Historical Evidence of Widespread Chytrid Infection in North American Amphibian Populations

MARTIN OUELLET,*†† IGOR MIKAELIAN,† BRUCE D. PAULI,‡ JEAN RODRIGUE,§ AND DAVID M. GREEN*

*Redpath Museum, McGill University, 859 Sherbrooke Street West, Montréal, Québec H3A 2K6, Canada
†IDEXX Veterinary Services, 2825 Kovr Drive, West Sacramento, California 95605, U.S.A.
‡Canadian Wildlife Service, National Wildlife Research Centre, Carleton University, Raven Road, Ottawa, Ontario K1A 0H3, Canada
§Service canadien de la faune, Environnement Canada, 1141 Route de l'Église, Sainte-Foy, Québec G1V 4H5, Canada

Abstract: *Emerging infectious diseases may be contributing to the global decline of amphibian populations. In particular, chytridiomycosis, a cutaneous fungal infection of amphibians caused by the chytrid* Batrachochytrium dendrobatidis*, gained attention in the 1990s as an apparently new pathogen. This fungus has been implicated as the causative agent of widespread mortalities among wild amphibians in geographically disparate parts of the world. To investigate the global distribution, historical occurrence, and infectiousness of this pathogen, we examined 3371 postmetamorphic and adult amphibians collected between 1895 and 2001 from 25 countries for the presence of chytrid fungi in the epidermis. Cutaneous chytrid infection was diagnosed in 383 of 2931 (13.1%) specimens of 12 common amphibian species from five Canadian provinces and seven American states, including 30 of 69 locations examined in the St. Lawrence River Valley of Québec. Chytrids were observed in 7.0% (46/655) of the adults collected in the 1960s, the earliest cases being two green frogs (*Rana clamitans*) collected in 1961 from Saint-Pierre-de-Wakefield, Québec. In recent studies, morbidity and mortality attributable to chytridiomycosis were not observed in infected amphibians from Québec despite a 17.8% (302/1698) prevalence of chytrid infection during the period 1990–2001. The prevalence of infection during this latter period was not statistically different when compared with the period 1960–1969. Epidermal chytrid infections were not observed in 440 amphibians examined from 23 other countries. In light of the fact that infection by* B. dendrobatidis *is widely distributed and apparently enzootic in seemingly healthy amphibians from eastern North America, lethal outbreaks of chytridiomycosis among amphibian populations appear to have complex causes and may be the result of underlying predisposing factors.*

Key Words: amphibian decline, amphibian disease, *Batrachochytrium dendrobatidis*, chytridiomycosis, emerging infectious disease, museum specimens

Evidencia Histórica de Quitridiomicosis Generalizada en Poblaciones de Anfibios de Norte América

Resumen: *Las enfermedades infecciosas emergentes pueden estar contribuyendo a la declinación global de poblaciones de anfibios. En particular, la quitridiomicosis, una infección fungosa cutánea en anfibios provocada por el quitridio* Batrachochytrium dendrobatidis*, atrajo la atención en la década de 1990 como un patógeno aparentemente nuevo. Este hongo ha sido implicado como el agente causal de extensas mortalidades en anfibios silvestres en sitios geográficamente dispares en el mundo. Para investigar la distribución global, la ocurrencia histórica y el nivel infeccioso de este patógeno, examinamos a 3371 anfibios postmetamórficos y adultos, recolectados entre 1895 y 2001 en 25 países, para detectar la presencia de hongos quitridios en la epidermis. Diagnosticamos quitridiomicosis cutánea en 383 de 2931 (13.1%) de especimenes de especies comunes de anfibios de cinco provincias Canadienses y siete estados de E. U. A., incluyendo 30 de 69 localidades*

††*email mouellet9@sympatico.ca*
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*examinadas en el Valle del Río San Lorenzo de Québec. Observamos quitridios en 7.0% (46/655) de los adultos recolectados en la década de 1960, los primeros casos correspondieron a dos ranas (*Rana clamitans*) recolectadas en 1961 en Saint-Pierre-de-Wakefield, Québec. En estudios recientes no se observó morbilidad y mortalidad atribuibles a la quitridiomicosis en anfibios infectados de Québec no obstante una prevalencia de 17.8% (302/1698) de quitridiomicosis durante el período 1990–2001. La prevalencia durante este período no fue estadísticamente diferente al compararla con el período 1960–1969. No se observaron quitridiomicosis epidérmicas en 440 anfibios provenientes de otros 23 países. A la luz del hecho de que la infección por* B. dendrobatidis *está ampliamente distribuida y que al parecer es enzoótica en poblaciones de anfibios del este de Norte América aparentemente sanas, las epidemias letales de quitridiomicosis en poblaciones de anfibios parecen tener causas complejas y pueden ser el resultado de factores de predisposición subyacentes.*

Palabras Clave: *Batrachochytrium dendrobatidis*, declinación de anfibios, enfermedad de anfibios, enfermedad infecciosa emergente, especimenes de museo, quitridiomicosis

Introduction

An infectious skin disease of amphibians and its associated pathology, chytridiomycosis, have been implicated as the proximate cause of the demise of wild amphibian populations in several parts of the world, including Central (Berger et al. 1998; Lips 1999; Lips et al. 2003), South (Bonaccorso et al. 2003; Ron et al. 2003), and North America (e.g., Bradley et al. 2002; Green et al. 2002; Davidson et al. 2003; Muths et al. 2003); Europe (Bosch et al. 2001); Africa (Hopkins & Channing 2003; Lane et al. 2003); and Australasia (Berger et al. 1998; Waldman et al. 2001). The infection has also been reported in captive amphibians in zoo and academic research collections and in commercial collections in aquaria and farms (Groff et al. 1991; Pessier et al. 1999; Mutschmann et al. 2000; Raverty & Reynolds 2001; Parker et al. 2002). Some amphibian populations have declined dramatically over the past few decades (Houlahan et al. 2000; Alford et al. 2001) and some species have become extinct. Habitat destruction and alteration (Dodd & Smith 2003), commercial exploitation (Niekisch 1986), introduced invasive species (Knapp & Matthews 2000), climate change (Pounds et al. 1999), acid precipitation (Beebee et al. 1990), increased ultraviolet B radiation (Blaustein et al. 1998), environmental contaminants (Sparling et al. 2001), and developmental abnormalities (Ouellet 2000) have all been proposed or demonstrated to account for amphibian population declines, but infectious diseases, particularly chytridiomycosis, may have a leading role in these declines in certain areas (Berger et al. 1998; Williams et al. 2002; Carey et al. 2003).

Chytridiomycosis is caused by a nonhyphal zoosporic chytrid fungus, *Batrachochytrium dendrobatidis* (phylum Chytridiomycota, class Chytridiomycetes, order Chytridiales), that infects keratinized cells of the amphibian epidermis (Longcore et al. 1999). Infections occur in both larval and postmetamorphic amphibians. Gross lesions are subtle and usually not apparent although areas of abnormal epidermal sloughing are sometimes reported in clinical examinations of dead or dying postmetamorphic animals. Histologically, irregular cell loss, erosions, and segments of markedly thickened stratum corneum containing chytrid thalli or zoosporangia are diagnostic (Carey et al. 2003). Few details are known about the physiology and life cycle of *B. dendrobatidis*, its survival in the wild, or factors that precipitate amphibian casualties (Piotrowski et al. 2004). Zoospores infect only keratinocytes and there is minimal inflammatory response in the skin. No consistent histologic findings other than skin lesions have been observed. Zoospores survive for up to 7 weeks in water in the absence of an amphibian host (Johnson & Speare 2003). Experiments seeking to fulfill Koch's postulates in dendrobatid frogs have been described by Nichols et al. (2001). The mechanism by which cutaneous chytridiomycosis becomes a fatal infection, however, is still unknown. Infection in tadpoles is restricted to the keratinized mouthparts (Fellers et al. 2001; Vredenburg & Summers 2001; but see Rachowicz 2002) and is not associated with illness or mortality.

B. dendrobatidis is hypothesized to be moving between countries via international trade (Daszak et al. 2003; Mazzoni et al. 2003; Pasmans et al. 2004) and spreading between regions as an epidemic wave (Laurance et al. 1996, 1997). The occurrence of the fungus in amphibians from widely dispersed areas around the world indicates an astonishingly rapid epidemic unless the organism has long been enzootic and previously unrecognized. Consequently, we searched for evidence of chytrid infections in preserved museum specimens and wild amphibians collected from Canada and many other countries around the world. We report that infections by a chytrid identifiable as *B. dendrobatidis* have been occurring with some regularity in apparently healthy amphibian populations from the St. Lawrence River Valley of Québec, Canada. We also present the distribution of chytrids in amphibians for several other Canadian provinces and American states and document that the infection has been occurring in North America since the 1960s.

Methods

Amphibian Collection

We investigated 3371 amphibians from 36 anuran and 7 salamander species for the presence of *B. dendrobatidis* (Table 1). All amphibians examined were postmetamorphic, wild-caught animals. Formalin-fixed, ethanol-stored specimens from the collections of the Natural Heritage Building of the Canadian Museum of Nature ($n = 1470$), Gatineau, Québec, and the Redpath Museum of McGill University ($n = 46$), Montréal, Québec, that were opportunistically collected between 1895 and 1990 were analyzed. We examined 1698 additional specimens captured alive in Québec between 1990 and 2001. We also evaluated tissue samples ($n = 157$) gathered in 1998 and 1999 from the Republic of Panama (Gray et al. 2002). The animals we examined were from the Mont Saint-Hilaire Biosphere Reserve ($n = 797$) and other locations in Québec ($n = 1201$), 10 other Canadian provinces or territories ($n = 623$), 21 American states ($n = 310$), and 23 other countries ($n = 440$). Geographic information of all locations examined is available from the authors upon request. All the live amphibians from Québec were examined immediately after capture for evidence of disease or physical abnormalities (as described in Ouellet et al. 1997). Except for 12 mudpuppies (*Necturus maculosus*) collected in the winter of 1998 (Marcogliese et al. 2000), no animals were sampled between November and February in this area. To our knowledge, none of the preserved animals we sampled from museum collections originated from areas with known historical die-offs or population declines.

Histological and Ultrastructural Examination

For all specimens, we clipped the fourth toe from the right hindlimb at the articulation proximal to the fourth phalange and stored it in 10% neutral buffered formalin for a minimum of 5 days before processing for histopathology. Paraffin-embedded toes were sectioned longitudinally at 4 μm and stained with haematoxylin-phloxine-saffron for examination under a light microscope. We diagnosed infection by *B. dendrobatidis* if chytrid thalli or zoosporangia were observed in the stratum corneum of the epidermis.

We confirmed identification of *B. dendrobatidis* by evaluating the ultrastructural features of thalli and/or zoospores (Longcore et al. 1999) in 10 infected specimens with transmission electron microscopy. Tissue samples were fixed in 2% glutaraldehyde fixative, stored in 0.085 M sodium cacodylate (pH 7.2), and postfixed in 1% osmium tetroxide buffered with 0.1 Zetterqvist buffer (pH 7.2). Ultrathin sections were stained with uranyl acetate followed by lead citrate. We also sent three asymptomatic American bullfrogs (*Rana catesbeiana*) collected in May 1999 from the Mont Saint-Hilaire Biosphere Reserve to J. E. Longcore to confirm the identity of *B. dendrobatidis* with multilocus sequence typing (Morehouse et al. 2003).

Data Analysis

We produced maps showing our sample locations and our data on current and historical chytrid distributions with a geographic information system, ArcView GIS (version 3.2, Environmental Systems Research Institute, Redlands, California). We used Pearson chi-square analyses (Zar 1999) to test for differences in infection occurrence between the 1960–1969 and 1990–2001 sampling periods for all amphibians investigated in Québec and between months for amphibians collected from the Mont Saint-Hilaire Biosphere Reserve and at all other Québec locations during the period 1960–2001. Statistical analyses were performed with SYSTAT (version 10, SPSS, Chicago).

Results

Cutaneous infection by chytrids occurred in 383 of 2931 (13.1%) amphibians from 12 common species collected throughout Canada and the United States (Table 1). All 440 specimens examined from 23 countries other than Canada and the United States tested negative for chytrid infection. In infected animals we observed typical *B. dendrobatidis* thalli or zoosporangia in the stratum corneum of the epidermis (Fig. 1a). The identification was confirmed by ultrastructural evaluation of thalli and/or zoospores (Fig. 1b) and by multilocus sequence typing (Morehouse et al. 2003). Thalli and developing, mature, and empty zoosporangia of *B. dendrobatidis* were restricted to the stratum corneum of the epidermis. Discharge papillae were present in some zoosporangia. Chytrid infection generally involved $< 1\%$ of the surface of the skin of the digit, with always < 30 organisms per microscopic field in the most affected portions of the epidermis (at 400X magnification). Histologic changes associated with chytrid infection were mild and included multifocal parakeratotic hyperkeratosis (thickening of the stratum corneum) and acanthosis (thickening of the stratum spinosum), occasionally associated with minimal spongiosis and granulocytic exocytosis through the epidermis.

In North America, *B. dendrobatidis* was recorded in wild-caught animals from five Canadian provinces (British Columbia, Ontario, Québec, New Brunswick, Nova Scotia) and seven American states (California, Wyoming, Minnesota, Wisconsin, Missouri, Indiana, Virginia) (Fig. 2). Chytrid infection occurred at 30 of 69 locations examined in the St. Lawrence River Valley of Québec (Fig. 3), with infection observed in nine anuran and two salamander species (Table 2). A prevalence of 17.8% (302/1698) was encountered in Québec from 1990 to 2001, despite the

Table 1. Summary information of the 3371 amphibians examined for *Batrachochytrium dendrobatidis* infection from 25 different countries.

Taxon	*Country*	*Sampling period*	*Prevalence**
Ambystoma laterale	Canada	1999–2000	0/120
Ambystoma maculatum	Canada	1998–2000	4/139
Bufo americanus	Canada, United States	1959–2000	4/156
Bufo boreas	Canada	1961–1977	0/24
Bufo cognatus	United States	1965–1969	0/27
Bufo danatensis	Kirghizia	1990	0/18
Bufo marinus	Barbados, Colombia, Federative Republic of Brazil, Fiji, Jamaica, Papua New Guinea, Puerto Rico, Trinidad & Tobago	1966–1973	0/86
Bufo punctatus	Mexico	1973	0/12
Bufo regularis	Kenya	1972	0/10
Bufo valliceps	United States	1961	0/15
Colostethus talamancae	Republic of Panama	1999	0/1
Dendrobates auratus	Republic of Panama	1998–1999	0/47
Desmognathus fuscus	Canada	1959	0/1
Eurycea bislineata	Canada	1999	0/2
Hyla versicolor	Canada	1964–2000	1/16
Leiopelma archeyi	New Zealand	1987	0/3
Leiopelma hamiltoni	New Zealand	1987	0/3
Leiopelma hochstetteri	New Zealand	1987–1989	0/11
Leptodactylus chaquensis	Argentine Republic	1987	0/4
Necturus maculosus	Canada	1998	0/12
Notophthalmus viridescens	Canada	1962–1999	24/217
Physalaemus pustulosus	Republic of Panama	1999	0/109
Plethodon cinereus	Canada	1964–1999	0/35
Pseudacris crucifer	Canada	1996–2000	0/9
Pseudacris triseriata	Canada	1993–2001	54/143
Rana amurensis	Russian Federation	1986	0/26
Rana boylii	United States	1975	8/20
Rana brevipoda	Japan	1988	0/14
Rana camerani	Armenia	1989	0/15
Rana catesbeiana	Canada, United States	1954–1999	81/295
Rana clamitans	Canada, United States	1900–1999	142/718
Rana esculenta complex	Latvia	1989–1990	0/5
Rana japonica	Japan, People's Republic of China	1984–1988	0/14
Rana occipitalis	Federal Republic of Nigeria	1971	0/5
Rana palustris	Canada	1917–2000	3/120
Rana pipiens	Canada, United States	1895–2000	40/528
Rana pleuraden	People's Republic of China	1987	0/7
Rana ridibunda	Armenia, Iran, Latvia	1962–1990	0/19
Rana rugosa	Japan	1988	0/6
Rana septentrionalis	Canada	1997–1999	10/121
Rana sphenocephala	United States	1988	0/30
Rana sylvatica	Canada, United States	1921–1998	12/183
Rana temporaria	Czech Federative Republic, Russian Federation, United Kingdom	1984–1990	0/25
Total			383/3371

**Number of individuals with chytrid infection/number of individuals examined.*

fact that no morbidity or mortality attributable to chytridiomycosis was observed in these populations (Table 3).

Infection in museum specimens from Canada and the United States was found in 7.0% (46/655) of the amphibians collected in the 1960s and 8.2% (20/245) of the specimens collected in the 1970s. The earliest cases of chytrid infection we found in North American museum specimens were in two green frogs (*R. clamitans*) collected in July 1961 from Saint-Pierre-de-Wakefield, Québec (45°42′N, 75°43′W). The epidermis in some specimens collected from 1895 to the late 1950s, however, was sometimes partly eroded because of prolonged storage in fixative solutions. Therefore, all old cases where upper layers of the epidermis had sloughed away were not included in this study. Because of this, it is difficult to say with certainty that chytrid infection was completely absent from these older samples, although we did not observe it in specimens collected before 1961.

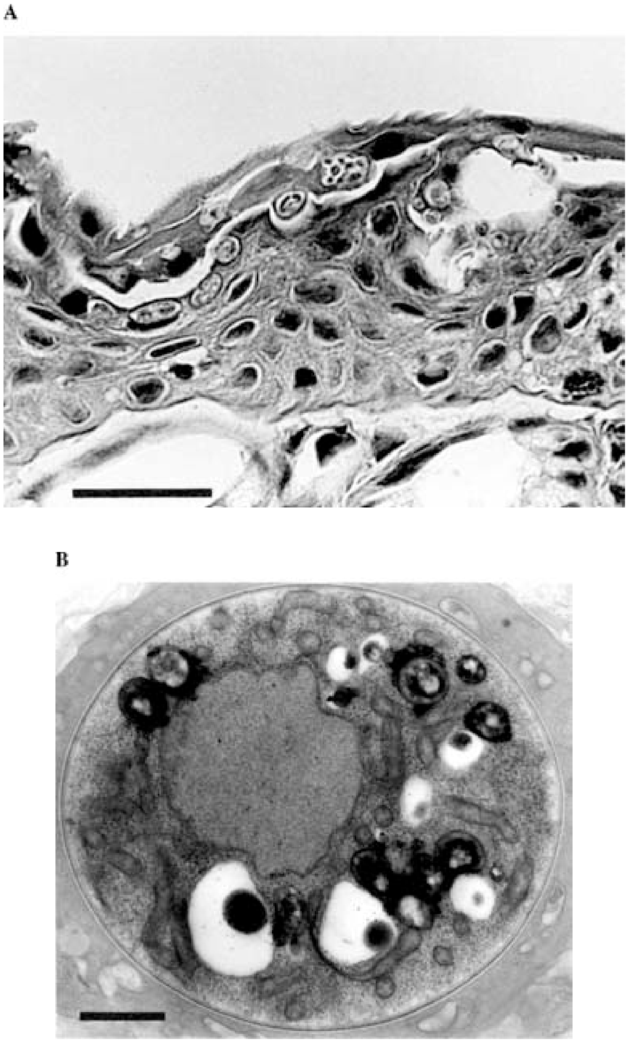

Figure 1. Batrachochytrium dendrobatidis *infection. (a) Histological section of the epidermis of a toe of a green frog (*Rana clamitans*) collected from the Mont Saint-Hilaire Biosphere Reserve, Québec, in 1999 (bar = 50 μm). The stratum corneum contains fungal thalli and zoosporangia. (b) Transmission electron micrograph of a thallus in a toe cross section of another naturally infected* R. clamitans *from the same location (bar = 1 μm).*

Infection occurrence did not differ significantly between the 1960–1969 and 1990–2001 sampling periods for all amphibians surveyed in Québec ($n = 1915$, $\chi^2 = 2.63$, df $= 1$, $p = 0.10$). There was variation, however, in the prevalence of chytrid infection depending on the month the amphibians were collected at both the Mont Saint-Hilaire Biosphere Reserve and all other locations in Québec for the period 1960–2001. Infection appeared to be more prevalent during the spring and fall months of April, May, June, and October than during the summer months of July, August, and September (Fig. 4). This temporal pattern was significant at each location (Mont Saint-Hilaire: $n = 796$, $\chi^2 = 50.73$, df $= 2$, $p < 0.0001$; Québec: $n = 1152$, $\chi^2 = 71.27$, df $= 2$, $p < 0.0001$). Twelve *N. maculosus* (not included in the analysis) sampled in the winter tested negative for *B. dendrobatidis*.

Discussion

Unexpectedly high prevalences of chytrid infection were observed in North American anuran and salamander populations. Even so, these detected levels of infection should be viewed as very conservative estimates because we assessed only toe-clips (one per animal) collected during field research from apparently healthy amphibians or removed postmortem from preserved museum specimens. The ventral abdominal skin (especially the pelvic patch), the hindlimbs, and the feet are the most consistently infected by *B. dendrobatidis* (Berger et al. 1998; Pessier et al. 1999). Furthermore, histological examination may not be sensitive enough to detect light chytrid infections on healthy specimens because only a small surface area of the skin is examined after toe embedding and sectioning. This may help explain why we did not observe chytrid infection in samples collected in countries where others have found chytridiomycosis (e.g., Republic of Panama, Berger et al. 1998). Newly developed diagnostic tests such as the use of Congo Red dye (Briggs & Burgin 2003) or immunohistochemical staining (Berger et al. 2002; Van Ells et al. 2003) might prove useful ancillary diagnostic aids. In addition, recent advances in molecular technologies are aiding the development of diagnostic tests with a high degree of sensitivity and specificity (Boyle et al. 2004).

The prevalence of chytrid infections was highest during spring and fall months in wild amphibians examined in Québec. The lower prevalence observed in the summer months might tentatively be explained by the fact that the amphibian immune system functions most effectively at warmer temperatures (Cooper et al. 1992; Maniero & Carey 1997; Carey 2000). Consistent with this hypothesis, housing captive amphibians at a high environmental temperature has been shown to eliminate the fungal pathogen (Woodhams et al. 2003) and, in laboratory broth cultures, *B. dendrobatidis* has been observed to grow best at cooler temperatures (Longcore et al. 1999). Our results are also in accordance with anecdotal observations that lethal chytrid infections in amphibians occur frequently at cold temperatures (Carey 2000). These observations suggest that variation in the immune status of amphibians in relation to temperature and the basic biology of *B. dendrobatidis* could increase the prevalence and the severity of infection during colder seasons. Consequently, amphibians may be most susceptible to chytrid infection during winter months, a time when most Canadian amphibians are difficult to find and study because they are hibernating. As a result, we are not sure whether

Figure 2. Distribution of Batrachochytrium dendrobatidis *infection in Canada and the United States. A total of 168 locations were investigated and cutaneous infection by chytrids was diagnosed in amphibians from five Canadian provinces and seven American states.*

mortalities of chytrid-infected amphibians occur in the winter. More research is required to understand the possible role of environmental correlates on chytrid epizootics.

We observed a widespread prevalence of chytrid infection in apparently healthy amphibians collected during recent research throughout the St. Lawrence River Valley of Québec. The reason these animals do not appear to be adversely affected by the fungus is unknown. The production of antimicrobial peptides in amphibian dermal granular glands might provide some natural resistance against *B. dendrobatidis* (Rollins-Smith et al. 2002). This innate defense capacity of amphibians would be a potential explanation of species-specific differences in susceptibility to skin infection. Although a consistent association between chytrid infection and epidermal lesions suggests these organisms should be considered parasitic rather than saprobic (i.e., an organism that lives by feeding on dead organic matter), the severity of infection in the wild-caught amphibians we studied was mild compared with that observed in captive frogs (genus *Dendrobates*) and in wild amphibians collected during mass mortality events in Australia and Central America (Berger et al. 1998; Pessier et al. 1999). In the latter cases, the stratum corneum showed marked hyperkeratosis and colonization by many *B. dendrobatidis*. This difference in infection severity may be the result of species-specific susceptibility to chytrid infection or factors such as adverse environmental conditions, coinfection by yet undetected agents, compromised immune function, or stress associated with captivity in the captive amphibians. It is unknown whether these cofactors may also be involved in the wasting syndrome associated with experimental transmission of chytrid infection in captive amphibians.

These factors may be absent in most declining populations of wild amphibians, at least in Québec, because mass mortality associated with chytrid infection has not been recorded over the past decade despite continuous monitoring of amphibian health in this area. In view of the low level of genetic variation among strains of *B. dendrobatidis* between our samples and others from North America, Central America, Africa, and Australia (Morehouse et al. 2003), chytridiomycosis does not appear to be a fatal disease for a large number of amphibian species in eastern North America.

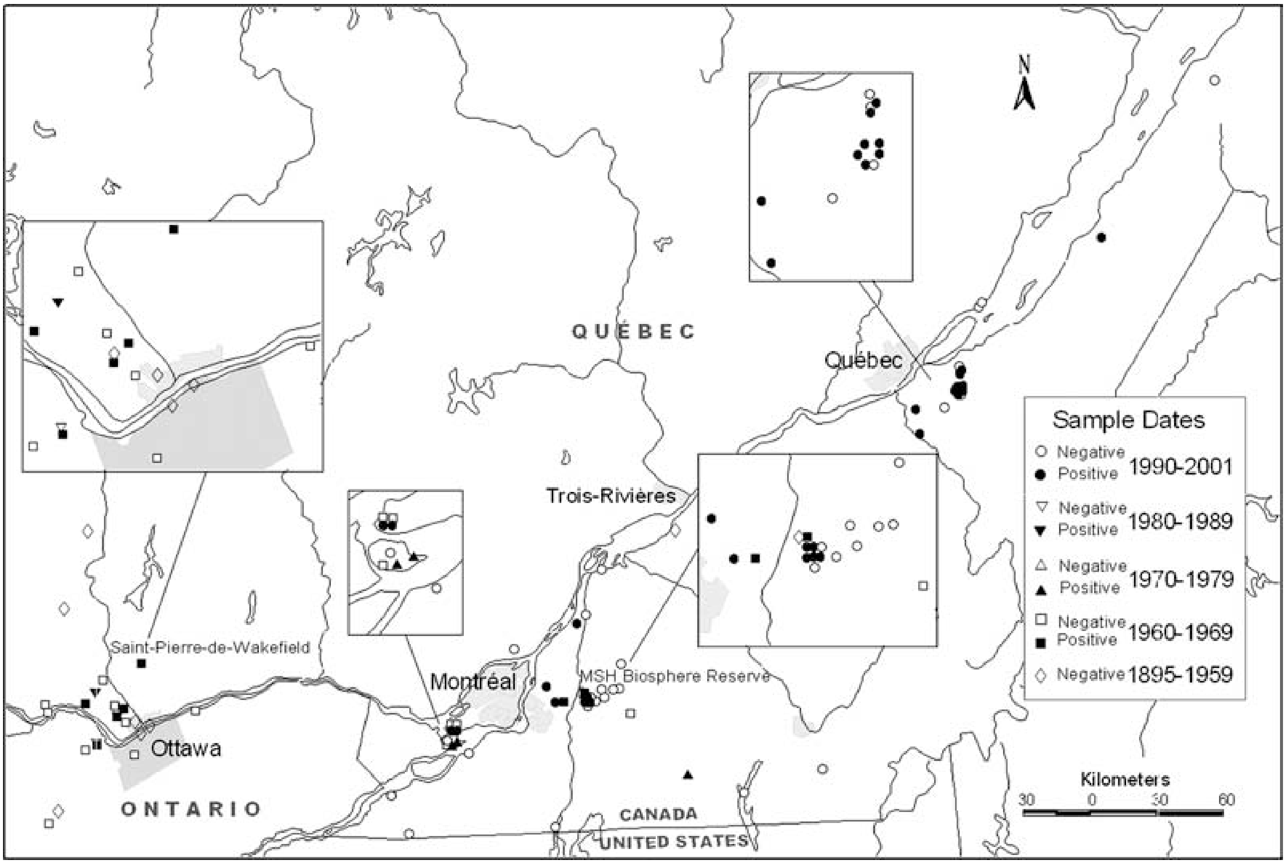

Figure 3. Distribution of Batrachochytrium dendrobatidis *infection in the St. Lawrence River Valley of Québec. Cutaneous infection by chytrids was diagnosed at 30 of the 69 locations investigated (MSH, Mont Saint-Hilaire). The earliest cases of chytrid infection were recorded in two green frogs (*Rana clamitans*) collected in 1961 from Saint-Pierre-de-Wakefield.*

Nevertheless, the presence of chytrid fungus infection in threatened populations of native amphibians is of great concern. One reason is that these populations may be less able to recover after population crashes. Caution is warranted, however, because chytrids may not necessarily be the proximate cause of population declines. Convincing demonstrations of causality are therefore required and suspicion must go beyond the simple observation of *B. dendrobatidis* from skin samples of declining amphibians. Retrospective discovery of chytrid thalli or zoosporangia in museum specimens from a previously unexplained amphibian decline likewise is not a demonstration of a direct causal relationship. Finally, the discovery of small numbers of sick, dying, or dead amphibians is not unusual in the field, whether or not chytrids are present in the skin. Although molecular methods have yielded evidence that this chytrid might be a recently emerged clone spreading through naïve populations (Morehouse et al. 2003), alternative hypotheses have not yet been addressed.

Our data suggest that naturally occurring chytrid infections in amphibians from eastern North America are expected to be widely distributed, and no single cause is likely to explain lethal outbreaks or population declines in the wild. For instance, the western chorus frog (*Pseudacris triseriata*) is now in serious decline in the Monteregian plain of Québec (Daigle 1997). This species is listed as vulnerable in Québec (Gazette Officielle du Québec 1999), yet morbidity and mortality associated with chytridiomycosis were not observed in this species despite a 37.8% prevalence of the infection in the Monteregian population. Land-use practices have undoubtedly contributed to the population decline (Société de la faune et des parcs du Québec 2002), and current habitat loss and fragmentation is still considered the most significant threat for this and other amphibian species in this area. Still, the enzootic nature of *B. dendrobatidis* in Québec calls for extreme prudence in any movements or translocations of amphibians and reinforces the importance of equipment disinfection between sites. Health monitoring of *P. triseriata* is advised but notoriously difficult because of its cryptic nature. In the United States, this species is widespread and chytridiomycosis has been reported at some sites (Carey et al. 2003; Rittmann et al. 2003).

Table 2. Prevalence of *Batrachochytrium dendrobatidis* infection in amphibians collected in Québec in 1960–2001 and comparison between the Mont Saint-Hilaire (MSH) Biosphere Reserve and all other locations sampled in Québec.*

Taxon	*MSH Biosphere Reserve*	*Québec*
Ambystoma laterale	0/120 (0)	–
Ambystoma maculatum	0/120 (0)	4/19 (21.1)
Bufo americanus	1/15 (6.7)	3/77 (3.9)
Eurycea bislineata	–	0/2 (0)
Hyla versicolor	1/12 (8.3)	0/4 (0)
Necturus maculosus	–	0/12 (0)
Notophthalmus viridescens	2/96 (2.1)	22/121 (18.2)
Plethodon cinereus	0/35 (0)	–
Pseudacris crucifer	0/5 (0)	0/4 (0)
Pseudacris triseriata	–	54/143 (37.8)
Rana catesbeiana	80/133 (60.2)	1/51 (2.0)
Rana clamitans	50/103 (48.5)	85/403 (21.1)
Rana palustris	3/99 (3.0)	–
Rana pipiens	1/5 (20.0)	20/191 (10.5)
Rana septentrionalis	–	10/121 (8.3)
Rana sylvatica	6/53 (11.3)	2/16 (12.5)
Total	144/796 (18.1)	201/1164 (17.3)

**Percentages are given in parentheses following the number infected/number examined.*

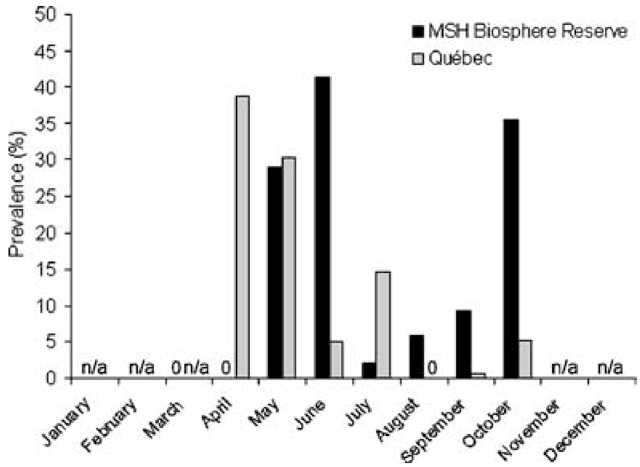

Figure 4. Seasonality of Batrachochytrium dendrobatidis *infection in the Mont Saint-Hilaire Biosphere Reserve and at all other Québec locations for a total of 1960 amphibians collected during 1960–2001 (MSH, Mont Saint-Hilaire; n/a, no account or insufficient data).*

Chytrid infection has been present among North American amphibian populations since at least the early 1960s. Our observations indicate that chytrid fungus infection is common and probably enzootic but previously unrecognized in wild amphibians. Furthermore, the prevalence of infection in Québec during the last decade is not statistically different when compared with the period 1960–1969. Because of the limitations of some of our older samples and our diagnostic techniques, the presence of chytrids in specimens collected before 1960 could be neither confirmed nor denied. As a result, it is difficult to determine how long North American amphibians have been infected with *B. dendrobatidis*. Although emerging infectious diseases such as chytridiomycosis have gained prominence as agents of the global decline of amphibians (Berger et al. 1999; Daszak et al. 2003), our evidence of the presence of *B. dendrobatidis* in museum specimens indicates that these diseases may not be new (also Carey et al. 1999; Mikaelian et al. 2000; Green & Kagarise Sherman 2001). It is likely that other amphibian diseases will be discovered, and our study emphasizes the value of museum collections for investigating the origin and historical range of newly recognized pathogens.

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Table 3. Prevalence of *Batrachochytrium dendrobatidis* infection in 3371 amphibians examined according to sampling period.[a]

Location	*1895–1959*	*1960–1969*	*1970–1979*	*1980–1989*	*1990–2001*
MSH Biosphere Reserve	0/1 (0)	1/30 (3.3)	–	–	143/766 (18.7)
Québec[b]	0/37 (0)	28/187 (15.0)	8/30 (26.7)	6/15 (40.0)	159/932 (17.1)
Canada[c]	0/136 (0)	16/316 (5.1)	2/126 (1.6)	0/45 (0)	–
United States	0/29 (0)	1/122 (0.8)	10/89 (11.2)	8/63 (12.7)	1/7 (14.3)
23 other countries[d]	–	0/43 (0)	0/74 (0)	0/127 (0)	0/196 (0)

[a]*Percentages are given in parentheses following the number infected/number examined; MSH is Mont Saint-Hilaire.*
[b]*Without the MSH Biosphere Reserve.*
[c]*Without the province of Québec.*
[d]*Listed in Table 1.*

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