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NOTE

Glove decontamination procedures to prevent pathogen and DNA cross-contamination among frogs

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ABSTRACT: Working with aquatic organisms often requires handling multiple individuals in a single session, potentially resulting in cross-contamination by live pathogens or DNA. Most researchers address this problem by disposing of gloves between animals. However, this generates excessive waste and may be impractical for processing very slippery animals that might be easier to handle with cotton gloves. We tested methods to decontaminate cotton or nitrile gloves after contamination with cultured *Batrachochytrium dendrobatidis* (*Bd*) or after handling heavily *Bd*-infected *Xenopus laevis* with layered cotton and nitrile gloves. Bleach eliminated detectable *Bd* DNA from culture-contaminated nitrile gloves, but gloves retained detectable *Bd* DNA following ethanol disinfection. After handling a *Bd*-infected frog, *Bd* DNA contamination was greatly reduced by removal of the outer cotton glove, after which either bleach decontamination or ethanol decontamination followed by drying hands with a paper towel lowered *Bd* DNA below the detection threshold of our assay. These results provide new options to prevent pathogen or DNA cross-contamination, especially when handling slippery aquatic organisms. However, tradeoffs should be considered when selecting an animal handling procedure, such as the potential for cotton gloves to abrade amphibian skin or disrupt skin mucus. Disposing of gloves between animals should remain the gold standard for maintaining biosecurity in sensitive situations.

KEY WORDS: Chytrid · Chytridiomycosis · Cross-detection · DNA clearance · qPCR assay · African clawed frog

1. INTRODUCTION

Professionals in many disciplines handle aquatic animals on a regular basis (e.g. field researchers, conservation biologists, land managers, zookeepers, and aquarists), potentially causing unintended transmission of infectious diseases between animals or populations (Gray et al. 2017, 2018). When the purpose of animal handling is to collect samples (e.g. skin swabs for DNA analysis), it is important to prevent cross-contamination by non-infectious material (e.g. intact DNA) that might reduce the integrity of data collection (Raffel et al. 2013, Gray et al. 2017). Researchers often address this problem by changing gloves between handling animals in field studies, especially when working with species threatened by emerging infectious diseases (Gray et al. 2017). This approach is widely considered the gold standard, especially when biosecurity is a major concern (Havlíková et al. 2015, Gray et al. 2017). However, disposing of gloves between individuals is costly and results in excessive waste (Gray et al. 2017), and needing to pull dispos-

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able gloves over wet hands can increase processing time when handling large numbers of animals in experimental studies or surveys (T. R. Raffel & J. E. Noelker pers. obs.). Hence, there is a need for validated methods to decontaminate gloved hands between handling individual animals.

Pathogen and DNA cross-contamination are of particular concern for researchers studying amphibian chytridiomycosis, a skin disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) (Raffel et al. 2013, 2015, Havlíková et al. 2015, Gray et al. 2017, 2018). Some researchers have addressed this issue via chemical sanitization of gloves (Cashins et al. 2008a, Raffel et al. 2013, 2015, Gray et al. 2017). Several chemical agents have been shown to effectively kill the infectious stages of chytrid fungi (Johnson et al. 2003, Webb et al. 2007, Becker & Gratwicke 2017, Van Rooij et al. 2017). Some of the most effective disinfectants are ethanol and bleach (active ingredient: sodium hypochlorite = NaOCl), both of which killed *Bd* within 30 s at high concentrations (70% ethanol and ⩾1% NaOCl; Johnson et al. 2003). Ethanol dries within seconds, leaving no toxic residue behind that might affect the health of subsequent animals. However, ethanol is commonly used to preserve DNA samples and may not prevent DNA cross-contamination. Bleach may prevent DNA cross-contamination by denaturing *Bd* DNA (Cashins et al. 2008a). However, bleach may also irritate researchers' skin, damage clothing and equipment, or leave behind chloride residues that may be toxic to amphibians (Green 2009). Raffel et al. (2013) addressed the latter concern by rinsing bleach-decontaminated gloves with a commercial dechlorinating agent. However, to our knowledge, the effectiveness of this procedure to prevent DNA cross-contamination has not been formally tested.

The choice of gloves is also important. Nitrile gloves and even bare hands have fungicidal properties (Mendez et al. 2008, Thomas et al. 2020), but nitrile or latex gloves may be directly toxic to amphibians (Gutleb et al. 2001, Cashins et al. 2008b). Nitrile exam gloves add a further complication when working with slippery aquatic organisms such as *Xenopus laevis*, by preventing researchers from securely gripping the animal (Green 2009). Researchers can improve their grip on slippery organisms (and add separation between animals and potentially toxic nitrile gloves) by using a dampened cloth or cotton glove (Horsberg 1994, Reed 2005). However, cotton gloves are more expensive to replace than nitrile exam gloves, increasing the incentive to decontaminate gloves for reuse. Here we validated procedures for decontaminating nitrile and cotton gloves following contamination with a *Bd* broth culture or after handling *Bd*-infected *X. laevis*.

2. MATERIALS AND METHODS

2.1. Expt 1: decontaminating cotton and nitrile gloves directly contaminated with cultured *Bd*

We compared the effectiveness of various methods to remove detectable *Bd* DNA from 100% cotton (Uline Cotton Inspection Gloves S-19283) or nitrile (Syngaurd Nitrile Exam Gloves NGPF 7002) gloves following direct contamination with a *Bd* broth culture. Experimental nitrile gloves were worn alone (without cotton gloves), and experimental cotton gloves were worn over nitrile gloves that were not swabbed for this test. To contaminate gloves, we pipetted 1 ml of a *Bd* broth culture (strain JEL423, grown for 18 d at 20°C) across the palm and fingers of each glove. Four replicate cotton gloves were swabbed immediately following each of 4 randomly assigned treatments: (1) negative control (1 ml ultrapure water), (2) *Bd* contamination, (3) autoclave sterilization, or (4) laundry sanitization (see Section 2.5). Four replicate nitrile gloves were swabbed immediately following 1 of 4 randomly assigned treatments: (1) negative control, (2) *Bd* contamination, (3) bleach sanitization, or (4) ethanol sanitization (see Sections 2.3 and 2.4).

2.2. Expt 2: decontaminating gloves after handling a *Bd*-infected frog

As part of a larger *Bd* transmission experiment in 2020, we tested the efficacy of using either a bleach or ethanol procedure to decontaminate nitrile gloves worn under cotton gloves while handling 12 *Bd*-infected *Xenopus laevis*. Frogs had been individually exposed to 3×10^6 *Bd* zoospores (strain JEL423) and held for 4 wk at 8°C in groups of 10 frogs housed in plastic bins containing 4 l of dechlorinated tap water, on a 12 h light:12 h dark cycle with weekly water changes. When collecting swab samples, 1 researcher swabbed, while a second researcher served as the animal handler (Fig. 1A). We first swabbed each frog, with the animal handler wearing a pair of cotton gloves over nitrile gloves (see Section 2.6). Next, we swabbed the contaminated cotton glove (Step 1). The animal handler then removed the outer cotton glove, and we swabbed the underlying nitrile glove (Step 2).

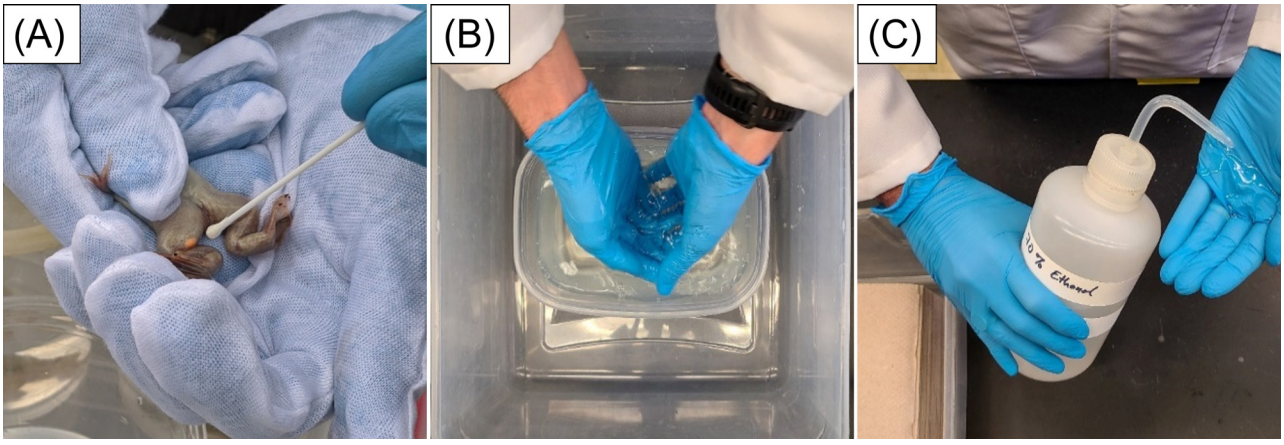

Fig. 1. Swabbing and decontamination procedures. (A) Collecting a skin swab with cotton over nitrile gloves. (B) Bleach—dechlorinate—rinse procedure, showing immersion of nitrile gloves in double-contained bleach. (C) Ethanol procedure. Photo credit: Andrea R. Nadjarian (A) and Declan S. McCrary (B,C)

The animal handler then decontaminated their nitrile gloved hands using either the bleach or ethanol sanitization procedure (see Sections 2.3 and 2.4), followed by a third swab sample (Step 3). The animal handler then donned a fresh pair of cotton gloves over the decontaminated nitrile gloves, followed by a final swab sample (Step 4). All animal use was conducted under Institutional Animal Care and Use Committee (IACUC) protocol 18011 and IBC protocol 2759-13.

2.3. Bleach decontamination of nitrile gloves

We tested a bleach—dechlorinate—rinse procedure to decontaminate nitrile gloves using 50% bleach (3% NaOCl; Fig. 1B). To sanitize contaminated nitrile gloves, the researcher immersed their gloved hands in 50% Pure Bright Liquid Germicidal Bleach (50% water; 0.11% NaOCl) solution for at least 30 s, an exposure time known to be 100% lethal to *Bd* (Becker & Gratwicke 2017). To deactivate chlorines remaining on the gloves following decontamination, gloved hands were immersed for 20 s in 30% dechlorination solution (300 ml Kordon AmQuel Plus Ammonia Detoxifier mixed with 700 ml water). This is >2000 times the manufacturer's recommended concentration to dechlorinate tap water. This was followed by a final rinse in aged tap water (Fig. 1B).

2.4. Ethanol decontamination of nitrile gloves

With goals of preventing cross-contamination by live *Bd* and reducing cross-contamination by *Bd* DNA, we tested a glove sanitization procedure using 70% ethanol (30% water). To sanitize contaminated nitrile gloves, the researcher used a squirt bottle to apply sufficient ethanol to fully wet their gloved hands, then lathered their hands for 20 s and dried them with an unbleached paper towel (Fig. 1C). Drying with a paper towel shortened the drying time and was hypothesized to physically reduce the amount of DNA-containing material.

2.5. Decontaminating cotton gloves

For the autoclave procedure, we used a liquid cycle with a 20 min sterilization period (Model 3AV-ADV-PRO Consolidated Sterilizer Systems). For the laundry procedure, we used a Giantex Model FW35-1508 laundry machine (30 l high-volume setting) with 400 ml concentrated bleach (final NaOCl concentration: 0.08%) and 72 ml laundry sanitizer (Lysol Laundry Sanitizer Additive; 2.4% quaternary ammonium compounds). This NaOCl concentration is sufficient by itself to kill *Bd* within 1 min (Becker & Gratwicke 2017). Washing involved 3 cold-water cycles (wash: 17 min; soak: 15 min; rinse: 16 min; spin: 7 min). Gloves were air-dried before reuse.

2.6. Swabbing procedures and *Bd* qPCR

We sampled each *Bd*-infected *X. laevis* following methods adapted from Raffel et al. (2013), by stroking a rayon fine-tip urethra swab (MW113, Medical Wire & Equipment Company) 5 times along the ventral

side of each thigh and foot. We sampled each glove by stroking a swab across the hand 5 times, starting at the palm and running down the length of each digit. We analyzed each swab by qPCR, using methods adapted from Hyatt et al. (2007). We generated a standard curve using serially diluted positive control plasmid DNA (Pisces Molecular) and calculated the number of zoospore genome equivalents (Z_e) per swab based on a published conversion factor for strain JEL423 (63.5 gene copies per zoospore; Altman & Raffel 2019). Assays were run in duplicate (1 well each at 1:10 and 1:100 dilution) for Expt 1, to guard against the possibility of qPCR inhibition at 1:10 dilution. We did not detect inhibition for Expt 1. We therefore assayed in singlicate at 1:10 dilution for Expt 2 to reduce costs (Kriger et al. 2006). We defined a positive result as $\geqslant 0.1$ Z_e based on the reported detection limit for this assay (Boyle et al. 2004). Results were analyzed in R (ver. 4.1.3) using the base package as well as 'Hmisc' and 'effsize' (Torchiano 2020, Harrell & Dupont 2021, R Core Team 2022). We log-transformed *Bd* qPCR data prior to statistical analyses to normalize model residuals ($\ln[Z_e + 1]$; $p > 0.05$ based on the Shapiro-Wilk test).

2.7. Nitrile glove decontamination cost–benefit analysis

To explore potential benefits of disinfecting gloves, we conducted an informal cost–benefit analysis for the mesocosm experiment referenced in Section 2.2. Within this study, a total of 180 frogs were each swabbed weekly over 9 wk. During each swabbing session, 1 animal handler and 1 swabber each used a single pair of nitrile gloves for each of 6 experimental blocks, using the bleach–dechlorinate–rinse procedure between individual animals. We calculated the total number, mass, and cost of nitrile gloves, and costs of materials used for the disinfection procedure (bleach, AmQuel, and paper towels), in comparison to what would have been needed if the animal handler changed gloves between individual frogs (costs based on vendor prices on January 3, 2024).

3. RESULTS

All negative-control gloves tested negative for both experiments. For Expt 1, contaminated swab samples from nitrile gloves exhibited 25 times higher *Bd* DNA levels (ln *Bd* load ± 95% CI = 11.51 ± 0.54) than swab samples from cotton gloves (8.29 ± 0.61). The autoclave sterilization and laundry sanitization procedure successfully eliminated all detectable DNA from culture-contaminated cotton gloves, as did the bleach–dechlorinate–rinse procedure for nitrile gloves. However, culture-contaminated nitrile gloves retained detectable DNA following ethanol decontamination (8.29 ± 0.76). For Expt 2, frogs with higher *Bd* loads tended to result in greater contamination of cotton gloves, though a Pearson's correlation test was non-significant ($r = 0.49$, $t_9 = 1.70$, $p = 0.123$). The level of *Bd* DNA contamination was reduced by each step of either the bleach or ethanol decontamination procedure (Fig. 2). Paired *t*-tests assuming unequal variances showed that removing contaminated cotton gloves significantly reduced the level of *Bd* contamination (Fig. 2; $t_8 = 6.17$, $p < 0.001$; Cohen's d ± 95% CI = 2.61 ± 1.36), as did the bleach decontamination procedure (Fig. 2; $t_{10} = 3.21$, $p = 0.009$; Cohen's $d = 1.37 \pm 0.99$). There were no detections of *Bd* DNA above our detection threshold ($Z_e \geqslant 0.1$) for nitrile gloves following the ethanol decontamination procedure or (fresh) cotton gloves pulled over decontaminated nitrile gloves (Fig. 2, Table 1). One out of 6 nitrile gloves had a low positive result (0.57 *Bd* Z_e) following the bleach decontamination procedure (Fig. 2, Table 1). Two-sample *t*-tests revealed no significant differences between gloves in the bleach versus ethanol decontamination treatments at any of the 4 stages of the glove decontamination procedure (Fig. 2; all $p > 0.3$).

Cost–benefit analysis found that using the bleach–dechlorinate–rinse procedure in our mesocosm experiment reduced nitrile glove waste by ~93.5%

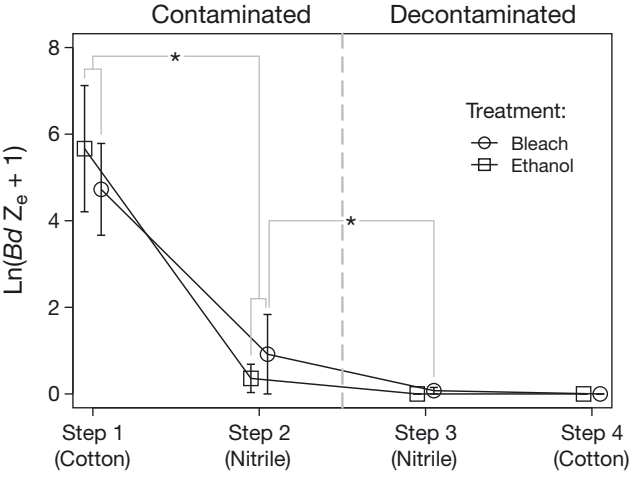

Fig. 2. *Batrachochytrium dendrobatidis* (*Bd*) zoospore equivalents (Z_e) detected on cotton and nitrile gloves before and after the bleach or ethanol decontamination procedure in Expt 2. Error bars: SE. *Significant reduction ($p < 0.05$)

Table 1. Detection of *Batrachochytrium dendrobatidis* (*Bd*) DNA on contaminated cotton or nitrile gloves from Expt 2, before or after each step of the decontamination procedure. We (1) swabbed 12 frog-contaminated cotton gloves (1 swab was removed due to a labeling error); (2) swabbed the underlying nitrile glove after removing the cotton glove (2 swabs were misplaced prior to qPCR); (3) swabbed the nitrile glove again after decontamination with either bleach or ethanol ('Treatment'; 6 gloves each); and (4) swabbed a fresh cotton glove after placing it over the decontaminated nitrile glove. We also swabbed 6 negative control gloves that were never exposed to *Bd* contamination (3 nitrile and 3 cotton). Swabs were counted as 'positive' if we detected ⩾0.1 zoospore equivalents (Z_e) of *Bd* DNA. na: not applicable

Glove type	Procedure step	Status	Treatment	Positive	Negative
Cotton	1	Contaminated	na	9	2
Nitrile	2	Contaminated	na	3	7
Nitrile	3	Decontaminated	Bleach	1[a]	5
Nitrile	3	Decontaminated	Ethanol	0	6
Cotton	4	Fresh glove	Bleach	0	6
Cotton	4	Fresh glove	Ethanol	0	6
Cotton	na	Negative control	na	0	3
Nitrile	na	Negative control	na	0	3

[a]This sample returned a value of 0.57 Z_e and could be a false positive

(0.68 vs. 10.5 kg) and overall decontamination costs by ~75.4% ($118 vs. $481), relative to if we had changed gloves between individual frogs.

4. DISCUSSION

We verified that cotton and nitrile gloves could be successfully decontaminated from *Bd* DNA using multiple procedures. Either autoclaving or laundry sanitization succeeded at eliminating detectable *Bd* DNA from cotton gloves, as did a bleach–dechlorinate–rinse procedure for nitrile gloves. An ethanol decontamination procedure reduced but did not eliminate *Bd* DNA from directly contaminated nitrile gloves; however, the ethanol rinse succeeded at lowering *Bd* DNA below the detection limit of our assay for nitrile gloves worn underneath contaminated cotton gloves. This appears to be because DNA-containing material was absorbed by the cotton gloves, resulting in decreased detectability of *Bd* DNA on contaminated cotton gloves relative to similarly contaminated nitrile gloves (Expt 1), and a great reduction in *Bd* DNA contamination for underlying nitrile gloves following removal of a contaminated cotton glove (Expt 2).

Our results show that removal of cotton gloves between handling animals can reduce DNA cross-contamination to subsequently handled animals, to the point where even a 70% ethanol rinse followed by drying hands with a paper towel was sufficient to reduce DNA below the 0.1 Z_e detection limit of a standard qPCR assay. The latter reduction was most likely due to physical removal of DNA by wiping with the paper towel, given that ethanol does not denature DNA. Future investigations could test these procedures with species that might be more sensitive to *Bd* infection than *Xenopus laevis*, to ensure that no transmission occurs following repeated handling with decontaminated gloves.

Cotton gloves may be useful for handling slippery aquatic animals and, like nitrile gloves, are generally considered safe for human use. However, researchers should consider potential negative effects of handling amphibians with fabric, which could possibly abrade skin or disrupt mucus coats (Horsberg 1994, Reed 2005). Researchers should certainly avoid overly thick, dry, or rough cotton gloves (Reed 2005). Fabric gloves should be avoided for smaller species that might be disproportionately at risk for injury (Horsberg 1994), or really any species that can be adequately handled with regular nitrile gloves. Nevertheless, the improved grip of cotton gloves may be worthwhile in certain circumstances, such as experimental settings where dropping a particularly slippery animal might cause injury or loss of biocontainment.

We have successfully used the bleach–dechlorinate–rinse procedure for decontaminating gloves in *Bd* infection experiments with multiple species (Raffel et al. 2013, 2015), including with cotton gloves for experiments with *X. laevis*, and observed no apparent negative health effects on individuals over several weekly swabbing sessions (T. R. Raffel & J. E. Noelker pers. obs,). These procedures resulted in substantial waste reduction and cost savings. We did not notice any problems with glove deterioration, consistent with a prior study that found minimal deterioration of

nitrile gloves after 20 disinfection cycles with either bleach or ethanol (Esmizadeh et al. 2021). These procedures may be useful for researchers interested in preventing cross-contamination by pathogens or DNA while working with aquatic organisms, and wishing to reduce costs and waste produced from experiments or surveys. Nevertheless, we believe changing gloves between animals (or populations) should remain the preferred option when biosecurity is a significant concern, such as when sampling multiple ponds or working with endangered species in a zoo setting.

Data availability. Code and data can be accessed here: https://github.com/jaynoelker/Noelker_etal_GloveDecontamination

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Author contributions. All authors helped conceive the project, complete experiments, and edit the manuscript; J.E.N., V.A.R., and T.R.R. conducted analyses; J.E.N. and T.R.R. wrote the manuscript.

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