Opinion – Infectious Salmon Anaemia (ISA) diagnostics on farmed salmon in British Columbia, July 14, 2011

Dr. Frederick S.B. Kibenge - Professional background and competence within the field.

Since July 1996, I have been Professor of Virology, and starting in June 2009, Chairman of Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward. Beginning in May 2004, I have served as OIE (World Organization for Animal Health) Expert for Infectious Salmon Anaemia (ISA). In this role, my laboratory confirmed the first occurrence of ISA in farmed Atlantic salmon in Chile in July 2007, and has worked very closely with government officials and the Atlantic salmon industry in Chile to characterize the virus responsible for the 2007-2011 ISA epizootic in Chile, and to provide training to the designated National Reference Laboratory for ISA in Chile. I have a Bachelor of Veterinary Medicine degree, BVM (Makerere University, 1978), a PhD in Animal Virology (Murdoch University, 1983), and extensive post-doctoral research experience in virology in UK (University of Liverpool) and USA (Washington State University and Ohio State University). I have published extensively on ISA virus (ISAV).

In this regard, only Dr. Alexandra Morton from British Columbia (BC) has contacted me about the possibility that ISA may be present in BC, and previously sent fish samples to my laboratory to test for ISAV.

Method of Undertaking

I first read the briefing written by Dr. Morton titled "Briefing – Two viruses, salmon farms and the Fraser sockeye" dated June 25, 2011, before reviewing the other material that was provided to me on July 6, 2011. Further clarification was sought from Dr. Morton to confirm that ISAV suspected cases in British Columbia have now been reported to CFIA, and whether the individually signed fish pathology reports were part of the farm audits performed by the British Columbia Ministry of Agriculture and Lands (BCMAL) in 2006-2010. Given the fact that ISAV has never been confirmed in British Columbia, the 37 signed reports by Dr. Gary Marty describing "Classic lesions associated with ISAV infection" were reviewed in detail, particularly with regards to accompanying and/or confirmatory laboratory diagnosis. To gain a further understanding, the OIE Manual of Diagnostic Tests for Aquatic Animals[1] was also consulted. I then prepared the report expressing my opinion on the two questions posed by Dr. Morton:

(1) Was proper reporting protocol followed given these diagnostics? And

(2) What is the potential risk to Pacific salmon given there are approximately 1,100 reports of ISA-type lesions, 82 of which were in Pacific salmon and 4 in Sablefish from 2006-2010?

(1) Was proper reporting protocol followed given these diagnostics?

It is evident that since 1992 there has been a significant decline in the Fraser sockeye (*Oncorhynchus nerka*) based on the number of adults returning to spawn. The pre-spawn mortality (with direct losses to fishermen estimated at more than Can$72 million in 2002) is suspected by DFO to be due to infection with a retrovirus (Salmon leukaemia virus, Marine

anaemia or Plasmacytoid leukaemia). Information from the BCMAL fish disease reports suggests presence of suspect ISA on some of the surrounding salmon farms, which might be transmitted to the migrating Fraser sockeye. While the retrovirus is known to be endemic to the region (has been diagnosed in Chinook salmon by salmon farmers since 1989, and Atlantic salmon are carriers), ISAV is considered an exotic virus as it has never been reported in British Columbia. Moreover, ISA is an OIE listed aquatic animal disease and OIE Member Countries have an obligation to report it to the OIE Central Bureau.

Dr. Gary Marty, the lead Provincial Veterinarian in Abbotsford, Animal Health Centre, BCMAL, who has conducted histopathology on fish sampled as part of the BC Auditing and Surveillance Program on the Fraser sockeye migration route (15 salmon farms/quarter from 2006-2010), has recorded the presence of classic lesions associated with ISAV infection (sinusoidal congestion in liver and/or interstitial haemorrhage/congestion in kidney) in 1011 cases in Atlantic salmon, 82 cases in Pacific salmon and 4 cases in Sablefish. The Database BCP002864 Histopathology FHAS 2006-2010a indicates PCR testing was also performed on the samples, but the results are not included in this database. In addition, approximately 37 reports signed by Dr. Marty describe the multifocal sinusoidal congestion in the liver of submitted cases of farmed salmon (Atlantic salmon, Chinook salmon, Coho salmon) as a differential diagnosis for ISAV infection. In some of the cases where RT-PCR testing for ISAV was performed, the result was negative, thereby ruling out ISAV infection (e.g., Cases 09-3272, 10-314, 10-799, 10-1368, and 10-1442). Virus isolation was attempted in other cases (cell lines used not mentioned) and was negative (e.g., Cases 07-1859, 09-2594, 09-2594, and 09-2492). In yet other cases, confirmatory diagnosis was obtained for viral haemorrhagic septicaemia virus (VHSV) (e.g., Cases 07-1353, 07-2120, 07-

2123 09-806, and 10-1034). Thus, it is not known if 22 of the 37 cases (~60%) with a differential diagnosis for ISAV infection were submitted for further laboratory testing. Additionally, in the 5 cases in which virus isolation was attempted and was negative, since the cell lines used were not mentioned, it not possible to say if this result ruled out ISAV infection.

The OIE Manual of Diagnostic Tests for Aquatic Animals[1] notes that "no pathology lesions are pathognomonic to ISA, although specific lesions are known to be consistent with ISA". The manual goes on to state that "...following the suspicion of fish infected with ISAV on a farm, an official investigation to confirm or rule out the presence of the disease will be carried out as quickly as possible, applying inspection and clinical examination, as well as collection and selection of samples and using the methods for laboratory examination as described in Section 4." The presence of the pathological changes recorded by Dr. Marty is sufficient to suspect Atlantic salmon of being infected with ISAV (i.e., ISA suspect case). Where RT-PCR testing for ISAV was also performed and the results were negative, this would rule out ISA in those cases.

Was proper reporting protocol followed given these diagnostics? It is not possible to answer this question without knowing if RT-PCR for ISAV was done and what results were obtained from the fish sampled. This information is not included in the 2 databases provided, although one of them (Database BCP002864 Histopathology FHAS 2006-2010a) includes an Animal Health Centre case number for PCR and another one for Histopathology. RT-PCR testing for ISAV is a two step process in that once the virus is detected, normally by using PCR primers targeting genomic segment 8, the ISAV genotype (North American or European) and HPR type of genomic segment 6 need to be determined by DNA sequencing. HPR stands for highly

polymorphic region (HPR) of the haemagglutinin-esterase (HE) (segment 6) gene; it is generally accepted that the HPR is a virulence marker[2]. The non-pathogenic variant ISAVs are called HPR0 viruses to indicate that they have "full-length HPR" because they have no amino acid deletions in their HPR. All ISAV isolated to date from clinical disease have deletions in HPR of HE gene relative to HPR0. It is therefore important to know the HPR type of any detected ISAV.

In case of the signed 37 reports, the apparent low profile of the pathology reports was primarily due to absence of significant mortality on the salmon farms and the fact that the cases that were tested by RT-PCR were always negative for ISAV, and because ISAV has never been confirmed in British Columbia. Testing by RT-PCR[3] and Immunohistochemistry (IHC) can still be done for the remainder of the cases with similar pathology (if samples are still available either frozen or in formalin or wax block) in order to confirm or rule out ISA. If British Columbia's marine-farmed Atlantic salmon are to be considered free from ISAV infection, data need to be provided on RT-PCR testing at regular intervals in addition to the regular health inspections and/or farm audits. If ISAV infection is detected, it has to be confirmed by the OIE Reference Laboratory for ISA (at AVC/UPEI in Charlottetown, PEI), and reported to the OIE Central Bureau by the OIE Delegate for Canada.

(2) What is the potential risk to Pacific salmon given there are approximately 1,100 reports of ISA-type lesions, 82 of which were in Pacific salmon and 4 in Sablefish from 2006-2010?

No natural outbreaks of ISA have been reported in fish other than Atlantic salmon (*Salmo salar*). Experimental infection of several Pacific salmon species chum (*Oncorhynchus keta*), steelhead

trout (*O. mykiss*), Chinook (*O. tshawytscha*) and coho salmon (*O. kisutch*) with ISAV by Rolland and Winton[4] resulted only in subclinical infection. The only *Oncorhynchus* spp conclusively shown to develop clinical disease and die due to ISAV infection is rainbow trout (*O. mykiss*), and this was following experimental infections with either highly pathogenic ISAV isolates[5] or using genetically susceptible juvenile rainbow trout[6]. From a detailed comparative study of ISAV-induced lesions in rainbow trout and Atlantic salmon, MacWilliams *et al.*[7] concluded that liver lesions were absent in live-sampled rainbow trout and that "....if ....lesions were viewed in rainbow trout under field conditions, it is unlikely that ISA would have been considered as a differential diagnosis." On this basis, the association by Dr. Marty of sinusoidal congestion in the liver with ISAV infection in the 82 cases in Pacific salmon might be unsubstantiated.

There is no published information of the presentation of ISA in Sablefish (*Anoplopoma fimbria*). Sablefish is not on the CFIA list of susceptible species of aquatic animals[8]. Therefore, the significance of ISA-type lesions in the 4 cases in Sablefish is not known.

ISAV was isolated from marine-farmed rainbow trout in Ireland in 2002, but the infection was subclinical[9,10]. The clinical presentation in diseased coho salmon in Chile in which we found ISAV was not characteristic of ISA[11]. The condition was subsequently named Icteric Syndrome, and the ISAV infection might have been coincidental[12] since the condition could be reproduced in the absence of ISAV and only in coho salmon[13].

In the 2007-2011 ISA epizootic in Chile, ISAV (virulent strain or HPR0) has been detected throughout the salmon industry, including in farmed rainbow trout and coho salmon, but clinical

disease similar to that seen in Atlantic salmon has not been reported. A recent analysis of 502 samples of fish, mollusks, crustaceans, sea lion faeces, and microplankton around farmed salmon cages in an estuary in southern Chile detected ISAV only in free-living *Salmo salar*, and the virus strain was 100% identical with the most commonly isolated strain, the most virulent strain in Chile, HPR7b[14]. The free-living salmon, which can be assumed to be escapees from salmon farms, had high virus loads of ISAV, but showed no clinical disease[14].

The potential risk of ISAV infection to Pacific salmon would be significant if infection were to be confirmed in British Columbia's marine-farmed Atlantic salmon. The spread of the virus to Pacific salmon would pause a challenge because the clinical presentation would be different (see MacWilliams *et al.*, 2007)[7] and may not be recognized without proper laboratory investigation.

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From: Alexandra Morton <gorbuscha@gmail.com>
To: kibenge@upei.ca
Date: 10/6/2011 8:08 PM
Subject: Your report
Attachments: Yellow pinksm.jpg; yellow brains sm.jpg

Dr Kibenge

I just want to give you a heads up that because I paid for your report, I own it and as such I feel that I can use it. I have received a legal opinion on this.

I hope this is not a problem for you, there really is not much in it and I don't understand why it would be of issue to you.

All the records sent to you are now public.

In all the records I examined I could not find any test of 60 fish that seem required by the Manual of Compliance to clear a population of 600,000 fish of any of the pathogens of concern noted in Dr. Marty's reports.

That the two lesions he notes as classic ISA - type are rising and falling together is of concern. Experts tell me that if it were to change by only a few nucleotides the PCRs would not capture it.

For your interest. There are now yellow salmon in the Fraser river. I picked up four like this in a casual survey of a few hours.

alex