DETECTION AND REPORTING OF RANAVIRUS IN AMPHIBIANS: EVALUATION OF THE ROLES OF THE WORLD ORGANISATION FOR ANIMAL HEALTH AND THE PUBLISHED LITERATURE

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ABSTRACT: Pathogens of wildlife can have direct impacts on human and livestock health as well as on biodiversity, as causative factors in population declines and extinctions. The World Organization for Animal Health (OIE) seeks to facilitate rapid sharing of information about animal diseases to enable up-to-date risk assessments of translocations of animals and animal products. The OIE also produces manuals of recommended methods to standardize diagnostic testing. Ranaviruses are important amphibian pathogens that may have spread through international trade, and infections became notifiable to OIE in 2009. We surveyed and reviewed published literature for data on sampling, diagnostic testing, and reporting of ranavirus during 2009–14. We also investigated attitudes and awareness of the OIE and its recommendations for best practice. We found that sampling effort is uneven and concentrated in the northern hemisphere. We also identified citizen science projects that have the potential to improve the quantity and quality of data on the incidence of ranavirus infection and the circumstances surrounding disease outbreaks. We found reporting of infection to be inconsistent: reporting was split between the published literature (where it was subject to a 2-yr lag) and the OIE with little overlap, results of negative diagnostic tests were underreported, and scientific researchers lacked awareness of the role of the OIE. Approaches to diagnostic screening were poorly harmonized and heavily reliant on molecular methods. These flaws in the mechanisms of ranavirus detection and reporting hamper the construction of a comprehensive disease information database.

Key words: Molecular diagnostics, OIE, ranavirus, surveillance, wildlife disease, World Organization for Animal Health.

INTRODUCTION

Infectious diseases of wildlife can drive declines and extinctions of wildlife populations and place significant burdens on the health of humans and domestic animals (Jones et al. 2008; MacPhee and Greenwood 2013). International cooperation in the control of animal diseases is facilitated by the World Organization for Animal Health (OIE). The main objective of OIE is to enable rapid information transfer about animal diseases between its 180 member states as well as nonmembers (OIE 2017). The World Animal Health Information System (WAHIS) was developed to facilitate this transfer of information.

The OIE member states are required to file regular updates of their national and regional status with respect to listed diseases (OIE 2016a). Laboratories undertaking diagnostic testing for notifiable diseases report positive results to their national veterinary authority, which then reports to the OIE (Jebara et al. 2012). The frequency of reports depends on the status (i.e., whether disease/infection is present or absent), with weekly updates required immediately following a new outbreak. The database of reports is intended to enable risk analysis of international trade in animal products or sanitary methods in the international food trade—limiting pathogen pollution (Cunningham et al. 2003) and reducing the risk of new disease outbreaks. In addition to sharing disease information, the OIE produces the Terrestrial and Aquatic Animal Health Codes, setting out standards for the improvement of animal health and welfare worldwide, and for safe international

trade in animals and their products (OIE 2016a). Manuals of Diagnostic Tests are published to standardize the use of diagnostic techniques for listed diseases (OIE 2016b), pointing laboratories to established methods.

Until recently, most OIE listed diseases could be described as having direct impacts on either human health (zoonotic pathogens) or food production (pathogens capable of affecting domestic food producing animals). However, two diseases linked to amphibian declines, and known to have been translocated by humans, were listed as notifiable to the OIE in 2009: chytridiomycosis, caused by the "chytrid" fungus *Batrachochytrium dendrobatidis* (Bd), and ranavirosis, caused by large double-stranded DNA viruses of the genus *Ranavirus* (Schloegel et al. 2010). Neither of these pathogens has zoonotic potential, and although they can affect domestic amphibians used in food production, they are primarily of global concern because of impacts on wild populations and communities of amphibians (Cunningham et al. 2003; Price et al. 2014).

Ranaviruses (genus *Ranavirus,* family *Iridoviridae*) have a patchy global distribution and broad host ranges, affecting amphibians, reptiles, and fish (Duffus et al. 2015). In amphibians, they are important pathogens capable of causing population decline and extinction, which may affect entire communities (Earl and Gray 2014; Price et al. 2014). Humans have contributed to the ongoing emergence of ranavirosis through the movement of amphibians in trade (Picco and Collins 2008; Schloegel et al. 2009). The potential for very severe host impacts and a likely role for international trade in facilitating emergence were major factors behind the OIE decision to make ranavirus notifiable (Schloegel et al. 2010).

We assessed the nature and extent of global detection and reporting of ranavirosis. We evaluated the role of the OIE in ranavirus surveillance with respect to its main objectives: to facilitate rapid sharing of information and to enable risk assessments of trade in animal products. We also evaluated the role of the published literature in reporting information about ranavirus distribution. We analyzed data on the motivations for undertaking sampling of amphibians for detection of ranavirus infection and the uptake and use of the recommended methods given in the OIE's Aquatic Manual. Finally, we used additional survey data to assess the potential for an enhanced role for citizen science projects in ranavirus surveillance.

MATERIALS AND METHODS

Systematic literature review

We searched the literature in January 2015 with the use of the bibliographic databases: BioMed Central, BIOSIS Citation Index, and CAB Abstracts. Variations of "amphibian" and related terms (amphib*, frog, toad, salamander, newt, caecilian, caudat*, anur*, gymnophion*) were searched for in conjunction with variations of "ranavirus" or "iridovirus" (ranavir* or iridovir*). Articles which did not describe original research involving testing of naturally infected amphibians for ranaviruses sampled since 2009 (i.e., review articles and articles describing testing of experimentally infected amphibians) were discarded. Data collection was restricted to 2009 or after because ranavirus infection became notifiable to the OIE in that year, and one of our objectives was to compare published studies and OIE data. All papers included in the review related to sampling carried out since 2009 and were published before January 2015.

To ensure that the literature search was exhaustive we checked our article list against resources made available by the Global Ranavirus Consortium (GRC 2014) and a table published by Miller et al. (2011) which detailed all instances of amphibian ranavirus infection known at that time by geographical region. These resources revealed only one additional reference, a thesis paper (Todd-Thompson 2010).

We collated data from published articles, including the geographic origin of the amphibian samples, whether captive or free-ranging hosts were tested, the number of samples tested, the diagnostic method(s) used, and the number of samples positive for ranavirus infection. Papers were categorized according to the period of time in which samples were collected (2009–11 or 2012–14) to enable comparisons with questionnaire data.

Questionnaires

We produced two surveys, approved by the Royal (Dick) School of Veterinary Studies Human Ethical Review Committee at the University of Edinburgh, with questions relating to ranavirus surveillance and diagnostic testing using Survey-

Monkey (2015). The first survey targeted laboratories or institutions undertaking diagnostic testing of amphibian samples for ranaviruses ("laboratory survey") to gather data on the amount, motivation, and methods of ranavirus surveillance (Supplementary Material S1). The second survey targeted nongovernmental organizations and citizen science projects ("NGO survey") and aimed to characterize the role and potential for such projects in surveillance (Supplementary Material S2).

We contacted the Global Ranavirus Consortium mailing list, International Union for Conservation of Nature Amphibian Specialist Group members, and authors of published papers on the subject of amphibian ranaviruses by email in late 2014 to invite them to respond to the questionnaires online. We also emailed links to the NGO survey to representatives of amphibian conservation groups. Links to both online surveys were published in the Amphibian Specialist Group blog, and an article highlighting the surveys was included in FrogLog (Black 2015). Maps summarizing data from both the systematic review and questionnaire data were generated in R (R Development Core Team 2013) using the World Borders Dataset (Sandvick 2009).

Both questionnaires contained a section for individual and organizational contact details, but respondents were assured that data would be categorized only by geographical region and reported anonymously. Personal details of respondents were used to avoid duplication between questionnaire data and published records.

The laboratory survey contained sections on the sources of samples, the purpose of testing, the number of samples tested between 2012 and 2014 inclusive, the number of positive results, and reporting methods. Questions focused on the preceding 3 yr (2012–14) for practical reasons relating to ease of access to records. The NGO survey contained sections on the type of organization, the recording of amphibian mortality events, and the facilitation of sample submission for diagnostic testing.

Participants in the laboratory survey were also asked about the specifics of diagnostic tests used as well as attitudes toward and use of the OIE Aquatic Manual. The Aquatic Manual currently recommends two molecular approaches for detection and identification of ranaviruses: PCR testing followed by either restriction endonuclease analysis or DNA sequencing (OIE 2016b). Follow-up of PCR with DNA sequencing is recommended to confirm that positive results yield from amplification of the desired target rather than some other nonspecific target and therefore confirm that the assay is robust.

In addition to responses to survey questions, we assessed how frequently the OIE-recommended PCR method was utilized via a search of the National Center for Biotechnology Information (NCBI) nucleotide databases (Clark et al. 2016). We compared the OIE-recommended primer set to PCR primers 4 and 5 of Mao et al. (1997), which target different regions of the same major capsid protein (MCP) gene. To obtain sequences for the PCR products produced by both assays, we extracted the nucleotide sequence at the genomic regions amplified by each primer set from the type species of ranavirus, frog virus 3 (Tan et al. 2004). To check how many sequences from each assay had been archived in GenBank, we used the frog virus 3 sequences as queries to search for similar sequences at the same genomic region. We performed megablast searches against the nr/nt databases with "max. target sequences" set to 1000, "expect threshold" set to 0.1, and remaining search parameters set to defaults (McGinnis and Madden 2004). Searches were limited to entries tagged "Ranavirus (taxid:10492)." To recover only sequences likely to originate from sequencing of PCR products we removed those hits with lower than 75% coverage of the full PCR products as well as removing hits to whole or partial genome sequences or the complete gene.

Phylogenetics

In addition to validating results of diagnostic tests, we hypothesized that information gained from DNA sequencing might convey important information about virus genotype that could be used to make management decisions. We used phylogenetics to assess the precision of virus genotype information gained from sequencing the product of a positive PCR screen for the MCP gene of ranavirus (see Supplementary Material S3 for detailed methods). We constructed a phylogenetic tree from sequences in the genomic region amplified by the MCP PCR primers 4 and 5 (Mao et al. 1997) and compared this to a tree constructed from the sequences of 26 complete genes, considered more reliable than candidate gene approaches for inferring relationships among virus isolates (Jancovich et al. 2010; Mavian et al. 2012). Both phylogenetic trees used nucleotide data from 18 ranavirus isolates with whole-genome data available in NCBI GenBank (isolate details in Table S1).

Statistical analysis

To evaluate the potential for bias in reporting the absence ranavirus through the published literature we performed a chi-squared test on the proportion of positive to negative records compared to the same type of data gathered through the lab survey.

RESULTS

Forty-three papers were included in the systematic review of literature (Supplementary Material Table S2). The laboratory survey was completed by 43 respondents and the NGO survey by 21 respondents. Not all respondents answered all questions.

Surveillance effort

Our survey of ranavirus reporting over the period 2009–14 showed that although the number of samples and sampling events per geographic area was extremely variable, all continents where amphibians occur were sampled except Australia (Fig. 1). Few sampling events were recorded in some regions. For example, Africa remained largely unsampled apart from Cameroon and Madagascar. Most effort was expended in the northern hemisphere; 79% (34/43) of published papers recorded sampling from North America and Europe but relatively few studies reported sampling in the tropics and in the southern hemisphere. Most records from the southern hemisphere occurred in the last 3 yr examined (Fig. 1c), suggesting that change may be occurring.

Scientific researchers made up 88% (30/34) of laboratory survey respondents who answered a question on the source of amphibian samples. Sixty-eight percent of them said samples were "always" or "often" the result of their own sampling with samples submitted by members of the public, government agencies, and veterinarians contributing to the remainder (Fig. 2a). Respondents listed ongoing surveillance for a previously found pathogen, routine scanning for a pathogen not previously present, investigations of mass mortality events, and studies to answer specific research questions as motivations for sampling amphibians for ranavirus with similar frequencies (Fig. 2b).

Citizen science

A total of 48% (10/21) of NGO survey respondents stated that they facilitated the submission of amphibian samples for labora-
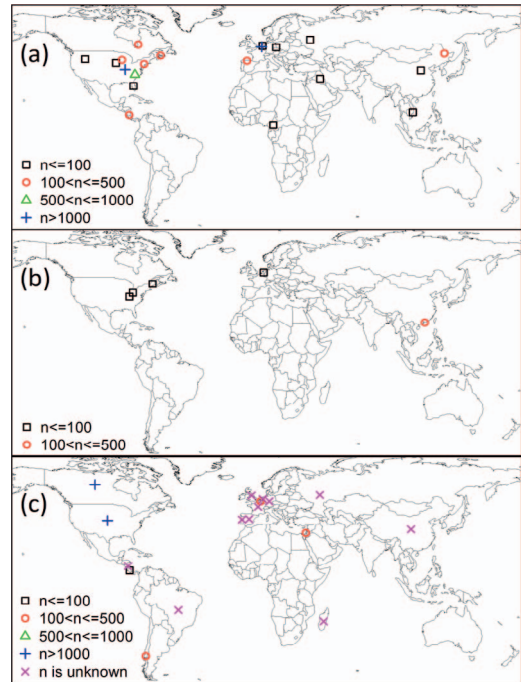

FIGURE 1. Geographical overview of sampling effort by researchers who performed diagnostic tests for *Ranavirus* using data from (a) published literature (in the period 2009–11); (b) published literature (2012–14); (c) responses to a survey of laboratories (2012–14). *n*=number of samples collected at each location.

tory testing. Data on amphibian mortality and disease distribution were also collected by citizen science projects: 67% of NGO survey respondents reported that their organization received data concerning sick or dead amphibians from members of the public.

Reporting

A total of 12% (5/43) of published papers reported no samples testing positive for ranavirus. In comparison, 33% (5/15) of respondents to the laboratory survey reported that they had obtained no positive results. A significantly higher proportion of published papers reported the presence of ranavirus compared to unpublished laboratory surveys (χ^2=9.11, $P<0.01$).

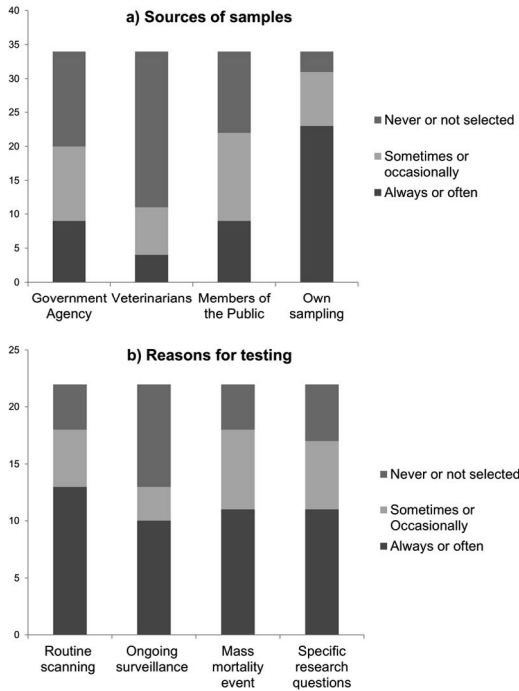Only 14% (6/43) of published papers conducted sampling between 2011 and 2014,
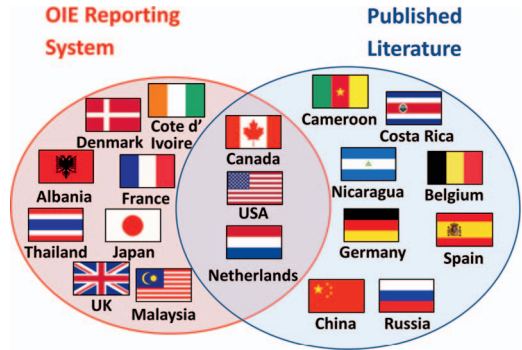
FIGURE 3. Comparison of the World Organization for Animal Health (OIE) reporting system and the published literature as sources of information about spatial and temporal trends in reports of *Ranavirus* infections and disease outbreaks between 2009 and 2014. The OIE reporting occurs at a national level so reports are summarized at that level and indicated by flags.

FIGURE 2. Sources of samples and reasons for undertaking diagnostic testing for ranaviruses. (a) Respondents to a survey of laboratories ($n=34$) indicated the frequency with which a list of individuals and organizations submitted amphibian samples to their laboratory (question 4, laboratory survey; see Supplementary Material S1). (b) Given a list of possible motivations, respondents ($n=22$) indicated the frequency with which amphibian ranavirus testing was carried out at their laboratories (question 20, laboratory survey; see Supplementary Material S1). Suggested motivations were "Routine scanning (for a pathogen not previously present)," "Ongoing surveillance (for a previously found pathogen)," "(To investigate a) mass mortality event" or "(To answer a) specific research question." Respondents were not required to select a frequency (always, often, sometimes, occasionally, or never) for every source/motivation on the lists. "Not selected" is used where no selection was made and is expected to correspond closely with "never" as, in all cases, respondents had selected other options as applying always, often, sometimes, or occasionally.

as compared with 86% between 2009 and 2011. Some of these changes may reflect actual changes in ranavirus diagnostic testing efforts; however, they may also be a consequence of the considerable time lag in publishing data. The mean lag time (date of last sampling subtracted from publication date) for publication was 2 yr (SD=1.2 yr), with some studies not published until 5 yr after the last sampling date.

The WAHIS database is not a comprehensive source of distribution data for infection with ranaviruses. Our laboratory survey and literature review revealed amphibian samples from Germany, Russia, Costa Rica, Nicaragua, and Cameroon that tested positive since 2009 (Fig. 3), but WAHIS has "no information available" for these countries (WAHID 2015). The disease was also listed by WAHIS as never having been reported in China, and "not reported in this period" in Belgium or Spain, although the published literature detailed ranavirus infections in each of these three countries since 2009 (Fig. 3).

Survey data revealed a general lack of awareness regarding the existence and function of the OIE: only 21% (7/34) of respondents who answered a question on the subject stated that they reported the results of their testing to the OIE. Results of testing were reported to bodies other than the OIE, with 32% of laboratory questionnaire respondents stating that they reported results to the sample submitter, national conservation bodies, or government entities.

FIGURE 4.   Summary of common techniques used in diagnostic testing for *Ranavirus* recorded (a) in published studies and (b) by respondents to a survey of laboratories. Where more than one test was used by a single respondent or study, the different methods used are each counted once—frequency is therefore able to exceed the number (*n*) of individual respondents/studies that contributed. PCR=conventional PCR assay; qPCR=real-time quantitative PCR; VI=virus isolation; other=any other diagnostic technique.

Diagnostic methods

In total, 56% (24/43) of published accounts and 68% (15/22) of survey respondents who answered questions on diagnostic methods, applied OIE recommended methods (cell culture, PCR with sequence analysis, or both). None described the use of PCR-restriction endonuclease analysis. Most studies used just one diagnostic method for ranavirus diagnostic testing (58% of published accounts and 45% of laboratory survey respondents). Both conventional and quantitative PCR approaches predominated. Only one study that applied a single technique used a non–PCR-based approach (virus isolation; Fig. 4a). The use of DNA sequencing was not done to confirm specific amplification of PCR targets in 47% (20/43) of published studies, and 50% (8/16) of laboratory survey respondents using conventional PCR also did not routinely carry out sequencing of PCR products.

The OIE manual of diagnostic tests was not referred to for best practice by 66% (22/33) of laboratory survey respondents. The failure to follow OIE recommendations stemmed from a lack of awareness, perceived capacity, and confidence in the methods. Twelve respondents had never heard of the Aquatic Manual, and cost (three respondents), time (two respondents), or lack of agreement with the recommendations (three respondents) were other reasons given.

A variety of published PCR protocols (Table 1) were cited by published papers and by laboratory survey respondents. None of these publications contained details of the diagnostic sensitivity or specificity of the protocol when used on amphibian samples. The most recent edition of the Aquatic Manual recommends the use of a specific PCR targeting the ranavirus MCP gene (580 base pairs [bp] sequence; OIE 2016b). However, we found that most investigators (94% of respondents) using a conventional PCR method followed the PCR protocol of Mao et al. (1997) which targets a different region of the same gene. An analysis of sequences that were present in the NCBI nucleotide sequence databases confirmed this preference for the Mao et al. (1997) assay over the recommended OIE protocol: there were 81 entries for the Mao et al. (1997) PCR product compared with only 30 entries for the OIE recommended PCR product (Supplementary Material S3). Unpublished protocols were used by 62% (8/13) of laboratory survey respondents who used quantitative real-time PCR (qPCR) and half of these stated that their protocol was either "not validated" or that they were unsure how it was validated.

Phylogenetics

To assess the quality of information lost through failure to follow OIE recommenda-

TABLE 1. PCR methods used in diagnostic screening for Ranavirus infection reported through the published literature and a questionnaire survey of laboratories. Details of original publication where method was described and the availability of validation (diagnostic sensitivity/specificity) data.

Citation	Validation data available?
Conventional PCR protocols	
Mao et al. (1997)	No
Hyatt et al. (2000)	No
Holopainen et al. (2009)	No
Bollinger et al. (1999)	No
Kattenbelt et al. (2000)	No
Real-time quantitative PCR protocols	
Picco et al. (2007)	Cites Brunner (2004)
Pallister et al. (2007)	No
Brunner and Collins (2009)	No
Brunner (2004)	Some validation data published later (Hoverman et al. 2011, citing Picco et al. 2007)
Forson and Storfer (2006)	No
Allender et al. (2013)	Yes (in turtles)

tions regarding sequencing of PCR products, we compared phylogenies constructed from 1) sequences in the genomic region covered by the product of the most commonly used PCR method (Mao et al. 1997), and 2) a concatenated alignment of 26 conserved ranavirus genes. The concatenated gene approach resulted in much more data for analysis: the sequence alignment was 38,793 bp in length versus 531 bp for the MCP PCR product alignment. However, both phylogenies supported very similar topologies, with the lack of monophyly of common midwife toad virus–like viruses in the MCP tree being the major difference. In spite of an overall loss of resolution resulting from the much smaller data set, the MCP PCR product tree offered a reasonable phylogenetic signal; that is, sequence data derived from this test could be used to assign virus isolates to the main virus types (Fig. 5).

DISCUSSION

We used a systematic literature review and questionnaire surveys to collect data on sampling of amphibians for ranaviruses, diagnostic methods, and reporting of results since 2009 when infection with these viruses was made notifiable to the OIE. We examined these data in the context of the OIE's objective to facilitate rapid sharing of information about infections with important animal pathogens. We found that OIE members were
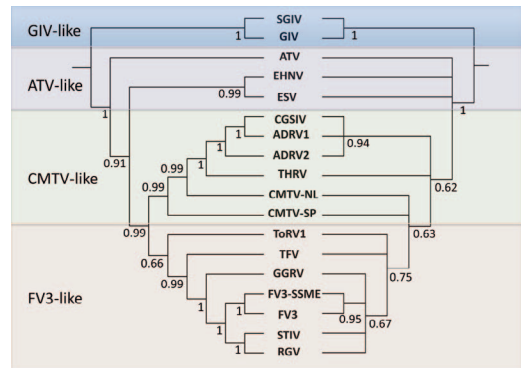

FIGURE 5. Phylogenetics was used to assess the amount of information on virus genotype (phylogenetic signal) obtained through DNA sequencing of a commonly generated PCR product by comparison to a multigene method of tree construction from complete virus genomes. Both trees contain the same virus isolates and are presented side by side with major clades highlighted. The phylogeny on the left utilizes data from 26 conserved genes (total alignment length was 38,793 base pairs). The phylogeny on the right utilizes sequences covering part of the major capsid protein gene corresponding to the region amplified by primers 4 and 5 (531 base pairs) from Mao et al. (1997). GIV=grouper iridovirus; ATV=ambystoma tigrinum virus; CMTV=common midwife toad virus; FV3=frog virus 3.

underreporting ranavirus infection to the OIE, resulting in a WAHIS database which did not reflect the full state of knowledge. We also found that recommendations set out in the Aquatic Manual were not routinely followed. Furthermore, failure to follow OIE advice to sequence PCR products has resulted in a loss of genetic data that could be used to characterize viruses with direct implications for management.

The published literature and OIE reporting system represent the two main reporting mechanisms for ranavirosis, but we found there to be very little overlap between the two. Of 19 nations reporting ranavirus infections in the period 2009–14, only three were present in both WAHIS and the published literature (though the French outbreak has been published recently; Miaud et al. 2016). Ranavirus reporting for the remaining nations was split evenly between the OIE system and the published literature. Efforts to control diseases with direct effects on domestic animals, coordinated by the OIE, have resulted in real successes such as the eradication of rinderpest (Njeumi et al. 2012). However, reporting of wildlife diseases can be opportunistic, as seen with avian influenza surveillance in wild birds (Machalaba 2015). Only half of laboratory survey respondents reported their results either directly to the OIE or to another body that may have assumed this responsibility.

We found that most samples tested for ranavirus were collected by research scientists, which in the context of the split in reporting between OIE reporting system and the published literature, suggests a lack of communication between the academic community and government agencies. This means that timely reporting of results is likely to remain a challenge. The primary output of academic research is publication in peer-reviewed journals, but we found weaknesses in the scientific literature as a reporting mechanism, with an average time lag between sampling and publication of 2 yr. We also found a significant bias toward the publication of positive results. Despite a move toward open-access data sharing, barriers to data publishing and reuse remain, including a lack of the time and resources but also a lack of explicit career rewards for such sharing (Swan and Brown 2008).

It is not clear whether ranavirosis is a typical case for wildlife diseases listed by the OIE but it is reasonable to think there will be comparable cases, because some of the laboratories we surveyed are centers for diagnostic testing of pathogens from diverse host species, and many of the surveyed NGOs were amphibian and reptile groups with an interest in multiple diseases. The fungal disease, Bd and ranavirus became OIE listed at the same time, and diagnostic testing for both pathogens often occurs in the same laboratories, so many of the same issues with reporting may be relevant to reporting of chytridiomycoses. A brief comparison of the databases of the open-source "Bd-maps" project and the OIE appeared to confirm this; e.g., Bd-maps recorded Bd in Chile after 2009 (Bourke et al. 2010) but the OIE database had no record from Chile.

In addition to these challenges surrounding reporting, we also found challenges around sampling for ranavirosis with a geographical bias toward the northern hemisphere. Amphibian mass-mortality events triggered sampling for ranaviruses (e.g., Reshetnikov et al. 2014), but our survey respondents indicated that diagnostic testing, ongoing surveillance, and the pursuit of answers to specific research questions were equally common motivations. The availability of funding is likely to be a key factor affecting the geographical distribution of sampling effort (Jones et al. 2008). However, although some countries conduct wildlife disease surveillance as a part of routine management, most still only address events in postoutbreak scenarios (Schwind et al. 2014).

The OIE has also been unsuccessful in standardizing diagnostic testing methods through its manuals; rather, we found many different tests in use. We found that a single assay is typically used in testing for ranaviruses, almost always a molecular method. Molecular methods are hugely valuable, but they only provide information on the presence

of a pathogen rather than disease. Rijks et al. (2016) have taken a more thorough approach, utilizing pathology in combination with molecular approaches. This is by no means a problem specific to ranavirus research, but a rigorous understanding of the limitations of commonly utilized molecular methods is required, especially the necessity for validation. By comparison, diagnostic testing of chytridiomycoses has been more standardized but similarly narrow in terms of the type of diagnostic approach used. For both Bd and *Batrachochytrium salamandravorans,* the rapid publication of a molecular method was followed by almost universal adoption (Boyle et al. 2004; Blooi et al. 2013). In the case of ranavirus, there have been periods of stability (e.g., the dominance of the Mao et al. [1997] PCR method) but many—often unvalidated—qPCR protocols are now in use.

The OIE does not include a qPCR method among its recommended methods, but this is likely because of a lack of available protocols at the time of the last release of its manual. In the absence of OIE recommendations, we urge caution in the interpretation of results. Methods are generally unvalidated or validated only in a limited way (Jaramillo et al. 2012). Miller et al. (2015) warn against reliance on high cycle threshold scores from qPCR assays in detection because of the risk of false-positive results. At the limit of detection (the lowest amount of the target detectable in a single reaction), results for identical samples from different laboratories may differ (Waiblinger et al. 2011). Despite this, recent studies have made strong conclusions based only on high or unreported cycle threshold scores from partially validated qPCR methods (Kolby et al. 2015; Warne et al. 2016).

The OIE recommends DNA sequencing of PCR products to support results from diagnostic testing, but we found that sequencing was not carried out in half of cases. In localities where infections have been confirmed previously, such repeated confirmation may be unnecessary. However, given the imperfect specificity of molecular methods and potential for contamination of samples, it seems a highly desirable step when testing for infections in new localities or hosts. We also showed that even small amounts of DNA sequence data can yield useful information about virus genotype, which might have important implications for management given that virus genotypes are somewhat spatially separated (e.g., common midwife toad virus–like viruses in mainland Europe and Asia) and that genotype may predict differences in virulence and host range (Teacher et al. 2010; Price et al. 2014). In many cases, we found that even when sequencing had occurred these data were not always archived in a public database, which limits the capacity to deliver the OIE objective of enabling the best possible risk assessments.

In addition to the OIE system of reporting, there are some disease-specific international reporting systems. The afore-mentioned "Bd maps" is an open-source geographical database to collate the results of diagnostic testing for Bd (Aanensen and Fisher 2016). The GRC recently launched a similar project for ranavirus reporting: the Global Ranavirus Reporting System (GRRS 2016). These systems rely on voluntary reporting of disease information. The GRRS has the potential to provide a more comprehensive source of information on ranavirus infection than currently exists. Certainly, the established Bd-maps project holds a large volume of data. The GRRS is heavily integrated within the academic community (who currently undertake the bulk of ranavirus diagnostic testing) through the GRC, and therefore might be able to cut the lag in reporting via academic literature by gaining permission to share basic information ahead of publication. This online system also uses a simple, open-access form that delivers a more straightforward reporting route than that currently utilized by the OIE.

Citizen science projects can make important contributions to wildlife disease surveillance (Lawson 2015). Indeed, approximately half of the respondents to our NGO survey had facilitated the submission of dead amphibians for diagnostic testing. Citizen science projects can also co-ordinate targeted disease surveillance efforts (Griffiths et al. 2015) and enable large-scale reconstructions of disease

emergence to test hypotheses about modes of spread (North et al. 2015; Price et al. 2016). Our NGO survey also showed that many amphibian groups recorded mortality and morbidity events, which can be used as a basis for syndromic surveillance. Projects in the Netherlands, the UK, and the US are currently monitoring the spatial and temporal distribution of amphibians and their mortality events, as well as facilitating postmortem examination and diagnostic testing of dead amphibians by liaising with members of the public (Lawson et al. 2015; Reptile, Amphibian, and Fish Conservation in The Netherlands 2016; US Geological Survey 2016).

If we are to meet the challenges presented by the rapidly evolving interactions between pathogens, wildlife, livestock, and humans, then the global scientific community must cooperate wherever possible to adopt a robust approach to diagnostic testing and rapid, consistent sharing of data. This should be done utilizing the best available platform, which may be either WAHIS or a disease-specific network such as the GRRS.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2016-08-176.

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