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Survival of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on bare hands and gloves: hygiene implications for amphibian handling

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ABSTRACT: Hygiene protocols for handling amphibians in the field and in laboratories have been proposed to decrease the transmission of chytridiomycosis caused by infection with the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, which is responsible for global amphibian declines. However, these protocols are mainly based on theoretical principles. The aim of this study was to develop an evidence-based approach to amphibian handling hygiene protocols by testing the survival of *B. dendrobatidis* on human hands and various gloves. Bare or gloved human fingers were exposed to cultured zoospores and zoosporangia of *B. dendrobatidis*. Survival of *B. dendrobatidis* on hands and gloves was tested for up to 10 min post-exposure by inoculation onto tryptone/gelatin hydrolysate/lactose (TGhL) agar plates. The effects of repeated hand washings with water and with 70% ethanol and of washing gloves with water were also tested. Bare human skin demonstrated a fungicidal effect on *B. dendrobatidis* by 2 min and killed 100% of cells by 6 min, but this killing effect was reduced by repeated washing with water and ethanol. Nitrile gloves killed all *B. dendrobatidis* on contact, but washing in water decreased this effect. Latex and polyethylene gloves had no killing effect, and *B. dendrobatidis* survived for over 6 min. The killing effect of vinyl gloves varied with brands and batches. These results support the use of an unused pair of gloves for each new amphibian handled in either the field or the laboratory, and if this is not possible, bare hands are a preferable, although imperfect, alternative to continual use of the same pair of gloves.

KEY WORDS: *Batrachochytrium dendrobatidis* · Human skin · Gloves · Hygiene · Amphibian handling

INTRODUCTION

Batrachochytrium dendrobatidis has been recognised in the last decade as one of the most significant pathogens of wild and captive amphibians worldwide (Berger et al. 1998, Daszak et al. 1999, Skerratt et al. 2007). This potentially lethal fungus continues to play a major role in the decline, and in some cases disappearance, of many amphibian populations around the globe (Berger et al. 1998, 1999, Daszak et al. 1999, Schloegel et al. 2005, Lips et al. 2006, Pounds et al. 2006, Skerratt et al. 2007). With the increased interest in declining amphibian populations within urban as well as remote and more pristine areas, hygiene protocols have been proposed to reduce the possibility that researchers and wildlife managers could spread the disease between individuals and populations. Growing knowledge of the biology of *B. dendrobatidis* and the effects of various physical and chemical treatments (Johnson et al. 2003, Webb et al. 2007) allowed the development of evidence-based field and laboratory hygiene protocols to minimise the risk of disease dissemination (Daszak et al. 2001, Johnson & Speare 2003, 2005, Department of Environment and Heritage 2006). Most hygiene proto-

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cols recommend single use of disposable gloves for handling either wild or captive frogs in order to avoid the potential transfer of zoospores between animals. A similar protocol is recommended when handling fomites potentially contaminated with *B. dendrobatidis* such as tanks, calipers, scales and so on. Such a precautionary practice acts as a physical barrier preventing indirect contamination between animals (New South Wales National Parks and Wildlife Service 2000, Lynch 2001, Department of Environment and Heritage 2006). The use of a fresh pair of gloves for each amphibian can add considerably to costs, which is a concern particularly when funds for research are scarce. It can also significantly add to time and complexity of catching frogs in often physically challenging field situations such as in fast-flowing streams. The Threat Abatement Plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis (Department of Environment and Heritage 2006) also advocates that, apart from using disinfectants such as sodium hypochlorite (0.4%) or ethanol (70%), bare hands, gloves and other non-disposable instruments could also be cleaned in the local water body. This recommendation was based on the theory that water at an infected site already contains *B. dendrobatidis* and that using this water for cleaning purposes will not put the frogs removed from that water body at a higher risk of infection. Subsequently, Kirshtein et al. (2007) were able to detect between 19 and 454 zoospore equivalents l^{-1} in water collected from 4 different sites associated with amphibians in the USA which tested positive for *B. dendrobatidis*. Walker et al. (2007) found 0.5 to 262 (median 31) zoospore equivalents l^{-1} in 64% of ponds at a site in Spain, with higher counts occurring in more turbid water. While washing with water is a cheap and convenient option, these hygiene practices have never been evaluated until now. In this study we tested the ability of bare human skin as well as various glove surfaces to transfer live *B. dendrobatidis* zoospores and zoosporangia to culture media.

While it has been established that *Batrachochytrium dendrobatidis* causes chytridiomycosis, a major disease of amphibians, very little is known about the amphibian chytrid's ecology and natural routes of transmission. Although it is believed that *B. dendrobatidis* is a pathogen that occurs in the environment (Department of Environment and Heritage 2006), it has been difficult to isolate (Kirshtein et al. 2007, Walker et al. 2007), and no animal reservoir other than amphibians has been found (Rowley et al. 2006, 2007). Amphibians become infected when the water-borne zoospores of *B. dendrobatidis* invade their epidermis (Berger et al. 1998, 2004, 2005, Longcore et al. 1999). *In vitro*, *B. dendrobatidis* grows optimally at 20 to 23°C, ceases growth at 29°C and dies at ≥30°C (Longcore et al. 1999, Berger 2001, Johnson et al. 2003, Piotrowski et al. 2004). At 37°C, it can survive for more than 2 h, but less than 4 h (Johnson et al. 2003). This means that, in theory, *B. dendrobatidis* should not grow on human hand skin, which averages 32.1°C (Kokubo et al. 1998), but could remain alive long enough to be transferred from one frog to another. To assist in the development of evidence-based hygiene protocols, we report here on a series of experiments that tested the survival of *B. dendrobatidis* on human skin and on gloves. These experiments were not designed to replicate conditions in the field, but were designed to assess potential survival of *B. dendrobatidis* on different surfaces and hence the potential for transmission of the amphibian chytrid by these surfaces.

MATERIALS AND METHODS

All experiments were carried out in a laminar flow cabinet (BH series, Biological Safety Cabinet Class II, Gelman Sciences) to avoid contamination of culture media. For each experiment, the *Batrachochytrium dendrobatidis* strain: Rockhampton-Lcaerulea-99-LB1, of various passages, was grown in 9 ml of tryptone/gelatin hydrolysate/lactose (TGhL) medium in flat-bottomed 25 cm^2 plastic sterile cell culture flasks (TPP or Sarstedt) for 4 to 6 d at 21 to 23°C. Presence and activity of zoospores in culture were assessed under an inverted microscope. The average zoospore concentration (60 000 zoospores ml^{-1}) was determined using a Neubauer haemocytometer (Blaubrand®) under a compound microscope at a magnification of ×400. All exposure-inoculation trials were done at an average room temperature of 24.5°C and ambient humidity ranging from 49 to 62%.

Expt 1. Bare hands. Before each replicate was carried out, 10 ml of liquid culture was poured into a large, sterile 92 mm diameter × 16 mm depth plastic Petri dish (Sarstedt). Exposure of bare hands was achieved by simultaneously dipping 8 fingers, not including the thumbs, for 2 s into the Petri dish of *Batrachochytrium dendrobatidis* culture. Survival of *B. dendrobatidis* was then tested by inoculation from finger tips onto agar. Each finger tip was pressed individually for 1 s onto the surface of a sterile TGhL agar plate (55 mm diameter × 16 mm depth) with antibiotics (200 mg l^{-1} of penicillin G (Sigma, Sigma-Aldrich) and 300 mg l^{-1} of streptomycin sulphate (Sigma, Sigma-Aldrich). The first digit was pressed immediately at time (T) = 0, then the second digit at T = 0.5 min, the third digit at T = 1 min and so on at times post-exposure T = 2, 3, 4, 5 and 6 min. These times were chosen after our pilot study (Speare et al. 2004) showed that *B. dendrobatidis* zoospores and zoosporangia did not survive on the surface of human skin beyond 2 min. For this experiment, 8 agar plates were used for each repli-

cate. The procedure was replicated 12 times. Before each replicate was performed, hands were washed with tap water and dried on paper towels. Replicates were carried out on different occasions with the same strain of *B. dendrobatidis* but with cultures of different passages and the hands of 4 volunteers.

Johnson et al. (2003) showed that *Batrachochytrium dendrobatidis* did not survive desiccation beyond 3 h at 22°C. To avoid the confounding effect of drying on the survival of *B. dendrobatidis*, hands were not kept in the laminar flow cabinet between inoculation times (in order to minimise the drying effect of the air flow). To avoid contamination, hands were held in the air and did not come into contact with any objects. To compensate for any movement or loss of liquid media through gravity or evaporation, each inoculation involved the wettest area of each finger.

Expt 2. Gloved hands. Four types of disposable gloves were tested using the same protocol as in Expt 1. Gloves used were: (Expt 2a) 100% nitrile gloves low powder (N-Dex®, Best Manufacturing Company) (N1), 20 replicates; (Expt 2b) latex gloves (Astra, Clorox Australia), 10 replicates; (Expt 2c) polyethylene gloves (Essentials, Hercules), 10 replicates; (Expt 2d) vinyl gloves (Livingstone International) (V1), 10 replicates. Nitrile and vinyl gloves were purchased from a laboratory supply specialist, while the latex and polyethylene gloves were purchased from a local grocery store. All gloves were bulk packaged in boxes of 100. Each replicate was carried out with a new pair of gloves.

Expt 3. Bare hands washed in water between each of 10 consecutive exposure-inoculation trials. A single volunteer was used to test the effect of repeated washing of hands in water using a protocol similar to Expt 1, but with slight modification of post-exposure times (0, 2, 3, 4, 5, 6, 7, 8 and 10 min), giving 9 agar plates per exposure-inoculation trial. In this experiment, the inoculating digit at $T = 10$ min post-exposure was the same as the one used at $T = 0$. Hands were washed in deionised sterile water 10 times sequentially on the same day after each exposure-inoculation trial. Hands were allowed to air dry for 3 min after washing before moving on to the next set of exposure-inoculation trials. The same culture was used for all 10 exposures. To avoid the introduction of added contaminants into the culture and agar plates, autoclaved water was used for hand washing.

Expt 4. Bare hands washed in 70% ethanol between each of 10 consecutive exposure-inoculation trials. Expt 3 was repeated, with water replaced by 70% ethanol as the cleaning medium between exposure-inoculation trials. Ethanol was sprayed on hands, rubbed in and allowed to air dry for 2 min.

Expt 5. Gloved hands washed in water between each of 10 consecutive exposure-inoculation trials. Expt 3 was repeated with different types of gloves using (1) 100% nitrile gloves low powder (N-Dex®, Best Manufacturing Company) (N1); (2) 100% nitrile textured and powdered gloves (Ni-Tek, Livingstone International) (N2); (3) vinyl gloves (Livingstone International) (V1); (4) vinyl gloves (Mediflex Industries) (V2). Times of inoculation post-exposure were as for Expt 1.

Agar plates were covered immediately after inoculation, and lids were secured using laboratory sealing film Parafilm M® (Pechiney Plastic Packaging). All plates were examined on Days 3, 10, 25 and 50 post-inoculation under an inverted microscope for presence of *Batrachochytrium dendrobatidis* growth, zoospore activity and potential contaminants such as bacteria or hyphal fungi. The criteria for growth of *B. dendrobatidis* were: zoosporangia observed to contain cellular structures and actively releasing motile zoospores that could also be observed free-living on the agar in great numbers at the edge of a growing colony. Plates were discarded after Day 50.

Focal areas of contamination could be removed, but plates with overwhelming contamination were discarded. In a few instances, *Batrachochytrium dendrobatidis* grew adjacent to a contaminant; however, this was not taken into account. Growth noted at any of the 4 observation times was recorded as a positive result. All results were expressed by the proportion (%) of uncontaminated plates showing growth of transferred *B. dendrobatidis* by the total number of uncontaminated plates. Growth was interpreted as successful transfer of viable zoospores or zoosporangia.

RESULTS

Expt 1. Bare hands

Transferred *Batrachochytrium dendrobatidis* grew on 100% of plates inoculated at 0.5 and 1 min post-exposure. Beyond this time, survival decreased to 50% at 2 min, 10% at 4 min and no survival was observed by 6 min post-exposure (Fig. 1). The media culture on the fingers of the volunteers did not dry while performing any of the replicates.

Expt 2. Gloved hands

The nitrile gloves used in this experiment were not textured and had a very light powder coating on the inside. Liquid media stayed trapped in glove folds, but also dried in some more exposed areas in less than 4 min. At the end of each replicate, it was noted that all remaining wet spots were foamy, indicating an interaction between the culture media and components of the

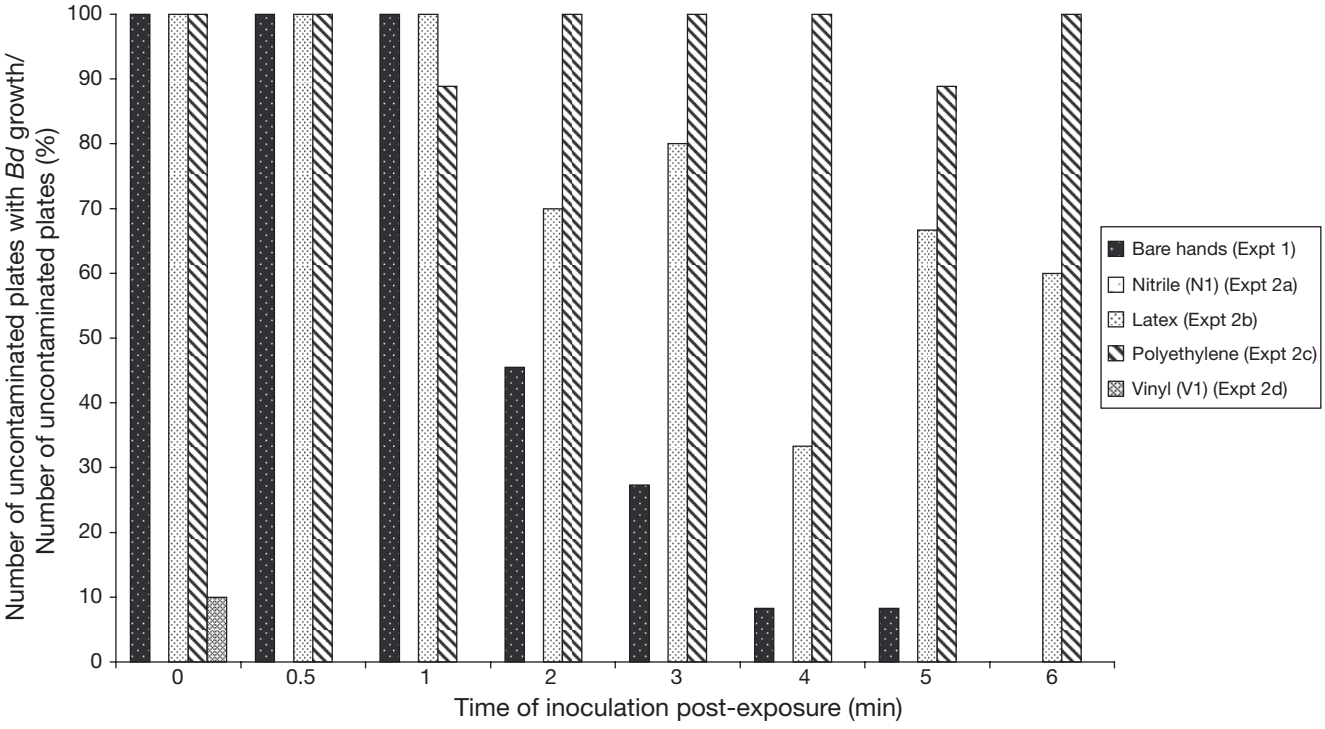

Fig. 1. *Batrachochytrium dendrobatidis*. Survival after inoculation of zoospores and zoosporangia from culture broth onto tryptone/gelatine hydrolysate/lactose (TGhL) agar plates by bare and gloved human digits at varying times post-exposure (Expts 1 and 2). No survival at any time when nitrile (N1) gloves were used. *Bd*: *B. dendrobatidis*

gloves. Despite repeating this experiment 20 times, growth of *Batrachochytrium dendrobatidis* was not observed at any time post-exposure (Fig. 1).

The latex gloves used in this experiment had a low level of powder coating on the inside. The distribution of the culture media on the latex surface appeared smooth and even in the areas of exposure. On some fingers, the droplet of culture medium dried in less than 4 min. The survival of zoospores and zoosporangia was very good on latex gloves (Fig. 1).

The polyethylene gloves had no powder coating. However, they had a textured surface on which the culture media 'beaded' (drops of liquid culture media did not spread on its surface). As a result, media collected by gloved fingers during exposure did not dry out throughout any of the replicates. Polyethylene gloves appeared to be even more efficient than the latex gloves at transferring live zoospores and zoosporangia (Fig. 1). Almost all transfers resulted in *Batrachochytrium dendrobatidis* growth.

The vinyl gloves used were not textured but were coated with powder on the inside. Some of the powder was also observed on the surface of the gloves. Culture media dried fairly quickly after exposure (in less than 3 min). Survival of *Batrachochytrium dendrobatidis* on vinyl gloves was very limited and growth was observed on one agar plate only at 0 min (Fig. 1).

Expt 3. Bare hands washed in water between each of 10 consecutive exposure-inoculation trials

In Expt 3 after 5 washes, the skin was feeling dry to the volunteer. Overall, survival of transferred cultures of *Batrachochytrium dendrobatidis* increased with the number of washes (Table 1). After the first wash, *B. dendrobatidis* survived up to 6 min. After the 5th wash *B. dendrobatidis* appeared to consistently grow up to at least 6 min, 4 more minutes than in our pilot study or Expt 1. The repeated washing increased survival of *B. dendrobatidis*, as shown by growth on more agar plates and for a longer time post-exposure (Table 1).

Expt 4. Bare hands washed in 70% ethanol between each of 10 consecutive exposure-inoculation trials

In Expt 4, the volunteer experienced an increased sensation of dryness of the skin after the second wash. Repeated use of 70% ethanol also increased the survival time of *Batrachochytrium dendrobatidis* on human skin after the first wash (Table 2). *B. dendrobatidis* survived up to 6 min after the first wash, and up to 8 min after the 6th wash (Table 2), which is a total of 6 extra minutes of survival on human skin compared to our pilot study and Expt 1.

Table 1. *Batrachochytrium dendrobatidis*. Growth after inoculation at varying times post-exposure of zoospores and zoosporangia from culture broth onto TGhL agar plates by bare human digits repeatedly washed in distilled sterile water before each new exposure (Expt 3). Shaded: growth; unshaded: no growth; X: contamination; *Bd*: *B. dendrobatidis*; % *Bd*: no. of uncontaminated plates with *Bd* growth/ no. of uncontaminated plates (%)

No. of washes	Time post-exposure (min) 0	2	3	4	5	6	7	8	10	%*Bd*
Baseline	X									0
1										22.2
2	X				X					14.3
3										22.2
4										33.3
5					X					25
6										44.4
7										55.5
8										55.5
9						X				50
%*Bd*	75	50	20	50	62.5	33.3	20	0	0	

Table 2. *Batrachochytrium dendrobatidis*. Growth after inoculation at varying times post-exposure of zoospores and zoosporangia from culture broth onto TGhL agar plates by bare human digits repeatedly washed in 70% ethanol before each new exposure (Expt 4). Shaded: growth; unshaded: no growth; X: contamination; *Bd*: *B. dendrobatidis*; % *Bd*: no. of uncontaminated plates with *Bd* growth/no. of uncontaminated plates (%)

No. of washes	Time post-exposure (min) 0	2	3	4	5	6	7	8	10	%*Bd*
Baseline			X							12.5
1										33.3
2										33.3
3										44.4
4									X	37.5
5										66.7
6									X	100
7										88.9
8					X				X	71.4
9										77.8
%*Bd*	100	90	88.9	50	44.4	50	40	30	0	

Expt 5. Gloved hands washed in water between each of 10 consecutive exposure-inoculation trials

The type and brand of nitrile gloves (N1) used in Expt 2 were used in Expt 5 as well as a different type of 100% nitrile glove (N2). The latter had textured fingertips and a higher level of powder coating on the inside, which was also visible on the outside of the gloves. As expected from the results obtained in Expt 2, *Batrachochytrium dendrobatidis* did not survive transfer on either of the brands of 100% nitrile gloves before the first wash (Tables 3 & 4). After washing, some live *B. dendrobatidis* was successfully transferred with both brands of gloves (Tables 3 & 4). However, the N1

Table 3. *Batrachochytrium dendrobatidis*. Growth after inoculation at varying times post-exposure of zoospores and zoosporangia from culture broth onto TGhL agar plates by gloved (Nitrile, N1) digits repeatedly washed in distilled sterile water before each new exposure (Expt 5, N1). Shaded: growth; unshaded: no growth; X: contamination; *Bd*: *B. dendrobatidis*; % *Bd*: no. of uncontaminated plates with *Bd* growth/no. of uncontaminated plates (%)

No. of washes	Time post-exposure (min) 0	0.5	1	2	3	4	5	6	%*Bd*
Baseline	X		X			X			0
1				X					14.3
2								X	42.9
3		X							28.6
4									12.5
5	X								0
6									25
7				X					0
8			X						14.3
9		X							0
%*Bd*	50	12.5	25	0	20	11.1	0	0	

Table 4. *Batrachochytrium dendrobatidis*. Growth after inoculation at varying times post-exposure of zoospores and zoosporangia from culture broth onto TGhL agar plates by gloved (Nitrile, N2) digits repeatedly washed in distilled sterile water before each new exposure (Expt 5, N2). Shaded: growth; unshaded: no growth; X: contamination; *Bd*: *B. dendrobatidis*; *Bd*: *B. dendrobatidis*; % *Bd*: no. of uncontaminated plates with *Bd* growth/no. of uncontaminated plates (%)

No. of washes	Time post-exposure (min) 0	0.5	1	2	3	4	5	6	%*Bd*
Baseline		X	X		X	X	X	X	0
1				X					57.1
2									50
3									62.5
4									75
5									100
6									50
7									87.5
8									75
9									75
%*Bd*	90	66.7	100	66.7	55.6	55.6	55.6	44.4	

gloves were less efficient in doing so, even after the 9th wash (Table 3). The total number of uncontaminated plates with *B. dendrobatidis* growth was almost 5 times higher in the experiment using N2 gloves than in the one using N1 gloves (Tables 3 & 4). *B. dendrobatidis* survived up to 4 min after the 2nd wash on N1 gloves while it survived up to 5 min after the 3rd wash and up to 6 min on N2 gloves after the 4th wash (Tables 3 & 4). Differences between brands may be due to the difference in fingertip texture. In both cases, the surfaces of the gloves foamed when first washed in water. The foaming completely stopped after the 3rd wash for N1 gloves and after the 5th wash for N2 gloves.

The type and brand of vinyl gloves (V1) used in Expt 2 were used in Expt 5. However, the gloves used in Expt 2 came from a box already opened in the laboratory, while the gloves used in Expt 5 came from a new, unopened box. The brand of vinyl gloves (V2) used in Expt 5 did not appear different in texture from V1 gloves and came from a new unopened box. Both V1 and V2 had a high level of powder coating on the inside, which was also visible on the outside of the gloves. Unexpectedly, we could not replicate the baseline results for unwashed vinyl gloves obtained in Expt 2. In this instance (Expt 5), both V1 and V2 gloves were very efficient in transferring live *Batrachochytrium dendrobatidis* (Tables 5 & 6). *B. dendrobatidis* survived up to 5 min before the first wash on the V1 gloves and up to 6 min on V2 gloves (Tables 5 & 6). After the 4th wash, survival of *B. dendrobatidis* also reached 6 min on gloves V1 (Table 5). Because in Expt 2 the V1 gloves came from an opened box, it is possible that the quality of the vinyl might have been altered over time, hence the inconsistent results between Expt 2 and the baseline in Expt 5.

Table 5. *Batrachochytrium dendrobatidis*. Growth after inoculation at varying times post-exposure of zoospores and zoosporangia from culture broth onto TGhL agar plates by gloved (Vinyl, V1) digits repeatedly washed in distilled sterile water before each new exposure (Expt 5, V1). Shaded: growth; unshaded: no growth; X: contamination; *Bd*: *B. dendrobatidis*; *Bd*: *B. dendrobatidis*; % *Bd*: no. of uncontaminated plates with *Bd* growth/no. of uncontaminated plates (%)

No. of washes	Time post-exposure (min) 0	0.5	1	2	3	4	5	6	%*Bd*
Baseline	■	■	■	■			■		62.5
1	■	■	■	■	■		■		75
2	■	■	■	■		■	■		75
3		■		■	■		■		50
4	■	■		■	■		■	■	80
5	■	■	■	■	■	■	■	■	100
6	■	■	■	■	■	■		■	87.5
7	■	■	■	■	■	■	■		87.5
8		■	■	■	■	■	■	■	87.5
9		■	■	■	■	■	■	■	87.5
%*Bd*	70	100	80	100	80	60	90	50	

Table 6. *Batrachochytrium dendrobatidis*. Growth after inoculation at varying times post-exposure of zoospores and zoosporangia from culture broth onto TGhL agar plates by gloved (Vinyl, V2) digits repeatedly washed in distilled sterile water before each new exposure (Expt 5, V2. Shaded: growth; unshaded: no growth; X: contamination; *Bd*: *B. dendrobatidis*; *Bd*: *B. dendrobatidis*; % *Bd*: no. of uncontaminated plates with *Bd* growth/no. of uncontaminated plates (%)

No. of washes	Time post-exposure (min) 0	0.5	1	2	3	4	5	6	%*Bd*
Baseline	■	■	■	■	■	■	X	■	100
1	■	■	■	■	■	■	■	X	100
2	■	■	■	X	■			■	71.4
3	■	■	■	■	■	■		■	87.5
4	■	■	■	■			■	■	75
5	■	■	■		■			■	62.5
6	■			■	■		■	■	62.5
7	■	■	■	■				■	62.5
8	■		■	■	■	■		■	75
9	■	■	■	■	■	■	■		87.5
%*Bd*	100	80	90	88.9	80	50	44.4	88.9	

DISCUSSION

These experiments were not designed to replicate working conditions in the field, but to assess potential survival of *Batrachochytrium dendrobatidis* on different surfaces and, hence, the potential for transmission of *B. dendrobatidis* by these surfaces. Human skin can kill *B. dendrobatidis* after a brief period of contact, but the killing effect decreased after repeated washing of hands with water or with 70% ethanol. Loss of killing effect by repeated washing suggests that the hands of a person catching frogs with bare hands in an aquatic environment will also lose their antichytrid properties. Nitrile gloves had a killing effect on *B. dendrobatidis*, but this was decreased by washing gloves with water. Latex gloves, which have been found to be lethally toxic to some amphibian larvae (Gutleb et al. 2001, S. Cashins pers. comm.), and polyethylene gloves had no significant killing effect, while the effect of vinyl gloves varied by batch.

Why did *Batrachochytrium dendrobatidis* die after contact with human skin? Although *B. dendrobatidis* is temperature sensitive, and human hand skin temperature of 32.1°C (Kokubo et al. 1998) is above the upper temperature limit for growth of *B. dendrobatidis*

(≥29ºC), the rapidity of the killing effect (2 min) is too quick to be explained by temperature. Since drying could not account for this effect, antifungal peptides may play a role. The skin of some amphibian species secretes peptides capable of inhibiting *B. dendrobatidis in vitro* (Rollins-Smith et al. 2003, 2005) and may give some protection against infection (Rollins-Smith et al. 2002, Woodhams et al. 2006). Since human skin is also capable of producing antifungal peptides (Braff et al 2005, Schröder & Harder 2006), survival of *B. dendrobatidis* on human epidermis might also be affected by the presence of antifungal peptides. The loss of the human skin killing effect after washing hands in water or repeated disinfection with ethanol could be explained by the removal of antifungal peptides from the skin surface.

Similarly, the killing effect on *Batrachochytrium dendrobatidis* of nitrile gloves and its disappearance after repeated washing could be explained by an antifungal agent present on the surface of the gloves, not nitrile per se, since a killing effect due to nitrile would not be decreased by washing. The manufacturers of the nitrile gloves do not claim any antifungal effect. The variation in killing effect between batches of vinyl gloves within the same brand highlights that the killing effect on *B. dendrobatidis* is probably related to an aspect of the manufacturing process that is not well regulated. Perhaps it is the nature of the powder on the surface of the gloves, as suggested for nitrile gloves. However, since none of the manufacturers provide details on the composition of this powder on their packaging, we are unable to comment further.

It could be argued that these experiments conducted in sterile *in vitro* conditions do not reflect what happens *in vivo* where *Batrachochytrium dendrobatidis* is mainly present intracellularly as zoosporangia in the keratinised layers of adult amphibian epidermis and keratinised mouth parts of amphibian larvae. As such, *B. dendrobatidis* zoosporangia might not transfer as readily as they do from culture media. However, their intracellular location in shed amphibian skin might allow them to survive longer on human skin and even fomites. Infected skin sloughs are infective to healthy amphibians (Berger et al. 1998). It is unclear what happens to zoospores released at the surface of amphibian skin. In theory, they are probably more easily transferred, but might not survive as long as the zoosporangia. Despite this uncertainty, the risk of transmission remains and so does the need for appropriate hygiene protocols.

These experiments show that single use of disposable gloves is the only handling technique providing 100% confidence that *Batrachochytrium dendrobatidis* will not be transmitted between amphibians. The concern about latex toxicity to tadpoles (Gutleb et al. 2001, S. Cashins pers. comm.) makes latex gloves less suitable than other disposable gloves. However, a single use of latex gloves for handling adult frogs is better than using bare hands. The results also suggest that multiple usage of disposable gloves poses a greater risk of transmission than bare hands. Consequently, in situations where availability and cost of disposable gloves may make surveys prohibitively expensive, use of bare hands is a preferable, although imperfect, alternative to the continued use of the same pair of gloves. Use of 70% ethanol to disinfect hands in this situation would, in theory, reduce risks of transmission between frogs by killing *B. dendrobatidis* contaminating the skin. However, the effectiveness of this treatment on transmission has not been tested and we recommend the single use of disposable gloves.

Another aspect not addressed by these studies is that handling amphibians with bare hands or multiple use gloves could transfer *Batrachochytrium dendrobatidis* DNA to subsequent amphibians, which may result in false positive PCR test results, although field work suggests this is uncommon (L. Skerratt pers. comm.). Hence, owing both to the risks of transmission of *B. dendrobatidis* and the potential transfer of PCR positivity when amphibians are handled with bare hands or multiple use of disposable gloves, we recommend only single use of disposable gloves for handling amphibians.

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