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Infectious Salmon Anemia (ISA): Update on the situation in North America

by

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Introduction

This paper updates information brought to the Working Group in 2000 about the Infectious Salmon Anaemia (ISA) virus in North America. This disease has caused major losses in the Atlantic salmon farming industry on both sides of the Atlantic Ocean, and was detected for the first time in 2000 in wild fish in Canada (New Brunswick), and Scotland. Escaped farmed fish in Canada also tested positive for ISA for the first time in 2000.

Status of the disease outbreak

Canada

Aggressive control measures taken by the salmon farming industry in Canada appear to be working. Of the smolts that were transferred to sea cages in spring 2000, up to December 31 2001 only one site had reported the disease. Three cages of fish were eradicated here, and so far in 2001 there have been no new cases of ISA reported.

Monitoring of disease status of wild and escaped-farmed salmon continued at the Magaguadavic River in 2000. None of the returning wild fish tested positive for ISA (N = 14, which was the river's entire wild adult salmon run in 2000). These fish were nonlethally sampled by using an rt-PCR test on swabs of gill mucous.

Of the thirty escaped-farmed salmon screened, all tested negative for the presence of ISA. These fish were all lethally sampled.

Additional positive rt-PCR tests for ISA in wild salmon in 2000 were recorded by DFO in the Margaree River in Nova Scotia (2 of 30 fish tested), the Morell River in Prince Edward Island (4 of 30 fish), and the St. John River New Brunswick (16 of 36 fish) (Gilles Olivier, DFO Fish Health Unit, Personal Communication). Follow up screening using cell cultures, which is considered the gold standard for ISA testing, failed to confirm the presence of the virus. It is plausible that these test results were false positives. Problems arising from the testing procedures are outlined later in the paper. At this time, it is probably premature to conclude that the disease and/or the virus is (are) present in these wild populations.

USA

The first reported case of the disease from the east coast salmon farming industry was announced in a press release issued 16 March 2001 by the Maine Department of Marine Resources. The diagnosis was confirmed on 15 February 2001 from a site in Cobscook Bay, Maine. Cobscook Bay is the most concentrated sector of the USA's salmon farming industry and is situated in close proximity to sites in the Canadian industry that had had the disease.

The Maine Aquaculture Association issued a press release on 13 March announcing that the Association's members had adopted an industry-wide ISA action plan. The plan includes independent testing at each farm site for ISA three times per year, and good husbandry measures including single year class grow-out sites, all in/out production strategies (no holdover of salmon

is permitted at a site after the culture cycle is finished. In New Brunswick, some sites are authorized to keep fish longer in order to give farmers the leeway to pick more favorable times to move fish to market), following, disinfection protocols, and slaughtering of fish in infected cages.

Problems with the tests used to detect the virus

Three tests are used to identify the presence of ISA virus in Atlantic salmon (Lovely *et al.* 1999). They are: 1) an ISAV specific immunofluorescence antibody test (IFAT), 2) a reverse transcriptase polymerase chain reaction (rt-PCR) test, and 3) a virology test which cultures the virus and tests for cytopathic effects (CPE's) on a cell line (salmon head kidney line SHK-1 is preferred, but others like Chinook salmon embryo line CHSE-214 can be used; Bouchard *et al.* 1999).

The IFAT test gives false positives, which is discouraging its use. The rt-PCR test provides the fastest result, but most investigators also prefer to have a final confirmation of the presence of the virus by a documentation of CPE's in a cell culture line. This, however, is time consuming and test results can take a month or more to obtain.

There is a growing suspicion that the rt-PCR test may be prone to false-positive results. In a number of instances, rt-PCR tests have indicated the presence of the virus, but the results have not been confirmed by subsequent cell culture tests. This may occur for two reasons: First, the rt-PCR methodology is extremely sensitive, but is based on a relatively short sequence of viral DNA. The DNA of other non-harmful and at-present unidentified viruses carried by salmon may contain this genetic sequence, and be responsible for the positive results. A second possibility is that the tests are picking up the presence of a small number of ISA virus particles (in theory, it could be as few as one) that the salmon's immune system is successfully combating.

Canadian and USA screening protocols will continue to use the rt-PCR test, but additional emphasis will be given on obtaining cell-culture results from any fish that test positive.

Strains of ISA

A recent comparison of a limited portion of the genome of a small number of ISA isolates from Norwegian and Canadian farmed Atlantic salmon showed that the Canadian version of the virus was about 80-90% identical with that of the Norwegian version (Blake *et al.* 1999). This is considered a fairly significant difference, and led the authors to suggest that the Canadian epidemic may not have resulted from a recent importation of the infection from Norway, but rather from a distinct North American genomic variant. The North American strain seems to be less lethal than the European variety.

Subsequently Krøssoy *et al.* (2001) have determined genome sequences from two regions of the DNA of the ISA virus from a Bay of Fundy site (North America), Loch Nevis (Scotland), and eight places in Norway. They compared base pair sequences, and the resulting amino acid substitutions that would result from the different codings. They concluded that the European isolates were 98 – 100% similar, whereas the Canadian isolate was only about 84-88% similar to

the European group. They estimated that the North American and European strains diverged from each other in about 1900, but cautioned that this was based on the limited data they had to calculate the rates of base pair substitutions. However, if this date were correct, it would correspond to a period of active transfers of salmonids from North America to Europe (Rainbow trout) and from Europe to North America (sea run brown trout). Both rainbow trout and brown trout have been shown to be asymptomatic hosts of the virus (Nylund and Jacobsen 1995, Nylund et al. 1997).

Krøssoy et al. (2001) speculated that the virus might have been spread from one continent to the other through these transfers, but their work could not determine where the original home of the virus was.

Canadian vaccine trials

Following an outbreak of ISA in vaccinated fish from the 1999 smolt year class shortly after they had been moved to the sea, the New Brunswick Salmon Health Committee and the New Brunswick Salmon Growers Association initiated an independent verification of the MULTIVaCC⁴ + ISA vaccine. Trials were carried out at the Department of Fisheries and Oceans St. Andrews Biological Station in a newly completed quarantine facility, and monitored by DFO and Huntsman Marine Science Center staff (B. Glebe, Personal Communication).

The MULTIVaCC⁴ is an oil-adjuvanted vaccine for protection against furunculosis, vibriosis and coldwater vibriosis. The company has added an autogenous ISAV vaccine made up of killed whole-cell virus to this mixture.

Salmon presmolts (average weight 90g) were injected with 0.2 ml of the vaccine formulation and acclimated to seawater. Control fish were sham injected with saline instead of vaccine. The success of the vaccine was judged by comparing the survival of control fish to the survival of vaccinated fish in a cohabitation challenge. In the only successfully completed trial to date, this consisted of exposing vaccinated after 780 degree and control fish to ISA infected fish (intraperitoneal injection) and recording subsequent mortalities. ISA virus as the cause of mortality in all dead fish was confirmed with rt-PCR and cell culture tests.

The vaccinated fish group suffered a 17.5% mortality compared to the 57.5% of the control fish that died. The results confirmed a significantly increased survival rate for fish that had been vaccinated, thus the vaccine contributes to the control of the disease. It is being widely employed in the North American industry.

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