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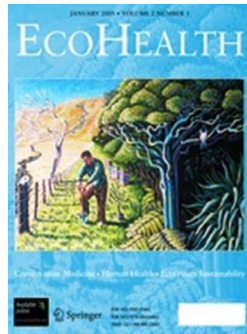
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Detection of *Batrachochytrium dendrobatidis* in amphibians imported into the UK for the pet trade.

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

3 **Abstract**

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

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

28 **Introduction**

29 Wildlife has been utilised as a commercial resource for thousands of years, and what was
30 primarily once a localised, subsistence activity is now an international, multi-billion dollar
31 industry (Broad et al., 2003). Modern advances in transport, coupled with transnational
32 commerce agreements have resulted in a vast trade network, and dramatically increased the
33 efficiency of within and between country animal movements. Wildlife trade is economically
34 important, perhaps more-so in developing countries where revenue generated from exports
35 can make up a significant part of their GDP (Roe et al., 2002). However, translocation of wild
36 animals (and domestic livestock) often poses significant threats through the facilitation of
37 pathogen spread (Karesh et al., 2005, Fèvre et al., 2006). Many pathogens cause emerging
38 infectious diseases (EIDs), so called due to their increase in incidence over the past 20 years
39 (Daszak et al., 2000, 2001). Historically, research has focused on zoonotic EIDs or those that
40 affect domestic livestock and until recently, the impacts on biodiversity have been largely
41 overlooked (Daszak et al., 2000). Wildlife EIDs are now being recognised for their ability to
42 cause population declines, local and global extinctions and subsequent ecosystem disruption
43 (Dobson and Fofopoulos, 2001, Cunningham et al., 2003). There are numerous examples of
44 epidemics caused by the movement of infected, non-native animals (Zepeda et al., 2001,
45 Swift et al., 2007, Gummow, 2010, MacDiarmid, 2011 and references therein), often with
46 economically and ecologically disastrous consequences. One such pathogen is the fungal
47 agent *Batrachochytrium dendrobatidis* (*Bd*) that infects amphibians. Sustained research since
48 the pathogen's discovery in 1997 (Berger et al., 1998) continues to provide evidence that *Bd*
49 is a major factor in the recent declines and extinctions of amphibian populations worldwide
50 (Lips et al., 2005, Pounds et al., 2006, Skerratt et al., 2007, Catenazzi et al., 2014). The trade

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3 51 in live amphibians is widely considered a mechanism for *Bd* dissemination (Fisher and
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5 52 Garner, 2007, Picco and Collins, 2008).

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7
8 53 Amphibians are traded on a surprisingly large scale both geographically and economically.
9
10 54 Schloegel et al. (2009) estimated that 5.07 million live amphibians are imported into the US
11
12 55 annually via three main ports. Amphibian uses vary widely from scientific research animals,
13
14 56 to culinary delicacies, to pets and garden pond embellishments; all involving the translocation
15
16 57 of large numbers of animals (Schlaepfer et al., 2005, Schloegel et al., 2009). The pet trade is
17
18 58 complex, involving an estimated six million amphibians globally per year from a wide range
19
20 59 of both captive bred and wild caught species, originating from multiple countries (OIE,
21
22 60 2006). Amphibians imported into the UK are primarily destined for the pet market and
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24 61 research industries, and approximately 85% of the recorded shipments (i.e. from countries
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26 62 outside the European Union) are processed at the Heathrow Animal Reception Centre
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28 63 (HARC) (R, Quest, pers. comm.). HARC is one of four UK border inspection posts (BIPs)
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30 64 licensed to handle amphibians, the others being Gatwick, Manchester and Edinburgh
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32 65 Airports. A recent study estimated that 130,000 amphibians are imported into the UK
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34 66 annually (Peel et al., 2012).

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40 67 There has been growing concern regarding the disease risk of non-native amphibian imports
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42 68 to the UK's native amphibian fauna (Cunningham et al., 2005, Garner et al., 2006, Smith,
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44 69 2013), and *Bd* has already been detected in some incoming shipments destined for the pet
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46 70 trade (Peel et al., 2012). *Batrachochytrium dendrobatidis* (*Bd*) was listed as a World
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48 71 Organisation for Animal Health (OIE) notifiable disease in 2008 (OIE, 2008, Schloegel et al.,
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50 72 2010). The OIE advocates the implementation of Disease Risk Analysis (DRA) and
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52 73 mitigation strategies for countries importing animals potentially infected with such pathogens
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54 74 (OIE, 2012), and provides a framework to aid DRA investigations. There are several
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56 75 difficulties to overcome however, when using these frameworks for amphibian imports.
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3 76 Firstly, there are virtually no data on the volume, species composition, or origins of
4
5 77 amphibian consignments entering the UK, as the trade is largely unregulated. Secondly, post-
6
7 78 import tracking is non-existent, therefore tracing the movements of these animals in-country
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9
10 79 is unachievable. Additionally, biosecurity standards of holding facilities (anywhere from zoos
11
12 80 to garden ponds) are highly variable. Monitoring *Bd* dissemination into wild populations
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14 81 would therefore require extraordinary effort. Given these problems, consignment point of
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16 82 entry is a practical place at which to attempt disease mitigation. However, this in turn has
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18 83 complications relating to regulatory deficiencies including lack of information regarding the
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20 84 husbandry, packing conditions of the animals prior to arrival and inconsistent documentation
21
22 85 of consignment arrivals. Additionally, financial (cost of sampling and processing, and
23
24 86 potential treatments) and time (man-hours and consignment turnover pressures) constraints,
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26 87 need to be considered in terms of designing feasible mitigation strategies.
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30 88 Here we report the findings of a collaborative study conducted at HARC over 30 months
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32 89 from December 2009 to June 2012. In this study we recorded the volume, species
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34 90 composition, country of origin, and *Bd* status of amphibian imports, in order to evaluate the
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36 91 risk of *Bd* being imported into the UK via HARC, and examine the feasibility of potential
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38 92 mitigation strategies.
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42 **Methods**

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45 94 Amphibians entering the UK through HARC typically arrive in either 'reptile' (terrestrial) or
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47 95 'fish' (aquatic) consignments. On arrival, HARC import inspectors recorded the consignment
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49 96 origin, amphibian species and number of individuals reported on the accompanying
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51 97 consignment invoice, for 'reptile' consignments entering the UK from December 2009 to
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53 98 June 2012. Although they may contain amphibians, 'fish' consignments were not sampled
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55 99 as it was not logistically feasible due to the rapid turnover of consignments and their complex
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3 100 packing (sealed, aerated, water filled bags contained within sealed insulated boxes). During
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5 101 routine inspection of consignment contents, amphibian skin swabs were collected by trained
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7 102 HARC staff.
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10 103 We used epidemiological sample size calculations based on binomial probabilities (Cannon
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12 104 and Roe, 1982) to determine sample sizes for each consignment. These calculations require
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14 105 values for: (1) population size (size of consignment), (2) desired confidence level (99% for
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16 106 the current study), and (3) baseline prevalence. Because baseline data are lacking, we
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18 107 assumed a baseline prevalence of 20% estimated from: pet shop prevalence data of 22%
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20 108 (Goka et al., 2009), opportunistic sampling of pet trade imports (3.2%) and laboratory
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22 109 animals (23.5% and 19%; Peel et al., 2012, Wombwell, 2008). Despite some variation in
23
24 110 packing conditions within consignments between species (for example, *Ceratophrys* sp. are
25
26 111 always packed individually whereas most other species are carried in conspecific groups), we
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28 112 assumed that all individuals in each consignment were equally likely to be exposed to *Bd*.
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30 113 Where multiple species were present, we sampled a representative number of each species,
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32 114 which was dependant on overall consignment size. Amphibians were randomly selected from
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34 115 containers, using a new pair of disposable powder-free nitrile gloves for each individual.
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36 116 When necessary the animal was rinsed with aged tap water to remove excess debris prior to
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38 117 swabbing. For each animal, the underside of the hind feet, drinkpatch, ventral and lateral skin
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40 118 surfaces were each swabbed with five strokes using a single use dry rayon-tipped swab
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42 119 (MWE: MW100 sterile tubed dry swab). The animal details (species, number of conspecifics
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44 120 in box, and any notable features) were recorded, and the amphibian was then placed in a
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46 121 separate container, and another individual selected. This was repeated until the specified
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48 122 number of animals had been swabbed. The swabs were stored in a refrigerator until they were
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50 123 processed.
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3 124 We processed the swabs following the protocol of Hyatt et al. (2007) with the exception of
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5 125 the addition of Bovine Serum Albumin (BSA), which was added to reduce PCR inhibition
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7 126 potentially caused by contaminants (Garland et al., 2010). Each sample was analysed in
8
9 127 duplicate and plates were re-run if R2 values were less than 0.9. Individual samples that
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11 128 returned a single positive result were re-run in duplicate until the same result was obtained in
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13 129 duplicate or up to five times, at which point they were reported to be negative.
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17 130 Total consignment volumes and composition were taken from shipment invoices. Data on
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19 131 consignments for import and for transit to third countries were compiled separately and used
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21 132 to determine trade routes and volumes. Consignment composition from different countries of
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23 133 origin was extracted, to determine number of animals and frequency of occurrence of genera.
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27 134 We contracted species data to genus level in order to reduce the impact of potential species
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29 135 misidentifications. A Sorenson Index was chosen to compare the genera composition between
30
31 136 all consignments, as the index is simple to interpret (constrained between 0 - 1) and uses
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33 137 presence/absence rather than abundance data (Kindt and Coe, 2005), as consignment size
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35 138 would otherwise influence the index. The Sorenson index is a measure of ecological distance
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37 139 where values of 0 indicate identical composition between two 'sites', and 1 indicates
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39 140 complete dissimilarity. The mean indices represent the within, and between, country
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41 141 differences in consignment composition. The similarity matrix produced was condensed by
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43 142 calculating the mean for indices of the same country, allowing an assessment of
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45 143 compositional similarity between and within countries.
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50 144 We performed a Monte Carlo style randomisation analysis to determine the probability of the
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52 145 observed pattern of positive samples amongst the consignments occurring by chance given
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54 146 the overall observed prevalence. A script in 'R' (R Core Development Team, 2014) was
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56 147 written to perform 10,000 iterations of a function to compare the variance of the observed
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3 148 distribution (ObsVar) of *Bd* presence, with the variance of a null distribution generated by
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5 149 randomising the *Bd* presence data across all consignments. As the variance of a clustered
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7 150 distribution is higher than that of a random distribution, the mean number of times the
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9 151 random variance was greater than the true variance is equal to the probability that that
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11 152 distribution occurred by chance.

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14 153 There were too few positive samples to produce converged generalised linear mixed models
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16 154 (GLMM's), to examine the importance factors such as 'country of origin', 'genus', 'number
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18 155 of species' and 'total number of amphibians in consignment', so Fisher's exact tests were
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20 156 performed to test the following hypotheses: 1) There is no association between country of
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22 157 origin and consignment *Bd* status, 2) There is no association between genus and detection of
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24 158 *Bd* in consignments.

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30 31 32 160 **Results**

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35 161 Data were collected for approximately 80% of terrestrial amphibian consignments (i.e.
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37 162 amphibians arriving in non-fish consignments) received by HARC. Information on the
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39 163 numbers of amphibians imported was available for 54 of the 59 consignments tested,
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41 164 resulting in a conservative estimate of 14492 amphibians received in 'reptile' consignments
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43 165 during the course of the study. Consignments arrived from 12 countries, with the USA
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45 166 exporting by far the largest number of animals to the UK, followed by Tanzania then Ghana
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47 167 (5600 in 15 consignments; 2880 in 11 consignments; 2106 in 12 consignments respectively).
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49 168 These made up 73% of all arrivals (Fig. 1). Numbers for individual consignments varied from
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51 169 19 to 1000 animals (mean = 272, SD = 239). On arrival at HARC, consignments were either
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53 170 checked then cleared for entrance into the UK ('imports') or checked and returned to a
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55 171 loading area for re-export ('transits') to a third country. Forty consignments were imported,
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3 172 equating to 10439 amphibians originating from 10 countries. Approximately 25% of
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5 173 consignments were in transit to a third country. We estimate that 7000 amphibians are
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7 174 arriving at Heathrow annually in 'reptile' consignments, 5050 of which are imported into the
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9 175 UK trade and the remainder re-exported to third countries. According to Peel et al. (2012), up
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11 176 to four times as many amphibians are imported in aquatic consignments, resulting in an
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13 177 overall estimate of 20000 amphibians imported annually into the UK through London
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15 178 Heathrow airport.

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19 179 Overall, 43 genera were recorded. The highest diversity of amphibians was imported from the
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21 180 USA, including a large number of genera not native to that country (Table 1). Whilst the most
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23 181 commonly imported genera originated from a variety of exporting countries, those being
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25 182 transited through Heathrow were predominantly African genera. Over 50% of all UK imports
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27 183 consisted of only four genera (*Hyla*, *Hyperolius*, *Bombina* and *Cynops*), all of which
28
29 184 originated from USA, Tanzania, Ghana and Hong Kong. The remaining imports comprised
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31 185 smaller numbers of a wider range of genera. Different consignments from the same country
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33 186 of origin were generally similar in composition, and grouping genera according to their
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35 187 natural geographic range showed that, with the exception of the US and Canada, exports
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37 188 consisted primarily of native species (Table 1).

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42 189 According to the Sorenson indices (Table 2), the majority of countries appear to export
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44 190 consignments with genera compliments distinct from those of other countries (values of, or
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46 191 near 1), with a degree of overlap between African countries. Canada and USA Chicago
47
48 192 exported exclusively *Xenopus* sp. and thus had an index of 0 (identical consignments). This
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50 193 indicates that country profiles may be useful in estimating the composition of imported
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52 194 amphibians over a number of consignments, but accurately predicting an individual
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54 195 consignment's content to a genus level is unlikely.

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3 196 Thirty-six (3.6%) of the 1010 skin swabs tested positive for *Bd*. Animals in seven (11.8%) of
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5 197 59 consignments were positive for *Bd*, and prevalence within a sample taken from a
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7 198 consignment with infected animals ranged from 5.0 - 85.7%. Extrapolating from the sample
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9 199 prevalence to estimate the number of infected amphibians within a consignment, the overall
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11 200 consignment prevalence was calculated (Table 3), which ranged from 1.0 - 85.7%.

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14 201 The randomisation test showed the observed variance (ObsVar = 0.02), was significantly
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16 202 higher ($p = 0.001$) than would be expected if *Bd* was randomly distributed amongst
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18 203 consignments ($\bar{x}(\text{ExpVar}) = 0.003$). This suggests that *Bd* was clustered within consignments
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20 204 and not evenly distributed amongst them. There appears to be further clustering of *Bd* within
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22 205 consignments at genus level (see Table 3), but sample sizes were too small for meaningful
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24 206 analysis.

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29 207 Although *Bd* was detected solely in consignments from the USA and Tanzania (4/17 and 3/11
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31 208 respectively), there was no significant association between country of origin and consignment
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33 209 *Bd* status (Fisher's exact test: $p = 0.69$). Of the 43 genera encountered during this study, *Bd*
34
35 210 was only detected in six: *Desmognathus* (1/4 animals tested), *Hyla* (10/53), *Hyperolius*
36
37 211 (17/207), *Leptopelis* (1/28), *Necturus* (6/7), and *Siren* (1/2). The presence of these genera in a
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39 212 consignment was not, however, associated with the presence of *Bd* (Fisher's exact test: $p =$
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41 213 0.15). All positive samples came from genera native to the country of export, but the small
42
43 214 overall number of positive samples precluded meaningful analysis of this.

44 45 46 47 48 215 **Discussion**

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51 216 In this study we showed that large numbers of individuals and number of genera, are
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53 217 imported annually into the UK via HARC. These numbers are undoubtedly underestimates as
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55 218 data could not be collected for all incoming consignments containing amphibians. When
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57 219 taking this into account, we estimate 5000 - 7000 individual terrestrial amphibians are
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3 220 imported through HARC each year, but this figure is lower than the 25000 calculated by Peel
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5 221 et al. (2012) for the year 2006. This inconsistency requires investigation as it may represent
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7 222 an overall decrease in trade, or may indicate a switch from Heathrow to other BIPs by
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9 223 importers.

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12 224 Imports were received from 11 geographically disparate countries and consisted of a large
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14 225 variety of species, highlighting the range of countries involved in the trade. With the
15
16 226 exception of those from the USA, consignments consisted of amphibians native to the
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18 227 country of export, suggesting that they were wild caught. Consignments from the USA were
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20 228 far more cosmopolitan with respect to genera, indicating a proportion of exported amphibians
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22 229 had been captive bred or re-exported from third-party countries.

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27 230 Species identification was not always achieved, and it is possible that some species were
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29 231 recorded incorrectly, e.g. *Hyperolius puncticulatus* (listed as 'Endangered' by the IUCN) is
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31 232 more likely to be *H. substriatus* (listed as 'Least Concern') (Schjøtz et al., 2008). Accurate
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33 233 identification is essential in order to monitor the conservation consequences of trade such as
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35 234 unsustainable harvesting, or trade in protected species, which could result in negative
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37 235 population impacts (Rosen and Smith, 2010). As consignment inventories produced in
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39 236 countries of origin can be unreliable (pers. obs.), personnel at importing BIPs should receive
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41 237 adequate training and resources to rectify consignment paperwork.

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45 238 Whilst there were notable differences in composition between consignments from different
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47 239 countries, there was also some variation of exports from the same country due to the high
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49 240 diversity of genera exported. Predicting the contents of a consignment prior to its arrival is,
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51 241 therefore, impossible and means that pathogen screening can only be achieved on an *ad hoc*,
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53 242 rather than pre-planned, basis. Our detection of *Bd* in imported and re-exported consignments
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55 243 supports recent evidence that the global trade in amphibians, coupled with the presence of
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244 non-native introduced species, is related to the global distribution of *Bd* in wild populations
245 (Garner et al., 2006, Schloegel et al., 2009, Liu et al., 2013, Kolby et al., 2014).

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

264 *Bd* DNA was detected exclusively in amphibians from the USA and Tanzania, both in
265 consignments for import and in transit. The country of origin, however, was not significantly
266 associated with probability of *Bd* detection, but this was likely due to low numbers of
267 consignments arriving from other countries, resulting in poor statistical power. Future studies
268 could include a collaborative survey from multiple European airports may provide sufficient

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3 269 data to identify countries with statistically higher probabilities of *Bd* positive exports. This
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5 270 would be a beneficial avenue to pursue as it would inform the implementation of targeted
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7 271 surveillance. *Bd* has recently been detected at higher prevalence (11.7%) in aquatic
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9 272 amphibians exported from Hong Kong (Kolby et al., 2014). Amphibians arriving in ‘fish’
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11 273 consignments were excluded from the current study for logistical reasons, but it would be
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13 274 prudent to investigate this possible route of incursion in the near future, as (1) a large number
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15 275 of amphibians are imported via this route (Peel et al., 2012), and (2) amphibians are more
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17 276 likely to test positive for *Bd* when aquatic than when terrestrial (Cunningham pers. obs.).

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21 277 The indication that *Bd* positive animals were clustered within specific components (specific
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23 278 genera) of specific consignments, is promising for two reasons. Firstly, if *Bd* is contained
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25 279 within components of a consignment, this could reduce the number of samples required to
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27 280 detect the presence of the pathogen, thus increasing cost effectiveness of any future *Bd*
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29 281 surveillance. Secondly, if *Bd* is contained within sub-sections of a consignment, the positive
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31 282 part (rather than the whole) of a consignment could be treated/disposed of, reducing
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33 283 economic losses associated with the detection of *Bd*-positive animals. This requires further
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35 284 investigation, e.g. through complete consignment swabbing, to fully understand the
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37 285 distribution of *Bd* within consignments, as low levels of *Bd* could have been missed in the
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39 286 current study.

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44 287 Of the 59 consignments investigated, approximately 25% were re-exported, making HARC
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46 288 an important hub in the amphibian trade network. Identification of such hubs is useful as they
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48 289 comprise logical places for targeting pathogen screening and possible mitigation measures.
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50 290 The turn-over of consignments at HARC is rapid as (1) space is at a premium, and there are
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52 291 limited facilities or manpower for longer-term care and housing; (2) wholesalers are keen to
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54 292 take ownership of their stock, and unpack the animals after their journey; and (3) re-exports
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56 293 have a short transit time between connecting flights. Current *Bd* detection methods are
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3 294 unsuited to preventing pathogen importation as the length of time necessary to obtain and
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5 295 process samples is not compatible with the processing time for shipments. Methods are been
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7 296 developed to sample and detect *Bd* in a time-scale realistic to that required at import points. It
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10 297 is likely that these advances in technology will continue to improve the feasibility of
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12 298 pathogen detection in traded amphibians at the point of importation. Continued surveillance
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14 299 of imported amphibians is warranted given the increasing reports of *Bd* in trade animals
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16 300 (Schloegel et al., 2009, Kolby et al., 2014).

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19 301 Findings from this study support the hypothesis that the international trade in amphibians has
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21 302 contributed to the global spread of *Bd*. Whilst research is on-going into the factors
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23 303 influencing the spread of *Bd* and other amphibian pathogens, much of this research has
24
25 304 focused on the trade in amphibians for food. This study highlights the presence of *Bd* in the
26
27 305 pet trade, and further research is required to evaluate to what extent this could impact on wild
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29 306 amphibian populations.
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36 310 **References**

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

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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	<i>Ambystoma</i>		29										
	<i>Anaxyrus</i>		6										
	<i>Desmognathus</i>		6										
	<i>Hyla</i>		65										
	<i>Necturus</i>		6										
	<i>Pseudacris</i>		6										
	<i>Siren</i>		6										
South America	<i>Calyptocephalella</i>		6										
	<i>Ceratophrys</i>		76										
	<i>Lepidobatrachus</i>		18										
	<i>Melanophryniscus</i>		6										
	<i>Osteopilus</i>		12										
	<i>Trachycephalus</i>		6										
	<i>Phyllomedusa</i>		6										
Africa	<i>Afrixalus</i>							9	100				
	<i>Amietophrynus</i>			67	45		67						
	<i>Breviceps</i>						67						
	<i>Chiromantis</i>						67		100				
	<i>Dyscophus</i>		30			100							
	<i>Hemisus</i>				9			9					
	<i>Heterixalus</i>					25							
	<i>Hyperolius</i>		6		73		67	73					
	<i>Kassina</i>		6	33	27		67	45					
	<i>Leptopelis</i>			33				45					
	<i>Mantella</i>					75							
	<i>Phrynomantis</i>				27		67	27					
	<i>Ptychadena</i>			33									
	<i>Pyxicephalus</i>		12	33			33	18					
	<i>Scaphiophryne</i>					50							
	<i>Schismaderma</i>						33						
	<i>Tomopterna</i>						33						
<i>Xenopus</i>		100	18										
Europe	<i>Pseudepidalea</i>			67	9								
	<i>Salamandra</i>									100			
Asia	<i>Bombina</i>		30										
	<i>Cynops</i>										100		
	<i>Kaloula</i>		18										
	<i>Litoria</i>											100	
	<i>Megophrys</i>												100
	<i>Pachytriton</i>									100			
	<i>Polypedates</i>											33	
	<i>Rhacophorus</i>										100	67	100
	<i>Rhinella</i>		6										

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444 **Table 2.**
 445 Mean Sorenson Indices.
 446 Indices were calculated using the presence or absence of genera for all consignments. Consignments were
 447 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

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473 **Table 3.**
 474 Estimates of numbers of infected individual amphibians, and overall consignment prevalence, entering the UK
 475 via HARC between December 2009 and June 2012. Estimated total number of infected individuals is calculated
 476 as, sample prevalence x volume. Estimated overall consignment prevalence is calculated as the sum of the
 477 estimated number of infected amphibians in the consignment, divided by the total number of amphibians in the
 478 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 <i>Afrivalus</i>	0 (0/0)	0	11.1
	550 <i>Hyperolius</i>	11.1 (1/9)	61	
	50 <i>Kassina</i>	0 (0/4)	0	
	300 <i>Leptopelis</i>	16.7 (1/6)	50	
16: TZ	25 <i>Hemisis</i>	0 (0/2)	0	22.9
	200 <i>Hyperolius</i>	40.0 (4/20)	80	
	100 <i>Leptopelis</i>	0 (0/5)	0	
	25 <i>Pyxicephalus</i>	0 (0/2)	0	
17: US Miami	70 <i>Ceratophrys</i>	0 (0/6)	0	48.8
	100 <i>Hyla</i>	83.3 (10/12)	83	
25: TZ	100 <i>Hyperolius</i>	85.7 (12/14)	86	85.7
38: US Miami	25 <i>Calyptocephalella</i>	0 (0/0)	0	2.8
	90 <i>Ceratophrys</i>	0 (0/6)	0	
	450 <i>Hyla</i>	0 (0/4)	0	
	25 <i>Necturus</i>	85.5 (6/7)	21	
	163 <i>Pseudacris</i>	0 (0/2)	0	
50: US Miami	200 <i>Bombina</i>	0 (0/4)	0	1.0
	100 <i>Ceratophrys</i>	0 (0/4)	0	
	50 <i>Dyscophus</i>	0 (0/4)	0	
	200 <i>Hyla</i>	0 (0/2)	0	
	25 <i>Kaloula</i>	0 (0/0)	0	
	25 <i>Lepidobatrachus</i>	0 (0/3)	0	
53: US Miami	12 <i>Siren</i>	50.0 (1/2)	6	1.7
	250 <i>Bombina</i>	0 (0/10)	0	
	72 <i>Ceratophrys</i>	0 (0/5)	0	
	24 <i>Desmognathus</i>	25.0 (1/4)	6	

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3 490 **Figure 1:** Routes, and volume of sampled amphibian imports into the UK through Heathrow Animal Reception
4 491 | Centre between December 2009 and June 2012.
5 492 | Importing countries with negative shipments shaded light grey, positive shipments shaded dark grey. Arrow
6 493 | thickness represents the total volume of amphibians imported.
7 494 | The proportion of *Bd* infected animals indicated in black.
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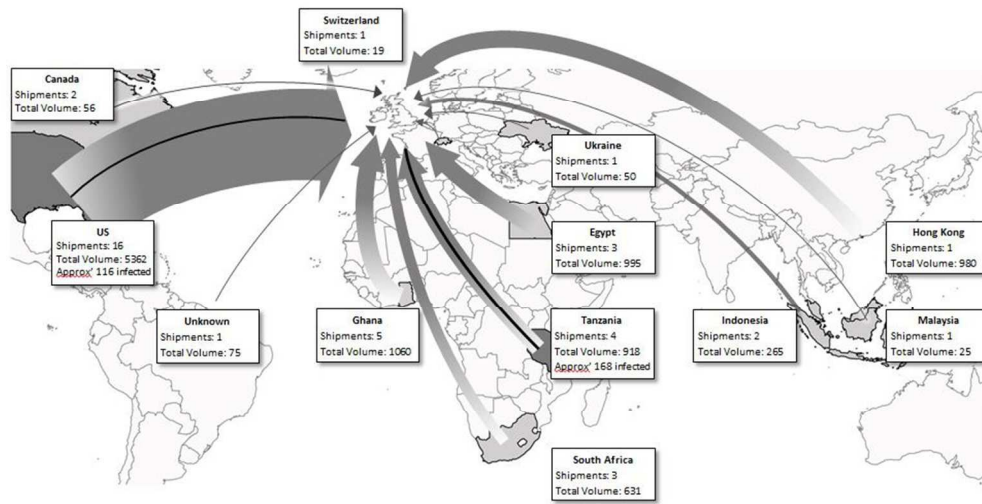


Figure 1: Routes, and volume of sampled amphibian imports into the UK through Heathrow Animal Reception Centre between December 2009 and June 2012. Importing countries with negative shipments shaded light grey, positive shipments shaded dark grey. Arrow thickness represents the total volume of amphibians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

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