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REPLY COMMENT

PCR testing for diagnosis of *Ichthyophonus hoferi*: Reply to LaPatra & Kocan (2013)

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ABSTRACT: LaPatra & Kocan (2013) critiqued our paper Hamazaki et al. (2013; Dis Aquat Org 105:21–25) for data not supporting the conclusions of 'PCR testing is as accurate as culture…', but they neither pointed out what part of our data did not support our conclusion, nor did they provide any contrary scientific evidence supporting their argument that PCR testing is less accurate than culture. In the absence of any contradictory data, we stand by our data and our conclusion: PCR test is as suitable as culture as a diagnostic and field surveillance tool.

KEY WORDS: *Ichthyophonus hoferi* · Explant culture · Polymerase chain reaction · Diagnosis · Confirmation

While LaPatra & Kocan (2013) critiqued that our dataset did not support our conclusion that 'PCR testing can be as accurate as culture for diagnosis of *Ichthyophonus hoferi* …' (Hamazaki et al. 2013), they did not dispute the validity of our data: greater then 90% sensitivity, greater than 97% specificity, and greater than 96% agreement between culture and conventional polymerase chain reaction (PCR, or more precisely cPCR) for diagnosing *Ichthyophonus* infection. Nor did they present any counter scientific evidence that cPCR testing is less accurate than culture testing. Instead, they criticised our paper for overstating the usefulness of the cPCR test as a suitable diagnostic and field surveillance tool; however, statistically identical estimates of *Ichthyophonus* infection prevalence between culture and cPCR tests (Kahler et al. 2011, Hamazaki et al. 2013) prove that the cPCR test is as suitable as culture as a field surveillance tool. We respond here to the specific criticisms: (1) possibility of cPCR and impossibility of culture for 'false positives', (2) evaluation of cPCR tests not strictly adhering to the World Organization for Animal Health (OIE) guideline protocol (OIE 2013), (3) our dismissal of Whipps et al. (2006) on low sensitivity of cPCR.

Every diagnostic technique can generate both 'false negatives' and 'false positives.' For a technique using assays, 'false positives' can come from a fish that was exposed to but not necessarily infected with a pathogen, or from a fish that had recovered from an infection. On the other hand, for direct visual techniques 'false positives' can come from misidentification of a pathogen, for which LaPatra & Kocan (2013) acknowledge use of cPCR as suitable for confirming identity of a pathogen, including identification of *Ichthyophonus* at the species level (Criscione et al. 2002). Acknowledging that every technique is prone to errors of both 'false positives' and 'false negatives', the major question is whether (or which) errors are problematic for a population of interest and surveillance objective. It is likely that a diagnostic technique suitable for one population and objective may not be suitable for other populations and other objectives.

Thus, under the guidelines of the World Organization for Animal Health (OIE 2013), evaluation of a diagnostic technique should ideally be conducted using known positive/negative reference samples from *the target population* (e.g. Yukon River Chinook

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salmon). Using laboratory samples (e.g. hatchery raised Chinook salmon artificially fed with *Ichthyophonus* infected prey) is not ideal because those samples may not represent the true infection status of the target population (OIE 2013, Chapter 1.1.2). For the Yukon River Chinook salmon, sources and routes of *Ichthyophonus* have not been identified. Acknowledging the difficulties of obtaining true ideal reference samples, the OIE guideline accepts using samples identified by another test of sufficiently high accuracy (OIE 2013, Chapter 1.1.2). Since culture has been used as a standard for diagnosis of *Ichthyophonus* (Kocan et al. 2011), using culture as reference samples (Hamazaki et al. 2013) is entirely in accord with the guideline not only of the OIE but also the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) Diagnostic Evaluation Expert Panel (Banoo et al. 2008).

We dismissed Whipps et al. (2006)'s results for low sensitivity of cPCR because of small sample size (n = 6 to 7) and a misleading definition of infection severity ('light' vs. 'heavy') that does not necessarily correspond to intensity of infection or pathogen load (Hamazaki et al. 2013). Results from small samples have no statistical confidence and validity. It is our opinion that their flawed data should not have been reported, let alone be generalized. For this reason we included datasets of Whipps et al. (2006) in our study (Table 2 in Hamazaki et al. 2013), so that readers can reproduce and evaluate the validity of our conclusions. Moreover, it is unclear why LaPatra & Kocan (2013) pay special attention to this Whipps et al. (2006)'s flawed and questionable finding, while they ignore Whipps et al. (2006)'s major findings of greater than 90% of sensitivity and specificity (n > 300) for diagnosing *Ichthyophonus* infection (Table 2 in Whipps et al. 2006).

Finally, LaPatra & Kocan (2013)'s critique of our overstatement of the usefulness of the cPCR test as a field surveillance tool is irrelevant because usefulness of a diagnostic test is determined by research objectives. For example, Zuray, Kocan and Hershberger (2012) employed a diagnostic criterion of 'presence of visible lesions on heart tissue' for surveillance of *Ichthyophonus* disease prevalence of the Yukon River Chinook salmon in the upriver. Obviously, this criterion is much less accurate than the culture or the PCR tests and subject to errors of both 'false positives' (e.g. visible lesions caused by other pathogens) and 'false negatives' (e.g. diseased fish not developing lesions). However, it can be reasonable for discerning a long-term trend of *Ichthyophonus* disease prevalence, given that the observed trend was similar to that at the mouth of the Yukon River using culture/PCR tests (JTC 2013). As this example illustrates, while employing the most accurate diagnostic test is preferable, the most important criteria is whether a diagnostic test can sufficiently meet research objectives.

In conclusion, in the absence of contrary scientific evidence, we stand by our data and conclusion that the PCR test is as good as culture for diagnosis of *Ichthyophonus* infection. It is our opinion that researchers should choose the most appropriate diagnostic tool based on the needs of their research objectives and field circumstances.

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