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COMMENT

PCR testing for diagnosis of *Ichthyophonus hoferi*: Comment on Hamazaki et al. (2013)

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ABSTRACT: It is our opinion that Hamazaki et al. (2013; Dis Aquat Org 105:21–25) overstate the usefulness of PCR as a field diagnostic technique and underestimate the accuracy and utility of *in vitro* explant culture. In order for field diagnostic studies to be meaningful they should accurately and dependably identify the infected individuals within a population, both subclinical and clinical cases. Although explant culture, like most techniques, can miss some infected individuals, 'false positives' are impossible, unlike for cPCR based methodologies.

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

Hamazaki et al. (2013) conclude that the conventional polymerase chain reaction (cPCR) is a suitable diagnostic and field surveillance tool for detecting *Ichthyophonus* in Chinook salmon from the Yukon River. However, the real value of the cPCR for *Ichthyophonus* involves its utility as a non-quantitative diagnostic tool that is particularly well suited for confirming the identity of the pathogen. From this perspective, cPCR effectively replaces older, more onerous, and less reliable methods including evaluation of morphological features, and serological assays. Although the cPCR is exceptionally suited as a confirmatory diagnostic tool, it has several shortcomings when applied as a field surveillance tool. Most importantly, as the authors acknowledge, these molecular-based methods detect segments of the *Ichthyophonus* genome and not the whole organism. Therefore, a positive PCR result may be returned from a fish that was exposed to, but not necessarily infected with the parasite. The authors attempt to refute this shortcoming by stating 'This disadvantage, however, is probably not an issue in our study because it is unlikely that Yukon River Chinook salmon carry non-living *Ichthyophonus*' (p. 22). This is an assumption on the part of the authors that is not supported by any scientific evidence.

Microscopic evaluation of *in vitro* explant culture is a commonly used field surveillance technique for determining the infection prevalence of *Ichthyophonus* in fish populations. Unlike cPCR, where a positive test indicates the presence of a portion of the *Ichthyophonus* genome (not evidence of infection), a culture-positive fish is unequivocally positive, as the live organism was isolated and visualized from the host tissues; consequently, false-positives are not possible. If, as the authors suggest, rapid results are the priority for the field surveillances, then the most rapid diagnostic tool for *Ichthyophonus* would be explant culture of suspect tissue followed by confirmation of the identity of the parasite. Regardless, the labor, cost, and laboratory processing time are greatly reduced with explant cultures compared to cPCR.

Additionally, detailed and standardized methods exist for evaluating the sensitivity and specificity of laboratory diagnostic tests. Such methods, including those described by the World Organization for Animal Health (OIE 2009), require the comparison of test

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results from fish with known exposure/infection profiles. Unfortunately, the true infection status of the wild Chinook salmon reported by Hamazaki et al. (2013) was unknown. To compensate for this the authors used culture-positive fish as a baseline of infection. Sometimes this returned more positive cPCR than culture (and vice versa). Therefore, true test specificity and sensitivity (false positives, false negatives, true positives, and true negatives) were impossible to determine with the experimental design, and the title of the manuscript 'PCR testing can be as accurate as culture…' is unsupported.

Regardless of the validity of the above arguments against using cPCR as a diagnostic technique, the data themselves do not support the implication of the title nor the authors' conclusions, and there are several incorrect assumptions and misinterpretations of the available data. As an example the authors downplay culture as impractical by citing the remoteness of the sampling locations, need for refrigeration, and slow processing time. To the contrary, explant cultures have been successfully employed in remote locations, including the Yukon River, where >3000 tissue explant cultures were evaluated on-site from 1999 to 2003 (Kocan et al. 2004). Additionally, there is no need to refrigerate either the culture medium or the explant cultures because all components of the medium are stable at room temperature and the parasite will survive and grow at temperatures between 0° and 30°C, thus negating the need for refrigeration or incubation. Because the majority of cultures are positive by 5 to 7 d, a diagnosis can be made within 1 wk of sample collection.

The authors also attempt to dismiss the conclusions of Whipps et al. (2006), who concluded that the 'Sensitivity of the [cPCR] test varied depending on the severity of the infection…' (p. 144) and 'the sensitivity of this [cPCR] test when detecting light infection in heart tissue was lower (range between 50 and 100%)…' (p. 144). Even lower sensitivities were reported for somatic muscle. However, Hamazaki et al. (2013) dismiss these results and further dismiss the Whipps et al. (2006) sensitivity results as an 'anomaly' without providing any supporting evidence.

It is our opinion that the conclusions presented in Hamazaki et al. (2013) are not supported by the scientific evidence. The real value of cPCR for *Ichthyophonus* involves its utility as a non-quantitative diagnostic tool that is particularly well suited for confirming the identity of the pathogen. Several shortcomings exist when attempting to extend cPCR's intended application as a confirmatory technique to a primary field surveillance tool and therefore microscopic evaluation of explant cultures followed by confirmation by molecular or other techniques should be the primary method for field surveillance and laboratory diagnosis of *Ichthyophonus*.

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