Author's response to reviews

Title: False rumours of disease outbreaks caused by infectious myonecrosis virus (IMNV) in the whiteleg shrimp in Asia

Authors:

Saengchan Senapin (saengchan@biotec.or.th)
Kornsunee Phiwsaiya (kornsunee.phi@biotec.or.th)
Warachin Gangnonngiw (warachin101@hotmail.com)
Timothy W Flegel (sctwf@mahidol.ac.th)

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Author's response to reviews: see over
Dear Sirs:

RE MS: False rumours of disease outbreaks caused by infectious myonecrosis virus (IMNV) in the whiteleg shrimp in Asia
By Saengchan Senapin, Kornsunee Phiwsaiya, Warachin Gangnonngiw and Timothy W. Flegel

We have revised the manuscript cited above as recommended by the reviewer. The specific changes are outlined below in response to the review comments and the modified passages in the text of the revised manuscript are shown in red typeface.

We hope that these changes will make the paper suitable for publication.

Yours truly,

Prof. T.W. Flegel
Centex Head

Response to review comments

1. On page 3, lines 68-69: "This strongly indicated that the source of the virus for the outbreak was living shrimp imported from Brazil, probably as broodstock for PL production." Is there any additional, external evidence in support of this?

   Response: We have added (lines 69-72 of the revised MS) a sentence referring to our previous publication, stating, “As previously reported [4], a contact in Indonesia who wished to remain anonymous related that P. vannamei broodstock had been smuggled onto Java island from Brazil for use in a commercial hatchery.”

   2. How were the samples tested in this study preserved during transit from the farms to the laboratory? Is it possible that the negative results were due to improper preservation? Especially for those samples that were shipped long distance and/or stored for a long time.

   Response: All of the samples delivered to our laboratory for RT-PCR testing consisted of pleopods collected from living shrimp and preserved immediately in 95% alcohol. The samples were delivered to our laboratory and processed within 7 days after collection, as was the original material we used to detect IMNV in samples from Indonesia. In addition, the IQ2000 detection kit includes an internal control based on shrimp actin that gives a band that must appear in negative tests to verify the integrity of the RNA template. This band appeared in all of our samples that gave negative test results. In lines 90-93 of the revised manuscript we have added, “All samples for RT-PCR testing consisted of pleopods collected from living shrimp and preserved in 95% ethanol. RNA was extracted and tested within 7 days of sample collection. This protocol was the same as that used for the original samples in which IMNV was detected from Indonesia.” We have also added at lines 97 to 99, “The IQ2000 negative tests all showed an internal control band at 680 bp (see Fig. 2) indicating that the RNA in each sample was intact, and the kit positive control lanes on the same gels gave the expected positive results.”