From:	Wright, Peter <wrightpf@dfo.mpo.com>
Sent:	Friday, December 2, 2011 5:28 AM
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Subject:	FW: Paper authored by Molly Kibenge et al.

FYI

Peter Wright PhD

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'Semper in excreta sumus solum profundum variat'

From: Kitching, Paul AGRI:EX [mailto:Paul.Kitching@gov.bc.ca]
Sent: December 1, 2011 7:24 PM
To: Wright, Peter
Cc: Callan, Tara AG:EX; Marty, Gary D AGRI:EX
Subject: FW: Paper authored by Molly Kibenge et al.

Hi Peter

Hope all is well with you and that Moncton is keeping you challenged. I asked a colleague to review the Molly Kibenge manuscript. I think he was pretty restrained in his comments, but these points alone would result in rejection . There were many other questions which i have about the primers etc. I wanted to ask you about the samples you received – i think you are 4 hours in front of BC, so can i give you a call at 12.30 your time, ½ hour before our conference call ?

You can share this review if you wish

paul

As you had requested I reviewed the Paper titled:
Asymptomatic infectious salmon anaemia in juvenile *Oncorhychus* species from the North West Pacific Ocean

This is a manuscript in the early stages of revision and as such was difficult to understand. However, in addition to the failure to culture this virus, there are issues dealing with the RT-PCR testing which are important to consider when assessing the strength of its argument that asymptomatic infectious salmon anemia virus is present in wild and farmed stocks of British Columbia Salmon.

This study is based on the use of RT-PCR testing which is very sensitive and can detect down to single a copy of a target sequence. This high level of sensitivity may lead to false positive results due to amplification of PCR product contaminating laboratory equipment. For this reason a second RT-PCR reaction targeting another gene sequence is often done on the extracted nucleic acids. Amplification of this second gene region would lend support to a positive result. The authors attempted to perform the second RT-PCR by amplification of a number of other genes - yet were consistently unsuccessful. This indicates the first results were false positives. It was only when the samples were sent to the AVC laboratory that some positivity was repeated. Interestingly, of the initial 116 positive tests only five samples were positive for the segment 7 gene and these were all from Chinook salmon. These RT-PCR segment 7 products had a very high level of genetic similarity (99.7%) to a strain from Norway suggesting a common source.

RT-PCR amplification of samples containing a large amount of chromosomal DNA may lead to nonspecific amplification due to the primers binding to homologous chromosome sites. This may result in the production of fragments which happen to be approximately the same size of the expected target. The sequence of these products must be determined. For the Sockeye samples from Cultus Lake, all 64 were found to be positive by RT-PCR based by the detection of the appropriate size fragment. When the authors attempted to sequence the products, only one PCR fragment could be cloned and it yielded a DNA sequence which “had identity to ISAV only in the primer sequence”, in other words it was a false positive result, yet it was still called a positive result. There is no proof that the other 63 samples from Cultus Lake were not false positives.

I hope this helps.