First report of Infectious Salmon Anaemia (ISA) in the United States

Deborah A. Bouchard, K. Brockway, C. Giray, W. Keleher and P. L. Merrill

Micro Technologies, Inc., 41 Main St., Richmond, ME 04357 USA

Abstract

The first documented appearance of infectious salmon anaemia (ISA) at a US salmon farm is described. Fish presented clinically with listless behavior, exophthalmia, pale gills, and fin and skin haemorrhages. Gross necropsy findings included dark livers, swollen spleens and petechial haemorrhaging of mesenteric fat and internal organs. Laboratory testing results consisted of cytopathic effect attributable to ISAV on cell culture, appropriately amplified cDNA fragments by rt-PCR, and positive-range immunochemistry results. Histopathology suggestive of ISA was observed in kidney and liver sections.

Infectious salmon anaemia (ISA) is an important disease of Atlantic salmon (Salmo salar). ISA (or Haemorrhagic Kidney Syndrome/HKS as it was also known) has previously been reported in cultured Atlantic salmon in marine netpens in Norway (Thorud & Djubvik 1988), New Brunswick (Byrne, P.J. et al, 1998; Mullins et al 1996), Scotland (Rodger et al 1998) and the Faroe Islands (2000). Conflicting reports surfaced in 2000 about the presence of ISA in Chile as well. The pathogen that causes ISA is infectious salmon anaemia virus (ISAV), a contagious virus most likely belonging to the orthomyxovirus family (Falk, et al. 1997). At least two ISAV strain types have been isolated (Krossoy, B. et al, 2001; Blake, et al 1999). The virus may result in substantial infection and mortality of cultured Atlantic salmon (Lovely, J.E. et al 1999). Primary diagnostic testing for ISAV is generally accepted to be by viral culture using the SHK-1 and/or CHSE-214 cell lines, with confirming tests consisting of reverse-transcriptase polymerase chain reaction (rt-PCR), indirect fluorescent antibody testing (IFAT) and histology (Bouchard, et al, 1999).

Clinical History

Approximately 350,000 Atlantic salmon S0 smolt of a composite mixed-Maine strain were stocked into 6 saltwater cages at a salmon farm in Cobscook Bay, Maine in April 2000. This marine site was within 5 km of a New Brunswick farm raising Atlantic salmon that had reportedly been experiencing clinical mortality from ISA. Because of this fact, biosecurity at the transfer site was practiced at a high level.

The transferred smolt originated from a certified Maine hatchery with an excellent fish health record. Of the 350,000 fish, all had been vaccinated with a commercially available tetravalent bacterin, and a subset of approximately 50,000 were also vaccinated with an
autogenous ISAV vaccine. For the cage relevant to this report (containing only non-ISAV-vaccinated fish), approximately 8% of the fish succumbed relatively quickly following saltwater transfer to osmoregulation complications attributable to poor smoltification. Otherwise, no health problems were reported for any fish on the site until December 2000, when icing, predator attacks and related stress resulted in the loss of approximately 10,000 more fish from the relevant cage. About three weeks after this episode, mortality in the same cage rose to around 150 fish per day, and a diagnostic investigation was triggered. Water temperature at the site was approx. 2°C at the time of this elevated mortality, and fish were in the 1200 gram range.

Clinical Findings
At the time of the diagnostic investigation, the site manager reported observing a number of lethargic, darkened fish swimming near the water surface. Several moribund fish were collected and submitted on ice to the authors’ laboratory for workup. Clinical signs collectively included some fin rot and skin sores, in some cases ulcerated into the muscle. Fish had pale gills, some ventral skin haemorrhaging, and bilateral exophthalmia. Internally, swollen spleens were apparent in several fish, and one fish had a darkened liver with sharply-defined margins. Multifocal-to disseminated petechial hemorrhaging was observed along the mesenteric fat, the swim bladder and the pyloric cecae in some fish. Kidney tissue was somewhat spongy. Samples were taken for cell culture, rt-PCR, IFAT testing, and histology.

Laboratory Findings
Virology
One five-fish pool of tissue extract (kidney, spleen, gill lamellae, and liver) was inoculated onto CHSE-214 and SHK-1 cell lines and incubated at 16°C. Cytopathic effect characteristic of ISAV was observed on both cell lines by day 12 post-inoculation. Supernatant from this pool was analyzed by rt-PCR and was found to be positive (see below).

rt-PCR
One mid-kidney direct tissue sample, and the 5-fish pooled cell-culture supernatant sample, were analyzed using ISAV-specific primers (Blake, et al 1999; Devold, et al 2000). Both samples were positive as evidenced by the presence of appropriate cDNA fragment bands.

IFAT Testing
Using mid-kidney slide impressions and the monoclonal antibody technique described by Dannevig et al (1995), one sample, corresponding to the positive rt-PCR analysis, was judged to have a positive fluorescent pattern (3+ out of a possible 4+).

Histopathology
Lesions included multifocal to coalescing interstitial haemorrhaging in the mid-kidney region, and an increase in diffuse vacuolization within hepatocytes. Some hepatocytes also had nuclei with enlarged nucleoli. Splenic congestion was also noted.

Based on the above findings, a positive diagnosis of infectious salmon anemia was made. This represents the first reported case of confirmed infectious salmon anemia in the United States.
References


