frontiers | Frontiers in Amphibian and Reptile Science

TYPE Original Research
PUBLISHED 08 December 2025
DOI 10.3389/famrs.2025.1698665

OPEN ACCESS

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RECEIVED 04 September 2025
ACCEPTED 30 October 2025
PUBLISHED 08 December 2025

CITATION
Viadanna PHdO, Brinkman A, Vimont B, Gray MJ, Warwick AR, Poudyal NC, Pearhill RAI and Brunner JL (2025) Prevalence of *Batrachochytrium dendrobatidis*, *B. salamandrivorans*, and *Ranavirus* in the US domestic pet amphibian trade. *Front. Amphib. Reptile Sci.* 3:1698665. doi: 10.3389/famrs.2025.1698665

Prevalence of *Batrachochytrium dendrobatidis*, *B. salamandrivorans*, and *Ranavirus* in the US domestic pet amphibian trade

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The global trade of live animals facilitates the spread of emerging pathogens, such as the deadly amphibian pathogens, *Batrachochytrium dendrobatidis* (*Bd*), *B. salamandrivorans* (*Bsal*), and *Ranavirus* spp (*Rv*). Yet little is known about their prevalence within domestic trade networks. We used an anonymous surveillance scheme to estimate the prevalence of *Bd*, *Bsal*, and *Rv* within and among businesses in the US domestic pet amphibian trade. We found that *Bd* was rare within affected businesses, but common among businesses, whereas *Rv* was common within affected businesses, but rare among them. However, the aggregate prevalence of both pathogens was very low: *Bd* prevalence was 1.9% and *Rv* was 2.9% among enclosures. *Bsal* was not detected in this or prior surveys among pet owners and is most likely absent. The rarity (or absence) of pathogens in the US domestic pet amphibian trade, in contrast to their relative pervasiveness at US borders, may be due in part to biosecurity practices common in the industry. Half of participants quarantined newly acquired animals and most used gloves. How practices and operation of the domestic pet amphibian trade magnify or, as our study suggests, reduce the risk of pathogen persistence or spread requires further study.

KEYWORDS

amphibian disease, biosecurity, pet trade, surveillance, disease dynamics

Introduction

The international trade of live animals has contributed to the spread and emergence of myriad pathogens that threaten humans, domestic animals, and wildlife alike (Cunningham et al., 2003; Daszak et al., 2000; Smith et al., 2009). For instance, the fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), linked to the decline and even extinction of hundreds

of amphibian species (Scheele et al., 2019), is thought to have spread within the amphibian trade (Liu et al., 2013). Its more recently discovered sister species, *B. salamandrivorans* (*Bsal*) (Martel et al., 2013), was likely introduced into northern Europe via the pet trade from Southeast Asia (Cunningham et al., 2015; Fitzpatrick et al., 2018; Sabino-Pinto et al., 2018), and spilled over into European fire salamanders (*Salamandra salamandra*), which have subsequently experienced massive declines (Spitzen-van der Sluijs et al., 2016; Stegen et al., 2017). Finally, viruses in the genus *Ranavirus* (*Rv*), which cause mass mortality events in amphibians, as well as bony fishes and reptiles, are thought to have spread with and spilled over from international trade (Kolby et al., 2014; Soto-Azat et al., 2016; Price et al., 2016; Herath et al., 2021).

These pathogens can be common in recently imported amphibians. For example, *Bd* was detected in 62% and *Rv* in 7.9% of American bullfrogs (*Lithobates catesbeianus*) imported into the US (Schloegel et al., 2009). Seventy percent of African clawed frogs (*Xenopus laevis*) exported from Hong Kong tested positive for *Bd* (Kolby et al., 2014). Although *Bd* prevalence was lower among ornamental pet amphibians, over half of them tested positive for *Rv* (Kolby et al., 2014). The assumption is that these pathogens escape from the trade network via the accidental or intentional release of infected animals or from improperly disinfected waste (Picco and Collins, 2008; Cunningham et al., 2015; Soto-Azat et al., 2016; Ribeiro et al., 2019; Martel et al., 2020).

This perspective, however, emphasizes the passive movement of pathogens across borders (Green et al., 2020; Connelly et al., 2023) and through domestic trade networks. It assumes these networks have little or no active role in shaping pathogen persistence and spread within a country, or in influencing the risk of pathogen spillover into wildlife. However, the US domestic pet amphibian trade network is comprised of diverse businesses, which vary a great deal in terms of their size, the diversity of species and morphs sold, as well as their values, price, knowledge of pathogens, and biosecurity practices employed (Cavasos et al., 2023a, b; de Oliveira Viadanna et al., 2025 *in review*), all of which may play a role in amplifying or diminishing pathogen prevalence. Indeed, routine biosecurity practices as well as active surveillance for pathogens should reduce the spread and persistence of pathogens in the network. For example, only 6-18% of pet amphibian businesses in the US reported *Bd* or *Rv* in their businesses at least once (Cavasos et al., 2023b), and less than 1% of pet owners reported having any issue with *Bd*, *Bsal*, or *Rv* (Cavasos et al., 2023a).

Estimates of pathogen prevalence within pet amphibians are generally low, but variable. For example, in Spain 2% of pet amphibians were positive for *Rv* and 10% for *Bd* (Thumsová et al., 2021). In the Czech Republic 5.1% of captive amphibians tested positive for *Bd* (Havlíková et al., 2015), and only 2.9% of captive amphibians from private owners, zoos, and laboratories in Belgium, the Netherlands, Germany, and France were positive for *Bd* (Sluijs et al., 2011). Infections are also far from ubiquitous among suppliers, too. In Singapore, only 11% of amphibians in pet stores were positive for *Bd* (Gilbert et al., 2012) and just five of 20 traders in Spain had *Rv* or *Bd* (Thumsová et al., 2021). Perhaps most surprisingly, while amphibian species capable of carrying *Bsal* have been (Gray et al., 2023), and continue to be, imported into the US (Connelly et al., 2023; Green et al., 2020) from regions where *Bsal* is endemic (Nguyen et al., 2017), this pathogen has never been detected in the US despite extensive surveillance efforts (Klocke et al., 2017). We posit that domestic pet amphibian trade networks play a key role in amplifying or diminishing the spread and persistence of pathogens, thus the risk of spillover over to naïve populations and species.

We set out to estimate the prevalence of *Bd*, *Bsal*, and *Rv* within and among businesses involved in the domestic US pet amphibian trade, and to determine if prevalence is associated with any particular amphibian species, types of businesses, or biosecurity practices they employ. We expanded a successful anonymous pathogen surveillance pilot program in which participating businesses were sent kits to screen amphibian housing enclosures, our unit of study, which were returned for pathogen screening (Pearhill et al., 2024). Participant businesses also provided information on their size, role in the pet amphibian trade network, and biosecurity practices. We then combined data from this and the pilot study to estimate the prevalence of each pathogen within and among businesses using a novel, zero-inflated model accounting for sampling without replacement.

Methods

We used several methods to recruit participants in this surveillance program, including pamphlets, in-person advertising at reptile expositions, and advertisements in social media outlets. To guarantee participant anonymity, we used a two-layer system (Figure 1). First participants answered a series of questions about their business in an online survey (Supplementary File 1), which generated a unique participant identity code. Their survey responses were anonymized by one of the researchers (ARW), who did not have access to any of their surveillance results. ARW would then forwarded to the researchers at Washington State University (WSU) the surveillance results. Participating businesses were then sent a pathogen surveillance testing kits through the Pet Advocacy Network (PAN), an advocacy organization for the US pet trade, which associated participant IDs with addresses and reshipped the kits to the participants (first layer of anonymity). If participants had questions, they were advised to contact PAN, who acted as an intermediary with the researchers so that businesses would remain anonymous. Participants returned their samples to WSU, using PAN's address in place of their own, so that WSU researchers would not know their identity. Each participant's surveillance results were posted on an encrypted online website accessible only with their unique participant ID and a personal identification number they created (second layer of anonymity). Thus, the researchers had access to the results and information about the businesses, but not the identity of the businesses, and PAN knew the business identity, but did not have access to the surveillance results.

Only participating businesses with complete information (sometimes after communication via PAN) and currently having

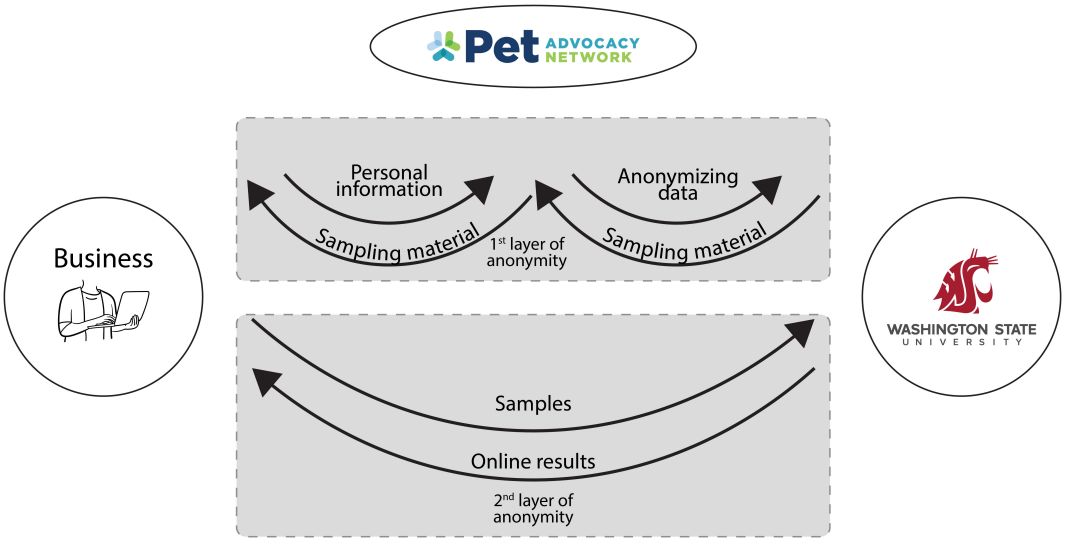

FIGURE 1
Diagram of the flow of information and materials between researchers at Washington State University (WSU) and participating businesses intended to ensure participant anonymity. The first layer of anonymity involved a third party (Pet Advocacy Network; PAN) acting as an intermediary for information and shipments of sampling materials so that researchers at WSU were unaware of participant identity. The second layer of anonymity involved sharing samples and results anonymously, without PAN's involvement. Businesses used PAN's address in place of their own when returning samples and results were posted online accessible with a unique participant ID and a PIN code.

pet amphibians in their facility were selected to participate. Participants with >10% of their amphibians coming from imports were shunted into a sister project focused on importers. Advertising for this study began in April 2023 and samples were accepted through June 2024.

Testing kits contained sampling materials (i.e., swabs for terrestrial habitats or eDNA filters and syringes for aquatic habitats, gloves, drying racks, Whirl-Pak bags, etc.), instructions (written materials and links to videos), a short questionnaire about biosecurity practices, and a pre-labeled, pre-paid return envelope (Supplementary File 2). Enclosures, or "habitats" in the instructions, were our units of sampling. They were defined as "tanks, containers, or groups of housing containers between which water or animals can move." We included sampling materials sufficient to sample up to 30 enclosures or all available enclosures in the facility, whichever was less. Participants were instructed to randomly select enclosures for sampling among all of their enclosures using an online random number generator. Participants swabbed the body (skin) of up to five animals per terrestrial enclosure (swabs were then pooled by enclosure) or filtered a ≥ 50 mL of water from aquatic enclosure through a 0.45 µm filter using a sterile 50 mL syringe. Swabs from each terrestrial enclosure were placed in a Whirl-pak bag with three 5 g desiccant pouches to preserve the collected DNA during storage and shipping. The filter from each aquatic enclosure was preserved with the addition of 2 ml of buffer ATL from a DNeasy Blood and Tissue kit (Qiagen) included in a separate syringe in the sampling kit, the ends of the filter housing sealed with plastic caps and placed in a whirl pack bag before shipment.

DNA was extracted from returned swab samples using an enzymatic lysis buffer, consisting of a solution of 20mM of Tris-Cl, 2mM of Na-EDTA, 1.2% Triton X-100, and 20 mg ml^{-1} of lysozyme (Abundo et al., 2021), followed by lysis with proteinase K and buffer AL, and DNA precipitation with 100% EtOH according to the Qiagen DNeasy Blood and Tissue Kits protocol. The mixture was then added to a spin column for DNA isolation and purification following the manufacturers' instructions. For the filter we added 0.2 ml of proteinase K to the 2 ml of buffer ATL included in the returned samples and incubated the filters, in their casing, for 12–24 hours at 56 °C. The solution in the filter housing was then transferred to a 5 mL tube by centrifugation. The whole lysate was filtrated using a QIAshredder spin column (Qiagen) and then extracted following the manufacturers' instructions.

Extracted DNA samples were screened for pathogen DNA using Taqman qPCR assays targeting *Bd* (Boyle et al., 2004), *Bsal* (Blooi et al., 2013), and *Rv* (Stilwell et al., 2018) in singleplex, triplicate 20 uL reactions with 5 uL of template DNA, for 45 cycles on a StepOnePlus thermocycler (Applied Biosystems, Waltham, MA, USA). Two dilutions of gBlock oligonucleotides (10^0 and 10^4 copies μL^{-1}) with the target sequences were used as positive controls. Samples were considered positive if at least two wells showed amplification.

We used a zero-inflated statistical model to account for the fact that we might not detect a pathogen in the samples from a given facility because: 1) the infection was not present in the facility or; 2) the infection was present in the facility, but not in our sample. The model estimates the prevalence of infection among enclosures within infected businesses, which we assume for simplicity is a constant p across infected businesses, and the prevalence of infection among businesses, θ. It uses a hypergeometric distribution, as opposed to the more common binomial likelihood, to account for the fact that we have samples from a

subset of habitats that were randomly selected, without replacement, from a finite number of enclosures in the facility. We assume that we would detect an infection if it were present within an enclosure because cohoused animals usually rapidly infect one another (Brunner et al., 2017; Malagon et al., 2020), and also that false positives did not occur.

Non-invasive, external samples, such as the skin swabs and eDNA samples we used here, might fail to detect *Rv*, which is a chiefly internal infection (as opposed to *Bd* and *Bsal*, which are skin infections). While prior work has shown that non-lethal methods can have comparable probabilities of detecting *Rv* as samples of internal organs (Ford et al., 2022), this is not always the case (Brunner et al., 2019). We were also relying on untrained personal to effectively swab sometimes very small amphibians. It is likely that at least low-level *Rv* infections might have gone undetected (Pearhill et al., 2024). However, low-intensity infections are also less likely to be transmitted (Brunner et al., 2025), so such infections may also be less consequential.

The zero-inflated model was implemented in a Bayesian framework, to accommodate our prior understanding of the relative rarity of pathogens in the US domestic trade (e.g., *Bsal* has not been detected in extensive surveillance), using Stan and the rstan package (Guo et al., 2024). See Supplementary File 3 for details of the model as well as justification for the priors we used. We fit this model to our data from this and the pilot study (Pearhill et al., 2024), combined. The sampling methods were very similar, and participants came from the same pool of businesses involved in the US pet amphibian trade. Just one participant in the current surveillance program reported having participated in the pilot project. This participant reported strong biosecurity practices and their samples did not test positive for any pathogens in the current project. Because of the anonymous nature of our data collection, their information cannot be matched between projects. In addition, even if some businesses participated in both years, these submissions occurred over a year apart. As a result, we treat these as unique and independent observations in our analyses.

Results

A total of 52 businesses volunteered to participate, but twelve were excluded (see Methods). Of the 40 included in the study, 15 participants (38%) returned samples for pathogen testing. The participants who returned samples were not obviously different from those who did not (nor statistically significantly different based on chi-square tests for categorical variables and *t*-test for numerical variables; results not shown). Most participating businesses reported that most of their amphibians came from wholesalers or distributors (median = 100%), though a small percentage came from importers, retail pet stores, wild-caught, or in-house breeding, and most sold directly to pet owners (median = 100%; Figure 2). Most businesses that returned samples had gross annual sale over $1,000,000 (53.33%), while the most common responses from businesses that did not return samples was sales between $200,001 and $1,000,000 (28%), with amphibians comprising less than 10% of their sale (40 and 44%, respectively) for both groups (Figure 3).

The questionnaire on biosecurity practices, completed alongside the sampling, showed that 7 out of 15 businesses (47%) quarantined newly acquired animals for periods ranging from 6 weeks to 3 months. One business reported that it "does not mix any animals," while three others indicated that quarantined animals were kept in separate rooms. Most (80%) businesses report that they used gloves when handling animals or cleaning enclosures (Figure 4). Of the 14 businesses that reported using gloves, 11 changed gloves between enclosures and three changed gloves between individual animals. Only one (6.6%) reported disinfecting wastewater and solid waste; it also quarantined new acquisitions for 3 months and used gloves for cleaning. One business reported that it did not use gloves but quarantined animals for 1–3 months.

We received a total of 107 samples from enclosures housing 470 individual animals across 44 amphibian species. Most species sampled were anurans, but caudates were the majority of the individuals in sampled enclosures (Table 1). Just one sample, an eDNA filter from an enclosure containing Iranian kaiser newts (*Neurergus kaiseri*), had detectable *Bd* DNA (cycle threshold = 38; estimated 0.59 copies ml^{-1}). This business was small ($5,000 to 50,000 in gross sales, 26% to 50% that came from amphibians), with a reported 50 enclosures in their facility. They received 50% of their animals from hobbyists, 49% from in-house breeding, and 1% from imports. They reported quarantining animals for one month and using gloves when handling animals. There was also just a single swab sample from an individually housed pacman frog (*Ceratophrys cranwelli*) with detectable *Rv* DNA (cycle threshold = 39; estimated 0.55 copies ml^{-1}). This business was large (> $1,000,000 in gross sales) but reported <10% of sales came from amphibians and had only two amphibian enclosures. Their animals came from hobbyists (20%), wholesalers (40%), imports (10%), and the wild (30%). They reported using gloves when handling and feeding animals, but not quarantining new acquisitions. *Bsal* was not detected in any samples.

In the combined data (this and the pilot project; Pearhill et al., 2024) three out of 23 businesses had *Bd* (one in our project, two in the pilot project), so intuitively the prevalence of *Bd* among businesses is 3/23 = 0.130. However, because *Bd* infections were rare within businesses ($\bar{p}$ = 0.052; 95% CI = 0.017 – 0.110), it is likely that our sampling missed infections in other businesses, especially those in which we sampled only a small fraction of the enclosures. Thus, our best estimate is that prevalence among businesses is much higher ($\bar{\theta}$ = 0.388; 95% CI = 0.128 – 0.730; Figure 5A). Collectively, our data suggests an aggregate prevalence —the probability that any randomly selected enclosure in the US pet trade is infected—of *Bd* of 0.019 (95% CI = 0.005 – 0.042).

Ranavirus was detected in just one facility—it was not found in the pilot project (Pearhill et al., 2024)—but in a facility that had only two enclosures. The estimated prevalence within businesses is thus somewhat higher than for *Bd* ($\bar{p}$ = 0.244) but with little confidence (95% CI = 0.033 – 0.557) in the estimate. The prevalence of infection among businesses is estimated to be lower than for *Bd* ($\bar{\theta}$ = 0.131, 95% CI = 0.022 – 0.346; Figure 5B). The aggregate

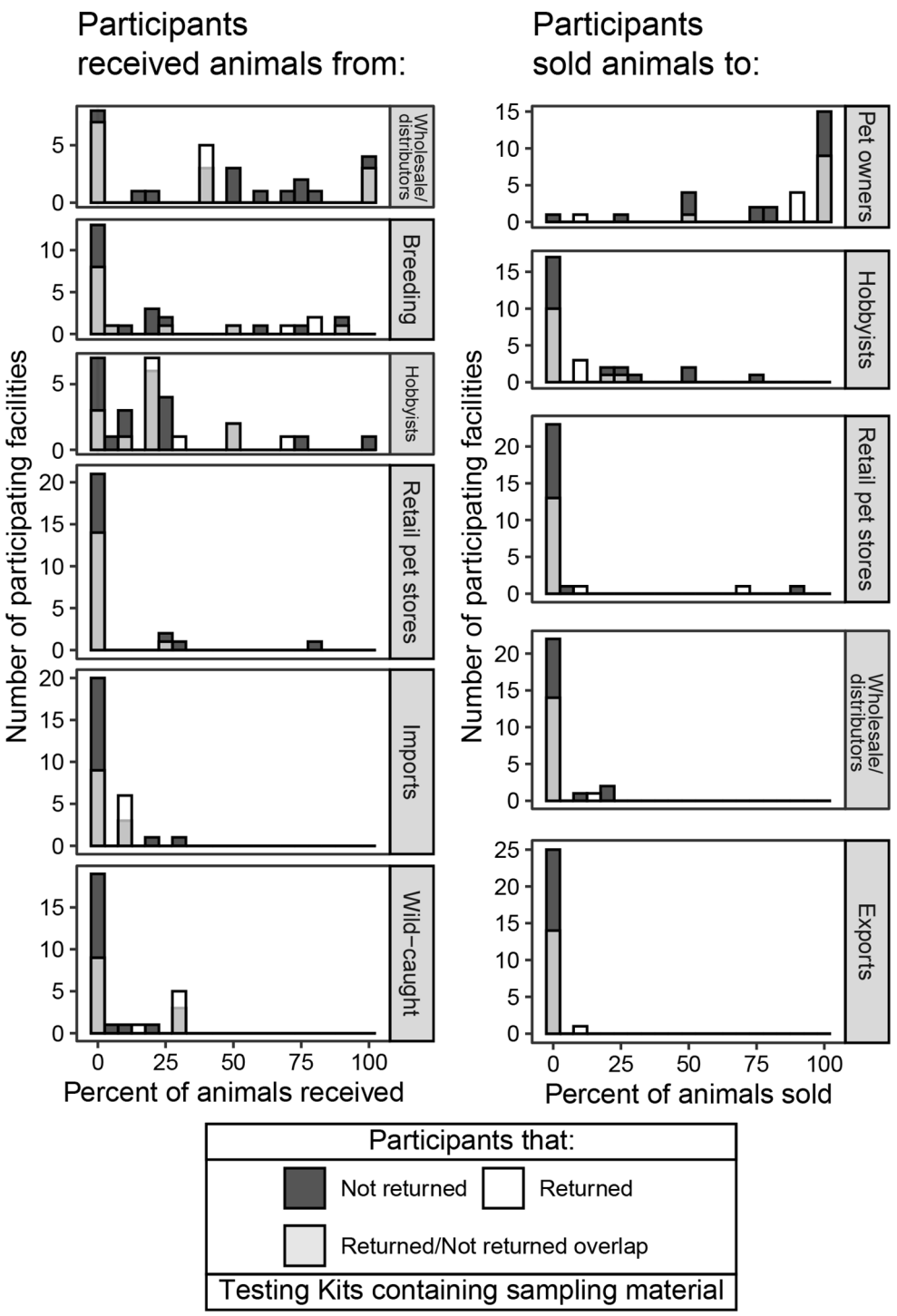

FIGURE 2
Graphic of the results of the online questionnaire from 40 participants showing the approximate percentage of pet amphibians received from each source and sold to each destination, from June 2023 to June 2024. We also included in the histogram the information regarding if participants returned or not the testing kits containing sampling material for the detection of pathogens, which are represented by the dark gray (not returned) and white (returned) box. Light-gray box was used to represent the overlapping of results from participants that did not return the kits with participants that returned the kits.

prevalence of *Rv* in the US pet trade is 0.028 (95% CI = 0.003 – 0.085).

Bsal was not detected in our samples. Our best estimate for prevalence within businesses is $\bar{p}$ = 0.086 (95% CI = 0.002 – 0.299), and among businesses is $\bar{\theta}$ = 0.013 (95% CI = 0 – 0.049; Figure 5C). These estimates, however, are jointly uncertain and so the boundaries changed by small amounts from our priors (Figure 5C). However, we can place an upper 95% CI on the

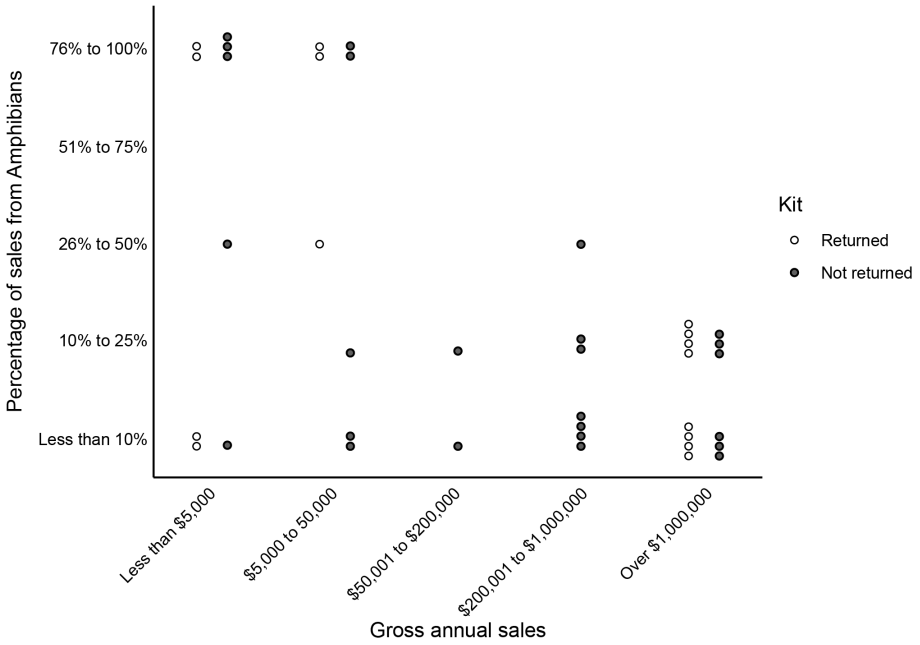

FIGURE 3
The approximate gross annual sales and percentage of those sales that came from amphibians were reported by the 40 participants in an online questionnaire, from June 2023 to June 2024. Participants that did and did not return testing kit samples are presented side-by-side within each category.

probability any randomly selected habitat in the US pet trade has *Bsal* at 0.004.

Discussion

Our aggregate estimates of prevalence—the probability of detectable infection in any randomly selected captive enclosure in a US business in the pet amphibian trade—was low across all three pathogens, essentially zero for *Bsal*. Our mean estimate of aggregate *Bd* prevalence (1.9%) in the US pet amphibian trade is largely consistent with prior estimates in other places. For instance, Havlíková et al. (2015) estimated a 5.1% aggregate prevalence in the pet amphibian trade in the Czech Republic, and van der Sluijs et al. (2011) a prevalence of 2.9% in Europe. A prior study of pet salamanders in the US found 1.1% *Bd* aggregate prevalence (Klocke et al., 2017). However, the aggregate prevalence of *Bd* was somewhat higher elsewhere: 10.4% among pet amphibians in trade fairs in

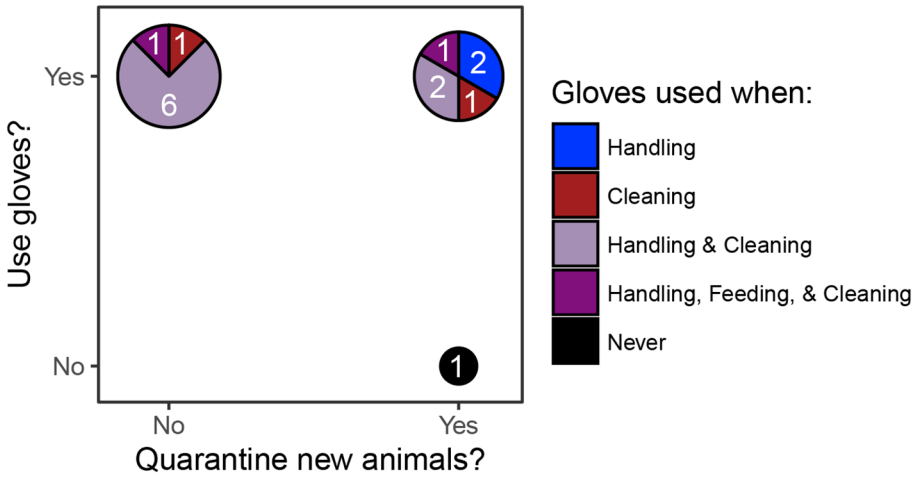

FIGURE 4
Self-reported use of quarantine and glove use among the 15 participants that returned samples for testing. Glove use if further divided into the categories of activities in which participants reported using gloves.

TABLE 1 Description number of species, number of enclosures and represented number of individuals of the received sampling material.

Species	N species	%	N enclosure	%	N individuals	%
Salamanders	19	43.2	53	49.5	297	63.2
Frogs	25	56.8	54	50.5	173	36.8
Total	44		107		470	

Spain and in Japan 6.9% in captive pet amphibians (Tamukai et al., 2014) and 21.9% in pet amphibians sold in eight stores, perhaps because many were wild imported animals (Goka et al., 2009).

Estimates of *Rv* in the domestic pet amphibian trade are limited compared to *Bd* and *Bsal* research (Thumsová et al., 2021), consisting mostly from imported population (e.g., Kolby et al., 2014; Schloegel et al., 2009), or from findings from wild or farmed sources (e.g., Brunner et al., 2019). So, our estimate of an aggregate *Rv* prevalence of 2.8% for the US pet amphibian trade is, to our knowledge, the first in the US. It is, however, consistent with the estimated 2.4% of amphibians in trade fairs in Spain positive for *Rv* (Thumsová et al., 2021).

Lastly, we did not find *Bsal* in the US domestic pet amphibian trade, nor has it been found in North America despite extensive testing (Govindarajulu et al., 2017; Klocke et al., 2017; Basanta et al., 2022). This is most consistent with the absence of *Bsal* in the US pet amphibian trade network. If it is present, it is exceedingly rare—the upper 95% CI on aggregate prevalence is just 0.4%. In European captive collections, *Bsal* also seems to be either absent (e.g., in Germany; Jung et al., 2020) or fairly uncommon, as in one study of 11 private collections in the United Kingdom, the Netherlands, and Spain linked to a *Bsal* outbreak, with an aggregate prevalence of ~8% (Fitzpatrick et al., 2018).

We think a more useful framework for understanding the potential for spread and spillover of established pathogens, such as *Bd* and *Rv*, is to separately estimate prevalence within and among businesses. The former can be seen as a signal of how infections are contained and suppressed or propagated within businesses, and thus the risk they present to businesses (and consumers) further down the chain of sales. The latter is perhaps most relevant for understanding the commonness of infections in the trade as a whole, as businesses can be thought of as nodes in a network. We might arrive at the same small aggregate prevalence through two scenarios.

First, a large fraction of businesses might each have a low prevalence of infection within them. We found that *Bd* may well fit this scenario: somewhere between 13% and 73% (mean = 39%) of businesses might reasonably be expected to have *Bd* in them, but within affected businesses, *Bd* was rare, with ~5% prevalence. The uncertainty in the estimated prevalence among businesses stems from the low prevalence of infection within businesses. These results are similar, if somewhat lower, than what was found in prior surveillance that targeted amphibians in pet stores (see Supplementary File 3 for discussion). This widespread, but low to moderate risk, suggests that from a businesses or consumer's point of view, it would be difficult to avoid buying animals from businesses with infected animals. However, even if animals came from any of these businesses, there is a good chance that the newly acquired animals would be infection free. It also implies that interventions would need to be broadly applied and focus on practices that reduce a low, but common risk.

Second, a pathogen might be uncommon among businesses but widespread within those businesses that are affected. This would suggest a very localized problem and strategies to minimize risk would involve identifying and targeting these few problematic businesses. The status of *Rv* appears to be in between these scenarios, although more like the second. *Rv* infections were rare among businesses, just 13% (95% CI = 2 – 35%), but more common

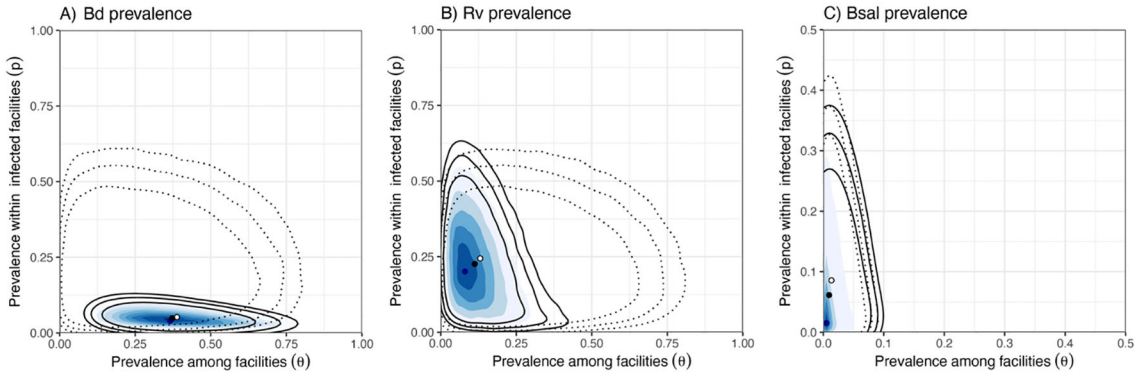

FIGURE 5
Estimative prevalence of *Bd* **(A)**, *Rv* **(B)**, and *Bsal* **(C)** within (*p*, y-axis) and among (*θ*, x-axis) businesses in the pet amphibian trade in the US. Prior (dotted lines) and posterior estimates (solid lines) are, from the outside to inside, central 80%, 90%, and 95% intervals. The blue shading provides a finer gradation of the posterior density. The dark blue points are the mode of the posterior, the black points the median, and the white points the mean.

among habitats within infected businesses (24%; 95% CI = 3 – 56%). However, infection was found in just one of two enclosures in a single business, so there is a great deal of uncertainty in our estimates.

In any case, both *Bd* and *Rv* seem to be less common in domestic trade than at US borders (Schloegel et al., 2009; Kolby et al., 2014). A similar pattern was observed in Japan, where 10.3% of imported pet amphibians had *Bd*, but only 6.9% of those in private collections and commercially bred pet amphibians (Tamukai et al., 2014). There are several possible explanations for this winnowing of prevalence.

First, we must acknowledge that our sample of participants may be biased towards businesses with greater knowledge of infectious diseases and biosecurity practices (Cavasos et al., 2023a), and thus less risk of infection.

Second, there are likely differences in the species imported, which are presumably more likely to carry infections, and those that are captive bred domestically, which may be lower risk. To the extent that the animals that were sampled in this study were a mix of imported and domestic, captive-bred animals, the prevalence of infection would, all else equal, be diluted. There is a great deal of information about the volume and identity of animals imported into the US (Eskew et al., 2020; Connelly et al., 2023), but very little about amphibians within the domestic trade (but see de Oliveira Viadanna et al., 2025, in review). There seems to be a growing emphasis, at least in the US trade, on captive-bred animals—most large reptile or exotic animal expositions and trade shows require animals to be captive-bred (Z. Brinks, pers. comm.)—though rare species and variants, which are usually supplied from the wild, still attract a great deal of interest among collectors in the US and European Union (Altherr and Lameter, 2020; Herrel and van der Meijden, 2014; Mohanty and Measey, 2019; Sinclair et al., 2021).

There may also be some active or passive selection against infected animals imported into the domestic trade. Infected animals are presumably more likely to die than uninfected individuals, but importers and distributors may also actively remove, treat, or cull obviously diseased animals. Either way, infected animals would be less likely to move on in the trade network and so the prevalence of infection would tend to be reduced without further transmission.

Lastly, conditions or practices within the domestic trade might reduce infections or prevent their spread. For instance, *Bsal* grows best between 10° and 15 °C, but not ≥25 °C (Martel et al., 2013), and temperatures within businesses may inhibit the growth, persistence, or transmission of *Bsal*. Similarly, businesses are frequently aware of risks from pathogens and employ biosecurity measures, such as quarantine, to prevent infections from being introduced and spreading within their facilities (e.g., glove use, disinfection; Cavasos et al., 2023b). Just under half of the businesses that returned samples reported quarantining newly acquired animals. All but one used gloves when feeding, handing, or cleaning animals. This same participant also reported that the gloves were changed in between enclosures. However, because infections were rare, there were no clear associations between infections at any particular host species, business types, or practices.

In our study, *Bd* was found in *Neurergus kaiseri*, which has five prior records of infection (Olson et al., 2021) (https://amphibiandisease.org/). *Rv* was detected in *Ceratophrys cranwelli*, which appears to be the first record in this species. *Bd* was found in a small business that quarantines animals, while *Rv* was found in a large business that does not quarantine.

It is clear from our research that *Bd* and *Rv* are rare pathogens circulating in the US domestic pet amphibian trade, and *Bsal* continues to be undetected. Indeed, it appears that infections are less common in the domestic trade network than at US borders. More studies are needed to understand which practices and aspects of the domestic pet amphibian trades are associated with the epidemiology of these pathogens, and to determine how customers and hobbyists can identify higher-risk businesses and avoid purchasing animals that could facilitate pathogen spillover into the environment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by the WSU Institutional Animal Care and Use Committee, ASAF #7087. The animal studies were conducted in accordance with the local legislation and institutional requirements. Consent was obtained from the owners for the participation of their animals in this study. The requirement of ethical approval for human studies was waived by the Washington State University Institutional Review Board. The human studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin.

Author contributions

PV: Writing – review & editing, Investigation, Writing – original draft, Methodology, Data curation, Conceptualization, Formal Analysis, Visualization. AB: Writing – original draft, Methodology. BV: Methodology, Writing – original draft. MG: Methodology, Writing – original draft, Funding acquisition. AW: Methodology, Writing – original draft. NP: Writing – original draft, Methodology. RP: Methodology, Writing – original draft. JB: Writing – original draft, Funding acquisition, Writing – review & editing, Data curation, Formal Analysis, Project administration, Methodology, Visualization, Conceptualization, Supervision.

Funding

The author(s) declare financial support was received for the research and/or publication of this article. National Science Foundation DEB grant 1754474.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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