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ABSTRACT: Frog virus 3 (FV3) and FV3-like viruses, are members of the genus *Ranavirus* (family *Iridoviridae*), and they have been associated with infectious diseases that may be contributing to amphibian population declines. We examined the mode of transmission of an FV3-like virus, and potential hosts and reservoirs of the virus in a local amphibian community. Using the polymerase chain reaction to detect infected animals, we found an FV3-like virus in south-central Ontario, Canada, amphibian communities, where it infects sympatric amphibian species, including ranid and hylid tadpoles (*Rana sylvatica, Hyla versicolor*, and *Pseudacris* spp.), larval salamanders (*Ambystoma* spp.), and adult eastern-spotted newts (*Notophthalmus viridescens*). The high prevalence of FV3-like infections in caudate larvae suggests that salamanders are likely to be both hosts and reservoirs. In laboratory FV3 challenges of *R. sylvatica*, the rate of infection was dependent on the amount of virus to which the animals were exposed. In addition, although vertical transmission was suspected, horizontal transmission through exposure to infected pond water is the most likely route of infection in tadpoles. Based on our observations, a simple model of FV3/FV3-like virus transmission postulates that, in aquatic amphibian communities, transmission of the virus occurs between anuran and urodele species, with ambystomatid salamanders the most likely reservoir for the ranavirus in our study.

Key words: Aquatic amphibian communities, frog virus 3-like infections, transmission, vectors.

INTRODUCTION

Amphibian populations are in decline on a global scale (Stuart et al., 2004). A number of factors are thought to be contributing to amphibian population declines, including habitat loss and modification, increasing ultraviolet radiation levels, predation, climate change, environmental contaminants, and emerging infectious diseases, as well as interactions between these factors (Alford and Richards, 1999). Although emerging infectious diseases are thought to be a contributing factor to global amphibian declines, anthropogenic environmental modification is considered to be, at least in part, responsible for their recent appearance or increasing virulence (Daszak et al., 2001; Pounds et al., 2006). An emerging infectious disease is defined as a disease caused by a pathogen that is currently increasing in geographical range, is infecting an increased diversity of hosts, and/or has recently evolved (Daszak et al., 2000). Examples of diseases in amphibian populations around the world that have recently gained attention include chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (Muths et al., 2003; Rachowicz et al., 2006), and the iridoviruses (family *Iridoviridae*) (Harp and Petranka, 2006). Iridoviruses have been associated with large-scale morbidity and mortality events of amphibians throughout North America and Europe. For example, of 44 amphibian mortality events studied in the United States between 1996 and 2001, iridovirus infection was the sole cause of mortality in 48% of these events, and it was thought to be a factor in 9% of the other recorded mortality events with multiple etiology (Green et al., 2002).

The distribution of iridoviruses in Ca-

nadian amphibians is poorly understood. The first reported iridovirus outbreak and mortality event in Canada was caused by the Regina ranavirus, which infected larval and adult tiger salamanders (*Ambystoma tigrinum diaboli*) in Saskatchewan (Bollinger et al., 1999). More recently, frog virus 3 (FV3) or an FV3-like virus, was identified in populations of wood frogs (*Rana sylvatica*), leopard frogs (*Rana pipiens*), and green frogs (*Rana clamitans*) in southern Ontario where it was associated with wood frog tadpole die-offs (Greer et al., 2005; Charbonneau, 2006).

Although the mechanism of FV3/FV3-like virus transmission remains unclear, both vertical (reproduction-dependent) and horizontal (nonreproduction-dependent) transmission may be possible. A horizontal transmission model has been proposed for another member of the *Ranavirus* genus, *Ambystoma tigrinum* virus (ATV), which can be passed to larval and recently metamorphosed Arizona tiger salamanders (*Ambystoma tigrinum nebulosum*) by exposure to free virus in water (Brunner et al., 2004). In addition, salamanders that were exposed to ATV as larvae could become ATV carriers as adults and pass the virus on to uninfected adults (Brunner et al., 2004). A recent and similar study examined potential transmission mechanisms of ranaviruses between wood frogs inhabiting geographically isolated ponds (Harp and Petranka, 2006). In this study, transmission within ponds was enhanced by tadpoles scavenging diseased, moribund, or dead tadpoles, and the authors concluded that the virus could be spread between local ponds as a result of the movement of contaminated sediment by animals or humans (Harp and Petranka, 2006).

Our study focused on an amphibian community centered on small ponds located near a field station maintained by Trent University at Bobcaygeon, Ontario, Canada (44°33′N, 78°33′W). From 1999 through 2001, virtually all of the wood frog tadpoles in one of these ponds (Oliver Pond) died shortly before metamorphosis (Greer et al., 2005). Field observations suggested that only wood frog tadpoles died during these events and that they were positive for FV3-like virus infections; clinical signs were not observed in other amphibian species in the pond, and they were not examined for the presence of FV3-like infections (Greer et al., 2005). Although these outbreaks provided no evidence of interspecies transmission of FV3, interspecies transmission of FV3 has not been documented between the three-spined stickleback (*Gasterostelus aculeatus*) and tadpoles of the red-legged frog (*Rana aurora*) (Mao et al., 1999). Because very little is known about the infection and transmission of ranaviruses, we examined the community-level patterns of infection in our study pond, and we investigated whether there is evidence of horizontal and/or vertical transmission of FV3 or FV3-like viruses in this community.

MATERIALS AND METHODS

Animal collection

Wood frog tadpoles and salamander larvae were collected from three small ponds (Oliver Pond, Parker Pond, and Crowe's Pond) near the Bobcaygeon site between 16 May and 9 July 2005. Wood frog tadpoles in both Oliver and Parker ponds previously have been shown to be infected by FV3 (Greer et al., 2005; Charbonneau, 2006). Wood frog tadpoles were sampled at five different dates in Oliver Pond, at eight dates in Parker Pond, and at five dates in Crowe's Pond. We collected tadpoles that ranged across almost all free-swimming developmental stages (Gosner stages 25–40; Gosner, 1960). We also collected a single sample of ambystomatid larvae (blue spotted or yellow spotted salamanders, *Ambystoma laterale* and *Ambystoma maculatum*), *Pseudacris crucifer* and *Pseudacris triseriata* tadpoles (spring peeper and western chorus frog, respectively), *Hyla versicolor* tadpoles (gray tree frog), and adult eastern spotted newts (*Notophthalmus viridescens*) from Parker Pond. A single sample of nine ambystomatid larvae was also collected from Oliver Pond. Sample size was 10 individuals in all cases except for the sample of eastern spotted newts collected at Parker Pond where only five animals could be retrieved. All animals were

euthanized using an immersion overdose of tricaine methanesulfonate (MS-222) and preserved in 70% ethanol for polymerase chain reaction (PCR) analysis for the presence of FV3.

Establishment of virus-negative wood frog eggs and care of wood frog eggs and tadpoles

We collected clutches of wood frog eggs (Gosner stage 1, before rotation) that had been deposited the previous night from the three ponds located within 15 km of Peterborough, Ontario (44°21′N, 78°17′W). We also collected wood frog clutches from Oliver and Crowe's ponds within 1 to 2 hr of being deposited (Gosner stages 0–1). To limit the risk of field contamination with FV3, as soon as possible after collection we transferred egg masses into municipal tap water that had been aged for 2 to 3 days. However, we cannot be certain that the eggs did not come into contact with the ranavirus through contaminated pond water or through virus potentially present in the jelly matrix of the brood. We confirmed that larvae from these clutches were not infected using PCR examination of a suite of reference tadpoles. However, neither the jelly from the egg masses nor the pond water was tested for the presence of ranavirus. Additionally, in early spring, we collected three pairs of wood frogs in terrestrial amplexus on a road near Crowe's Pond (Duffus and Ireland, in press). Although we cannot confirm that the amplexing adults had not been in contact with pond water that year, any clutches produced by these pairs subsequent to collection would not have been exposed to pond water. Thus, for the larvae produced by these pairs, we have assumed that contact with pathogen-contaminated pond water was eliminated as a source of infection. The three pairs still in amplexus were placed in approximately 4 l of aged tap water in covered aquaria and maintained in an environmental chamber with a constant temperature of 15 C and dark conditions to facilitate breeding in captivity. All three females produced eggs. Each egg mass was removed from the tank that contained its parents and transferred to a 19-l aquarium, partially filled with aged tap water. Two of the three egg masses were viable, developing normally with a hatching rate of >90% (data not shown). The parents were euthanized with an overdose of MS-222 and preserved in 70% ethanol for analysis for the presence of FV3/ FV3-like virus.

Once hatched, the tadpoles were raised in 19-l tanks in an environmental chamber held at 15 C. Water was changed every 3 days after the tadpoles began feeding to avoid water fouling. Tadpoles were fed shredded, boiled spinach ad libitum. At approximately Gosner stage 25, the temperature in the chamber was raised to 20 C, to more closely follow natural pond temperatures.

Assessment of vertical transmission

Samples ($n=5$) of the eggs from the most recently deposited broods collected from each of the ponds and a sample from one of the broods produced in captivity were preserved immediately after oviposition. The remaining eggs were left to develop as described above. During the course of development, we sampled each brood three times, taking up to five animals for each sample (samples were taken on 16–29 April [Gosner stage 1], 2–26 May and 5–29 June [Gosner stages 24–34], and 15–19 July [Gosner stages 34–45]). Tadpoles were euthanized and preserved in 70% ethanol for PCR analysis for the presence of FV3/FV3-like virus. Because the eggs were in contact with potentially infected pond water for a couple of hours before collection, presence of the virus in these animals is assumed to be from a parental source. Data from April and May, and June and July, were pooled.

Horizontal transmission experiment

Virus stock culture: The FV3, originally obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA), was grown in fathead minnow (*Pimephales promelas*, ATCC). The number of plaque-forming units (PFU) per milliliter was determined and the final viral concentrations in the treatments were approximately 67, 670, and 6,700 PFU/ml. These virus concentrations were achieved by the addition of the appropriate volume of virus culture to the exposure water.

Pilot experiment: Wood frog tadpoles that hatched from a brood laid in the laboratory by ranavirus-negative frogs that were collected during terrestrial amplexus (tadpoles later tested negative for FV3/FV3-like virus) were used in an experiment to assess the potential for horizontal transmission. Tadpoles were exposed to FV3 at a concentration of 670 PFU/ml of the virus for 5 days via bath exposure. The tadpoles ranged between Gosner stage 25 and 30. After exposure the animals were euthanized, preserved in 70% ethanol, and screened for the presence of FV3.

Assessment of horizontal transmission: To determine the effect of virus concentration on

the infection rate of FV3 in tadpoles and juvenile frogs, they were exposed to FV3 at different concentrations via their holding water. When treated, the tadpoles ranged in development from Gosner stage 25 to 42/43 (by the end of the experiment, there were stage 45 juveniles present in some treatments). Each treatment consisted of two replicates of 15 individuals. For analysis, the trials were pooled and 20 individuals were screened from each treatment. To ensure maximal exposure to FV3, the tadpoles were placed into 1-l beakers containing 150 ml of aged tap water with the appropriate amount of FV3 and left overnight. The water level was increased to 350 ml the next morning to increase the water volume for the tadpoles, dilute waste products, and provide more oxygen to the tadpoles. Because aeration may inactivate the virus, reducing the live virus count in the water, the beakers were left unaerated for 5 to 6 hr at the beginning of the experiment to increase the likelihood of viral transmission, but they were aerated for the remainder of the experiment. The tadpoles were fed boiled spinach, and the water was changed every 72 hr. No virus was added to the water after the water changes occurred; therefore, the total length of the exposure was 3 days with a recovery/resting period of 6 days. Animals that died over the course of the experiment were removed and preserved in 70% ethanol for analysis for the presence of FV3. At day 9, the experiment was terminated and the remaining tadpoles were euthanized and placed into 70% ethanol.

Virus detection

We used PCR to detect FV3/FV3-like virus infection in our amphibians. Livers were removed from field-collected and experimental animals and placed into 500 µl of lysis buffer (4 M urea, 0.2 M NaCl, 0.5% n-lauroyl sarcosine, 10 mM 1,2-cyclohexanediaminetetraacetic acid, and 0.1 M Tris-HCl, pH 8.0). To prevent cross-contamination, dissection instruments were cleaned with a high concentration ($>50\%$) solution of DECON 75 (Decon Laboratories Limited, East Sussex, UK) and rinsed well with water. We also sampled lysis buffer every five or 10 samples as negative controls. Tissue samples were incubated at 37 C overnight, and then 15 U of proteinase K (Roche Diagnostics, Manheim, Germany) was added, and samples were incubated for 2 hr at 65 C. An additional aliquot of 15 U of proteinase K was added before a final incubation at 37 C overnight. DNA digests were extracted following the QIAamp DNeasy extraction kit protocol (QIA-GEN, Mississauga, Ontario, Canada) following Greer et al. (2005). DNA obtained from the hepatic tissue was assessed by PCR for the presence of FV3 using primers that amplify a 500-base pair (bp) region of the major capsid protein of FV3 (Greer et al., 2005). The PCR volume of 10 µl contained 2 µl of extracted DNA, 1 U of Taq DNA polymerase, $1\times$ PCR buffer, 1.5 mM $MgCl_2$ (all from Invitrogen, Burlington, Ontario, Canada), 0.2 µM each dNTP (GE Healthcare, Piscataway, New Jersey, USA), and 0.2 µM each primer. PCR was performed using the following cycles: initial denaturation at 94 C for 5 min; followed by 35 cycles of 30 sec at 94 C, 30 sec at 60 C, and 30 sec at 72 C; followed by a final extension of 2 min at 72 C (Greer et al., 2005). PCR products were run on a 1.5% agarose electrophoresis gel stained with ethidium bromide. Presence of a 500-bp band was considered to indicate the presence of FV3 in samples.

RESULTS

FV3 is found in natural populations of amphibians in Southeastern Ontario

In an effort to understand the natural infectious cycle of FV3 in natural populations, we examined a number of ponds in southeastern Ontario to determine whether FV3 infections were present. Of the 50 wood frog tadpoles collected from Oliver Pond, 16 tested positive for FV3, and the Gosner stages for the infected tadpole can be found in Table 1. However, none of the tadpoles showed clinical signs of disease. In Parker Pond, we found 20% of tadpoles collected early in the season (Gosner stages 25–26) infected with FV3 (Table 1). However, when tadpoles were collected later in the season from Parker Pond (Gosner stages 38–40), none were found to be infected (Table 1). Finally, we were unable to detect FV3 infection in Crowe's Pond (Table 1).

Because our data confirmed previous observations that wood frog tadpoles are infected with FV3, we decided to extend these observations by examining whether other populations of amphibians in our study area are also infected by FV3. We identified FV3 infections in two of nine ambystomatid larvae (blue or yellow spot-

TABLE 1.   Frog virus 3 (FV3)-like infections in wild-collected wood frog tadpoles during 2005 according to collection date and Gosner stage.

	16 May	26 May	6 June	13 June	17 June
Crowe's Pond					
Gosner stages	25–26	25–29	28–34	37–39	39–40
Infection rate (%)[a]	0	0	0	0	0
Oliver Pond					
Gosner stages	25	25–27	30–32	35–37	37–39
Infection rate (%)	80	40	10	30	0
Parker Pond[b]					
Gosner stages	25–26	26–28	30–38	31–38	38–40
Infection rate (%)	20	0	0	0	0

[a] To determine the presence of an FV3-like infection, a sample of liver was used for the polymerase chain reaction screen for the major capsid protein of FV3 ($n=10$/date).

[b] Parker Pond was sampled on three additional dates, because all animals tested negatively for the virus; the results were not included here.

ted salamanders, or both) collected from Oliver Pond (data not shown). At Parker Pond, 60% of the ambystomatid larvae were infected with FV3, although none showed clinical signs of infection (Table 2). In addition, 14% of *Pseudacris* spp. (spring peeper, chorus frog, or both) tadpoles, 5% of gray tree frog tadpoles, and 20% of the aquatic adult eastern spotted newts were infected with FV3 at Parker Pond (Table 2).

Vertical and horizontal transmission of FV3 in wood frogs

Because FV3 infections have been found in a wide range of amphibian species within a community, we next examined how the virus may spread among community members. We first examined whether FV3 could be transmitted directly from parent to offspring (vertical transmission). As described above, we were fortuitously able to collect three terrestrially amplexing pairs of wood frog adults that had probably been unexposed to pond water since the mating season of the previous year. These animals produced egg clutches we designated as A, B, and C. Both parents of egg clutches A and B tested negative for FV3, and none of the tadpoles or eggs from clutches A or B tested positive for FV3. However, the

TABLE 2.   Prevalence of frog virus 3 (FV3)-like virus infections present in other amphibian species in the aquatic community found in Parker Pond.

Amphibian	Date sampled						
	13 June	17 June	21 June	25 June	29 June	30 June	9 July
Ambystomatids[a]	50%[b]	90%	50%	40%	70%		
Gosner stages[c]*Pseudacris* spp.	35–39	33–41	34–43	30–41	30–40		
	0%	60%	0%	10%	0%		
Gosner stages *H. versicolor*						31–40	31–40
						10%	0%

[a] These animals were not staged.

[b] To determine the presence of an FV3-like infection, a sample of liver was used for the polymerase chain reaction screen for the major capsid protein of FV3 ($n=10$/date).

[c] The anuran tadpoles were staged according to Gosner (1960).

TABLE 3.   Assessment for the potential of vertical transmission of the ranavirus in wood frogs through the examination of eggs and lab-raised tadpoles.

		Eggs	April–May	June–July
Country Road 6	Gosner stages	1	25	25–44
	No. infected/no. tested[a]	0/5	0/4	0/6
Oliver Pond	Gosner stages	1	24–27	27–44
	No. infected/no. tested	1/4	0/5	0/6
Crowe's Line Road	Gosner stages	1	24–25	24–45
	No. infected/no. tested	1/5	1/3	0/8
Division at Donwood	Gosner stages	1	24–25	25–43
	No. infected/no. tested	1/5	0/4	0/6
Barb's Marsh	Gosner stages	1	24–29	24–44
	No. infected/no. tested	1/5	0/5	0/9
Known parents A	Gosner stages	1	24–25	25–44
	No. infected/no. tested	0/5	0/5	0/8
Known parents B[b]	Gosner stages	1		
	No. infected/no. tested	0/5		
Known parents C[c]	Gosner stages	1		
	No. infected/no. tested	1/5		

[a] To determine the presence of frog virus 3 (FV3)-like particles, eggs were removed from the jelly and the whole egg was tested, whereas in the tadpoles, a piece of liver tissue was used for the polymerase chain reaction screen for the major capsid protein of FV3.

[b] Data past the egg stage were not collected because the tadpoles were to be used in another experiment.

[c] The clutch was not viable, and no tadpoles developed.

male parent of brood C tested positive for FV3 and one of five embryos from this brood was infected with FV3 (Table 3). The eggs present in brood C were not fertilized; therefore, analyses of further developmental stages were not possible. In addition to the egg clutches that were deposited in the laboratory, clutches collected from natural populations were also analysed via PCR. The FV3 was detected in four of the five clutches, including one clutch that later produced tadpoles that tested positive for an FV3/FV3-like infection (Table 3). However, except for these tadpoles from one clutch, no other infected tadpoles were detected, and later stage tadpoles when tested showed no evidence of FV3 infection. This may indicate that infected eggs/larvae either recovered from the infection or were killed by the infection resulting in the absence of later stage FV3-infected tadpoles (i.e., above Gosner stage 26; Table 3).

Although we have identified in this study that vertical transmission maybe a potential mechanism of spread of FV3, horizontal transmission seems the most likely means of transmission of FV3. Therefore, to examine the susceptibility of tadpoles becoming horizontally infected with FV3, we exposed wood frog tadpoles/juveniles (Gosner stages 25–46) to varying concentrations of FV3 present in their holding water. We found that as the concentration of FV3 increased, the percentage of infected individuals increased (Fig. 1). These results suggest that tadpoles can become infected with FV3 through exposure to virus-contaminated
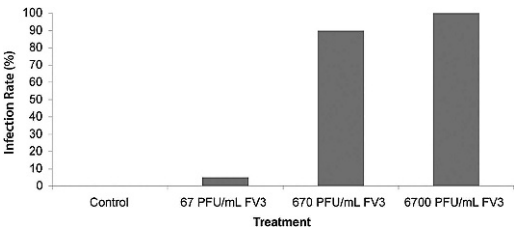

FIGURE 1.   Dose-dependent infection of wood frog tadpoles through contact with FV3 in water. (n=20 tadpoles/treatment).

water and firmly support the hypothesis that the virus can be spread horizontally.

DISCUSSION

We set out to determine the extent of FV3/FV3-like infected amphibian species present in ponds in southeastern Ontario and the mechanism of virus transmission. In the three ponds where wood frog tadpoles were monitored for FV3/FV3-like infections, the extent of infection ranged from extensive (Oliver Pond) to minimal (Parker Pond) and absent (Crowe's Pond). In wood frogs, infections occurred in eggs, early-to-late tadpole stages, and in an adult. It should be noted that the ponds were not monitored through tadpole metamorphosis. Furthermore, although infections were detected with PCR, we never observed visible signs of FV3/FV3-like infections in field-collected animals, although previous observations suggest that extensive mortality occurs in wood frog tadpoles infected with FV3/FV3-like viruses (Green et al., 2002; Harp and Petranka, 2006), including those studied at one of our study sites (Greer et al., 2005). Our results also concur with those of Harp and Petranka (2006) in that they indicate that in some cases, wood frog tadpoles can carry the virus without exhibiting signs of infection. Interestingly, no late stage tadpoles collected from Oliver Pond were infected. Although this may be a result of the small sample size, it is also possible that the wood frog tadpoles were able to clear the FV3 infection, as seen in *Xenopus laevis* (Gantress et al., 2003). Alternatively, infection-induced mortality may have reduced the number of infected individuals from the wood frog population before our sampling of later stage tadpoles, which would not have been detected by our analysis.

In Oliver Pond, FV3/FV3-like virus infections of wood frog tadpoles had been recorded as early as 6 yr before this study, but extensive die-offs of late stage tadpoles, including those exhibiting signs of

FV3 infection, only occurred in the first 3 yr. Although the infection rate of FV3 in adult wood frogs remains unknown, it is possible that a portion of the current population of breeding adult wood frogs at Oliver Pond are subclinical carriers of FV3. There is also the possibility that the virus has become less virulent over time, resulting in reduced incidence of mortality.

The absence of infected wood frog tadpoles at Crowe's Pond, less than 1 km from Oliver Pond, supports observations suggesting that FV3/FV3-like virus outbreaks that cause mortality and recur for several years are usually localized to a small geographical area, and possibly restricted to only one pond in an area (e.g., Carey et al., 1999; Greer et al., 2005). As the dispersal ability of adult wood frogs is poor (Homan et al., 2004), it is unlikely that the geographic movement of the virus occurs through movement of wood frogs. An alternative explanation, hypothesized by Harp and Petranka (2006), is that virus spread between ponds could occur through the movement of virus-contaminated sediment by humans or animals. Since we have found extensive infection of ambystomatid salamanders with FV3 or an FV3-like virus in our pond communities, it is possible that virus movement between ponds is mediated by salamanders or other amphibians living in these pond communities. It has been suggested that, for *Ambystoma tigrinum* virus (ATV), a ranavirus related to FV3, infected Arizona tiger salamander adults can act as intraspecies reservoirs for ATV (Brunner et al., 2004). However, this does not explain the geographical heterogeneity of ranavirus infections in a continuous landscape.

The possibility of interspecies transmission from possible reservoir species suggests one mechanism by which FV3/FV3-like viruses may circulate in ponds. Another mechanism by which wood frogs could become infected is through vertical transmission. We have presented data that supports the hypothesis that vertical trans-

mission of FV3/FV3-like viruses from parent to eggs in wood frogs is possible. In our study, eggs were laid in captivity from a pair of wood frogs in which the male was positive and the female was negative. Therefore, the male parent represented the only potential source of FV3/FV3-like virus. There are two ways in which the eggs could have become infected, through water contaminated by the infected male or through infected semen/gametes. It is possible that virus shed into the water by the male could have infected the eggs through the incorporation of infected water into the jelly matrix; thus, this is a form of pseudovertical transmission. The second potential method is through infection via the sperm or seminal fluid of the male. The reproductive tract in amphibians shares common anatomy with the urinary and gastrointestinal tracts, there are several opportunities for sperm or eggs to come into contact with tissue potentially infected with FV3. An iridovirus associated with salamander mortality events in Utah was cultured and isolated from the testes of affected salamanders (Docherty et al., 2003). This virus was also isolated from the gut, liver and kidneys of salamanders in the same mortality event (Docherty et al., 2003). It is possible that the testes of the infected wood frog were also infected with FV3 or an FV3-like virus and that the initial exposure to the virus may have occurred during spermatogenesis. Further exposure to the virus could occur as a result of the shared ureter/gonaduct. Upon fertilization, the sperm comes into direct contact with the egg before the egg membranes absorb water and the virus could pass from the sperm/seminal fluid into the egg. However, although our results support the possibility of vertical transmission, more investigation is needed to ascertain whether in fact it does occur and which routes are possible.

It has been hypothesized that salamanders, which are chronically and sublethally infected with a ranavirus, can reintroduce the virus into their breeding pond upon their return the next breeding season and can therefore infect the next generation of larvae (Brunner et al., 2004). Brunner et al. (2004) provided evidence of infection in returning salamanders (two of 30), but they provided no mechanism for the transfer of the virus between these adults and larvae. From our observation that vertical transmission of an FV3-like ranavirus may be a possible route of infection. Evidence that nondensity-dependent parent-offspring transmission of the virus is provided. It is also possible that this reintroduction of the virus between years completes the interyear infection loop for the FV3-like virus present in Ontario, providing that the infected adult has breeding site fidelity.

Along with the evidence for vertical transmission of FV3/FV3-like viruses in wood frogs, it is clear that wood frog tadpoles can be infected by contact with contaminated water. The horizontal transmission demonstrated in this study support those of Harp and Petranka (2006), who infected wood frog tadpoles simply by holding them in the same water as tadpoles with signs of disease. This also seems to be true with salamanders and ATV; infection rates of tiger salamander larvae and metamorphs increased with water concentrations of ATV up to 10^4 PFU/ml, after which there was no increase in the infection rate (Brunner et al., 2005). Wood frog tadpoles exhibited a similar trend when they were exposed in the present study to different concentrations of FV3. At a concentration of 670 PFU/ml or more, the infection rate was close to or at 100%, but at lower FV3 concentrations (6.7 PFU/ml), the infection rate was correspondingly low. Harp and Petranka (2006) saw a similar trend where wood frog infection rates increased when the tadpoles were allowed to scavenge on the bodies of moribund and dead infected tadpoles, as opposed to being only exposed through contaminated water or sediment. Despite the fact that waterborne transmission of the virus would

seem to be the most likely route, the low infectivity observed at the low virus challenge concentration in the present study further supports the possibility that vertical transmission is a viable mode of transmission in wood frogs; it is unlikely that the concentration of the virus is high enough in the water of southeastern Ontario breeding ponds in early spring to infect the eggs.

The FV3-like virus that is present in Ontario fulfils the criteria for two different factors of disease-induced extinction as detailed by de Castro and Bolker (2005). First, there is nondensity-dependent transmission of the virus in the form of vertical transmission. Second, there is a mechanism of disease-induced extinction through infection by exposure to sympatric reservoir species (de Castro and Bolker, 2005). In the FV3-like virus present in Ontario, there are two different potential groups representing both intra- and interspecies reservoirs of the virus. As demonstrated here with wood frogs, there is the potential for vertical transmission of the virus from year to year between individuals of the same species through transmission of infection from parent to offspring. In terms of interspecies reservoirs, because of the fact that there are detectable infection rates of the FV3-like virus throughout the aquatic amphibian community we studied, it is likely that the same strain of the virus is present in multiple species. This suggests a potential for interspecies transmission of the virus between different species of amphibian larvae in aquatic systems. A potential mechanism for such movement relates to movement of amphibians collected as bait for sport fishing. Although the introduction of this virus to areas where "naïve" or sensitive species exist could potentially cause extirpation of local amphibian species, there have been no sustained declines or declines on large geographical scales documented in ranid frogs or ambystomatid salamanders that have been attributed to ranavirus infections.

The mode of FV3/FV3-like virus transmission among species composing aquatic amphibian communities is likely to be more complex than a single species interaction (e.g., only a single species acting as a reservoir). Nevertheless, a simple model can be proposed to help explain virus distribution in an aquatic amphibian community. A proposed model (Fig. 2) is inspired by a model of viral dynamics developed by Day and Proulx (2004). In this model, we have made a number of assumptions: 1) the same strain of FV3 is present in all of the amphibian species; 2) this strain of FV3 can be transmitted between amphibian species; 3) vertical transmission of FV3 can occur, at least in one species of amphibians; 4) horizontal transmission of the virus is likely in all cases; 5) there are no external stressors such as desiccation, density, or human-induced transmission of the virus between ponds (but see Harp and Petranka, 2006); 6) amphibian species vary in their susceptibility to the virus; 7) FV3 transmission is not limited to density-dependent factors; 8) carriers of the virus, showing no signs of infection, are present in all infected amphibian species; 9) caudates are a likely reservoir of the virus in amphibian communities; and 10) the virus can be spread between individuals by scavenging of dead and/or moribund infected animals (Harp and Petranka, 2006). None of our observations contradict these assumptions (Fig. 2).

In each population of each amphibian species in a pond infected by the virus, there may be a susceptible group of individuals, an infected group of individuals, and a resistant group of individuals. Because of interspecies transmission of FV3/FV3-like viruses, it is possible to combine the susceptible groups of all species into one larger, multispecies group, all infected individuals into a second similar group, and all resistant individuals into a third group. Individuals that carry the virus and can transmit the virus to susceptible individuals, regardless of
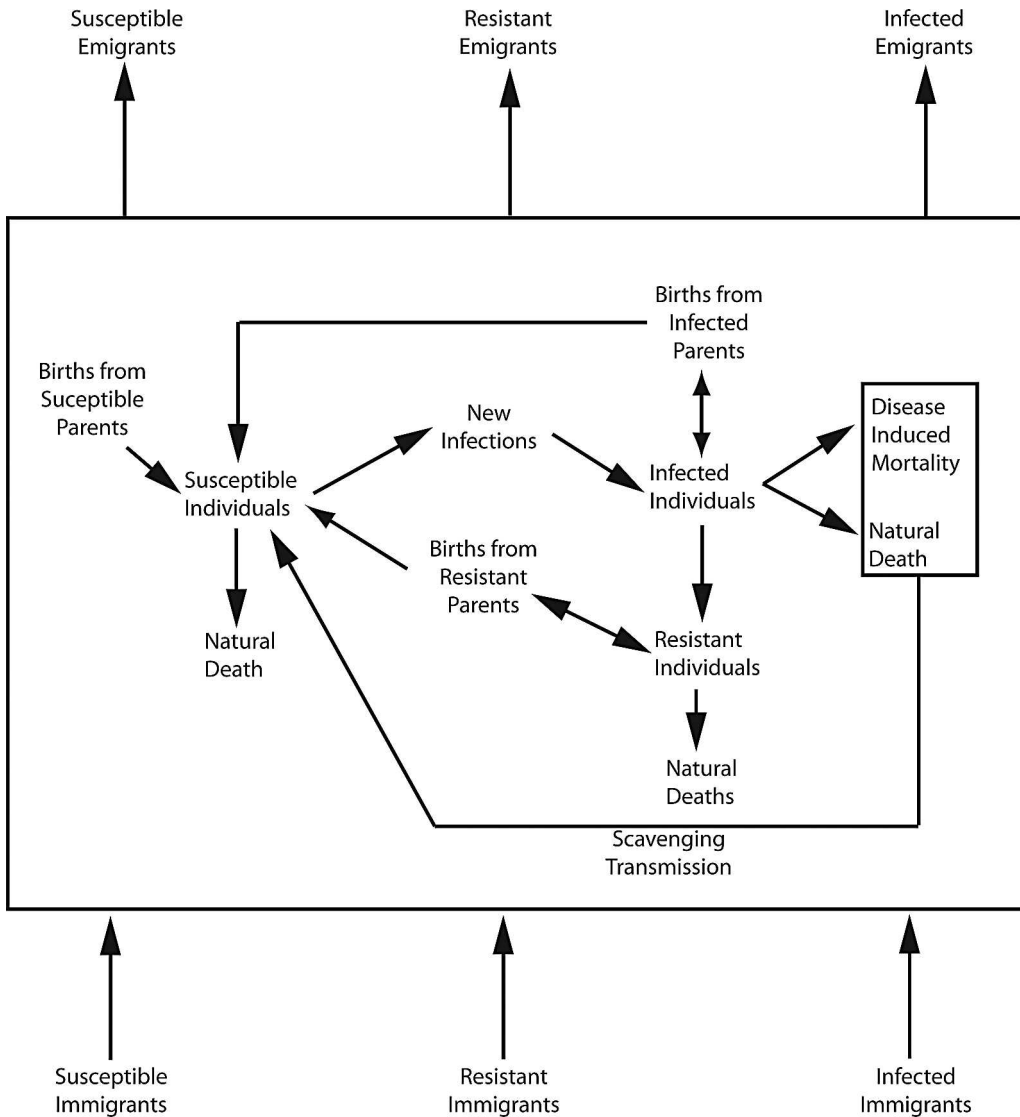
FIGURE 2. A generalized model for the transmission of FV3 in aquatic amphibian communities. The large box represents the boundary of the aquatic amphibian community and arrows indicate the direction in which the infection is traveling. Within the model, natural deaths and disease-induced mortality of infected individuals are boxed together because scavenging from either of these groups may result in the transmission of the virus.

whether they exhibit signs, are considered to be a part of the pool of infected individuals. In a natural population, the situation is made more complex because of births and deaths. Deaths may be due to viral infections (disease-induced mortality) or may result from predation and other natural causes such as natural developmental abnormalities. It is not known whether vertical transmission can occur in all amphibian species that are affected by FV3/FV3-like viruses. Where vertical transmission does occur, if an infected individual mates with a susceptible individual, it is likely that at least some of the offspring will be infected. If a resistant individual mates with a susceptible individual, the result depends on the mech-

anism of resistance. If the resistance has been acquired through exposure to the virus, the offspring will be susceptible. However, if the resistance is innate, it is likely that at least some of the offspring will be resistant to the virus. A similar situation occurs when a resistant individual and an infected individual mate. If the resistance is acquired through exposure, then it is likely that some or all of the offspring will be infected. If the resistance is genetic, some, if not all, of the offspring may be resistant to the virus; those offspring not resistant to the virus may be infected with the virus through the infected parent, or they may be susceptible to later viral infection. Because communities are not closed to immigrants and emigrants, immigration and emigration of individuals from infected, susceptible, and resistant groups must be included.

There are still large gaps in our knowledge about the FV3/FV3-like virus that is present in Ontario, requiring further investigation into the strain of the virus present, its distribution and means of spread, and the mode and extent of transmission both within and between amphibian species. More research is needed into the potential of vertical transmission of FV3-like viruses, including where the virus is present on or in developing eggs. Because individuals of all infected species may carry the virus but not exhibit external signs of infection, subsequent analyses should use real-time PCR to assess viral load, and test the prediction that individuals exhibiting clinical signs of infection are the only ones infected by the virus.

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