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Research article

Assessing the effects of biosecurity measures in terrarium management

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Abstract

As wild populations of amphibians and reptiles are threatened by habitat loss and emerging diseases, the importance of captive populations serving as survival assurance colonies and stock for reintroduction programmes increases. As does the need for adequate biosecurity procedures to reduce risks of pathogen spread within captive populations. This study documents the pathways of pathogens induced during some of the daily husbandry procedures performed by zookeepers, and how they can be mitigated. The study compares the effectiveness of two different biosecurity measures, individually and combined, at reducing pathogen transfer. Ten zookeepers performed daily husbandry routines on 10 simulated terrariums using no biosecurity measures, or using designated tools, disposable gloves, or a combination of the two. The effectiveness of these measures to avoid pathogen spread was investigated through the use of a UV tracer, allowing detection of contamination of subsequent enclosures. The study documented a significant difference between the degree of contamination in the four trials ($P<0.0001$), with the combination of gloves and dedicated tools providing the lowest degree of contamination ($P<0.0001$ compared to the control scenario). Although there was a tendency for gloves to reduce contamination, neither gloves nor dedicated tools alone significantly decreased contamination. The study clearly demonstrates the dramatic effect of simple biosecurity measures for reducing pathogen spread among animal enclosures and introduces a simple yet effective tool to the field of zoo management.

Introduction

Increasing globalisation and anthropogenic movement spreads new pathogens, such as the devastating fungus *Batrachochytrium dendrobatidis*, around the globe at an alarming rate (Pessier and Mendelson 2010). Zoos are engaged in countering species loss through captive breeding but may also serve as melting pots where pathogens move from one species to another. When novel pathogens enter an animal collection, humans can be effective vectors of pathogens (Reiss and Woods 2011).

Without biosecurity measures to mitigate transfer of infectious agents, diseases may spread among enclosures leading to clinical illness, reproductive failure and death. In extreme cases, reintroduction programmes may back-fire when reintroduced animals infect native populations with novel pathogens (Walker et al. 2008). Cleaning procedures, protective clothing and personal protective equipment (PPE) are expected to be effective biosecurity measures, but very little documentation of their effectiveness exists. Studies from the healthcare sector show that biosecurity measures such as PPE can mitigate the spread of contaminated matter during simulation scenarios (Drew et al. 2016) and that fluorescent markers may be useful in tracking contamination in these scenarios (Bell et al. 2015).

This study aimed to document pathogenic spread during routine management of simulated animal enclosures and assessed the efficacy of two different biosecurity measures:

the usage of PPE (disposable gloves) and the usage of equipment dedicated to each enclosure. The hypothesis was that the application of these two measures individually, would decrease the amount of contamination, and when combined, would near completely abolish pathogen spread.

Materials and methods

Study design

In a dimly lit room, 10 similar enclosures were set up on a table, each measuring 18x25x16 cm and simulating terraria commonly used for amphibians and smaller reptiles. Each enclosure was equipped with substrate, a water bowl, a hide box and a piece of banana intended to simulate faeces. In one enclosure, a thin layer of UV detectable melamine resin (plastic) powder (Glo Germ Company, Moab, Utah, USA), was scattered to simulate pathogenic contamination. A small amount of calcium carbonate powder was also scattered in all enclosures, as the UV detectable powder had a faint visible white appearance. This way, it was impossible for the participants to detect which enclosure had been contaminated.

Ten zookeepers were tasked with performing a series of routine management procedures on each enclosure: removing faeces (banana), lifting the hide box to check on hiding animals (of course no animals were present in the simulated enclosures), and emptying a water bowl, scrubbing it with a cleaning sponge and refilling it with water from a watering can. The test subjects were asked to perform the procedures with a normal level of awareness, but the manner in which they performed the procedures was left to the subjects to decide.

The subjects were instructed to service the enclosures in the same order, from right to left, in each of four scenarios: first, with gloves (but not changing them); second, using a new set of disposable gloves for each enclosure; third, using gloves (but not changing them) and dedicated equipment in the form of a dedicated cleaning sponge for each enclosure; and fourth, a combination of the two measures, both using and changing disposable gloves and using a cleaning sponge dedicated to each enclosure. The order in which the subjects performed the trials was randomised.

After each trial, an ultraviolet lamp (model UVL 100, Glo Germ Company, Moab, Utah) was used to detect the fluorescent contamination. Based on a pilot study, contamination was divided into four levels: No contamination (score 0); Low degree (score 1) of contamination was registered as an area covering more than single spots but <5 cm^2; Medium degree (score 2) was registered as an area covering 5–10 cm^2; and High degree (score 3) was registered as an area covering >10 cm^2. Contaminated areas were constituted of cumulative areas of contamination registered. Single spots and scattered single spots of contamination were not registered due to being possible accidental contamination created during set-up. The contamination level was assessed on four surfaces: the interior walls of the enclosure, the animal hide box, the substrate in the enclosure and the water bowl containing fluid. Thus, the total score for each enclosure could vary between 0 and 12. As the third enclosure was set up as the source of contamination for each trial, it was possible to detect the spread of contamination to seven enclosures (total maximal score 84), while the first two served as controls.

Between each scenario, the entire setup was cleaned and searched thoroughly with the UV lamp to reveal any remaining contamination that could affect future results. Then, a new identical scenario was prepared. Subjects were instructed to service the 10 enclosures in the same order each time.

Test subjects

The study group consisted of five female and five male, fulltime, formally trained zookeepers. The female test subjects' age range was 28–51 years (mean age: 37 years) and their seniority range was 3–30 years (mean seniority: 12.4 years). The male test subjects' age range was 29–42 years (mean age: 35.4 years) and their seniority range was 5–13 years (mean seniority: 9 years). To avoid bias, none of the keepers witnessed another keeper performing the simulations.

Statistics

After assessing normality using D'Agostino and Pearson omnibus normality test (GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA), the total scores for each scenario were compared using a one-way ANOVA followed by Tukey's multiple comparisons test. Total score, seniority and age

Figure 1. Photograph showing setup of 10 enclosures mimicking small terraria each containing a hide and a water dish.

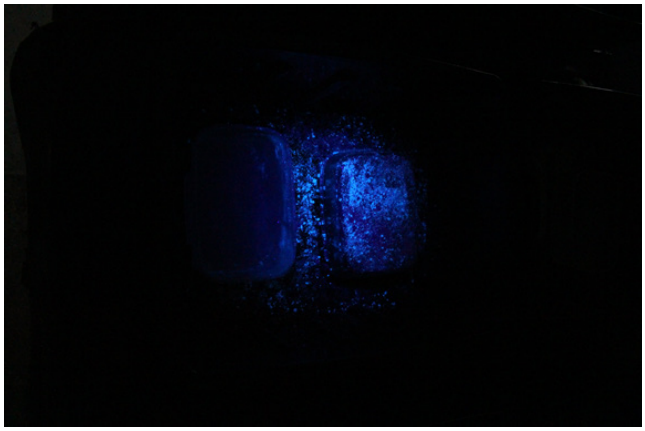

Figure 2. Example of contamination with the fluorescent Glo Germ product detected with UV light.

were compared between the two sexes using a student's t-test, and the correlation between total score and age and seniority, respectively, was investigated using simple correlation.

Results

Figure 3 illustrates the degree of contamination for each of the biosecurity measures. The scores were normally distributed, and there was a significant difference between the degree of contamination in the four trials ($P<0.0001$) with the combination of gloves and dedicated tools providing the lowest degree of contamination ($P<0.0001$ compared to the control scenario). Using gloves decreased the degree of contamination (mean score 18.7 vs 29.3), but this difference was not significantly different ($P=0.1012$). No significant difference was detected between control and dedicated tools ($P=0.9878$).

No effect of sex was seen in age, seniority or total score ($P=0.76$, 0.43 and 0.17, respectively), and no correlation between age or seniority and total score ($R^2=0.0382$ and 0.0134, respectively).

Discussion

In a comparable study, healthy tadpoles were handled with and without glove changes, in between the handling of individuals infected with a FV3 ranavirus. The study showed that not changing gloves between the handling of individuals drastically increased mortality risk of previously uninfected tadpoles (Gray et al. 2018).

The results of this study compare to those of a healthcare study in a simulated emergency department. That study showed that between test persons working within a scenario with a simulated spread of an infectious disease, applying partial PPE proved to be significantly inferior to applying all PPE available (Drew et al. 2016).

Based on the findings in this study, the application of disposable gloves has a measurable effect when it comes to mitigating pathogenic pathways, during routine management of enclosures, an effect that would likely have been statistically significant had more subjects or trials been included. While it was hypothesised that using dedicated cleaning sponges would have a similar effect on its own, this was not observed to be the case, while a combination of the two measures resulted in an almost complete reduction of contamination. It may be speculated that the dedicated sponge caused subjects to concentrate contamination that was then carried on to the next enclosures on their hands. This study clearly demonstrates how simple biosecurity measures may have massive impact on the risk of disease transmission, providing professionals working with animal management in captive enclosures evidence upon which informed decisions on implementing such measures can be made.

However, the study also reveals that even with highly effective biosecurity measures, transmission of disease still occurred, and this could prove fatal when dealing with an infectious pathogen. It could be hypothesised that the efficiency of biosecurity measures depends on the level of training and attention to detail of application by the zookeeper. As the results of the study shows, sex, age and seniority do not necessarily prove to be contributing factors in improving use of biosecurity measures. The study highlights that, even when using effective biosecurity measures, one must still anticipate the risk of spreading new disease, as biosecurity is a risk-based process.

The Glo Germ product is marketed as a training aid to assess efficacy of hand washing and surface cleaning, and to avoid cross-contamination, specifically in regard to transmission of microbes (Glo Germ Company, Moab, Utah, USA). With a particle size of 5 µm or smaller, this powder realistically mimics bacteria, and hence is capable of simulating bacterial transmission (Guo et al. 2014). Particle size is similar to *Mycobacteria marinum* that has a size of up to 4 µm (Aubry et al. 2017), and zoospores of *Batrachochytrium dendrobatidis* that are 3–5 µm in diametre (Berger et al. 2005); but is not similar to enveloped ranavirus FV3 with a diametre of 0.16–0.2 µm (Goorha et al. 1999). The small particle size, however, also means that the powder may become airborne. Single spots and scattered single spots of contamination were not registered as true contamination. This low density of contamination could have several causes, including contamination during new setups, and airborne spread in between management procedures not related to the actual experiment. These spots were detected in the control enclosures, and they were therefore not included in data analysis.

This study aimed to simulate a zookeeper working in enclosures and performing routine husbandry procedures. Although in a regular workday, the design and size of enclosures will differ compared to those used in the study, and there may be different approaches to these enclosures and their inhabitants, the main husbandry procedures are roughly the same as they would be in a real zoo setting. It is therefore suggested that the results are applicable to routine animal management in zoos.

A potential flaw to the study could be its low sample size in the number of participating test subjects. A larger test base would provide a larger sample of results to assess. Future studies could include more realistic scenarios of husbandry practices, by having more varied cleaning and maintenance tasks, along with varied enclosure sizes and interior designs. Lids and locks are frequently used for animal enclosures, and may act as significant contamination surfaces, yet neither of these were applied to the enclosures in the study. Other possible disease vectors could be included in a future study, including a zookeeper's working accessories, such as the keys used for several enclosures or departments and communication devices.

Nonetheless, this study introduces an easily applicable tool for simulating and understanding contamination and pathogen transfer in animal enclosures, and the results will be able to guide professionals working with animal management in captive enclosures to make informed decisions on the implementation of biosecurity measures.

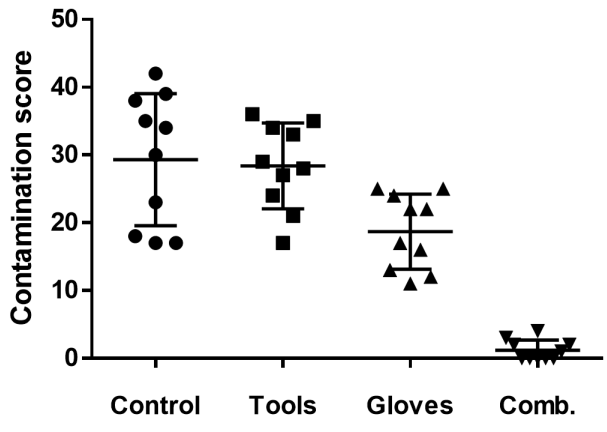

Figure 3. Mean contamination score of 10 zookeepers performing routine management procedures on 10 enclosures with no biosecurity measures (control), designated tools (tools), disposable gloves (gloves), and a combination of tools and gloves (comb.)

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