranaviral DNA. Upon
168   detection, a sero-molecular diagnostic approach could be implemented for screening aquatic or
169   water associated reptiles inhabiting the waterway to estimate infection and serological
170   prevalence and incidence. Quantitative PCR methods and IHC could be used to help describe
171   the ranaviral pathogenesis of any diseased animals detected during the survey. Finally, the
172   MCP genes of any isolated virus could be sequenced to allow phylogenetic classification.
173   Depending on the goals of the project, next generation sequencing can be used to get a better
174   understanding of the isolated virus.

Distribution and host range

Ranaviruses are distributed around the globe, overlapping the geographic distribution of reptiles on all continents (Duffus et al., 2015**)** (The Reptile Database http://www.reptile-database.org, 2017). Ranaviruses have been detected in over 12 families of the orders Testudines (turtles, tortoises and terrapins) and Squamata (lizards and snakes). It is important to note, however, that some reptile populations are naïve to these viruses and that particular ranavirus species are only found in specific areas. There have been several studies reporting the negative results of epidemiological surveys (Hanlon et al., 2016; Kolesnik et al., 2017; Winzeler et al., 2018). These results are extremely valuable as they also help describe the reptilian ranavirus distribution and emergence patterns.

Distribution and host range - Testudines (turtles, tortoises and terrapins)

Koch's postulates have been confirmed for a *Ranavirus* isolate (genus *Ranavirus*) in Testudines with the *Burmese star tortoise* (Johnson et al., 2007). The first reported cases of ranaviral infections in Testudines were identified microscopically during the 1980s in Hermann's turtles (*Testudo hermanni*) in Switzerland (Heldstab and Bestetti, 1982). Following this, ranaviruses were predominantly isolated from box turtles (*Terrapene carolina*) and were identified as the aetiological agent of 'red neck disease' in the soft-shelled turtle (*Trionyx sinensis*) (Chen et al., 1999). In the last decade, several new reports of ranaviral infections in Testudines have been published (Allender et al., 2006; Johnson et al., 2008; Johnson et al., 2010; Belzer and Seibert, 2011; Allender, 2012; Stohr et al., 2015; Perpiñán et al., 2016; Butkus et al., 2017, Agha et al., 2017; Archer et al., 2017). Despite the increasing number of reports of infections in the Testudines, ranaviral disease in these reptiles is still likely to be underreported due to a lack of awareness, an incomplete understanding of the pathology caused by the disease, few long-term studies and minimal population monitoring (Duffus et al., 2015). Sea turtles are a group of reptiles that have received little attention from ranavirus researchers, despite the existence ranavirus infections of marine fish species (Whittington et al. 2010).

Distribution and host range - Squamata (lizards and snakes)

The first reports of Ranavirus infection in squamates came after several green tree pythons were seized during an attempt to illegally import them into Australia from Indonesia. Hyatt et al. (2002) reported that these snakes were infected with a FV3-like *Ranavirus* isolate. In 2005 Marschang et al. (2005) reported the first ranaviral infection in lizards (Marschang et al. 2005). Recently the reports of ranavirus infections in squamates have been, for the most part, restricted to captive lizard populations (Alves de Matos et al., 2011; Stohr et al., 2013; Behncke et al., 2013; Stohr et al., 2015; Tamukai et al. 2016). There is little evidence of the role of ranavirus infection in wild squamate populations.

Distribution and host range - Rhynchocephalia (tuatara), Archosauriformes (crocodiles, birds)

Animals from the other groups of the class Reptilia, namely the Rhynchocephalia (tuatara) and the Archosauriformes (crocodilians and birds) have not been documented as having ranavirus infections. The tuatara only inhabit parts of New Zealand, where ranaviruses are believed to be present (i.e. short-finned eel ranavirus, Bovo et al., 1999). However, no studies have been

219   published on the presence of ranavirus in tuatara. While yearling Australian freshwater
220   crocodiles (*Crocodylus johnstoni*) were exposed to ranavirus (BIV) under laboratory conditions;
221   this challenge did not result in any adverse effects on the yearlings and the virus could not be
222   re-isolated (Ariel et al., 2015). A serosurvey of wild freshwater crocodiles did show evidence of
223   anti-ranaviral antibodies, indicating that wild populations are likely exposed (Ariel et al., 2017). It
224   is important to continue to study apparently resistant species, like crocodiles, as they may give
225   insights into the correlates of immunity. Birds and reptiles are closely related; crocodiles are
226   genetically more closely related to birds than they are to lizards. There are no reports of birds
227   infected by ranaviruses (probably relate to endothermy), despite this, birds may still play a role
228   in ranaviral transmission. It has been hypothesised that migratory birds, acting as mechanical
229   vectors, are responsible for some of the geographic transmission of ranaviruses (Whittington et
230   al., 1996).
231
232   **Pathology**
233   The clinical signs and pathogenesis of natural ranaviral infection in reptiles can be extremely
234   variable. Mortality during infection can range from 0-100% and the effect on a host can vary
235   from quite mild to extremely severe, requiring immediate veterinary attention or euthanasia
236   (Miller et al., 2015). There is evidence for reptiles being asymptomatic carriers of ranavirus and
237   for quiescent viral reactivation in amphibians that have recovered from infection (Stohr et al.,
238   2013; Robert et al., 2014). The complex presentation and inconsistency in the pathogenesis of
239   ranaviral infection in reptiles may occur because of the influence of host physiology and life
240   history, varying degrees of viral virulence, stressors, and temperatures acting on the course and
241   outcome of infection (see Susceptibility section).
242
243   Descriptions of pathogenesis in reptiles infected with a variety of ranaviral strains in several host
244   species under experimental, natural, and captive conditions are summarised in Table 1. Despite
245   differences in these epizootic events and challenge experiments, there are still some common
246   patterns of ranaviral pathology that emerge from these reports.
247
248   General lethargy and inappetence are associated with most cases of ranaviral infection in
249   reptiles; however, such clinical signs are common to many diseases and are not pathognomonic
250   for ranaviral infection. Turtles often present with respiratory signs, including nasal and oral
251   discharge. Oedema, especially of the neck, limbs and eyes, is also commonly associated with
252   this infection. The clinical signs of ranaviral infection in Squamates are scarcely described. This
253   is partially due to the lack of experimental infection trials in this group, which would help
254   describe pathogenesis markers. Maclaine et al (2018) recently described the pathogenesis of
255   ranaviral infection in an Australian lizard species, documenting that pathogenesis varies with
256   inoculation route. With increasing descriptions of ranaviral infected lizards over the last decade,
257   an emerging trend suggests that skin lesions may be a common occurrence (Stohr et al., 2013).
258
259   Ranaviral infections are systemic, and there is often extensive damage to multiple organs during
260   infection, especially the liver and spleen in reptiles. Liver lesions are also very common in the
261   pathogenesis of ranaviruses in amphibian and fish species (Miller et al., 2015). Histopathology
262   is frequently characterised by multifocal necrosis in multiple organs, and is often associated with

263 hematopoietic tissue (Ariel et al., 2015). Reptilian hosts of *ranaviruses* experience a range of
264 histological changes including necrosis and inflammation of the upper respiratory tract,
265 interstitial pneumonia, conjunctivitis, necrosis of endothelial cells and the submucosa of the
266 gastrointestinal tract, multifocal hepatic necrosis, fibrinoid vasculitis centred on splenic
267 ellipsoids, intracytoplasmic inclusion bodies, and necrotizing myositis (see Table 1). Evidence
268 from epizootics in reptiles indicates that ranaviral infection can be accompanied by secondary
269 pathogens that may exacerbate the disease and mask clinical signs of ranaviral infection (Stohr
270 et al., 2013; Sim et al., 2016; Archer et al., 2017).
271
272 **Transmission**
273 The natural route of transmission of ranaviruses in wild populations of reptiles is still debated,
274 although experimental data suggest multiple transmission routes are possible (Brunner et al.,
275 2015). During an experimental challenge of adult red-eared sliders (*Trachemys scripta elegans*),
276 Johnson et al. (2007) found that the orally exposed animals were refractory to infection while
277 animals challenged with the same dose via intramuscular injection developed severe disease. In
278 another study, exposure to ranavirus in water *via* cohabitation resulted in infection in red-eared
279 slider hatchlings (*T. scripta elegans*), although the route of infection was not determined, and
280 the concentration of virus in the water was not quantified (Brenes et al., 2014). Ariel et al. (2015)
281 found that adult freshwater turtles (*Emydura krefftii* and *Elseya latisternum*), freshwater
282 crocodiles (*C. johstoni*) and several species of snakes were refractory to infection irrespective
283 of the route of exposure. The hatchlings of both species of freshwater turtles were susceptible to
284 infection *via* intra-coelomic exposure although oral inculcation was not attempted. Juvenile
285 Australian eastern water dragons (*Intellagama lesueurii lesueurii*) can develop ranaviral disease
286 from all exposure routes tested (oral, intramuscular, and cohabitation) (Maclaine et al., 2018).
287
288 Studies report on different species of *Ranavirus* often found in divergent hosts, which may
289 account for apparent differences in susceptibility for different types of exposure. However,
290 differences in susceptibility *via* different routes of exposure may reflect real differences in
291 natural transmission routes. More experimental studies using a variety of species, life stages
292 and routes of transmission are needed to resolve this.
293
294 Amphibians are highly susceptible to ranaviral infection *via* all tested forms of inoculation (water
295 bath, skin contact, oral inoculation or injection) (Miller et al 2015). Fish are also susceptible *via*
296 multiple inoculation routes, although it appears to be species-dependent (Jensen et al., 2009;
297 Gobbo et al., 2010; Jensen et al., 2011). Differences in viable transmission routes result in
298 different epidemiologies, and thus research from other host classes may not accurately reflect
299 risks and susceptibility of reptilian populations. It is therefore important to account for variation
300 among reptile species when developing statistical models for reptilian disease.
301
302 **Vectors**
303 Humans are contributing to the global spread of ranaviruses, primarily though global animal
304 trade (Kolby et al., 2014; Duffus et al., 2015; Stöhr et al., 2015). Although there are reports of
305 ranaviral infection in traded reptiles (Hyatt et al, 2002; Stohr et al., 2013), no systematic survey
306 of ranaviral infection in traded reptiles has been conducted. There have been some ranaviral

307 disease outbreaks in private reptile collections and zoos (Marschang et al., 2005; Sim et al.,
308 2016), but the full extent of disease prevalence is hard to assess, both because of inapparent
309 infections, and lack of reporting of dead animals amongst reptile breeders and collectors.
310
311 Ranaviral DNA sequences have been identified in mosquitoes associated with an outbreak of a
312 *Ranavirus* in box turtles, providing evidence for vector transmission (Kimble et al., 2014).
313 Leeches are common ectoparasites of aquatic reptile species and can act as vectors for blood-
314 borne diseases (Siddall & Desser, 1992; Watermolen, 1996; Readel et al., 2008). There has
315 been at least one report of a ranavirus-positive leech (PCR for MCP) associated with an
316 infected amphibian host although there are no reports for reptiles (Hardman et al., 2013).
317 Despite these indicators of the possible involvement of vectors in ranavirus transmission, no
318 experimental studies have been published that support or refute this hypothesis.
319
320 **Reservoirs**
321 Ranaviral virions are extremely stable, they are capable of withstanding high and low pH and
322 temperatures, and are resistant to desiccation (Granoff et al., 1965; Langdon et al. 1986,
323 Langdon, 1989; Nazir et al., 2012). These qualities enable ranaviruses to remain infective after
324 exposure to a range of environments, such as mud, soil and water (Nazir et al., 2012).
325 Compared with other tortoise viruses (*Herpesvirus* and *Picornavirus*), ranaviruses are more
326 stable in a variety of environments e.g. drinking water, lake water, and soil (Reinauer et al.,
327 2005). The highly stable nature of the virions plays an important role in the transmission
328 dynamics of ranaviruses (Gray et al., 2009). Dead and decaying animals are also thought to
329 play a role in ongoing transmission in ranavirus epizootics, as they continue to release virions
330 and might be consumed by other susceptible animals (Gray et al., 2015).
331
332 **Correlates of susceptibility**
333 Reptiles are ectotherms and so their physiology is strongly influenced by the temperature of
334 their surrounding environment. By extension, the innate and adaptive immune response of
335 reptiles is also linked to available environmental temperatures (Zimmerman et al., 2010).
336 Ranavirus-infected reptiles, such as turtles, exhibit temperature-dependent pathogenesis
337 (Allender et al., 2013) similar to that observed in fish and amphibians (Brand et al., 2016;
338 Brunner et al., 2015); however, the replication efficacy of the virus is also linked to temperature
339 (Ariel et al., 2009). Thus, it is difficult to determine the degree to which temperature-dependent
340 pathogenesis is a result of the effect of temperature on the replication of the virus or on the
341 immune system of the turtles. Several studies have quantified the temperature dependent
342 activity of the innate immune system of reptiles (Merchant et al., 2006; Ferronato et al., 2009;
343 Merchant et al., 2012). In many experimental infections using ranaviruses temperature is
344 uncontrolled (reported as 'room temperature'). However, Allender et al. (2013) suggests that an
345 environmental temperature change of 6°C is enough to significantly reduce ranavirus loads and
346 halve morbidity in infected turtles. However, this study used a very small sample size. Thus, it is
347 important for future studies to quantify, and potentially control environmental temperature when
348 designing experiments that involve ranaviral infection in reptiles.
349

350 The effect of stressors on reptilian ranaviral disease is poorly understood (Polakiewicz et al.,
351 2013). Several studies in amphibians directly examined the effects of stressors on disease in
352 experimental infections (Haislip et al., 2012; Reeve et al., 2013) and epidemiological studies
353 have looked for correlations between environmental stressors and ranaviral prevalence
354 (Brunner et al., 2015). The immunosuppressive effects of some anthropogenic stressors (e.g.
355 pesticides, herbicides and heavy metals) on the reptile immune system suggest a possible
356 mechanism of environmental influence on susceptibility and future epidemiological studies
357 should consider this further (Keller et al., 2006; Soltanian, 2016).
358
359 **Immunology**
360 Studies of ranaviral host immunity and immune evasion in amphibians are extensive, while
361 similar work in reptiles is limited (Grayfer et al., 2015). The immunology section in the 2015
362 *Ranavirus* book, although comprehensive on amphibians, only mentions reptiles in passing
363 (Grayfer et al., 2015). Immunology is an area in which a great number of unknowns remain for
364 ranaviruses and reptiles.
365
366 **Immunology - Innate**
367 Antimicrobial peptides (AMPs) are involved in amphibian ranaviral defence. Amphibian
368 antimicrobials such as E2P and R2P are cable of inactivating ranaviral virions through direct
369 interaction at all temperatures tested (0-26°C) (Chinchar et al., 2001). Reptile species also
370 possess a range of antimicrobial peptides, such as crocosin, pelovaterin, omwaprin, etc.
371 (Preecharram et al., 2010; van Hoek, 2014). Homologs of the anti-ranaviral peptides in
372 amphibians (class-four AMPs) have not been found in reptiles, although defensin-like peptides
373 from the albumin of marine turtles possessed antiviral activity against enveloped rhabdoviruses
374 (Chattopadhyay, 2006). No reptilian AMPs have, however, been assayed for anti-ranaviral
375 activity. Very few studies have looked at the role of cytokines in reptile immunity against
376 ranaviruses and should be investigated in future studies. One study found that IFN-γ appears to
377 have some antiviral activity in ranavirus infected soft-shelled turtles cells, although the
378 mechanisms are unclear (Fu et al., 2014).
379
380 The reptile serum complement system is capable of inhibiting viral replication (Merchant et al.,
381 2005). Serum from American alligators (Alligator mississippiensis) exhibits antiviral activity
382 against human immunodeficiency virus type-1, which has been attributed to action of the
383 complement system (Merchant et al., 2005). However, the effect of reptilian compliment on
384 ranaviral replication efficiency has not been investigated.
385
386 Extensive work has attempted to elucidate the complex role of amphibian macrophages in
387 ranaviral infection (Grayfer et al., 2015). This is another avenue of enquiry that should be
388 explored further for reptiles.
389
390 **Immunology - adaptive**
391 Adaptive immunity in reptiles (and the majority of ectothermic vertebrates) has been studied
392 (Zimmerman et al., 2010). Much less is known about the reptilian adaptive response than the
393 innate response system (Zimmerman et al., 2010). Studies of the role of the adaptive immune

394   system in clearing ranaviral infection have been almost exclusively restricted to amphibians and
395   fish (Chen & Robert, 2011; Grayfer et al., 2015).

396

397   In reptiles, only two classes of immunoglobulins have been discovered in contrast to the five
398   classes of mammalian immunoglobulins (Zimmerman et al., 2010). Reptilian IgM is homologous
399   to the IgM found in all jawed vertebrates and is the first immunoglobulin produced in response to
400   an antigen. IgY, the other class of reptilian immunoglobulin, is believed to be the predecessor of
401   mammalian IgG and IgE as it shares many functions with both (Zimmerman et al., 2010). IgY is
402   produced as a long-lasting and specific adaptive response to infection and is the preferred
403   target of reptilian serological assays (Johnson et al., 2010; Zimmerman et al., 2010; Ariel et al.,
404   2017). Serological surveys for anti-ranaviral antibodies have mostly been limited to wild turtle
405   populations (see diagnostics section). However, the neutralising ability of anti-ranaviral
406   antibodies detected in reptile populations has not been determined.
407

408   **Treatment**
409   For treatment of acute ranviral infection, several antivirals have been identified (acyclovir,
410   valacyclovir and DNA aptamers) (Allender, 2012; Li et al., 2015). However, there are few
411   examples of their successful use to treat clinical cases (Johnson et al., 2010; Allender, 2012;
412   Miller et al., 2015). Ranavirus-infected reptiles that present at veterinary centres are often
413   already in a critical condition and antiviral therapy is ineffective at reviving them (Johnson et al.,
414   2008). Pharmacological studies of the effectiveness of different antivirals at different severities
415   and durations of ranaviral infection in reptiles have not been conducted, but would be extremely
416   useful for guiding the treatment of acute ranaviral infection in reptiles. As with any disease, early
417   application of treatment through accurate and quick diagnosis will likely improve prognosis.
418

419   Ranaviral vaccine development has been limited to the aquaculture industry (Miller et al., 2015).
420   Frogs can produce long-lasting FV3-specific neutralising antibodies on second exposure
421   (Maniero et al. 2006), suggesting it would be possible to develop vaccines for them. Reptiles
422   can produce anti-ranaviral antibodies during infection (Johnson et al., 2010), and vaccines have
423   been developed for other reptilian pathogens with varying success (Mohan et al., 1997;
424   Marschang et al., 2001; Yang et al., 2007). Vaccine research and development are extremely
425   costly, and more epidemiological research is required to determine if the development of a
426   ranaviral vaccine would be efficacious for wild reptilian populations. However, there are several
427   instances where a vaccine could be useful on a small scale. For example: in zoo collections, for
428   valuable broodstock, and for endangered or at risk populations. Epidemiological studies may be
429   able to feasibly identify and prophylactically treat animals most at risk.
430

431   It has long been known that environmental temperature has a substantial effect on the humoral
432   immune systems (e.g. antibody production) of ectotherms (Widal, 1897), which opens up the
433   possibility of influencing the outcome of an infection *via* control of environmental temperatures
434   (see susceptibility section). There is room for further investigation to determine the optimal
435   temperature for increasing survival from a ranaviral infection.
436

437   **Future research and conclusions**

438 The field of ranavirus research is dominated by studies on fish and amphibians, these studies
439 can serve as a guide for the tremendous number of directions ranaviral research in reptiles
440 could take. An increase in the number of epidemiological studies and surveys of ranaviruses in
441 reptile populations is required to understand the distribution of these viruses in the class
442 Reptilia, and to identify at-risk populations. Pathogenesis and transmission of ranaviruses in
443 reptiles are still poorly understood and will require elucidation before this disease can be
444 correctly modelled and appropriately managed in reptile populations. Reptile ranaviral host
445 immunity and immune evasion strategies are under-represented in the literature. From predator
446 to pollinator to prey, reptiles play vital roles in the ecosystems they inhabit, but like amphibians,
447 reptiles are experiencing global declines. It is, therefore, imperative that research continues to
448 expand our understanding of reptiles and ranaviruses to help protect this valuable part of
449 biodiversity.

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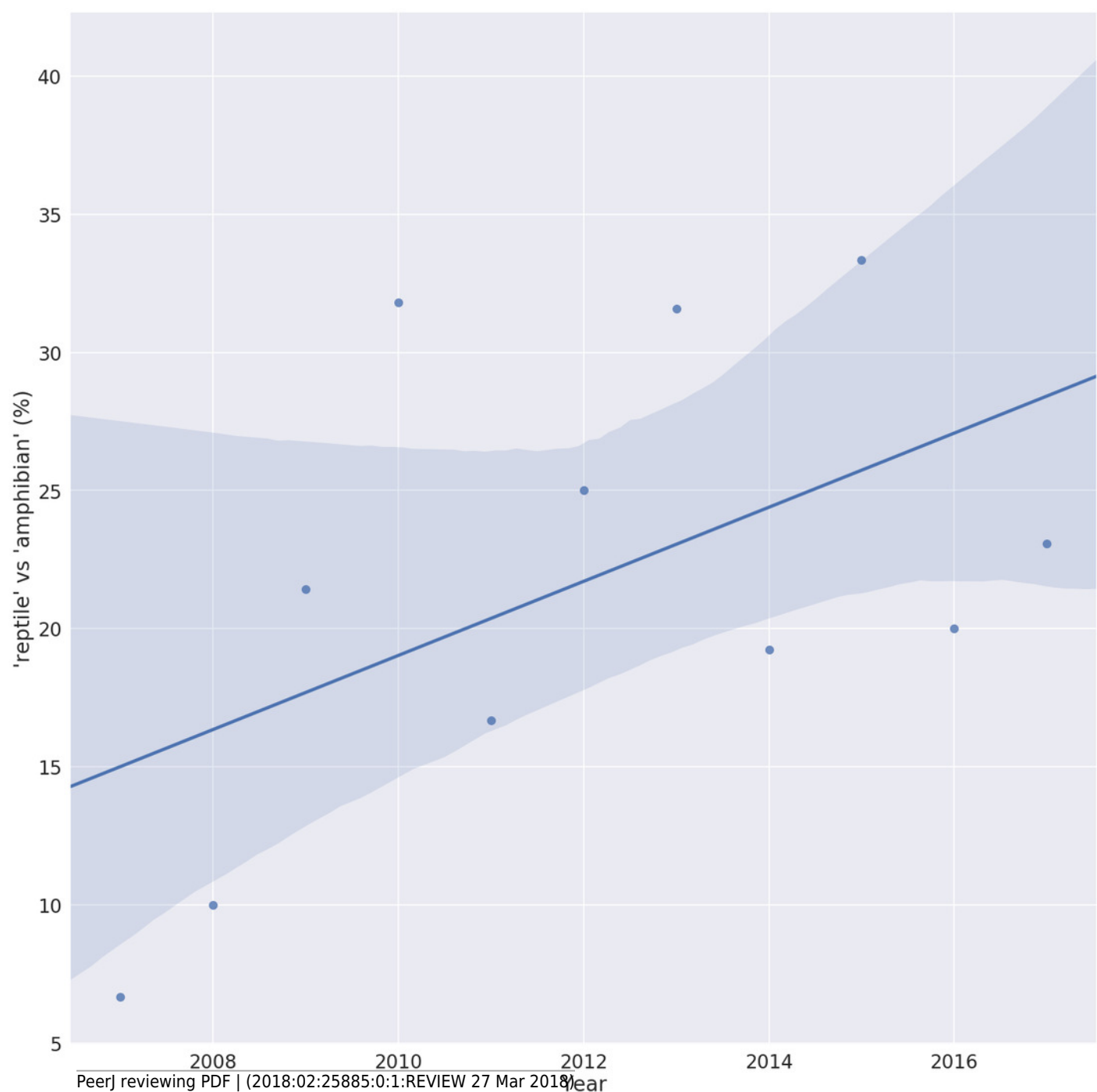
Figure 1

Figure 1. Trend in the number of ranavirus papers referring to reptiles.

Ratio of ranavirus papers using the term 'reptile' to 'amphibian' in their title or abstract, showing the increase in the relative percent of publications referring to reptiles. Solid line is the linear trend line fitted to the plot with 95% confidence interval (shaded area).

Representative reptilian ranaviral pathogenesis.

L = liver, Gi = gastrointestinal tract, Kidney = spleen, P = pancreas, M = Muscle, Fat, Ln = Lung, R = Respiratory tract, Sk = skin. * = experimental challenge, † = wild population, Δ = captive population.

Order	Genus	Species		Clinical Signs	Gross Pathology	Histopathology	Reported Diseased Organs	Reference
Testudines	*Emydura*	*macquarii krefftii*	*	Lethargy and anorexic		Multifocal necrosis in multiple organs, centered around blood vessels, with a prominent involvement of endothelial cells and the submucosa of the gastrointestinal tract	L, GI, K, S, P, M	Ariel et al., 2015
Testudines	*Geochelone*	*platynota*	Δ	nasal discharge, conjunctivitis, and severe subcutaneous edema of the neck		Stomatitis, esophagitis, splenitis, hematopoietic tissue necrosis, gastritis, conjunctivitis, glomerulonephritis, vasculitis	S, GI	Johnson et al., 2009
Testudines	*Pelodiscus*	*sinensis*	*Δ	"Redneck disease": Neck swelling and haemorrhage	Petechial haemorrhages on the surface of the liver		L	Chen et al., 1999
Testudines	*Trachemys*	*scripta elegans*	*	Nasal and ocular discharge, oral plaques, Leg Swelling, Skin abscess, conjunctivitis, lethargy, and anorectic.	Petechiae in several organs, including the glottis, liver, pancreas, and fat. The cecum and colon hemorrhage. Gastrointestinal tract thickened and edematous. Petechiae on the surface of the pancreas and congestion in the stomach.	Splenic haemorrhaging, fibrin and heterophils, multifocal hepatic necrosis including thrombi in small vessels and in sinusoids, interstitial pneumonia, fibrinoid vasculitis centered on splenic ellipsoids, and multicentric fibrin thrombi in the glomerular capillary loops, and pulmonary capillaries and necrotizing myositis.	L, P, F, GI, Ln, K, M	Allender et al., 2013; Johnson et al., 2007
Testudines	*Terrapene*	*carolina carolina*	†	palpebral edema, ocular discharge, and fluid draining from the mouth.		Stomatitis, esophagitis, splenitis, hematopoietic tissue necrosis, vasculitis	S	Johnson et al., 2008
Testudines	*Terrapene*	*ornata ornata*	*	Ocular discharge, lethargy and anorexic		Lesions centered on the splenic ellipsoids	S	Johnson et al., 2007
Squamata	*Morelia*	*viridis*	Δ	Lethargy and anorexic		Ulceration of the nasal mucosa, hepatic necrosis and severe necrotizing inflammation of the pharyngeal submucosa.	L, R	Hyatt et al., 2002
Squamata	*Uroplatus*	*fimbriatus*	Δ	Anorexic	Granuloma on the tongue, enlarged liver, friable spleen	Ulcerative necrotizing glossitis with bacterial colonies in the tongue and focal necrosis with peripheral bacterial colonies in the liver.	L	Marschang et al., 2005
Squamata	*Japalura*	splendida	Δ	Central nervous disorders, and lethargy	Haemorrhagic, oedematous gastrointestinal tract, and ecchymotic haemorrhages in the fatty tissue and the liver	Granulomatous and necrotising inflammation of the skin, vacuolar tubulonephrosis of the distal renal tubules, hyperaemia and liver necrosis, eosinophilic intranuclear and basophilic intracytoplasmic inclusion bodies in liver.	L, K, Sk	Behncke et al., 2013