OPEN ACCESS Freely available online

PLOS ONE

Transmission of Ranavirus between Ectothermic Vertebrate Hosts

Roberto Brenes[1]*, Matthew J. Gray[2], Thomas B. Waltzek[3], Rebecca P. Wilkes[4], Debra L. Miller[2,4]

1 Department of Biology, Carroll University, Waukesha, Wisconsin, United States of America, **2** Center for Wildlife Health, University of Tennessee, Knoxville, Tennessee, United States of America, **3** College of Veterinary Medicine, University of Florida, Gainesville, Florida, United States of America, **4** College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, United States of America

Abstract

Transmission is an essential process that contributes to the survival of pathogens. Ranaviruses are known to infect different classes of lower vertebrates including amphibians, fishes and reptiles. Differences in the likelihood of infection among ectothermic vertebrate hosts could explain the successful yearlong persistence of ranaviruses in aquatic environments. The goal of this study was to determine if transmission of a Frog Virus 3 (FV3)-like ranavirus was possible among three species from different ectothermic vertebrate classes: Cope's gray treefrog (*Hyla chrysoscelis*) larvae, mosquito fish (*Gambusia affinis*), and red-eared slider (*Trachemys scripta elegans*). We housed individuals previously exposed to the FV3-like ranavirus with naïve (unexposed) individuals in containers divided by plastic mesh screen to permit water flow between subjects. Our results showed that infected gray treefrog larvae were capable of transmitting ranavirus to naïve larval conspecifics and turtles (60% and 30% infection, respectively), but not to fish. Also, infected turtles and fish transmitted ranavirus to 50% and 10% of the naïve gray treefrog larvae, respectively. Nearly all infected amphibians experienced mortality, whereas infected turtles and fish did not die. Our results demonstrate that ranavirus can be transmitted through water among ectothermic vertebrate classes, which has not been reported previously. Moreover, fish and reptiles might serve as reservoirs for ranavirus given their ability to live with subclinical infections. Subclinical infections of ranavirus in fish and aquatic turtles could contribute to the pathogen's persistence, especially when highly susceptible hosts like amphibians are absent as a result of seasonal fluctuations in relative abundance.

Citation: Brenes R, Gray MJ, Waltzek TB, Wilkes RP, Miller DL (2014) Transmission of Ranavirus between Ectothermic Vertebrate Hosts. PLoS ONE 9(3): e92476. doi:10.1371/journal.pone.0092476

Editor: Rick Edward Paul, Institut Pasteur, France

Received January 25, 2014; **Accepted** February 22, 2014; **Published** March 25, 2014

Funding: Funding for this research was provided by The University of Tennessee (UT) Institute of Agriculture through an AgResearch Access and Diversity Fellowship, UT College of Agricultural Sciences and Natural Resources via Hazelwood and the UT-ESPN Scholarships, and the Society of Wetland Scientists, Student Research Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: rbrenes@carrollu.edu

Introduction

The persistence of an infectious disease in the environment is directly related to the availability of suitable hosts and the likelihood of pathogen transmission. In aquatic environments, pathogens can be transmitted between hosts via direct contact, ingestion of infected tissue (e.g., predation), or by indirect waterborne contact [1,2]. The route and magnitude of transmission depends on host density and environmental factors such as water temperature and pH [1–3]. When host densities are high, direct transmission via close contact, such as bumping or fighting, can occur. Conversely, when host densities are low or fluctuating in aquatic environments, indirect transmission through water may be most efficient [2,3]. Many pathogens that inhabit environments with fluctuating host densities are able to infect several host species that have different levels of susceptibility, with some individuals maintaining subclinical infections thereby contributing to the persistence of the pathogen [1,4].

Ranaviruses are large DNA viruses in the Iridoviridae family, a diverse group of viruses known to infect lower vertebrate hosts including amphibians [5–9], fish [10–13], and reptiles [14–17]. High variation in susceptibility of amphibians, fish and chelonians to ranaviruses has been reported [8,12,13,18–20]. Differences in host susceptibility to ranaviruses create an ideal scenario for the pathogen to move between hosts, utilizing highly susceptible species for amplification and low susceptible hosts for persistence [21]. Reservoirs composed of subclinically infected hosts might explain the yearlong persistence of ranaviruses in the environment [22]. Many aquatic communities where ranaviruses emerge are composed of multiple species from different ectothermic vertebrate classes [23].

Given the variability in susceptibility to ranavirus for host species within and among ectothermic vertebrate classes, presumably one class could function as a reservoir for another class. In experiments that we performed [24], we demonstrated that interclass transmission was possible via exposure to ranavirus inoculum in water. Although water bath exposure to a standard concentration of ranavirus is useful to understand basic transmission mechanisms, it does not connote natural transmission between hosts. Thus, our goal was to test whether interclass transmission of ranavirus was possible between ectothermic vertebrate classes using previously exposed hosts as the source of the virus. These results are fundamental to understanding the epizootiology of ranaviruses.

Methods

To determine the occurrence of ranavirus transmission between ectothermic vertebrate classes (fish, reptiles and amphibians), we set up experimental challenges between three sympatric species known to be susceptible to ranavirus infection: mosquito fish (*Gambusia affinis*; [24]), red-eared slider turtle (*Trachemys scripta elegans* [25]), and Cope's gray treefrog (*Hyla chryscocelis* [9]). We used a Frog Virus 3 (FV3)-like ranavirus that was isolated from a pallid sturgeon (*Scaphirhynchus albus*) during a die-off in an aquaculture facility [26]. From previous single species challenges, the species we used were known to be susceptible to this isolate, exhibiting 85%, 35% and 5% mortality in Cope's gray tree frog tadpoles [MJG, TBW, and DLM, unpubl. data], red-eared slider hatchlings [MJG, TBW, and DLM, unpubl. data], and mosquito fish [24], respectively, when exposed to the virus in water.

The mosquito fish used for the experiment were obtained as fingerlings (ca. 5–10 cm length) from a commercial hatchery and acclimated in the laboratory prior to the experiment for a week in a 1200-L tank with constant, flow-through water (75.7 L/s) that was dechlorinated and maintained at 25°C. Turtles were obtained as hatchlings (ca. 5 cm) from a commercial retailer (Turtle Shack, Port Richey, FL) and acclimated in a 1200-L tub for a week with floating platforms and lights for thermal and UV exposure (Zoo Med Powersun UV Self-Ballasted Mercury Vapor UVB Lamp, San Luis Obispo, CA). During the acclimation period, fishes and turtles were fed daily a commercial high protein fish food (Purina Mills Aquamax Pond Fish 4000 Catfish Food Pellets, Gray Summit, MO) *ad libitum*. Amphibian larvae were collected as egg masses from local wetlands, hatched and raised in 324-L wading pools. Tadpole acclimation, maintenance, and feeding protocols were identical to Hoverman et al. [9]. Upon purchase and arrival to the University of Tennessee, a random sample of five individuals of every species was humanely euthanized by immersion in benzocaine hydrochloride (100 mg/L; [27]), and tested for ranavirus infection using real-time quantitative PCR (qPCR; see methods below); all qPCR results were negative.

To test interclass transmission of the pathogen, we paired one virus-exposed individual with one unexposed individual of a different species. Virus-exposed individuals were exposed to 10^3 plaque forming units (PFU)/mL of ranavirus in water [9], while unexposed individuals were exposed to the same volume of virus-free media (i.e., minimum essential media, MEM Eagle Sigma-Aldrich, Seelze, Germany). Exposure occurred individually in 2-L containers filled with 1-L of dechlorinated water for 3 d [9]. Thereafter, individuals were randomly selected and paired in the following arrangement: 1) exposed turtle and unexposed tadpole, 2) exposed tadpole and unexposed turtle, 3) exposed fish and unexposed tadpole, 4) exposed tadpole and unexposed fish, and 5) exposed tadpole and unexposed tadpole. An identical complement of paired control treatments (i.e., both species unexposed) also was included. Individuals were paired in 15.5-L containers divided in half by a 2000-µm plastic mesh (design adapted from Harp and Petranka [28])The tubs were placed on shelving units (122×244-cm) at two heights in a randomized block design, with shelf height as the blocking variable. There were 20 experimental units per treatment. Room temperature in the laboratory was maintained at 25°C and the photoperiod was set at 12:12 day:night to emulate natural conditions [29].

After the inoculation period and every three days thereafter, water was changed (100% of volume) to maintain water quality during the experiment [9]. Amphibian larvae were fed ground fish food (TetraMin, Blacksburg, VA) at a ratio of 12% of their body mass after each water change [9]. Turtles and fish were fed high protein catfish pellets (Purina Mills Aquamax Pond Fish 4000 Catfish Food Pellets, Gray Summit, MO) every other day at a ratio of 3% of their body mass [30]. Five non-experimental individuals per species that were treated identical to controls were weighed for food ration estimates so not to introduce stress to experimental animals [8].

During the experiment, all individuals were monitored twice daily for morbidity. Individuals that exhibited morbidity consistent with ranaviral disease (i.e., petechial hemorrhages, edema, and loss of equilibrium) for >24 hours were humanely euthanized by double pithing [31]. Bodies of directly exposed individuals that were pithed were returned to the tub until the next water change to allow normal virus shedding post mortem. Conversely, unexposed individuals that were pithed were removed immediately from the containers. The duration of the experiment was four weeks (28 days), which is sufficient time to observe morbidity in ectothermic vertebrates exposed to ranavirus [8,12,32]. Any surviving individuals were humanely euthanized at the end of the experiment. Sections of liver and kidney were collected from all individuals for virus detection by qPCR. All husbandry and euthanasia procedures followed approved University of Tennessee IACUC protocol #2018.

We extracted genomic DNA (gDNA) from liver samples using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). We used a Qubit fluorometer and the Quant-iT dsDNA BR Assay Kit to quantify the concentration of gDNA in each sample (Invitrogen Corp., Carlsbad, CA, USA). Our qPCR procedures and primers were identical to Picco et al. [33]. All extracted DNA samples were run in duplicate and an individual was declared infected if the qPCR cycle threshold (CT) was <30 for both samples. The CT was determined for our PCR system (ABI 7900Fast Real-Time PCR System; Life Technologies Corporation, Carlsbad, CA) by developing a standard curve using known quantities of ranavirus. Four controls were included in each qPCR assay: DNA extracted from a ranavirus-positive tadpole, DNA extracted from a ranavirus-negative tadpole, DNA extracted from our ranavirus isolate, and DNA grade water.

We were interested in determining if transmission occurred between paired hosts of different vertebrate classes, and if so, did percent transmission differ depending on the host. We also were interested in whether individuals survived with subclinical infections. Thus, we performed a chi-square test of homogeneity to test for the differences in infection prevalence and mortality for the unexposed individuals among the paired-host treatments. All analyses were performed using SAS 9.3 [34] at $\alpha = 0.05$.

Results

We found that ranavirus could be transmitted between different vertebrate classes, and that infection prevalence ($\chi^2_4 = 24.76$, $P < 0.001$) and mortality ($\chi^2_4 = 36.40$, $P < 0.001$) differed among unexposed individuals depending on the paired species (Figure 1). Susceptibility of directly (i.e., inoculum exposed) and indirectly (shed by co-inhabitant) exposed individuals varied among host species. Amphibian larvae were the most susceptible vertebrate class with an average infection of 73% for individuals directly exposed to ranavirus, and 40% average infection for individuals exposed indirectly by other infected hosts (Figure 1). Ranavirus transmission from infected amphibian larvae to naïve hosts was observed in 60% of conspecifics and 30% of turtles, resulting in 100% and 0% mortality, respectively (Figure 2). No fish were infected or died after 28 days, despite being housed with a large percentage (70%) of infected amphibian larvae (Figures 1–2).

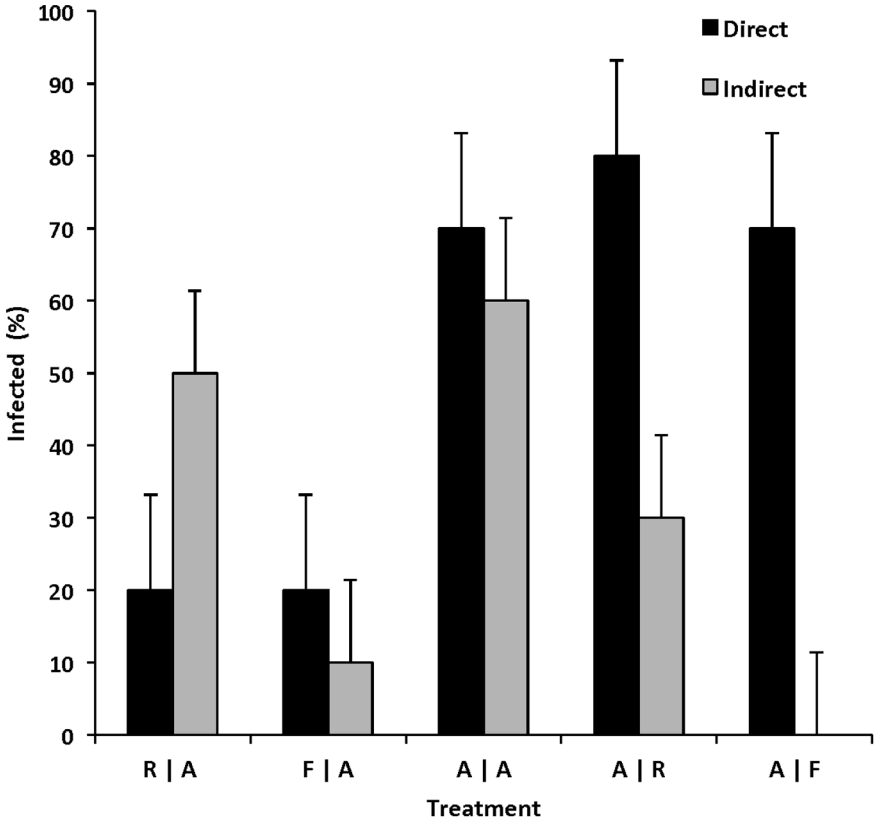

Figure 1. Infection of individuals exposed to ranavirus directly or indirectly. Infection prevalence between individuals exposed to ranavirus inoculum (direct) or via shedding (indirect) by a paired host. Treatments were paired individuals ($n = 20$ per bar) of different ectothermic vertebrate classes (A = amphibian, R = reptile, F = fish); thus, A|F = amphibian paired with fish. Infection of indirectly exposed individuals is evidence of waterborne transmission by directly exposed individuals.
doi:10.1371/journal.pone.0092476.g001

After 28 days, 20% of directly exposed turtles were infected while 50% of amphibians that were housed with them became infected and died (Figures 1 and 2), suggesting that at least 30% of turtles cleared the virus before the end of the experiment. Directly exposed fish showed low susceptibility to ranavirus (20% infection, 0% mortality). These individuals transmitted the virus to 10% of the co-inhabitant amphibian larvae – all of which died. No mortality occurred in our control treatments.

Discussion

The objective of our study was to determine if ranavirus could be naturally transmitted between ectothermic vertebrate classes, which had not been demonstrated previously. Virus-exposed mosquito fish and red-eared sliders were able to transmit ranavirus to Cope's gray treefrog tadpoles and cause 10% and 50% mortality, respectively. Exposed gray treefrog tadpoles were able to transmit ranavirus to red-eared sliders (30% infection), but none of the turtles died after 28 days. Exposed gray treefrog tadpoles were unable to transmit ranavirus to mosquito fish. Alternatively, mosquito fish may have become infected when exposed to infectious tadpoles but cleared the virus within 28 days when the experiment ended and surviving individuals were euthanized and tested for infection. Exposed gray treefrogs efficiently transmitted ranavirus to conspecifics (60% infection); all infected conspecifics died.

These results demonstrate that interclass transmission of ranavirus is possible through water by virion shedding from an infected individual. Previous studies [24,35] have inferred interclass transmission by exposing a host in one vertebrate class to a ranavirus isolated from a different vertebrate class. Our results also suggest that larval amphibians might be amplification hosts as demonstrated by high infection prevalence and mortality; whereas, fish and aquatic turtles may function as reservoir species due to lower susceptibility [21]. Our experiment was conducted with only one species from each ectothermic vertebrate class. Experiments are needed with additional species to determine if this trend holds.

Our study was conducted with an FV3-like ranavirus initially isolated from pallid sturgeon. Several studies have demonstrated that different FV3-like isolates have different pathogenity [8,36], and presumably transmission dynamics. Additionally, the role of fish, amphibians and reptiles as reservoirs or amplification hosts may be dependent on ranavirus species. For example, *Ambystoma tigrinum* virus (ATV) causes minimal infection and disease in anurans and fish [6,37]. Thus, although our results demonstrate that our isolate can be transmitted among ectothermic vertebrate classes and amphibians appear to function as amplification hosts, more studies are needed with other isolates before broad inferences can be made on the role of vertebrate classes in the persistence and emergence of ranaviruses.

Levels of mortality observed in our study were slightly lower than individual species challenge studies performed by others. For example, Hoverman et al. [9] reported 80% mortality of Cope's gray treefrog tadpoles exposed to an FV3-like ranavirus inoculum in a water bath. We found that 10% of mosquito fish became infected with half of those individuals dying when exposed to the

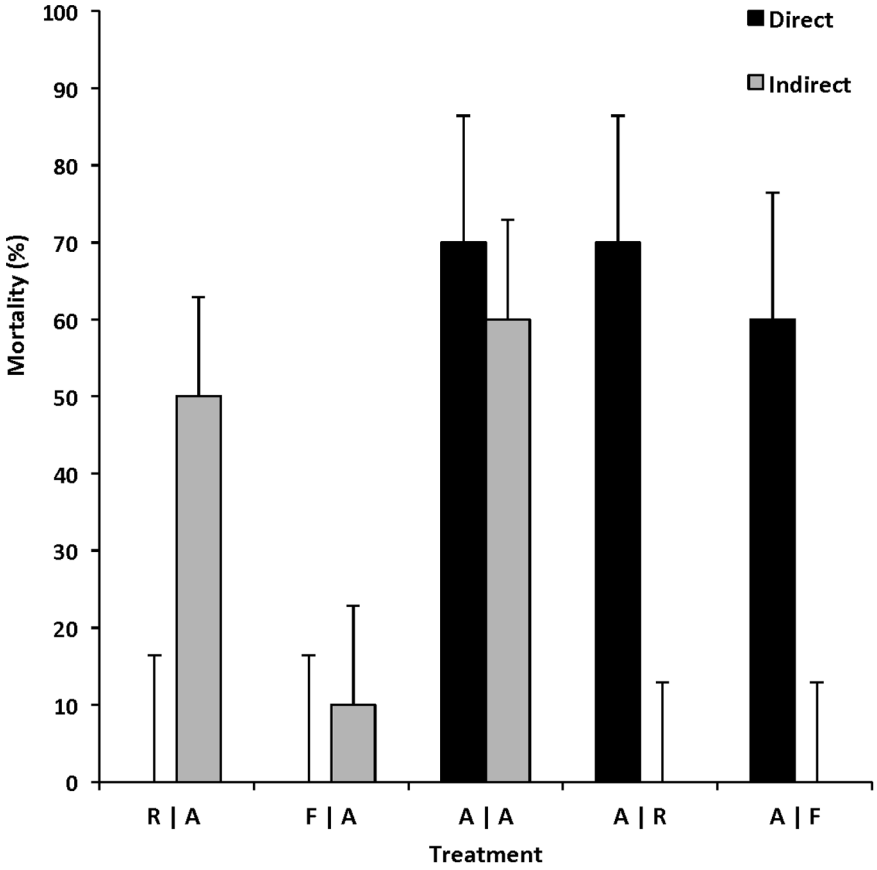

Figure 2. Mortality rate of individuals exposed to ranavirus directly or indirectly. Mortality between individuals exposed to ranavirus inoculum (direct) or via shedding (indirect) by a paired host. Treatments were paired individuals ($n = 20$ per bar) of different ectothermic vertebrate classes (A = amphibian, R = reptile, F = fish); thus, A|F = amphibian paired with fish.
doi:10.1371/journal.pone.0092476.g002

same virus [24]. MJG, DLM, and TBW (unpubl. data) found that 35% of red-eared sliders became infected and died when exposed to the same isolate used in our experiment. The differences in mortality rates may be a consequence of virion concentration in the water. The aforementioned studies exposed hosts to 10^3 PFU/mL of ranavirus, while unexposed individuals in our study were exposed to one individual that was previously exposed to ranavirus inoculum at 10^3 PFU/mL. Individuals exposed to the inoculum may not have become infected or perhaps shed virions at a concentration $<10^3$ PFU/mL when housed with a naïve individual. Dose dependency of ranavirus pathogenicity has been reported [38,39]. Also, within a species, direct exposure of ranavirus inoculum resulted in greater infection and mortality than indirect exposure of ranavirus shed from a paired individual. These results suggest that challenge experiments that use ranavirus inoculum at 10^3 PFU/mL may overestimate waterborne transmission dynamics among hosts.

Our study was conducted under controlled laboratory conditions; thus, may not represent true transmission dynamics in the wild. For example, the unsterile conditions of pond water and differences in temperature can affect the viability of ranavirus outside the host [40]. Other factors in the wild such as natural and anthropogenic stressors, direct contact of individuals, and necrophagy or predation can facilitate transmission of ranavirus [22]. To improve our understanding of interclass transmission dynamics in the wild, we recommend that future studies use outdoor aquatic mesocosms similar to Brenes [36] and Reeve et al. [41]. We also acknowledge that transmission dynamics during our study were followed for only 28 days. Longer duration studies will be useful in understanding the functional role of ranavirus hosts throughout the annual cycle.

Although transmission of ranavirus from an infected to naïve amphibian larvae via water bath has been documented previously [28], our results represent the first observation of high level of infection (60%) and mortality (50%) of amphibian larvae exposed to the pathogen solely by cohabitation with infected hosts. Harp and Petranka [28] reported low levels of infection (25%) and no mortality of naïve larval wood frogs (*Lithobates sylvaticus*) after 111 hours of cohabitation with infected conspecifics, attributing the low infection and lack of mortality to low levels of viral load shed by the moribund tadpoles. Although duration of cohabitation was longer in our study, Robert et al. [42] demonstrated transmission of ranavirus could occur as quickly as 3 hours in a water bath.

The capacity of subclinically infected fish and aquatic turtles to transmit ranavirus to amphibians has important implications regarding the persistence of this pathogen in aquatic environments. Reports of ranavirus outbreaks, particularly affecting amphibian communities, have been well documented [43–48]. In many cases, these outbreaks have been reported to be seasonally recurrent [44,49,50]. Most reports of recurrent ranavirus outbreaks in amphibian communities describe high levels of disease and mortality when amphibian larvae are highly abundant followed by a significant decrease of disease as the abundance of amphibian larvae decreases [51]. During these

periods when amphibian density is low or completely absent from the water bodies, ranavirus appears to be absent, but ranavirus prevalence can increase rapidly as soon as the next generation of amphibians returns to the aquatic ecosystems [44,49,50]. It has been hypothesized that ranavirus can persist in aquatic environments via biological reservoirs [52]. Considering that interclass transmission is possible, ranaviruses might persist in fish and aquatic turtles when the availability of highly susceptible hosts like amphibian larvae is reduced [1,39,53].

According to Cronin et al. [54], ideal reservoir species are those that can harbor subclinical infections of pathogens without suffering impairment in their biological or ecological functions until the right conditions arrive and the pathogen can be released into the environment where it can invade new hosts more suitable for its replication. For this to occur, the pathogen must exhibit three basic characteristics [54–56]. First, it should display different levels of infectivity among hosts either by being able to infect different species at different rates or by infecting different ages or developmental stages of the same species at different rates. In the case of ranaviruses, differences in susceptibility ranging from low to high susceptibility has been described for amphibians species [8,9] and life stages [57] as well as for fish [11,12,58] and reptiles species [16,20,32,59]. Second, the pathogen must be able to be transmitted efficiently among hosts. Ranaviruses can be transmitted among hosts by contact [2], consumption (predation or cannibalism; [2]), and via water exposure [40,60,61]. Third, host availability should fluctuate through time between high and low susceptible species. Because of the complex life cycle and breeding phenology of amphibians, fluctuations in abundance and composition of amphibian communities is common [62,63].

These three characteristics of the ranavirus-host system might facilitate the pathogen's persistence. We hypothesize that ranaviruses persist at low prevalence in low susceptible hosts, such as aquatic turtles and fish, and emerge when highly susceptible hosts, such as many species of larval amphibians, become abundant. Thus, aquatic turtles and fish may function as reservoirs for ranavirus, while amphibian larvae may function as amplification hosts [52]. Moreover, if low susceptible hosts are highly mobile, they may contribute to overland transport of ranaviruses.

More research is needed on the susceptibility of other ectothermic vertebrates, especially turtles and fish, to understand the complex dynamics of ranaviruses in the environment throughout the year. Identification of amplification and reservoirs species will facilitate modeling of ranavirus transmission dynamics, and development of tools that could predict likelihood of ranavirus outbreaks. Knowledge of potential ranavirus reservoirs also could assist formulation of conservation strategies for areas where outbreaks have been documented. For example, removal of a fish reservoir might be a disease intervention strategy.

Acknowledgments

We would like to thank S. Roon, J. Tucker, and L. Henderson for their help during the experiments. We thank the UT East Tennessee Research and Education Center for use of laboratory facilities, and the UT Institute of Agriculture for logistic support.

Author Contributions

Conceived and designed the experiments: RB MJG. Performed the experiments: RB. Analyzed the data: RB. Wrote the paper: RB. Contributed reagents/materials and performed PCR analysis: DLM MJG. Isolated the virus: TBW. Replicated the virus for the experiment: RPW.

References

1. Haydon DT, Cleaveland S, Taylor LH, Laurenson MK (2002) Identifying reservoirs of infection: A conceptual and practical challenge. Emerging Infectious Diseases 8: 1468–1473.
2. Brunner JL, Schock DM, Collins JP (2007) Transmission dynamics of the amphibian ranavirus Ambystoma tigrinum virus. Diseases of Aquatic Organisms 77: 87–95.
3. Breban R (2013) Role of environmental persistence in pathogen transmission: a mathematical modeling approach. Journal of Mathematical Biology 66: 535–546.
4. Woolhouse MEJ, Haydon DT, Antia R (2005) Emerging pathogens: the epidemiology and evolution of species jumps. Trends in Ecology & Evolution 20: 238–244.
5. Duffus ALJ, Pauli BD, Wozney K, Brunetti CR, Berrill M (2008) Frog virus 3-like infections in aquatic amphibian communities. Journal of Wildlife Diseases 44: 109–120.
6. Schock DM, Bollinger TK, Chinchar VG, Jancovich JK, Collins JP (2008) Experimental evidence that amphibian ranaviruses are multi-host pathogens. Copeia: 133–143.
7. Schock DM, Bollinger TK, Collins JP (2009) Mortality rates differ among amphibian populations exposed to three strains of a lethal ranavirus. Ecohealth 6: 438–448.
8. Hoverman JT, Gray MJ, Haislip NA, Miller DL (2011) Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. Ecohealth 8: 301–319.
9. Hoverman JT, Gray MJ, Miller DL (2010) Anuran susceptibilities to ranaviruses: role of species identity, exposure route, and a novel virus isolate. Diseases of Aquatic Organisms 89: 97–107.
10. Moody NJG, Owens L (1994) Experimental demostration of the pathogenicity of a frog virus, Bohle Iridovirus, for a fish species, *Barramundi lates calcarifer*. Diseases of Aquatic Organisms 18: 95–102.
11. Bang-Jensen B, Ersboll AK, Ariel E (2009) Susceptibility of pike *Esox lucius* to a panel of Ranavirus isolates. Diseases of Aquatic Organisms 83: 169–179.
12. Bang-Jensen B, Holopainen R, Tapiovaara H, Ariel E (2011) Susceptibility of pike-perch *Sander lucioperca* to a panel of ranavirus isolates. Aquaculture 313: 24–30.
13. Whittington RJ, Becker JA, Dennis MM (2010) Iridovirus infections in finfish - critical review with emphasis on ranaviruses. Journal of Fish Diseases 33: 95–122.
14. Hyatt AD, Williamson M, Coupar BEH, Middleton D, Hengstberger SG, et al. (2002) First identification of a ranavirus from green pythons (*Chondropython viridis*). Journal of Wildlife Diseases 38: 239–252.
15. De Voe R, Geissler K, Elmore S, Rotstein D, Lewbart G, et al. (2004) Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (*Terrapene carolina carolina*). Journal of Zoo and Wildlife Medicine 35: 534–543.
16. Johnson AJ, Pessier AP, Wellehan JFX, Childress A, Norton TM, et al. (2008) Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. Journal of Wildlife Diseases 44: 851–863.
17. Marschang RE (2011) Viruses Infecting Reptiles. Viruses-Basel 3: 2087–2126.
18. Ariel E, Owens L (1997) Epizootic mortalities in tilapia *Oreochromis mossambicus*. Diseases of Aquatic Organisms 29: 1–6.
19. Johnson AJ, Pessier AP, Childress AL, Jacobson ER (2006) Red-eared sliders (*Trachemys scripta elegans*) as a model of ranavirus infection in chelonians. In: Baer CK, editor. Proceedings of the Association of Reptilian and Amphibian Veterinarians, Thirteenth Annual Conference, Baltimore, Maryland, USA, 23–27 April, 2006. 73–74.
20. Allender MC, Abd-Eldaim M, Schumacher J, McRuer D, Christian LS, et al. (2011) PCR prevalence of ranavirus in free-ranging eastern box turtles (*Terrapene carolina carolina*) at rehabilitation centers in three southeastern US states. Journal of Wildlife Diseases 47: 759–764.
21. Paull SH, Song SJ, McClure KM, Sackett LC, Kilpatrick AM, et al. (2012) From superspreaders to disease hotspots: linking transmission across hosts and space. Frontiers in Ecology and the Environment 10: 75–82.
22. Gray MJ, Miller DL, Hoverman JT (2009) Ecology and pathology of amphibian ranaviruses. Diseases of Aquatic Organisms 87: 243–266.
23. Seigel RA, Farnsworth SD (2013) Responses, movements, and survival of relocated box turtles during the construction of the inter-county connector highway in Maryland. Journal of the Transportation Research Board No. 2362.
24. Brenes R, Miller DL, Waltzek TB, Wilkes RP, Tucker JL, et al. (2014) Susceptibility of fish and turtles to three ranaviruses isolated from different ectothermic vertebrate classes. Journal of Aquatic Animal Health 26:In Press.
25. Allender MC, Mitchell MA, Torres T, Sekowska J, Driskell EA (2013) Pathogenicity of frog virus 3-like virus in red-eared slider turtles (*Trachemys scripta elegans*) at two environmental temperatures. Journal of Comparative Pathology 149: 356–367.

26. Waltzek TB, Miller DL, Gray MJ, Drecktrah B, Briggler JT, et al. (In review) Isolation of Frog virus 3 from pallid sturgeon (*Scaphirhynchus albus*) suggests an interclass 1 host shift. Diseases of Aquatic Organisms.
27. Iwama GK, Ackerman PA (1994) Anaesthetics. In: Hochachka PW, Mommsen TP, editors. Biochemistry and molecular biology of fishes: Analytical techniques. Amsterdam, The Netherlands: Elsevier. 1–17.
28. Harp EM, Petranka JW (2006) Ranavirus in wood frogs (*Rana sylvatica*): Potential sources of transmission within and between ponds. Journal of Wildlife Diseases 42: 307–318.
29. Relyea RA, Werner EE (1999) Quantifying the relation between predator-induced behavior and growth performance in larval anurans. Ecology 80: 2117–2124.
30. Budy P, Baker M, Dahle SK (2011) Predicting fish growth potential and identifying water quality constraints: A spatially-explicit bioenergetics approach. Environmental Management 48: 691–709.
31. Baer CK (2006) Guidelines for euthanasia of nondomestic animals. Yulee, FL: American Association of Zoo Veterinarians 111p p.
32. Johnson AJ, Pessier AP, Jacobson ER (2007) Experimental transmission and induction of ranaviral disease in western ornate box turtles (*Terrapene ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*). Veterinary Pathology 44: 285–297.
33. Picco AM, Brunner JL, Collins JP (2007) Susceptibility of the endangered California tiger salamander, *Ambystoma californiense*, to Ranavirus infection. Journal of Wildlife Diseases 43: 286–290.
34. SAS (2012) SAS 9.3. 9.1 ed. Cary, NC: SAS Institute, Inc.
35. Bayley AE, Hill BJ, Feist SW (2013) Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts. Diseases of Aquatic Organisms 103: 171–183.
36. Brenes R (2013) Mechanisms contributing to the emergence of ranavirus in ectothermic vertebrate communities [Dissertation]. Knoxville, TN: University of Tennessee. 131 p.
37. Picco AM, Karam AP, Collins JP (2010) Pathogen host switching in commercial trade with management recommendations. Ecohealth 7: 252–256.
38. Pearman PB, Garner TWJ, Straub M, Greber UF (2004) Response of the Italian agile frog (*Rana latastei*) to a ranavirus, frog virus 3: A model for viral emergence in naive populations. Journal of Wildlife Diseases 40: 660–669.
39. Brunner JL, Richards K, Collins JP (2005) Dose and host characteristics influence virulence of ranavirus infections. Oecologia 144: 399–406.
40. Nazir J, Spengler M, Marschang RE (2012) Environmental persistence of amphibian and reptilian ranaviruses. Diseases of Aquatic Organisms 98: 177–184.
41. Reeve B, Crespi E, Whipps C, Brunner J (2013) Natural stressors and ranavirus susceptibility in larval wood frogs (*Rana sylvatica*). EcoHealth 10: 190–200.
42. Robert J, George E, Andino FD, Chen GC (2011) Waterborne infectivity of the Ranavirus frog virus 3 in *Xenopus laevis*. Virology 417: 410–417.
43. Weng SP, He JG, Wang XH, Lu L, Deng M, et al. (2002) Outbreaks of an iridovirus disease in cultured tiger frog, *Rana tigrina rugulosa*, in southern China. Journal of Fish Diseases 25: 423–427.
44. Greer AL, Berrill M, Wilson PJ (2005) Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. Diseases of Aquatic Organisms 67: 9–14.
45. Une Y, Sakuma A, Matsueda H, Nakai K, Murakami M (2009) Ranavirus outbreak in North American bullfrogs (*Rana catesbeiana*), Japan, 2008. Emerging Infectious Diseases 15: 1146–1147.
46. 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: 310–310.
47. Balseiro A, Dalton KP, del Cerro A, Marquez I, Parra F, et al. (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. Veterinary Journal 186: 256–258.
48. Hoverman JT, Gray MJ, Miller DL, Haislip NA (2012) Widespread occurrence of ranavirus in pond-breeding amphibian populations. Ecohealth 9: 36–48.
49. Cunningham AA, Hyatt AD, Russell P, Bennett PM (2007) Experimental transmission of a ranavirus disease of common toads (*Bufo bufo*) to common frogs (*Rana temporaria*). Epidemiology and Infection 135: 1213–1216.
50. Teacher AGF, Cunningham AA, Garner TWJ (2010) Assessing the long-term impact of ranavirus infection in wild common frog populations. Animal Conservation 13: 514–522.
51. Todd-Thompson M (2010) Seasonality, variation in species prevalence, and localized disease for ranavirus in Cades Cove (Great Smoky Mountains National Park) amphibians. [Thesis]. Knoxville, TN: University of Tennessee. 43 p.
52. Gray MJ, Miller DL (2013) Rise of ranavirus: An emerging pathogen threatens ectothermic vertebrates. Wildlife Professional 7: 51–55.
53. Brunner JL, Schock DM, Davidson EW, Collins JP (2004) Intraspecific reservoirs: Complex life history and the persistence of a lethal ranavirus. Ecology 85: 560–566.
54. Cronin JP, Welsh ME, Dekkers MG, Abercrombie ST, Mitchell CE (2010) Host physiological phenotype explains pathogen reservoir potential. Ecology Letters 13: 1221–1232.
55. Anderson RM, May RM (1979) Population biology of infectious diseases. Nature 280: 361–367.
56. May RM, Anderson RM (1979) Population biology of infectious diseases. Nature 280: 455–461.
57. Haislip NA, Gray MJ, Hoverman JT, Miller DL (2011) Development and disease: How susceptibility to an emerging pathogen changes through anuran development. PloS one 6: e22307.
58. Gobbo F, Cappellozza E, Pastore MR, Bovo G (2010) Susceptibility of black bullhead Ameiurus melas to a panel of ranavirus isolates. Diseases of Aquatic Organisms 90: 167–174.
59. Allender MC, Abd-Eldaim M, Kuhns A, Kennedy M (2009) Absence of ranavirus and herpesvirus in a survey of two aquatic turtle species in Illinois. Journal of Herpetological Medicine and Surgery 19: 16–20.
60. Jancovich JK, Davidson EW, Parameswaran N, Mao J, Chinchar VG, et al. (2005) Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. Molecular Ecology 14: 213–224.
61. Cunningham AA, Hyatt AD, Russell P, Bennett PM (2007) Emerging epidemic diseases of frogs in Britain are dependent on the source of ranavirus agent and the route of exposure. Epidemiology and Infection 135: 1200–1212.
62. Vignolia L, Bolognaa MA, Luisellib L (2007) Seasonal patterns of activity and community structure in an amphibian assemblage at a pond network with variable hydrology. Acta Oecologica 2007: 185–192.
63. Werner EE, Yurewicz KL, Skelly DK, Relyea RA (2007) Turnover in an amphibian metacommunity: the role of local and regional factors. Oikos 116: 1713–1725.