Chytridiomycosis: a global threat to amphibians

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Summary

Chytridiomycosis, which is caused by *Batrachochytrium dendrobatidis*, is an emerging infectious disease of amphibians. The disease is one of the main causes of the global decline in amphibians. The aetiological agent is ubiquitous, with worldwide distribution, and affects a large number of amphibian species in several biomes. In the last decade, scientific research has substantially increased knowledge of the aetiological agent and the associated infection. However, important epidemiological aspects of the environment-mediated interactions between the aetiological agent and the host are not yet clear. The objective of the present review is to describe chytridiomycosis with regard to the major features of the aetiological agent, the host and the environment.

Keywords

Aetiological agent – Amphibian – *Batrachochytrium dendrobatidis* – Chytridiomycosis – Environment – Epidemiology – Host.

Introduction

Concern about the decline of amphibian populations in several biomes around the world is not recent. Habitat degradation and loss, the presence of environmental pollutants, drought and overexploitation, among other factors, have been the main causes associated with amphibian decline. Currently, amphibians are considered to be more threatened than birds and mammals (1).

In recent decades, the emergence of infectious diseases has been related to episodes of mass mortality of amphibians worldwide. In particular, chytridiomycosis, a cutaneous infection of amphibians which is water transmissible and caused by *Batrachochytrium dendrobatidis*, is currently regarded as a major cause of the global decline in amphibians.

Amphibian population decreases and extinctions attributed to chytridiomycosis have been more severe at high altitudes (1, 2), especially in the Neotropics and Australia (1, 3, 4), where dramatic losses in species diversity and abundance have been reported. The global dispersion of *B. dendrobatidis* has been attributed to international animal trade routes (5, 6, 7).

Despite the large amount of information available on the microbiology of the aetiological agent and on some host species, little is known about the epidemiology of the disease. Thus, epidemiological research is urgent, both in the field of descriptive epidemiology (with reference to the time–place–host relationship) and within the framework of analytical epidemiology, according to the determinants of health and the disease process (the aetiological agent–host–environment relationship).

The aetiological agent

Batrachochytrium dendrobatidis is the first chytrid fungus (*Phylum Chytridiomycota*) to have been recognised as a parasite of vertebrates. It is the aetiological agent of chytridiomycosis, an emerging infectious disease (8, 9) of amphibians, which has been related to episodes of mass mortality in several amphibian species worldwide (10, 11).

At present, the two original hypotheses about the emergence of the pathogen and its uneven distribution in amphibian populations in different biomes remain valid (9, 12, 13, 14). The first hypothesis – the novel pathogen hypothesis – suggests that it is a new aetiological agent, recently emerged and tending to undergo a rapid and recent geographical expansion (14).

The second hypothesis – the endemic pathogen hypothesis – assumes that the aetiological agent has coexisted endemically with amphibians in nature, as a commensal or symbiont (14). Environmental disturbances are thought to have triggered some alterations in the agent–host relationship, causing changes in the pathogenicity and virulence of the agent and in the ability of the host to raise an immunological response or to adapt to the new challenge. This hypothesis is mainly supported by evidence that the infection was widely distributed in amphibian populations from different biomes before the reports of mass mortality episodes (15, 16).

Characteristics of the aetiological agent related to the infection and disease

Infectivity

The infection caused by *B. dendrobatidis* is limited to the skin; it has not been demonstrated that internal organs are involved (17, 18, 19). The disease develops in an isolated or colonial form, especially on the ventral abdomen (13, 18, 20). The infecting form is the zoospore, the primary means of dispersion of the fungus (19), and in experimental infection the infecting dose can be extremely low (21).

Pathogenicity and virulence

The infectivity, pathogenicity and virulence of the fungus have been examined by the application of Koch's postulates (13, 19, 22). *Batrachochytrium dendrobatidis* invades only the surface skin layers of the host, mainly on the ventral part of the abdomen, the hindlimbs (thigh and groin), feet and toes, and rarely on the dorsal surface (12, 13, 18, 19, 23).

The injuries produced by the fungal parasite seriously impair skin functions such as water absorption, osmotic regulation and respiration (22, 24). When it affects larvae, the infection is limited to the external oral region, the only region provided with a keratinised epithelium, and the lesions are relatively mild (25).

Mortality can be attributed to the breakdown of the cutaneous homeostatic function, the action of mycotoxins, or a combination of both mechanisms (24, 26, 27). In infected larvae death may occur during larval development or, most frequently, after metamorphosis, when the amphibian skin becomes keratinised and the infection of the epidermis becomes generalised (28, 29).

The virulence varies with the amphibian host species (8) and the strain of *B. dendrobatidis* (20, 30, 31).

Immunogenicity

Although a large number of species are considered to be susceptible to the infection, not all manifest the disease, and not all infections result in the eventual death of the host (12, 30, 32, 33, 34). Recent studies provide some evidence supporting the role of acquired immunity against chytridiomycosis (32, 35). Several studies have indicated that amphibians have innate immune defences, although with significant inter- and intra-species differences (34, 36, 37, 38, 39, 40). However, those defences are not always efficient enough to protect the host (37, 40, 41, 42).

Some *in vitro* studies have demonstrated the first line of defence against the infection to be the inhibitory action of antimicrobial peptides, secreted by the skin of amphibians, on the growth of the fungus (37, 39, 43).

However, the magnitude of this immune response under natural conditions, and what proportion of the amphibian population produces an immune response, are still unclear. Some species showing an immune response to *B. dendrobatidis* under experimental conditions (occasionally with high efficiency) have suffered mass mortality episodes under natural conditions, resulting in significant population decline (44).

Antigenicity

Specific studies have demonstrated that the activation of innate immunity is necessary for the induction of acquired immunity (35). *Batrachochytrium dendrobatidis* is capable of stimulating an immediate immune response and probably induces the formation of antibodies, according to recent but not conclusive studies (35).

Mussmann *et al*. (45) performed a study on the genome of *Xenopus laevis* and identified an immunoglobulin, IgX, which could be considered analogous to the mammalian secretory immunoglobulin IgA. Detection of IgX on the skin and determination of its function open a fresh research perspective (35).

Variability

It seems that the species *B. dendrobatidis* consists of multiple and deeply divergent lineages, although inter-lineage hybridisation is possible (46, 47). Thus, it is possible that contact between allopatric populations, through the international trade of amphibians, has allowed heterologous recombination, resulting in the emergence of more virulent lineages, with rapid and catastrophic worldwide dispersion (46, 47, 48).

Viability

Indirect evidence points to the ability of *B. dendrobatidi*s to survive as a saprophyte in water under environmental conditions (9), which could explain its persistence in the environment as a viable fungus in the absence or after the death of the main host (13, 49). Under experimental conditions, *B. dendrobatidis* can remain viable in water, wet soil or non-amphibian sources of keratin (50, 51). Also experimentally, zoospores can remain mobile in water for up to 24 h after their release, after which they rapidly form a cyst or die (52).

Batrachochytrium dendrobatidis therefore appears to remain viable in the environment in the absence of a host, as a free-living organism (27), through a still unknown strategy of survival. Awareness of the ability of the fungus to persist in the environment in the absence of a host is crucial in understanding the epidemiology of the infection (53).

The host

The preferred hosts of *B. dendrobatidis* are post-metamorphosis amphibians, although larvae are susceptible to the infection and can even suffer from the disease. A wide range of amphibian species are under threat or have become extinct as a consequence of chytridiomycosis (12, 54, 55, 56, 57, 58).

However, epidemiological research on this disease is difficult, owing to the characteristics of the host taxon. The large variety of species, with ontogeny through metamorphosis and varying degrees of susceptibility to infection, are major obstacles to understanding the epidemiological cycle. These characteristics hinder the primary objective of epidemiological study, which is to determine the morbidity and mortality rates associated with the disease without employing estimated data.

Host-related characteristics and susceptibility

Species

Batrachochytrium dendrobatidis infection is common in at least two out of three orders of the class Amphibia (10). The infection affects a large number of amphibian species worldwide and several of these species have experienced rapid population declines (1, 10, 11, 59).

The infection is water transmissible, and diffusion of *B. dendrobatidis* into the aquatic environment is rapid. Therefore, species with aquatic activity at some stage of their life cycle are more likely to be affected than terrestrial species (60, 61). However, strictly terrestrial species of non-aquatic ecosystems are also at risk of infection, although the extent of infection in these species remains unknown (60, 61).

Some species are highly susceptible to the disease and show episodes of high mortality with catastrophic consequences for their conservation status, often suffering local extinction (8, 12, 55, 56, 59, 62). Other species show moderate susceptibility, experiencing an intense population decline once infected, but without suffering a complete loss of individuals (59, 62, 63). A few species endure the infection, showing no overt clinical signs and eliminating the infection successfully, with their conservation status remaining apparently unchanged (59, 62, 63, 64).

Some host-related factors may interact with the characteristics of the fungus to increase the risk of illness, e.g. high-altitude living, large host population availability, low geographical dispersion, low fecundity, aquatic reproduction and life cycle, and high habitat specialisation (8, 9, 56, 65, 66). Characteristics of the fungus that increase the risk of infection in the host population include: preference for low temperatures, low species-specificity, high virulence, potential survival ability in the absence of the host, and the ability to infect larvae that could act as reservoir (8, 59).

Sex

There is no evidence of differences in host susceptibility related to sex. However, the behaviour of males and females, especially during the reproductive period, may lead to greater exposure for one of the sexes to *B. dendrobatidis*, without implying a difference in the sex-associated susceptibility (67, 68, 69).

Age

There is no epidemiological evidence demonstrating age differences in susceptibility. However, recently metamorphosed and juvenile individuals seem to be more vulnerable to the disease (63, 70).

Larval stage

Larvae usually show a relatively mild infection, with few pathological alterations. Given this, larvae may act as secondary hosts of the infection and as an epidemiological reservoir, enabling the maintenance of the infection and increasing the activity of the fungus in the environment (9, 12, 29, 71). Like post-metamorphosed individuals, larvae also show differentiated intra- and inter-specific susceptibility (29, 71).

Nevertheless, in some species, larval infections are very virulent and the subsequent illness leads to death (6, 29, 71). Even milder infections can cause impaired metamorphosis and a significant decrease in body size, thus leading to higher mortality rates, reproductive inefficiency and behavioural changes (6, 21, 72, 73).

Resistance

Research studies have demonstrated that some species can be considered infection resistant (8, 16, 56, 63, 64, 74, 75); others are susceptible to the infection but resistant to the disease (64, 71, 76).

Differences in animal behaviour or in immune function, both between and within species, can influence the susceptibility to infectious diseases (43, 44, 71, 77). Some species that have suffered a severe population decrease because of chytridiomycosis have recovered afterwards and now coexist with the infection (33, 44, 78, 79, 80, 81). This may suggest that changes have occurred in the susceptibility of the host and, thus, in the epidemiological status of the disease (77).

Several studies have demonstrated a tendency for the recovery of a few amphibian species which had been considered practically extinct as a result of chytridiomycosis (3, 80). In certain ecosystems, in spite of the activity of the aetiological agent in the environment, no mass mortality episodes have been recorded (15, 64, 70, 79, 82, 83). Other research has indicated a decrease in the frequency of infection in certain ecosystems, which suggests a change in the epidemiological status of the disease from epidemic to endemic (33, 78, 81).

Physiological status of the host

The physiological status of the host may interfere with individual susceptibility to chytridiomycosis. Alterations in biotic and abiotic factors may induce stress or other physiological conditions in amphibians that could influence individual susceptibility to the disease (76, 84, 85, 86). The thermoregulatory behaviour of amphibians threatened by chytridiomycosis may decrease the frequency of the disease under natural conditions (87, 88).

Density

The population density of the host is an intrinsic factor in the dispersal of infectious diseases (89), especially for models that involve respiratory, vector and direct contact transmission. Chytridiomycosis is propagated by direct contact and water transmission; hence, it is considered that host density could play an important role in its propagation. Briggs *et al*. (90) demonstrated, by applying a mathematical model, the importance of population density in the spread of infection. However, there is no epidemiological evidence that clearly demonstrates the influence of host density on the incidence or spread of chytridiomycosis (91).

Characteristics of the environment that may mediate the relationship between the aetiological agent and the host

Temperature and humidity

Physical characteristics of the environment, such as temperature, humidity and orography, play an important role in the biology of the aetiological agent and the host (8, 9, 65, 70, 86). Conditions of temperature and humidity are fundamental for the survival of *B. dendrobatidis* and determine its geographical distribution (67, 86). It seems that temperature is the most important environmental factor associated with the presence and viability of the fungus (52) and with the sensitivity or resistance of amphibians to the infection (37, 92).

The infection occurs during the year in a clear cyclical pattern, although it is not typically seasonal. Lower infection frequencies during warm months and higher frequencies during cold months have been observed under natural conditions. This cyclical pattern has been demonstrated in Australia (33, 59, 77, 78, 93) and the Neotropics (15, 31, 55).

Orography

The infection occurs within a broad altitudinal range in which mass mortality episodes in amphibians have been reported, with apparently the same intensity (94). The fungus and the infection have been reported from sea level (59) to extreme elevations (95).

However, several authors have demonstrated the impact of chytridiomycosis on amphibian species in alpine and similar regions, i.e. in high-altitude habitats where temperatures drop below freezing during autumn and winter (54, 96). Biotic or abiotic environmental factors which are still unknown may have an influence on the viability of *B. dendrobatidis* in environments that are apparently inhospitable to its development.

Sources of infection and transmission

Although there is insufficient information, it is assumed that the epidemiological model of the transmission of chytridiomycosis is horizontal and propagated, and that the main sources of infection are infected hosts and contaminated water. Thus, transmission may occur by direct contact between infected and susceptible animals and, more frequently, by exposure to contaminated water (18, 21, 25, 50, 51, 52, 55, 56). Hypothetically, therefore, species having greater water dependency should be more vulnerable to the infection (8, 9, 56, 60, 65, 66).

Transmission by direct contact results in slow increases in case frequency. Water is more efficient in enabling dispersion of the aetiological agent, and results in faster transmission. Thus, large numbers of individuals can be contaminated at the same time, which may explain the epidemic waves reported in tropical outbreaks of chytridiomycosis, and the high mortality rates that characterise this disease (3, 5, 10, 11, 12, 28, 50, 76, 86).

The emergence and dispersion of chytridiomycosis at a global scale is probably due to human intervention, both directly (5, 8, 86, 95, 97, 98, 99, 100) and indirectly by means of the global impact of climate change (84, 101, 102, 103). On a smaller scale, and in addition to anthropogenic factors, the infection may be spread by other mechanical vectors, such as the feathers of aquatic and terrestrial birds or wet substrates (50). This could significantly increase the distribution of the disease agent.

Reservoirs

In addition to its ability to survive in the environment under saprophytic conditions, *B. dendrobatidis* may use other survival strategies outside the preferential host, using ecological or epidemiological reservoirs. Ecological reservoirs are secondary hosts that are not susceptible to the disease and suffer a mild but sufficient infection to allow the reproduction and subsequent transmission of the fungus.

The existence of ecological reservoirs was demonstrated by McMahon *et al*. (27), who found the infection and the disease in crayfish in the Colorado and Louisiana wetlands. Johnson and Speare (50) demonstrated that non-amphibian keratin sources could act both as mechanical vectors of the infection and as ecological reservoirs. These findings could indicate the possibility that other mechanical vectors or ecological reservoirs are involved in the maintenance and diffusion of the fungus in the environment. From the epidemiological point of view, it is fundamental to identify possible ecological reservoirs in order to adopt sanitary measures to break the biological cycle of *B. dendrobatidis* in the environment outside the main host.

Epidemiological reservoirs are secondary or alternative hosts that are susceptible to the infection and, in many cases, to the disease but will not undergo significant depopulation when compared with the main hosts. Larvae and resistant species could be considered as epidemiological reservoirs (although they do not entirely fit the definition), at least to differentiate them from ecological reservoirs.

Several authors have referred to larvae as possible reservoirs of infection in the environment. Infection in the oral region, typical of chytridiomycotic larval infection, has been observed some months before the beginning of adult mortality episodes (9, 104). Among the species that are probable reservoirs involved in the dissemination of the fungal infection at a global scale is the African frog *Xenopus laevis*. As far as the natural history of the disease is concerned, it is speculated that the worldwide trade in *X. laevis* during the 1940s and 1950s (as a tool used in human pregnancy diagnosis) corresponded to the worldwide spread of *B. dendrobatidis* (16, 105).

Lithobates catesbeianus is currently the species most commonly cited as a possible reservoir and carrier of infection (74, 98). This species is commonly raised on farms for commercial purposes worldwide, and its international trade is considered an important means of dissemination of chytridiomycosis (75). Other species have also been considered as reservoirs and potential carriers of infection (33, 63). Nevertheless, there is insufficient epidemiological information to corroborate these hypotheses.

Risk factors

Many authors agree that environmental changes play an important role as risk factors in the appearance and distribution of chytridiomycosis (3, 57, 84, 86, 95, 101, 103, 106). Climatic changes may trigger unusual temperature variations in ecosystems, thus favouring the fungus (84), altering nutrient availability (14, 107), negatively affecting the physiological and immune state of the host, or increasing the number and duration of dry periods (64), which have grave consequences for amphibian population dynamics. Climatic variation could directly or indirectly alter the growth and viability of the pathogen, enabling disease transmission and affecting the susceptibility and immune response of host species (84).

Many environmental, biotic and abiotic factors are potential risk factors for the emergence and dissemination of infectious diseases, including chytridiomycosis (62, 99, 101, 106). Unfortunately, insufficient data are available regarding causal relationships, and their respective magnitudes, to allow the prediction of space–time and population profiles of chytridiomycosis outbreaks.

As mentioned above, a significant risk factor for chytridiomycosis is undoubtedly the international trade in amphibians (46, 47), especially for food purposes; the volume of this trade is considered to be large (99). For this reason, the World Organisation for Animal Health (OIE) (99) has included chytridiomycosis on the list of notifiable diseases. This measure commits OIE Member Countries to regularly provide information about the disease situation within their borders. This officially generated information could provide sufficient basis for the Member Countries of the World Trade Organization to carry out risk analyses of the introduction or reintroduction of the infection into unharmed or free areas, and to demand negative tests for chytridiomycosis as a biosafety measure for international transportation and trade of amphibian species.

Final comments

Direct and/or indirect anthropogenic contribution to the emergence and worldwide spread of chytridiomycosis seems quite probable. The epidemics of chytridiomycosis in amphibian populations have complex causes and could be the result of underlying predisposing factors (15) that are present in the habitat or artificially introduced. Although the ecological parameters affecting the susceptibility of amphibians to the fungus in nature are still unknown (71), research has increased our understanding of the epidemiology of chytridiomycosis in different locations and populations, based on the interactions and relationships among the aetiological agent, the host and the environment.

References

1. Stuart S.N., Chanson J.S., Cox N.A., Young B.E., Rodrigues A.S.L., Fischman D.L. & Waller R.W. (2004). – Status and trends of amphibian declines and extinctions worldwide. *Science*, **306** (5702), 1783–1786.

2. Lips K.R., Mendelson III J.R., Muñoz-Alonso A., Canseco-Marquez L. & Mulcahy D.G. (2004). – Amphibian population declines in Montane southern Mexico: resurveys of historical localities. *Biol. Conserv*., **119**, 555–564.

3. La Marca E., Lips K., Lötters S., Puschendorf R., Ibáñez R., Rueda-Almonacid J.V., Schulte R., Marty C., Castro F., Manzanilla-Puppo J., García-Pérez J.E., Bolaños F., Chaves G., Pounds J.A., Toral E. & Young B.E. (2005). – Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica*, **37** (2), 190–201.

4. Lips K.R., Green D.E. & Papendick R. (2003). – Chytridiomycosis in wild frogs from southern Costa Rica. *J. Herpetol*., **37** (1), 215–218.

5. Cunningham A.A., Daszak P. & Rodríguez J.P. (2003). – Pathogen pollution: defining a parasitology threat to biodiversity conservation. *J. Parasitol*., **89** (Suppl.), S78–S83.

6. Garner T.W.J., Walker S., Bosch J., Leech S., Rowcliffe J.M., Cunningham A.A. & Fisher M.C. (2009). – Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos*, **118**, 783–791.

7. Picco A.M. & Collins J.P. (2008). – Amphibian commerce as a likely source of pathogen pollution. *Conserv. Biol.*, **22** (6), 1582–1589.

8. Daszak P., Berger L., Cunningham A.A. & Hyatt A.D. (2003). – Infectious disease and amphibian population declines. *Divers. Distrib*., **9** (2), 141–150.

9. Daszak P., Berger L., Cunningham A.A., Hyatt A.D., Green D.E. & Speare R. (1999). – Emerging infectious diseases and amphibian population declines. *Emerg. infect. Dis*., **5** (6), 1–23.

10. Fisher M.P., Garner T.W.J. & Walker S.F. (2009). – Global emergence of *Batrachochytrium dendrobatidis* and amphibian

chytridiomycosis in space, time, and host. *Annu. Rev. Microbiol.*, **63**, 291–310.

11. Skerratt L.F., Berger L., Speare R., Cashins S., McDonald K.R., Phillott A.D., Hines H.B. & Kenyon N. (2007). – Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, **4**, 125–134.

12. Berger L., Speare R., Daszak P., Green D.E., Cunningham A.A., Goggin C.L., Slocombe R., Ragan M.A., Hyatt A.D., McDonald K.R., Hines H.B., Lips K.R., Marantelli G. & Parkes H. (1998). – Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl Acad. Sci. USA*, **95** (15), 9031–9036.

13. Longcore J.E., Pessier A.P. & Nichols A.P. (1999). – *Batrachochytrium dendrobatidis* gen. et *sp. nov.*, a chytrid pathogenic to amphibians. *Mycologia*, **91** (2), 219–227.

14. Rachowicz L.J., Hero J.M., Alford R.A., Taylor J.W., Morgan J.A.T., Vredenburg V.T., Collins J.P. & Briggs C.J. (2005). – The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. *Conserv. Biol.*, **19** (5), 1441–1448.

15. Ouellet M., Mikaelian I., Pauli B.D., Rodríguez J. & Green D.M. (2005). – Historical evidence of widespread chytrid infection in North American amphibian populations. *Conserv. Biol.*, **19** (5), 1431–1440.

16. Weldon C., Du Preez L.H., Hyatt A.D., Muller R. & Speare R. (2004). – Origin of the amphibian chytrid fungus. *Emerg. infect. Dis.*, **10** (12), 2100–2105.

17. Berger L., Speare R. & Skerratt L.F. (2005). – Distribution of *Batrachochytrium dendrobatidis* and pathology in the skin of green tree frogs *Litoria caerulea* with severe chytridiomycosis. *Dis. aquat. Organisms*, **68** (1), 65–70.

18. Pessier A.P. (2002). – An overview of amphibian skin disease. *Semin. avian exotic Pet Med.*, **11** (3), 162–174.

19. Pessier A.P., Nichols D.K., Longcore J.E. & Fuller M.S. (1999). – Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). *J. vet. diagn. Invest.*, **11**, 194–199.

20. Berger L., Marantelli G., Skerratt L.F. & Speare R. (2005). – Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. *Dis. aquat. Organisms*, **68** (1), 47–50.

21. Carey C., Bruzgul J.E., Livo L.J., Walling M.L., Kuehl K.A., Dixon B.F., Pessier A.P., Alford R.A. & Rogers K.B. (2006). – Experimental exposures of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). *EcoHealth*, **3**, 5–21.

22. Nichols D.K., Lamirande E.W., Pessier A.P. & Longcore J.E. (2001). – Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. *J. Wildl. Dis.*, **37** (1), 1–11.

23. North S. & Alford R.A. (2008). – Infection intensity and sampling locality affect *Batrachochytrium dendrobatidis* distribution among body regions on green-eyed tree frogs *Litoria genimaculata*. *Dis. aquat. Organisms*, **81** (3), 177–188.

24. Voyles J., Rosenblum E.B. & Berger L. (2011). – Interactions between *Batrachochytrium dendrobatidis* and its amphibian hosts: a review of pathogenesis and immunity. *Microbes Infect.*, **13** (1), 25–32.

25. Marantelli G., Berger L., Speare R. & Keegan L. (2004). – Distribution of the chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole development. *Pacific conserv. Biol.*, **10**, 173–179.

26. Voyles J., Young S., Berger L., Campbell C., Voyles W.F., Dinudom A., Cook D., Webb R., Alford R.A., Skerratt L.F. & Speare

R. (2009). – Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science*, **326**, 582–585.

27. McMahon T.A., Brannelly L.A., Chatfield M.W., Johnson P.T., Joseph M.B., McKenzie V.J., Richards-Zawacki C.L., Venesky M.D. & Rohr J.R. (2013). – Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proc. Natl Acad. Sci. USA*, **110**, 210–215.

28. Rachowicz L.J., Knapp R.A., Morgan J.A.T., Stice M.J., Vredenburg V.T., Parker J.M. & Briggs C. (2006). – Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology*, **87** (7), 1671–1683.

29. Rachowicz L.J. & Vredenburg V.T. (2004). – Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. *Dis. aquat. Organisms*, **61**, 75–83.

30. Fisher M.P., Bosch J., Yin Z., Stead D.A., Walker J., Selway L., Brown A.J.P., Walker L.A., Gow N.A.R., Stajich J.E. & Garner T.W.J. (2009). – Proteomic and phenotypic profiling of the amphibian pathogen *Batrachochytrium dendrobatidis* shows that genotype is linked to virulence. *Molec. Ecol.*, **18**, 415–429.

31. Retallick R.W.R. & Miera V. (2007). – Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. *Dis. aquat. Organisms*, **75**, 201–207.

32. Fisher M. (2008). – Molecular toolkit unlocks life cycle of panzootic amphibian pathogen *Batrachochytrium dendrobatidis*. *Proc. Natl Acad. Sci. USA*, **105** (45), 17209–17210.

33. Retallick R.W.R., McCallum H. & Speare R. (2004). – Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biol.*, **2** (11), 1965–1971.

34. Woodhams D.C., Voyles J., Lips K.R., Carey C. & Rollins-Smith L.A. (2006). – Predicted disease susceptibility in Panamanian amphibian assemblage based on skin peptide defences. *J. Wildl. Dis.*, **42** (2), 207–218.

35. Richmond J.Q., Savage A.E., Zamudio K.R. & Rosenblum E.B. (2009). – Toward immunogenetic studies of amphibian chytridiomycosis: linking innate and acquired immunity. *BioScience*, **59** (4), 311–320.

36. Rollins-Smith L.A. (2009). – The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. *Biochim. biophys. acta*, **1788**, 1583–1599.

37. Rollins-Smith L.A., Ramsey J.P., Pask J.D., Laura K., Reinert L.K. & Woodhams D.C. (2011). – Amphibian immune defenses against chytridiomycosis: impacts of changing environments. *Integr. Comp. Biol.*, **51** (4), 552–562.

38. Tennessen J.A., Woodhams D.C., Chaurand P., Reinert L.K., Billheimer D., Shyr Y., Caprioli R.M., Blouin M.S. & Rollins-Smith L.A. (2009). – Variations in expressed antimicrobial peptide repertoire of northern leopard frog (*Rana pipiens*) populations suggest intraspecies differences in resistance to pathogens. *Dev. comp. Immunol.*, **33**, 1247–1257.

39. Woodhams D.C., Ardipradja K., Alford R.A., Marantelli G., Reinert L.K. & Rollins-Smith L.A. (2007). – Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. *Anim. Conserv.*, **10**, 409–417.

40. Woodhams D.C., Rollins-Smith L.A., Carey C., Reinert L., Tyler M.J. & Alford R.A. (2006). – Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. *Oecologia*, **146**, 531–540.

41. Rollins-Smith L.A. & Conlon J.M. (2005). – Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations. *Dev. comp. Immunol.*, **29**, 589–598.

42. Rosenblum E.B., Poorten T.J., Settles M., Murdoch G.K., Robert J., Maddox N. & Eisen M.B. (2009). – Genome-wide transcriptional response of *Silurana* (*Xenopus*) *tropicalis* to infection with the deadly chytrid fungus. *PLoS ONE*, **4** (8), e6494.

43. Woodhams D.C., Alford R.A. & Marantelli G. (2003). – Emerging diseases of amphibians cured by elevated body temperature. *Dis. aquat. Organisms*, **55**, 65–67.

44. Briggs C.J., Vredenburg V.T., Knapp R.A. & Rachowicz L.J. (2005). – Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. *Ecology*, **86** (12), 3149–3159.

45. Mussmann R., Du Pasquier L. & Hsu E. (1996). – Is *Xenopus* IgX an anolog of IgA? *Eur. J. Immunol.*, **26** (12), 2823–2830.

46. Farrer R.A., Weinert L.A., Bielby J., Trenton W.J., Garner T.W.J., Balloux F., Clare F., Bosch J., Cunningham A.A., Weldon C., du Preez L.H., Anderson L., Pond S.L.K., Shahar-Golan R., Daniel A., Henk D.D. & Fisher M.C. (2011). – Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proc. Natl Acad. Sci. USA*, **108** (46), 18732–18736.

47. Schloegel L.M., Toledo L.F., Longcore J.E., Greenspan S.E., Vieira C.A., Lee M., Zhao S., Wangen C., Ferreira C.M., Hipolito M., Davies A.J., Cuomo C.A., Daszak P. & James T.Y. (2012). – Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. *Molec. Ecol.*, **21** (21), 5162–5177.

48. Goka K., Yokoyama J., Une Y., Kuroki T., Suzuki K., Nakahara M., Kobayashi A., Inaba S., Mitzutani T. & Hyatt A.

(2009). – Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. *Molec. Ecol.*, **18** (23), 4757–4774.

49. Wixson J.G. & Rogers K.B. (2009). – Detecting *Batrachochytrium dendrobatidis* in the wild when amphibians are absent. *Herpetol. Rev.*, **40** (3), 313–316.

50. Johnson M.L. & Speare R. (2005). – Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. *Dis. aquat. Organisms,* **65** (3), 181–186.

51. Johnson M.L. & Speare R. (2003). – Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. *Emerg. infect. Dis.*, **9** (8), 922–925.

52. Piotrowski J.S., Annis S.L. & Longcore J.E. (2004). – Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, **96** (1), 9–15.

53. Mitchell K.M., Churcher T.S., Garner T.W.J. & Fisher M.C. (2008). – Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. *Proc. Roy. Soc. Lond., B, biol. Sci*., **275**, 329–334.

54. Bosch J., Martínez-Solano I. & García-París M. (2001). – Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biol. Conserv*., **97**, 331–337.

55. Lips K.R., Brem F., Brenes R., Reeve J.D., Alford R.A., Voyles J., Carey C., Livo L., Pessier A.P. & Collins J.P. (2006). – Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Natl Acad. Sci. USA*, **103** (9), 3165–3170.

56. Lips K., Reeve J.D. & Witters L.R. (2003). – Ecological traits predicting amphibian population declines in Central America. *Conserv. Biol.*, **17** (4), 1078–1088.

57. Murray K.A., Skerratt L.F., Speare R. & McCallum H. (2009). – Impact and dynamics of disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. *Conserv. Biol.*, **23** (5), 1242–1252.

58. Ryan M.J., Lips K.R., Michael W. & Eichholz M.W. (2008). – Decline and extirpation of an endangered Panamanian stream frog population (*Craugastor punctariolus*) due to an outbreak of chytridiomycosis. *Biol. Conserv*., **141**, 1636–1647.

59. Berger L., Speare R., Hines H.B., Marantelli G., Hyatt A.D., McDonald K.R., Skerratt L.F., Olsen V., Clarke J.M., Gillespie G., Mahony M., Sheppard N., Williams C. & Tyler M.J. (2004). – Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust. vet. J*., **82** (7), 434–439.

60. Kriger K.M. & Hero J.M. (2007). – The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Divers. Distrib*., **13**, 781–788.

61. Pearl C.A., Bull E.L., Green D.E., Bowerman J., Adams M.J., Hyatt A. & Wente W. (2007). – Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. *J. Herpetol*., **41** (1), 145–149.

62. Collins J.P. & Storfer A. (2003). – Global amphibian decline: sorting the hypotheses. *Divers. Distrib*., **9** (2), 89–98.

63. Kriger K.M. & Hero J.M. (2006). – Survivorship in wild frogs infected with chytridiomycosis. *EcoHealth*, **3**, 171–177.

64. Daszak P., Scott D.E., Kilpatrick A.M., Faggioni C., Gibbons J.W. & Porter D. (2005). – Amphibian population declines at Savannah River site are linked to climate, not chytridiomycosis. *Ecology*, **86** (12), 3232–3237.

65. Hero J.M., Williams S.E. & Magnusson W.E. (2005). – Ecological traits of declining amphibians in upland areas of eastern Australia. *J. Zool.*, **267**, 221–232.

66. Hero J.M. & Morrison C. (2004). – Frog declines in Australia: global implications. *Herpetol. J.*, **14**, 175–186.

67. Muths E., Pilliod D.S. & Livo L.J. (2008). – Distribution and environmental limitations of an amphibian pathogen in the Rocky Mountains, USA. *Biol. Conserv.*, **141**, 1484–1492.

68. Rowley J.J.L. & Alford R.A. (2010). – Factors affecting interspecific variation in susceptability to disease in amphibians. Chapter 3. *In* Amphibian biology (H. Heatwole & J.W. Wilkinson, eds). Vol. 8. Amphibian decline: diseases, parasites, maladies, and pollution. Surrey Beatty and Sons, Baulkham Hills, Australia, 3053–3066.

69. Rowley J.J.L. & Alford R.A. (2007). – Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. *Dis. aquat. Organisms*, **77**, 1–9.

70. Longcore J.A., Longcore J.E., Pessier A. & Halteman (2007). – Chytridiomycosis widespread in anurans of Northeastern United States. *J. Wildl. Manag.*, **71** (2), 435–444.

71. Blaustein A.R., Romansic J.M., Scheessele E.A., Han B.A., Pessier A.P. & Longcore J.E. (2005). – Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. *Conserv. Biol.*, **19** (5), 1460–1468.

72. Parris M.J., Reese E. & Storfer A. (2006). – Antipredator behaviour of chytridiomycosis-infected northern leopard frog (*Rana pipiens*) tadpoles. *Can. J. Zool.*, **84**, 58–65.

73. Parris M.J. & Cornelius T.O. (2004). – Fungal pathogen causes competitive and developmental stress in larval amphibian communities. *Ecology*, **85** (12), 3385–3395.

74. Daszak P., Strieby A., Cunningham A.A., Longcore J.E., Brown C.C. & Porter D. (2004). – Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. *Herpetol. J.*, **14**, 201–207.

75. Garner T.W.J., Walker S., Bosch J., Hyatt A.D., Cunningham A.A. & Fisher M.C. (2005). – Chytrid fungus in Europe. *Emerg. infect. Dis*., **11** (10), 1639–1641.

76. Mendelson J.R., Brodie Jr E.D., Malone J.H., Acevedo M.E., Baker M.A., Smatresk N.J. & Campbell J.A. (2004). – Factors associated with the catastrophic declines of a cloud forest frog fauna in Guatemala. *Rev. Biol. trop*., **52** (4), 991–1000.

77. Woodhams D.C. & Alford R.A. (2005). – Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conserv. Biol.*, **19** (5), 1449–1459.

78. McDonald K.R., Méndez D., Müller R., Freeman A.B. & Speare R. (2005). – Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. *Pacific conserv. Biol.*, **11**, 114–120.

79. Pearl C.A., Bowerman J., Adams M.J. & Chelgren N.D. (2009). – Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon spotted frogs (*Rana pretiosa*). *EcoHealth*, **6**, 209–218.

80. Puschendorf R., Bolaños F. & Chaves G. (2006). – The amphibian chytrid fungus along altitudinal transects before the first reported declines in Costa Rica. *Biol. Conserv*., **132**, 136–142.

81. Voordouw M.J., Adama D., Houston B., Govindarajulu P. & Robinson J. (2010). – Prevalence of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in an endangered population of northern leopard frogs, *Rana pipiens*. *BMC Ecol.*, **10**, 6.

82. Kielgast J., Rödder D., Veith M. & Lötters S. (2010). – Widespread occurrence of the amphibian chytrid fungus in Kenya. *Anim. Conserv.*, **13** (Suppl. 1), 1–8.

83. Murphy P.J., St-Hilaire S., Bruer S., Corn P.S. & Peterson C.R. (2009). – Distribution and pathogenicity of *Batrachochytrium dendrobatidis* in boreal toads from the Grand Teton area of Western Wyoming. *EcoHealth*, **6**, 109–120.

84. Pounds J.A., Bustamante M.R., Coloma L.A., Consuegra J.A., Fogden M.P.L., Foster P.N., La Marca E., Masters K.L., Merino-Viteri A., Puschendorf R., Ron S.R., Sánchez-Azofeifa G.A., Still C.J. & Young B.E. (2006). – Widespread amphibian extinction from epidemic disease driven by global warming. *Nature*, **439** (7073), 161–167.

85. Reading C.J. (2007). – Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia*, **151**, 125–131.

86. Ron S.R. (2005). – Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the new world. *Biotropica*, **37** (2), 209–221.

87. Richards-Zawacki C.L. (2010). – Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proc. Roy. Soc. Lond., B, biol. Sci.*, **277**, 519–528.

88. Rowley J.J.L. & Alford R.A. (2013). – Hot bodies protect amphibians against chytrid infection in nature. *Sci. Rep.*, **3**, 1515.

89. Smith M.J., Telfer S., Kallio E.R., Burthe S., Cook A.R. & Lambin X. (2009). – Host–pathogen time series data in wildlife support a transmission function between density and frequency dependence. *Proc. Natl Acad. Sci. USA*, **106** (19), 7905–7909.

90. Briggs C.J., Knapp R.A. & Vredenburg V.T. (2010). – Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Natl Acad. Sci. USA*, **107**, 9695–9700.

91. Rachowicz L.J. & Briggs C.J. (2007). – Quantifying the disease transmission function: effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog *Rana muscosa*. *J. anim. Ecol*., **76**, 711–721.

92. Andre S.E., Parker J. & Briggs C.J. (2008). – Effect of temperature on host response to *Batrachochytrium dendrobatidis* infection in the mountain yellow-legged frog (*Rana muscosa*). *J. Wildl. Dis*., **44** (3), 716–720.

93. Kriger K.M. & Hero J.M. (2007). – Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *J. Zool*., **271**, 352–359.

94. Kriger K.M. & Hero J.M. (2008). – Altitudinal distribution of chytrid (*Batrachochytrium dendrobatidis*) infection in subtropical Australian frogs. *Austral Ecol*., **33**, 1022–2032.

95. Seimon T.A., Seimon A., Daszak P., Halloy S.R.P., Schloegel L.M., Aguilar C.A., Sowell P., Hyatt A.D., Konecky B. & Simmons J.E. (2007). – Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biol*., **13**, 288–299.

96. Scherer R.D., Muths E., Noon B.R. & Corn P.S. (2005). – An evaluation of weather and disease as causes of decline in two populations of boreal toads. *Ecol. Applic*., **15** (6), 2150–2160.

97. Fisher M. & Garner T.W.J. (2007). – The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduction of amphibian species. *Fung. Biol. Rev*., **21**, 2–9.

98. Garner T.W.J., Perkins M.W., Govindarajulu P., Seglie D., Walker S., Cunningham A.A. & Fisher M. (2006). – The emerging

amphibian *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biol. Lett.*, **2** (3), 455–459.

99. Schloegel L.M., Picco A.M., Kilpatrick A.M., Davies A.J., Hyatt A.D. & Daszak P. (2009). – Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biol. Conserv.*, **142**, 1420–1426.

100. Walker S.F., Bosch J., James T.Y., Litvintseva A.P., Valls J.A.O., Piña S., García G., Rosa G.A., Cunningham A.A., Hole S., Griffiths R. & Fisher M.C. (2008). – Invasive pathogens threaten species recovery programs. *Curr. Biol.*, **18** (18), R853–R854.

101. Carey C. & Alexander M.A. (2003). – Climate change and amphibian declines: is there a link? *Divers. Distrib.*, **9** (2), 111–121.

102. Laurance W.F. (2008). – Global warming and amphibian extinctions in eastern Australia. *Austral Ecol.*, **33**, 1–9.

103. Williams E.S., Yuill T., Artois M., Fischer J. & Haigh S.A. (2002). – Emerging infectious diseases in wildlife. *In* Infectious diseases of wildlife: detection, diagnosis and management (Part One) (R.G. Bengis, ed.). *Rev. sci. tech. Off. int. Epiz.*, **21** (1), 139–157.

104. Symonds E.O., Hines H.B., Bird P.S., Morton J.M. & Mills P.C. (2007). – Surveillance for *Batrachochytrium dendrobatidis* using *Mixophyes* (*Anura*: *Myobatrachidae*) larvae. *J. Wildl. Dis.*, **43** (1), 48–60.

105. Weldon C., De Villiers A.L. & Du Preez L.H. (2007). – Quantification of the trade in *Xenopus laevis* from South Africa, with implications for biodiversity conservation. *Afr. J. Herpetol.*, **56** (1), 77–83.

106. Alford R.A., Bradfield K.S. & Richards S.J. (2007). – Global warming and amphibian losses. *Nature*, **447** (31), E3–E4.

107. Johnson P.T.J., Townsend A.R., Cleveland C.C., Gilbert P.M., Howarth R.W., McKenzie V.J., Rejmankova E. & Ward M.H. (2010). – Linking environmental nutrient enrichment and disease emergence in humans and wildlife. *Ecol. Applic*., **20** (1), 16–29.