Title

Detection of *Batrachochytrium dendrobatidis* in amphibians imported into the UK for the pet trade

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Running Head: Bd in amphibians imported into the UK

Key Words: Amphibians, Pet trade, *Batrachochytrium dendrobatidis*.

Word Count: 3606

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Acknowledgments:

This study was funded by the UK government's Department for Environment, Food and Rural Affairs, grant FC1195. EW was supported by a Natural Environment Research Council and Economic and Social Research Council Interdisciplinary PhD studentship.

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Abstract

There is increasing evidence that the global spread of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has been facilitated by the international trade in amphibians. *Bd* was first detected in the UK in 2004, and has since been detected in multiple wild amphibian populations. Most amphibians imported into the UK for the pet trade from outside the European Union enter the country via Heathrow Animal Reception Centre (HARC), where *Bd* positive animals have been previously detected. Data on the volume, diversity and origin of imported amphibians were collected for 59 consignments arriving at HARC between November 2009 and June 2012, along with a surveillance study to investigate the prevalence of *Bd* in these animals. Forty three amphibian genera were recorded, originating from 12 countries. It was estimated that 5000 – 7000 amphibians are imported through HARC into the UK annually for the pet trade. *Bd* was detected in consignments from the USA and Tanzania, in six genera, resulting in an overall prevalence of 3.6%. This suggests that imported amphibians are a source of *Bd* within the international pet trade.

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Introduction

Wildlife has been utilised as a commercial resource for thousands of years, and what was primarily once a localised, subsistence activity is now an international, multi-billion dollar industry (Broad et al., 2003). Modern advances in transport, coupled with transnational commerce agreements has resulted in a vast trade network, and dramatically increased the efficiency of within and between country animal movements. Wildlife trade is economically important, perhaps more-so in developing countries where revenue generated from exports can make up a significant part of their GDP (Roe et al., 2002). However, translocation of wild animals (and domestic livestock) often poses significant threats through the facilitation of pathogen spread (Karesh et al., 2005, Fèvre et al., 2006). Many of these pathogens cause emerging infectious diseases (EIDs), so called due to their recent resurgence or rapid large scale spread (Daszak et al., 2000, 2001). Historically, research has focused on zoonotic EIDs or those that affect domestic livestock and until recently, the impacts on biodiversity have been largely overlooked (Daszak et al., 2000). Wildlife EIDs are now being recognised for their ability to cause population declines, local and global extinctions and subsequent ecosystem disruption (Dobson and Foufopoulos, 2001, Cunningham et al., 2003). There has been a plethora of examples of epidemics caused by the movement of infected, non-native animals (Zepeda et al., 2001, Swift et al., 2007, Gummow, 2010, MacDiarmid, 2011 and references therein), often with economically and ecologically disastrous consequences. One such pathogen is the fungal agent *Batrachochytrium dendrobatidis* (*Bd*) that infects amphibians. Sustained research since the pathogen's discovery in 1997 (Berger et al., 1998) continues to provide evidence that *Bd* is a major factor in the recent declines and extinctions of amphibian populations worldwide (Lips et al., 2005, Pounds et al., 2006, Skerratt et al., 2007, Catenazzi et al., 2014). The trade in live amphibians is widely considered a major mechanism for *Bd* dissemination (Fisher and Garner, 2007, Picco and Collins, 2008).

Amphibians are traded on a surprisingly large scale both geographically and economically. Schloegel et al. (2009) estimated that 5.07 million live amphibians are imported into the US annually via three main ports. Amphibian uses vary widely from scientific research animals, to culinary delicacies, to pets and garden pond embellishments; all involving the translocation of large numbers of animals (Schlaepfer et al., 2005, Schloegel et al., 2009). The pet trade is complex, involving an estimated six million amphibians globally per year from a wide range of both captive bred and wild caught species, originating from multiple countries (OIE, 2006). Amphibians imported into the UK are primarily destined for the pet market and research industries, and approximately 85% of the recorded shipments (i.e. from countries outside the European Union) are processed at the Heathrow Animal Reception Centre (HARC) (R, Quest, pers. comm.). HARC is one of four UK border inspection posts (BIPs) licensed to handle amphibians, the others being Gatwick, Manchester and Edinburgh Airports. A recent study estimated that 130000 amphibians are imported into the UK annually (Peel et al., 2012).

There has been growing concern regarding the disease risk of non-native amphibian imports to the UK's native amphibian fauna (Cunningham et al., 2005, Garner et al., 2006, Smith, 2013), and *Bd* has already been detected in some incoming shipments destined for the pet trade (Peel et al., 2012). *Batrachochytrium dendrobatidis* (*Bd*) was listed as a World Organisation for Animal Health (OIE) notifiable disease in 2008 (OIE, 2008, Schloegel et al., 2010). The OIE advocates the implementation of Disease Risk Analysis (DRA) and mitigation strategies for countries importing animals potentially infected with such pathogens (OIE, 2012), and provides a framework to aid DRA investigations. There are several difficulties to overcome however, when using these frameworks for amphibian imports.

Firstly, there are virtually no data on the volume, species composition, or origins of amphibian consignments entering the UK, as the trade is largely unregulated. Secondly, post-import tracking is non-existent, therefore tracing the movements of these animals in-country is unachievable. Additionally, biosecurity standards of holding facilities (anywhere from zoos to garden ponds), are highly variable. Monitoring *Bd* dissemination into wild populations would therefore require extraordinary effort. Given these problems, consignment point of entry is a practical place at which to attempt disease mitigation. However, this in turn has complications relating to regulatory deficiencies including lack of information regarding the husbandry or packing conditions of the animals prior to arrival and very little notice of consignment arrivals. Additionally, financial (cost of sampling and processing, and potential treatments) and time (man-hours and consignment turnover pressures) constraints, need to be considered in terms of designing feasible mitigation strategies.

Here we report the findings of a collaborative study conducted at HARC over 30 months from December 2009 to June 2012. In this study we recorded the volume, species composition, country of origin, and *Bd* status of amphibian imports, in order to evaluate the risk of *Bd* being imported into the UK via HARC, and examine the feasibility of potential mitigation strategies.

Methods

Amphibians entering the UK through HARC typically arrive in either 'reptile' (terrestrial) or 'fish' (aquatic) consignments. On arrival, HARC import inspectors recorded the consignment origin, amphibian species and number of individuals reported on the accompanying consignment invoice, for 'reptile' consignments entering the UK from December 2009 to June 2012. Although they may contain amphibians, 'fish' consignments were not sampled as it was not considered logistically feasible due to the rapid turnover of consignments and their complex packing (sealed, aerated, water filled bags contained within sealed insulated boxes). During routine inspection of consignment contents, amphibian skin swabs were collected by trained HARC staff.

We used epidemiological sample size calculations based on binomial probability formulae (Cannon and Roe, 1982) to determine sample sizes for each consignment. These calculations require values for: (1) population size (size of consignment), (2) desired confidence level (99% for the current study), and (3) baseline prevalence. The lack of comparative pre-existing data resulted in us using a baseline prevalence of 20% estimated from: pet shop prevalence data of 22% (Goka et al., 2009), opportunistic sampling of pet trade imports (3.2%) and laboratory animals (23.5% and 19%) (Peel et al., 2012, Wombwell, 2008). Despite some variation in packing conditions within consignments between species (for example, *Ceratophrys* sp. are always packed individually whereas most other species are carried in conspecific groups), we assumed that all individuals in each consignment were equally likely to be exposed to *Bd*. Where multiple species were present, we sampled a representative number of each species, which was dependant on overall consignment size. Amphibians were randomly selected from containers, using a new pair of disposable powder-free nitrile gloves for each individual. When necessary the animal was rinsed with aged tap water to remove excess debris prior to swabbing. For each animal, the underside of the hind feet, drinkpatch, ventral and lateral skin surfaces were each swabbed with five strokes using a single use dry rayon-tipped swab (MWE: MW100 sterile tubed dry swab). The animal details (species, number of conspecifics in box, and any notable features) were recorded, and the amphibian was then placed in a separate container, and another individual selected. This was repeated until the specified number of animals had been swabbed. The swabs were stored in a refrigerator until they were processed.

We processed the swabs following the protocol of Hyatt et al. (2007) with the exception of the addition of Bovine Serum Albumin (BSA), which was added to reduce PCR inhibition potentially caused by contaminants (Garland et al., 2010). Each sample was analysed in duplicate and plates were re-run if R2 values were less than 0.9. Individual samples that returned a single positive result were re-run in duplicate until the same result was obtained in duplicate or up to five times, at which point they were reported to be negative.

Total consignment volumes and composition were taken from shipment invoices. Data on consignments for import and for transit to third countries were compiled separately and used to determine trade routes and volumes. Consignment composition from different countries of origin was extracted, to determine number of animals and frequency of occurrence of genera.

We contracted species data to genus level in order to reduce the impact of potential species misidentifications. A Sorenson Index was chosen to compare the genera composition between all consignments, as the index is simple to interpret (constrained between 0 - 1) and uses presence/absence rather than abundance data (Kindt and Coe, 2005), as consignment size would otherwise influence the index. The Sorenson index is a measure of ecological distance where values of 0 indicate identical composition between two 'sites', and 1 indicates complete dissimilarity. The mean indices represent the within, and between, country differences in consignment composition. The similarity matrix produced was condensed by calculating the mean for indices of the same country, allowing an assessment of compositional similarity between and within countries.

We performed a Monte Carlo style randomisation analysis to determine the probability of the observed pattern of positive samples amongst the consignments occurring by chance given the overall observed prevalence. A script in 'R' (R Core Development Team, 2014) was written to perform 10,000 iterations of a function to compare the variance of the observed distribution (ObsVar) of *Bd* presence, with the variance of a null distribution generated by randomising the *Bd* presence data across all consignments. As the variance of a clustered distribution is higher than that of a random distribution, the mean number of times the random variance was greater than the true variance is equal to the probability that that distribution occurred by chance.

There were too few positive samples to produce converged generalised linear mixed models (GLMM's), to examine the importance factors such as 'country of origin', 'genus', 'number of species' and 'total number of amphibians in consignment', so Fisher's exact tests were performed to test the following hypotheses: 1) There is no association between country of origin and consignment *Bd* status, 2) There is no association between genus and detection of *Bd* in consignments.

Results

Data were collected for approximately 80% of terrestrial amphibian consignments (i.e. amphibians arriving in non-fish consignments) received by HARC. Information on the numbers of amphibians imported was available for 54 of the 59 consignments tested, resulting in a conservative estimate of 14492 amphibians received in 'reptile' consignments during the course of the study. Consignments arrived from 12 countries, with the USA exporting by far the largest number of animals to the UK, followed by Tanzania then Ghana (5600 in 15 consignments; 2880 in 11 consignments; 2106 in 12 consignments respectively). These made up 73% of all arrivals (Fig. 1). Numbers for individual consignments varied from 19 to 1000 animals (mean = 272, SD = 239). On arrival at HARC, consignments were either checked then cleared for entrance into the UK ('imports') or checked and returned to a loading area for re-export ('transits') to a third country. Forty consignments were imported, equating to 10439 amphibians originating from 10 countries. Approximately 25% of consignments were in transit to a third country. We estimate that 7000 amphibians are arriving at Heathrow annually in 'reptile' consignments, 5050 of which are imported into the UK trade and the remainder re-exported to third countries. According to Peel et al. (2012), up to four times as many amphibians are imported in aquatic consignments, resulting in an overall estimate of 20000 amphibians imported annually into the UK through London Heathrow airport.

Overall, 43 genera were recorded. The highest diversity of amphibians was imported from the USA, including a large number of genera not native to that country (Table 1). Whilst the most commonly imported genera originated from a variety of exporting countries, those being transited through Heathrow were predominantly African genera. Over 50% of all UK imports consisted of only four genera (*Hyla*, *Hyperolius*, *Bombina* and *Cynops*), all of which originated from USA, Tanzania, Ghana and Hong Kong. The remaining imports comprised smaller numbers of a wider range of genera. Different consignments from the same country of origin were generally similar in composition, and grouping genera according to their natural geographic range showed that, with the exception of the US and Canada, exports consisted primarily of native species (Table 1).

According to the Sorenson indices (Table 2), the majority of countries appear to export consignments with genera compliments distinct from those of other countries (values of, or near 1), with a degree of overlap between African countries. Canada and USA Chicago exported exclusively *Xenopus* sp. and thus had an index of 0 (identical consignments). This indicates that country profiles may be useful in estimating the composition of imported amphibians over a number of consignments, but accurately predicting an individual consignment's content to a genus level is unlikely.

Thirty-six (3.6%) of the 1010 skin swabs tested positive for *Bd*. Animals in seven (11.8%) of 59 consignments were positive for *Bd*, and prevalence within a sample taken from a consignment with infected animals ranged from 5.0 - 85.7%. Extrapolating from the sample prevalence to estimate the number of infected amphibians within a consignment, the overall consignment prevalence was calculated (Table 3), which ranged from 1.0 - 85.7%.

The randomisation test showed the observed variance (ObsVar = 0.02), was significantly higher (p = 0.001) than would be expected if *Bd* was randomly distributed amongst consignments ($\bar{x}($ExpVar) = 0.003). This suggests that *Bd* was clustered within consignments and not evenly distributed amongst them. There appears to be further clustering of *Bd* within consignments at genus level (see Table 3), but sample sizes were too small for meaningful analysis.

Although *Bd* was detected solely in consignments from the USA and Tanzania (4/17 and 3/11 respectively), there was no significant association between country of origin and consignment *Bd* status (Fisher's exact test: p = 0.69). Of the 43 genera encountered during this study, *Bd* was only detected in six: *Desmognathus* (1/4 animals tested), *Hyla* (10/53), *Hyperolius* (17/207), *Leptopelis* (1/28), *Necturus* (6/7), and *Siren* (1/2). The presence of these genera in a consignment was not, however, associated with the presence of *Bd* (Fisher's exact test: p = 0.15). All positive samples came from genera native to the country of export, but the small overall number of positive samples precluded meaningful analysis of this.

Discussion

In this study we showed that large numbers of individuals and number of genera, are imported annually into the UK via HARC. These numbers are undoubtedly underestimates as data could not be collected for all incoming consignments containing amphibians. When taking this into account, we estimate 5000 - 7000 individual terrestrial amphibians are imported through HARC each year, but this figure is lower than the 25000 calculated by Peel et al. (2012) for the year 2006. This inconsistency requires investigation as it may represent an overall decrease in trade, or may indicate a switch from Heathrow to other BIPs by importers.

Imports were received from 11 geographically disparate countries and consisted of a large variety of species, highlighting the range of countries involved in the trade. With the exception of those from the USA, consignments consisted of amphibians native to the country of export, suggesting that they were wild caught. Consignments from the USA were far more cosmopolitan with respect to genera, indicating a proportion of exported amphibians had been captive bred or re-exported from third-party countries.

Species identification was not always achieved, and it is possible that some species were recorded incorrectly, e.g. *Hyperolius puncticulatus* (listed as ‘Endangered’ by the IUCN) is more likely to be *H. substriatus* (listed as ‘Least Concern’) (Schiøtz et al., 2008). Accurate identification is essential in order to monitor the conservation consequences of trade such as unsustainable harvesting, or trade in protected species, which could result in negative population impacts (Rosen and Smith, 2010). As consignment inventories produced in countries of origin can be unreliable (pers. obs.), personnel at importing BIPs should receive adequate training and resources to rectify consignment paperwork.

Whilst there were notable differences in composition between consignments from different countries, there was also some variation of exports from the same country due to the high diversity of genera exported. Predicting the contents of a consignment prior to its arrival is, therefore, impossible and means that pathogen screening can only be achieved on an *ad hoc*, rather than pre-planned, basis. Our detection of *Bd* in imported and re-exported consignments supports recent evidence that the global trade in amphibians, coupled with the presence of non-native introduced species, is related to the global distribution of *Bd* in wild populations (Garner et al., 2006, Schloegel et al., 2009, Liu et al., 2013, Kolby et al., 2014).

The overall prevalence of *Bd* in imported amphibians (3.6%) was consistent with results of a smaller study (109 animals tested) conducted on imported amphibians at HARC in 2006, where 3.2% of samples were positive for *Bd* on qPCR (Peel et al., 2012). Taken together, these results suggest that there has been an ongoing - if rather low level - regular incursion of *Bd* into the UK through the amphibian pet trade. Whilst strict biosecurity measures make it unlikely that dissemination of *Bd* into wild populations occurs at the point of import (HARC), the level of pathogen containment post-import is unknown, and is potentially poor. As different *Bd* strains have differential impacts across amphibian species (Farrer et al., 2011, Bielby et al., 2013, Balaz et al., 2014), the inadvertent importation of strains more-virulent to native species than those *Bd* strains already present in a country should be minimised. Equally, other pathogens, such as *Batrachochytrium salamandrivorans*, which is highly pathogenic to many newts and salamanders (Martel et al., 2013) and novel ranaviruses with large host ranges (e.g. Price et al., 2014) can be introduced via the amphibian pet trade (Martel et al., 2014). In order to protect native wild amphibians, we recommend that border sanitary controls are implemented to minimise the risk of introducing new strains of *Bd*, and other pathogens. These could be adopted according to a cost-benefit analysis and following consultation with stakeholders, such as conservation biologists, herpetologists and commercial amphibian importers.

Bd DNA was detected exclusively in amphibians from the USA and Tanzania, both in consignments for import and in transit. The country of origin, however, was not significantly associated with probability of *Bd* detection, but this was likely due to low numbers of consignments arriving from other countries, resulting in poor statistical power. Future studies could include a collaborative survey from multiple European airports may provide sufficient data to identify countries with statistically higher probabilities of *Bd* positive exports. This would be a beneficial avenue to pursue as it would inform the implementation of targeted surveillance. *Bd* has recently been detected at higher prevalence (11.7%) in aquatic amphibians exported from Hong Kong (Kolby et al., 2014). Amphibians arriving in ‘fish’ consignments were excluded from the current study for logistical reasons, but it would be prudent to investigate this possible route of incursion in the near future, as (1) a large number of amphibians are imported via this route (Peel et al., 2012), and (2) amphibians are more likely to test positive for *Bd* when aquatic than when terrestrial (Cunningham pers. obs.).

The indication that *Bd* positive animals were clustered within specific components (specific genera) of specific consignments, is promising for two reasons. Firstly, if *Bd* is contained within components of a consignment, this could reduce the number of samples required to detect the presence of the pathogen, thus increasing cost effectiveness of any future *Bd* surveillance. Secondly, if *Bd* is contained within sub-sections of a consignment, the positive part (rather than the whole) of a consignment could be treated/disposed of, reducing economic losses associated with the detection of *Bd*-positive animals. This requires further investigation, e.g. through complete consignment swabbing, to fully understand the distribution of *Bd* within consignments, as low levels of *Bd* could have been missed in the current study.

Of the 59 consignments investigated, approximately 25% were re-exported, making HARC an important hub in the amphibian trade network. Identification of such hubs is useful as they comprise logical places for targeting pathogen screening and possible mitigation measures. The turn-over of consignments at HARC is rapid as (1) space is at a premium, and there are limited facilities or manpower for longer-term care and housing; (2) wholesalers are keen to take ownership of their stock, and unpack the animals after their journey; and (3) re-exports have a short transit time between connecting flights. Current *Bd* detection methods are unsuited to preventing pathogen importation as the length of time necessary to obtain and process samples is not compatible with the processing time for shipments. Even within the time-frame of this study, however, methods have been developed to sample and detect *Bd* in a time-scale realistic to that required at import points. Indeed, it is now possible to detect *Bd* 'in the field' with the use of portable PCR machines at relatively low cost (<£3 per sample). It is likely that these advances in technology will continue to improve the feasibility of pathogen detection in traded amphibians at the point of importation. Continued surveillance of imported amphibians is warranted given the increasing reports of *Bd* in trade animals (Schloegel et al., 2009, Kolby et al., 2014).

Findings from this study support the hypothesis that the international trade in amphibians has contributed to the global spread of *Bd*. Whilst research is on-going into the factors influencing the spread of *Bd* and other amphibian pathogens, much of this research has focused on the trade in amphibians for food. This study highlights the presence of *Bd* in the pet trade, and further research is required to evaluate to what extent this could impact on wild amphibian populations.

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Table 1: Percentage of consignments from exporting countries containing amphibian genera imported into the UK. CA – Canada, US – United States of America, EG – Egypt, GH – Ghana, MG – Madagascar, ZA – South Africa, TZ – Tanzania, CH – Switzerland, UA – Ukraine, HK – Hong Kong, ID – Indonesia, MY – Malaysia.

Continent		North America		Africa					Europe		Asia		
Country of origin		CA	US	EG	GH	MG	ZA	TZ	CH	UA	HK	ID	MY
Total No. consignments		2	17	3	11	4	3	1	1	1	1	3	1
North America	*Ambystoma*		29										
	Anaxyrus		6										
	Desmognathus		6										
	Hyla		65										
	Necturus		6										
	Pseudacris		6										
	Siren		6										
South America	*Calyptocephalella*		6										
	Ceratophrys		76										
	Lepidobatrachus		18										
	Melanophryniscus		6										
	Osteopilus		12										
	Trachycephalus		6										
	Phyllomedusa		6										
Africa	*Afrixalus*							9	100				
	Amietophrynus			67	45		67						
	Breviceps						67						
	Chiromantis						67		100				
	Dyscophus		30			100							
	Hemisus				9			9					
	Heterixalus					25							
	Hyperolius		6		73		67	73					
	Kassina		6	33	27		67	45					
	Leptopelis			33				45					
	Mantella					75							
	Phrynomantis				27		67	27					
	Ptychadena			33									
	Pyxicephalus		12	33			33	18					
	Scaphiophryne					50							
	Schismaderma						33						
	Tomopterna						33						
	Xenopus	100	18										
Europe	*Pseudepidalea*			67	9								
	Salamandra									100			
Asia	*Bombina*		30										
	Cynops										100		
	Kaloula		18										
	Litoria											100	
	Megophrys												100
	Pachytriton										100		
	Polypedates											33	
	Rhacophorus										100	67	100
	Rhinella		6										

Table 2.
Mean Sorenson Indices.
Indices were calculated using the presence or absence of genera for all consignments. Consignments were grouped according to country of origin, and departure airport where possible.

	CA	US C	US M	EG	GH	TZ	ZA	MG	ID
Canada	0.00								
US Chicago	0.00	0.00							
US Miami	1.00	1.00	0.49						
Egypt	1.00	1.00	0.99	0.37					
Ghana	1.00	1.00	0.98	0.81	0.52				
Tanzania	1.00	1.00	0.98	0.87	0.67	0.53			
South Africa	1.00	1.00	0.97	0.84	0.76	0.79	0.36		
Madagascar	1.00	1.00	0.92	1.00	1.00	1.00	1.00	0.22	
Indonesia	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50

Table 3.
Estimates of numbers of infected individual amphibians, and overall consignment prevalence, entering the UK via HARC between December 2009 and June 2012. Estimated total number of infected individuals is calculated as, sample prevalence x volume. Estimated overall consignment prevalence is calculated as the sum of the estimated number of infected amphibians in the consignment, divided by the total number of amphibians in the consignment.

Infected Consignment (ID and origin)	Consignment content (genus and volume)	Sample prevalence (%)	Estimated total No. of infected individuals	Estimated overall consignment prevalence (%)
11: TZ	100 *Afrixalus*	0 (0/0)	0	11.1
	550 *Hyperolius*	11.1 (1/9)	61	
	50 *Kassina*	0 (0/4)	0	
	300 *Leptopelis*	16.7 (1/6)	50	
16: TZ	25 *Hemisus*	0 (0/2)	0	22.9
	200 *Hyperolius*	40.0 (4/20)	80	
	100 *Leptopelis*	0 (0/5)	0	
	25 *Pyxicephalus*	0 (0/2)	0	
17: US Miami	70 *Ceratophrys*	0 (0/6)	0	48.8
	100 *Hyla*	83.3 (10/12)	83	
25: TZ	100 *Hyperolius*	85.7 (12/14)	86	85.7
38: US Miami	25 *Calyptocephalella*	0 (0/0)	0	2.8
	90 *Ceratophrys*	0 (0/6)	0	
	450 *Hyla*	0 (0/4)	0	
	25 *Necturus*	85.5 (6/7)	21	
	163 *Pseudacris*	0 (0/2)	0	
50: US Miami	200 *Bombina*	0 (0/4)	0	1.0
	100 *Ceratophrys*	0 (0/4)	0	
	50 *Dyscophus*	0 (0/4)	0	
	200 *Hyla*	0 (0/2)	0	
	25 *Kaloula*	0 (0/0)	0	
	25 *Lepidobatrachus*	0 (0/3)	0	
	12 *Siren*	50.0 (1/2)	6	
53: US Miami	250 *Bombina*	0 (0/10)	0	1.7
	72 *Ceratophrys*	0 (0/5)	0	
	24 *Desmognathus*	25.0 (1/4)	6	

Figure 1: Routes, and volume of sampled amphibian imports into the UK through Heathrow Animal Reception Centre between December 2009 and June 2012. Importing countries with negative shipments shaded light grey, positive shipments shaded dark grey. Arrow thickness represents the total volume of amphibians imported. The proportion of *Bd* infected animals indicated in black.