Epidemiological investigation of infectious hematopoietic necrosis virus in salt water net-pen reared Atlantic salmon in British Columbia, Canada

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Abstract

An epidemiological study of infectious hematopoietic necrosis viral disease (IHN) in farmed Atlantic salmon in British Columbia was conducted to better understand the management of this disease. The study consisted of a descriptive retrospective investigation of 18 IHN outbreaks on farms between 1992 and 1996, and a prospective surveillance program for the viral disease, after an area management plan was implemented to reduce the viral load around farms and farm-to-farm spread of the virus. The crude cumulative mortality associated with IHNV in Atlantic salmon was high (average 47%), and outbreaks lasted 5.8 months on average. On the two farms where the virus was detected during the surveillance program, IHNV was confirmed in all pens within 1 month. On two of three sites where fish were kept on farms after the initial disease outbreak subsided, IHN reoccurred within 30 weeks. The presentation of IHNV on farms, the spatial and temporal patterns of the outbreaks between 1992 and 1996, and the genetic similarity between isolates collected from nine outbreaks spanning a 5-year period, all supported the plausibility of farm-to-farm spread of the

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virus. Furthermore, the marked decrease in the incidence rate of IHN in farmed Atlantic salmon after the implementation of an area-based management plan aimed at reducing farm-to-farm spread of the virus also supported this hypothesis. Although the source of IHNV for the index case was not determined in this study, secondary spread of the virus between farms via management practices, such as movement of fish, co-habiting naïve fish with survivors of the viral disease, and movement of equipment, likely accounted for some farm outbreaks. This suggested that many cases of IHN may be preventable using good on-farm biosecurity.

1. Introduction

Infectious hematopoietic necrosis (IHN) is an endemic viral disease commonly seen for over 20 years in sockeye salmon (Oncorhynchus nerka) in the Pacific Northwest (Rucker et al., 1953; Amend et al., 1969; Wolf, 1988). In 1992, the virus was reported for the first time in Atlantic salmon (Salmo salar) in salt water net-pen sites in British Columbia, Canada (Armstrong et al., 1993). Within 4 years of the first report of IHN in farmed Atlantic salmon, the disease was reported on 13 additional sites within an 11-nautical-mile radius of the index case. In 1996, the companies farming in the area implemented a plan to mitigate disease impact and determine the viability of the affected area for future Atlantic salmon farming (J, Constantine, Ministry of Agriculture, Food and Fisheries, Animal Health Branch, Courtney, BC, 1996, pers. comm.).

The first step of this plan involved fallowing affected sites, by removing all farmed fish and disinfecting nets and, in some cases, barges. The simultaneous fallowing of infected sites within a designated area is a strategy used in Norway to control the farm-to-farm spread of infectious salmon anemia, and in Scotland and Ireland to control sea lice (Stewart, 1998). One site in the affected area was designated as a holding site. Fish from nine other sites were either harvested, culled, or moved to this site and eventually harvested. For a period of 2 months, 9 of 14 sites in the affected area were simultaneously fallowed. After this time Atlantic salmon were re-introduced to some sites. Some producers in the area chose to introduce chinook salmon (Oncorhynchus tshawytscha), a species that is less susceptible to IHN than Atlantic salmon (Traxler et al., 1993) for one grow-out cycle.

Another mitigative measure was to continue using an autogenous killed vaccine for all Atlantic salmon smolts placed into the affected area. This vaccine had been in use since the summer of 1995 (J, Constantine, Ministry of Agriculture, Food and Fisheries, Animal Health Branch, Courtney, BC, 1996, pers. comm.).

This study reports the results of an epidemiological investigation of IHN outbreaks that occurred between 1992 and 1996 and a surveillance program for the viral disease. The purpose of the investigation was to determine the duration and severity of IHN outbreaks in Atlantic salmon in net-pens, and the spatial and temporal distribution of farm outbreaks. The purpose of the surveillance program was to determine whether the industry's action plan was effective at preventing new outbreaks of IHN in the area and, if not, investigate these outbreaks as they occurred.

2. Methods

2.1. Epidemiological investigation (retrospective)

The study area was located in an archipelago between Vancouver Island and mainland British Columbia, Canada. All salmon producers who farmed Atlantic salmon within an 11 nautical mile radius of the index case, between 1992 and 1996, were contacted during the summer of 1996 and all participated in the epidemiological study. Producers were given a personal interview to determine their site's rearing history, the approximate number of fish in each year class on the site, if and when infectious hematopoietic necrosis virus (IHNV) was isolated, and the age of the fish at the time of the IHN outbreak.

For the purpose of this investigation a farm was considered to be undergoing an IHN outbreak if there was a laboratory isolation of IHNV from any of its fish in cell culture and confirmed by an IHNV-specific test (serum neutralization test (Department of Fisheries and Oceans, 1984) or reverse transcriptase polymerase chain reactions (RT-PCR) (Arakawa et al., 1990)). Veterinary records were used, with the consent of the producers, to verify the laboratory confirmation of IHN for farms participating in the study.

Existing farm mortality records were used to calculate crude weekly or monthly mortality rates. This was done by dividing the number of fish that died by the number of fish present at the start of each week or month. When there were 2-year classes of fish on the site we obtained separate mortality data for each year class. The month and year when the mortality curve associated with the isolation of IHNV started to increase was determined to be the onset of the outbreak. Outbreaks of IHN were mapped by location and date (month and year) of disease onset.

The duration of each outbreak was determined to be the time period when the mortality rate started to increase until it subsided to a level similar to the mortality rate prior to IHN. The mean average duration of IHN outbreaks was calculated for fish that had been in salt water for less than 1 year, and for fish that had been in salt water more than 1 year. The crude cumulative mortality during the IHN outbreak was calculated by dividing the number of fish that died during the IHN outbreak by the number present at the start of the outbreak. An adjustment for fish destroyed or harvested during the course of the outbreak was made by subtracting half of the number of withdrawals from the denominator (Martin et al., 1987). All deaths from the start to the end of the IHN mortality curve were included in the cumulative mortality calculation. The average crude cumulative mortality for the outbreaks identified in this study was calculated by summing the cumulative mortality of all outbreaks and dividing it by the number of outbreaks contributing to the numerator. All averages were expressed as percentages.

In three separate cases (site C, M and N) fish were maintained on farms after the initial IHN outbreak was over. In all three of these cases farm mortality records were obtained for the entire salt water life cycle of the fish.

Characteristics such as age of fish at onset of IHN and month of onset of IHN were summarized for all IHN outbreaks. Descriptive statistics were calculated using STATISTIX (version 4.1, analytical Software, Tallahassee, FL, 1985). All graphs were generated in Excel (Microsoft 97, Seattle, WA).

2.2. Surveillance study

A surveillance program that consisted of three separate components—two serological surveys (one at the start and the other at the end of the program) and continual disease monitoring—was implemented between February 1997 and November 1998. The methods and results of the serological surveys, as well as the results of the first 6 months of virus monitoring on sites with a history of the viral disease, are reported in St-Hilaire et al. (2001a).

All Atlantic salmon farms within an 11 nautical mile radius of the index outbreak of IHN were invited to participate in the program (Fig. 1). In addition, one other Atlantic salmon site (Q), located just outside the periphery of this area, and site B with rainbow trout (Oncorhynchus mykiss) were included in the study. Farms that had survivors of IHN still present on their site were excluded from the study, as were farms raising only chinook salmon. All 11 sites in the area that were eligible for the study participated in passive surveillance and 10 of these sites were also monitored using active surveillance.
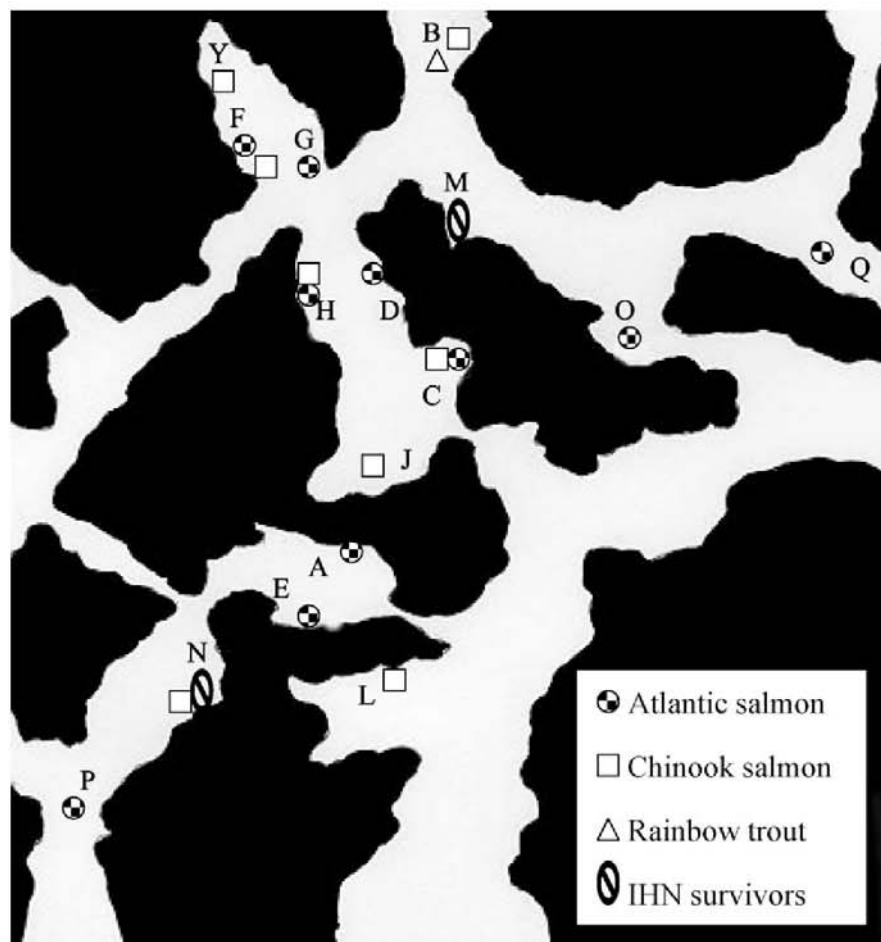

Fig. 1. Locations of sites in the study area between February 1997 and January 1998 during our surveillance program. Included are the species of fish on site and whether the fish had survived an IHN.

2.2.1. Active surveillance for IHN

Visits were made to the farms participating in the surveillance program within 2 months of the start of the program or within 2 months of the introduction of the new Atlantic salmon year class to the site, and every 2 to 4 months thereafter.

During site visits dead fish were collected and tested for IHNV by virus isolation, as described in Traxler et al. (1997). The only modifications to the protocol used by Traxler et al. (1997) were that the overlay used in this study did not contain 1% methyl cellulose, and the viral titres were not calculated. Samples that tested positive on virus isolation were confirmed with a nested RT-PCR test. An assay was considered positive if cytopathic effects (clusters of rounded up cells) were observed on the cell monolayer during a 7-day incubation period. The RT-PCR protocol used in this study was provided by William Batts at the Western Fisheries Research Center, United States Geological Survey, Biological Resources Division, Seattle, WA, USA, and is described in St-Hilaire et al. (2001b).

Depending on the time available and their size fish were either dissected on the farm or at the laboratory to remove the anterior kidney. Kidney samples were grouped according to the pen from which the fish originated. Each kidney sample was cultured for virus. No disinfection was done between samples collected from fish that originated in the same pen, but disinfection procedures were followed between samples that were collected from different pens. An effort was made to sample as many fish as possible during these site visits (minimum 2 and maximum 41); however, if there were too many dead fish (>30), then a subgroup of fish from every pen with dead fish were tested. To increase the likelihood of detecting disease, fresh fish were preferred as samples over fish showing more advanced post-mortem changes, and fish with pathological signs consistent with IHN (petechial haemorrhages in the visceral cavity), or other diseases, were preferred over fish that only had pathological lesions consistent with predator attacks. On eight occasions (three times on site A, once on site D, and four times on site Q), we requested the producer to send dead fish to the laboratory for viral testing to save time.

On the two sites where IHNV was detected during the course of this surveillance program, weekly farm visits were made to determine the spread of virus within the farms. Pen mortality records were monitored and dead fish from pens were tested to confirm the presence of the virus within individual pens. To determine the proportion of dead fish with the virus during an outbreak gross post-mortem findings were noted, within 10 weeks of the IHN diagnosis, for 330 fish on site O and 168 fish on site C. To determine whether gross post-mortem findings typical of IHN (petechial haemorrhage in the visceral cavity) were good indicators of the viral infection once the virus was confirmed on a site, 59 fish on site O, selected based on post mortem signs, were individually tested for IHNV using the virus isolation technique described above. Test results were compared to gross post-mortem findings using a Chi-square test (Martin et al., 1987).

The farm log books were also reviewed to determine any transfers of fish or equipment to the affected farms within 2 months of the outbreaks.

2.2.2. Passive surveillance for IHN

Farm veterinarians were notified about the surveillance program and their client participation, and were asked to inform us of suspected IHN cases. On two sites (site P and, after August 1997, site Q) passive surveillance was the only surveillance method used because

site visits could not be arranged. Producers were also encouraged to monitor their fish for pathological changes that were consistent with IHN (petechial hemorrhage in the visceral cavity) and notify us of suspect fish. All viral testing for the participating farms was financed through our surveillance program to encourage testing by the producers and veterinarians.

2.2.3. Analysis

During the surveillance program a farm was considered to have an outbreak of IHN if the virus was isolated from any of its fish on two separate occasions. If IHNV was isolated from fish on a farm a subsequent visit to the site was scheduled immediately.

The incidence rate of IHN outbreaks was determined for 3-month intervals between 1997 and 1998 by counting the number of sites where IHN was detected during each interval and dividing it by the number of months each 'susceptible' farm was observed during that time period (Martin et al., 1987). A farm was considered to be susceptible to IHNV if it contained Atlantic salmon or rainbow trout that had no known history of IHN.

Site Q (Fig. 1), which was outside an 11 nautical mile radius of the index case, was excluded from the incidence rate calculations, as was site E which was a new site in the area. Excluding sites Q and E from the 3-month interval IHN incidence rate calculations enabled comparison of the incidence rate of IHN before and after the implementation of the area management plan in the same group of farms. Data on the disease status of farms between 1992 and 1996 were obtained from the retrospective epidemiological investigation. It was assumed that all farms in the study area between 1992 and 1996 were susceptible to the virus until they were confirmed with the viral disease. Three-month incidence rates were calculated in the same manner as described above.

2.2.4. Ribonuclease protection assay

Three virus isolates from each of the two outbreaks detected during the surveillance program were compared using a ribonuclease protection assay (RPA) (Winter et al., 1985; Anderson et al., 2000). These isolates were also compared to isolates collected from the two sites with IHN survivors (sites M and N), and five other archived IHNV isolates from Atlantic salmon including an isolate collected during the first reported farm outbreak of IHN in 1992. Another IHNV isolate collected in October 1997 from a stray post-spawned sockeye salmon found in a stream approximately 6 nautical miles from site C was also included in the RPA for comparison.

The RPA protocol followed is described in Anderson et al. (2000). The samples were analyzed at the Western Fisheries Research Center, United States Geological Survey, Biological Resources Division, in Seattle, Washington, USA. The probe used in this assay assessed the entire IHNV glycoprotein gene (1610 nt) of the target IHNV isolate for heterogeneity (Anderson et al., 2000). This probe was made from a cDNA clone of an IHNV isolate collected from rainbow trout (Oncorhynchus mykiss) in 1975 at Round Butte hatchery, in Oregon, USA.

Visual pairwise comparisons of the fragment patterns were made between each of the isolates to look for pattern similarity. The percentage of similar bands or fragments between two test isolates was calculated by dividing the number of shared bands in the two RPA patterns by the total number of bands minus the number shared. This number was then multiplied by 100 and expressed as a percentage.

3. Results

3.1. Retrospective epidemiological investigation

Eighteen outbreaks of IHN on 14 sites were identified between 1992 and 1996. For 4 of the 18 outbreaks of IHN (on two different sites) mortality data were either not maintained or not available for summary. During four of the outbreaks there were two year classes of fish present on the farm. Fish were considered of different year classes if there was more than 8 months between their salt water entry dates. The crude cumulative mortalities were similar in the two year classes on two of the sites (X and H), but different on the other two sites (M and N) (Table 1). Data from both year classes were included separately in the summary statistics. There were two other sites that had both smolts and 1-year-old fish on site at the time of the IHN outbreak, but data were not included for the older fish either because they were not available or the fish were harvested shortly after IHN was diagnosed.

Table 1

Summary of the estimated time of onset of IHN, cumulative mortality during IHN outbreaks, time in salt water prior to the onset of IHN, and the duration of the IHN outbreak for 18 reported outbreaks of the disease in Atlantic salmon on 14 salt water net-pen sites in British Columbia, Canada, between 1992 and 1996

Site	Time of IHN onset	Time in salt water (months)	Duration of outbreak (months)	Cumulative mortality[a]
X	July 92	1 to 2	6	46%
		12	Unknown	18%[b]
A[c]	Dec. 92	Unknown	Unknown	Unknown
A[c]	Apr. 94	Unknown	Unknown	Unknown
D	Apr./May 94	1 to 4	5	66%
C	Apr./May 94	1 to 4	5	63%
H	Jan. 95	1 to 2	6	78%
		12	6	28%
K	Feb. 95	12	7	23%
J	Feb./Mar. 95	12	9	21%
X	Feb./Mar. 95	2	3	72%
F	Feb./Mar. 95	12	Not available	Not available
A[c]	Mar. 95	Unknown	Unknown	Unknown
I	June 95	6 to 8	6	75%
G	July 95	1 to 4	5	30%[b]
D	July 95	1 to 6	5	69%
B	Nov. 95	8 to 10	7	26%[b]
N	Jan./Feb. 96	1 to 2	6	49%
		12		54%[b]
M	Feb. 96	1 to 2	5	31%
		12	5	29%
L	Jan./Feb. 96	12 +	6	59%

[a] All information in this table was determined from the farm weekly or monthly mortality data surrounding the time of the IHN diagnosis. If farms had a two year classes of fish on their site at the time of IHN the cumulative mortality was reported separately for the different age groups.

[b] Fish were transferred, harvested, or culled during the course of the IHN outbreak.

[c] Records were not available to confirm any dates.

In total, mortality records from 18 groups of fish (single year class), from 14 outbreaks of IHN on 12 farms, were available for summary statistics (Table 1).

The crude cumulative mortality associated with IHNV for 18 groups of fish affected by the virus, between 1992 and 1996, ranged from 18% to 78% (mean = 46.5% and SD = 20.9) (Table 1). The average crude cumulative mortality in fish that were less than 1 year in salt water before developing the disease was 55% (SD = 19.4). The average crude cumulative mortality for fish that developed IHN after they had been in salt water for 1 year or more
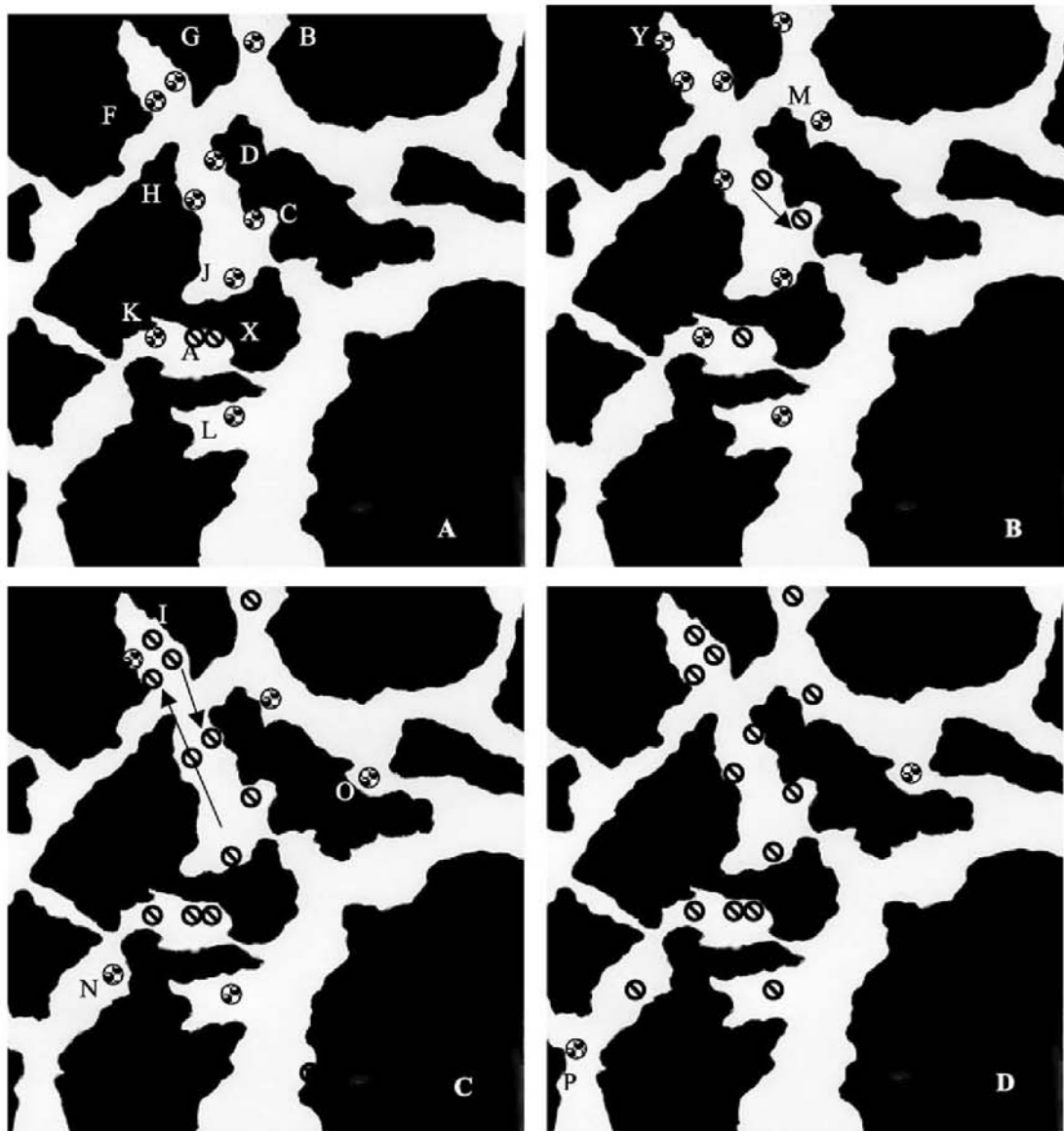

Fig. 2. The infectious hematopoietic necrosis viral disease status of sites in the study area in (A) December 1992, (B) December 1994, (C) December 1995 and (D) February 1996. ⊛ Indicates a farm with Atlantic salmon but no IHN. ⊘ Indicates a farm with Atlantic salmon that have been diagnosed with IHN. Arrows indicate direction of transfer of fish.

was 33% (SD = 16.5). The duration of the IHN outbreaks ranged from 3 to 9 months with an average and SD of 5.8 and 1.3 months, respectively.

Two of the three sites (M, N, and C in 1994) that kept survivors on their site for an extended period of time after the first outbreak of IHN had a second increase in mortality associated with the virus. The third farm (C) also had a second increase in mortality, but did not test for IHNV. The first outbreak of IHN on sites M and N occurred approximately 10 and 14 weeks after the fish were transferred to salt water, respectively. Mortality records from site M indicated the outbreak lasted approximately 5 months for both the first and the second outbreak of IHN on this site. On site N, the mortality curve for the first outbreak lasted between 6 and 10 months. The second increase in mortality associated with IHNV on this site only appeared to last 1 ½ months. The period between the two peaks in mortality on sites M and N was approximately 6 and 8 months, respectively.

3.2. Spatial and temporal distribution of farm outbreaks of IHN

The number of farms in the study area varied from 11 to 17 between 1992 and 1996 (Fig. 2a–d). With the exception of site L, all sites in the study area were located within four channels. Mapping of farm outbreaks by time and space revealed a systematic outward expansion of the viral disease. Infectious hematopoietic necrosis viral disease was reported on two of the 11 farms in the study area in 1992 (Fig. 2A). No new cases were reported in 1993; however, diseased fish and survivors of IHN remained present on two sites (X and A). In 1994, three outbreaks of IHN were reported in the study area (sites A, C and D) (Fig. 2B). Records indicated a transfer of fish from site D to site C at approximately the same time as the mortality associated with IHNV on site D was starting to increase. The new year class of fish on site A were also reported to have IHN in 1994 shortly after their introduction to salt water. Records indicated there were still survivors of the disease from 1992 on site at the time of the introduction. In 1995, several new outbreaks of IHN were reported (Fig. 2C). This coincided

Table 2
Summary of site visits and viral testing for farms in the IHN surveillance program between February 1997 and November 1998

Site	D	DS	V	VT
A	Dec. 96	Feb. 97	9 (3)	166
D	Apr. 97	Apr. 97	26 (1)	402
C	Nov. 96	Feb. 97	6[a]	20
H	Aug. 98	Oct. 98	1	3
F	Aug. 98	Oct. 98	1	30
G	Apr. 98	Apr. 98	6	104
B	Dec. 96	Feb. 97	4	42
O	Nov. 95	Feb. 97	3[a]	20
P	Mar. 96	June 97	1[b]	5
Q	Jan. 97	Feb. 97	3 (4)	54
E	Jan. 98	Jan. 98	3	37

D: date fish were introduced to the farm, DS: date surveillance was started, V: number of visits (and number of times when fish were sent to the laboratory by the producer), VT: number of viral tests performed.
[a] Only includes the number of site visits prior to the diagnosis of IHN on these farms.
[b] Relied on passive surveillance.

Table 3
Categorization of pathological lesions in dead fish on sites C and O during IHN outbreaks

Site	Weeks post IHN diagnosis	Number of fish examined	Number of fish with no lesions	Number of fish with granuloma-like lesions	Number of fish with petechial hemorrhage F other lesions
C	1	14	2	2	10 (71%)
	2	34	11	1	22 (64%)
	4 ½	45	6	2	37 (82%)
	5 ½	48	3	1	44 (92%)
	7	27	4	0	23 (85%)
O	0	19	5	12	2 (10.5%)
	1	12	5	6	1 (8.3%)
	1 ½	14	3	3	8 (57.1%)
	2	11	1	5	5 (45.5%)
	3	16	1	7	8 (50%)
	4 ½	42	4	6	30 (71.4%)
	6	66	7	7	52 (78.8%)
	8	69	14	0	55 (79.7%)
	10	81	2	1	78 (96.3%)

with an overall increase in the number of Atlantic salmon in the study area from approximately 1.7 million in 1992 to approximately 2.7 million in 1995. Four additional sites were stocked with Atlantic salmon and farms were generally stocked with more fish. Records on one site (A) where IHN was diagnosed indicated that new fish were introduced to the farm while IHN survivors were still present. Farm records also indicated the movement of fish from site G to site D and site J to site F at the start of their IHN outbreaks (Fig. 2C). On one of these sites (D) there was a history of IHN, but the site had been fallowed for approximately 1 month prior to the introduction of the new fish. At the end of 1995 the only sites not affected by IHN, with the exception of a low salinity site (Y) that was used to adapt fish to salt water, were located at the periphery of the study area (sites L, M, N, O, and P). By the end of February 1996 IHN was diagnosed on three of these sites (Fig. 2D).

3.3. Characteristics of the outbreaks

All reported outbreaks of IHN were in Atlantic salmon. The onset of disease in 50% (9/18) of the reported outbreaks was between the months of January and March (Table 1).

Table 4
Virus isolation results for 59 dead fish with different lesions. Data were collected during an IHN outbreak on site O

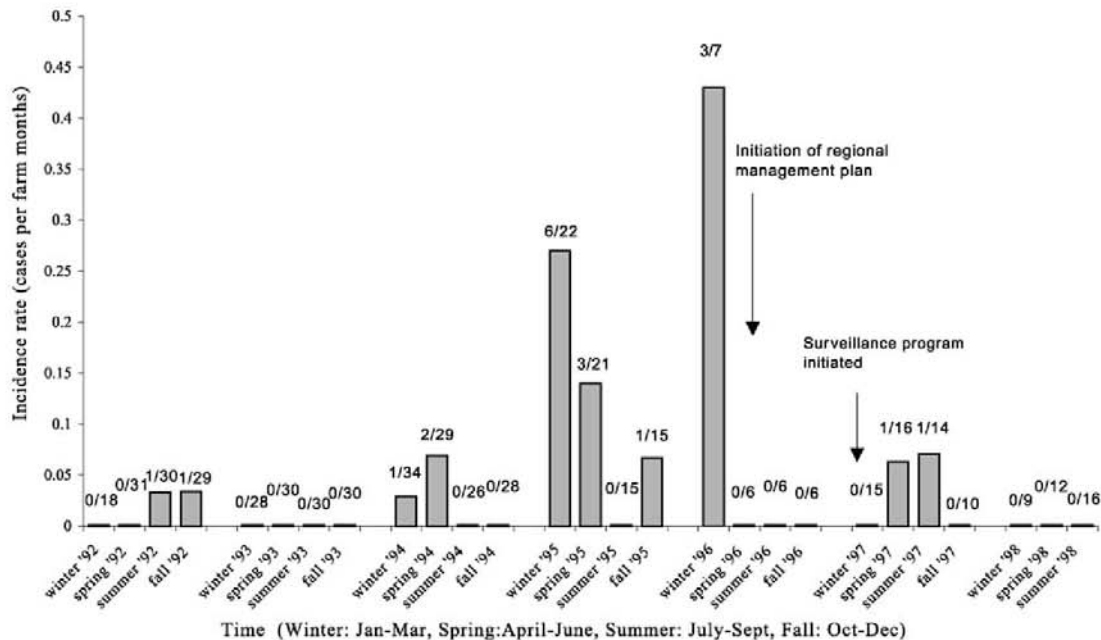| Pathological lesions	Positive virus isolation	Negative virus isolation	Total
No post mortem lesion	5 (45%)	6 (55%)	11
Granuloma-like lesions in visceral organs	14 (56%)	11 (44%)	25
Petechial hemorrhage in visceral cavity F other lesions	20 (87%)	3 (13%)	23
Total	39	20	59

$v^2 = 7.70$, $p = 0.02$, df $= 2$.

Fig. 3. Farm level incidence rate of IHN outbreaks for 3-month intervals between 1992 and 1998 in the study area. Numbers above the columns indicate the number of farms with IHN/the number of farm-months in the interval.

The fish in 12 of the 18 IHN outbreaks had been in salt water for less than 2 months before mortality associated with IHNV started. In the other six outbreaks, fish had been in salt water for at least 6 months, with no history of transfers prior to noticeable mortalities associated with IHNV (sites K, J, F, I, B, and L) (Table 1).

3.4. Surveillance study

3.4.1. Study area

Fig. 1 illustrates the location of all sites in the study area during the course of the surveillance study (including some not participating in the surveillance program). Nine farms were not included in the surveillance program, as they either remained fallow throughout the duration of the project (sites X, K, and I not shown in Fig. 1), only had chinook salmon on their sites (sites Y, J, L, and N), or had Atlantic salmon survivors on their site and did not re-introduce naïve stock (sites M and N) (Fig. 1).
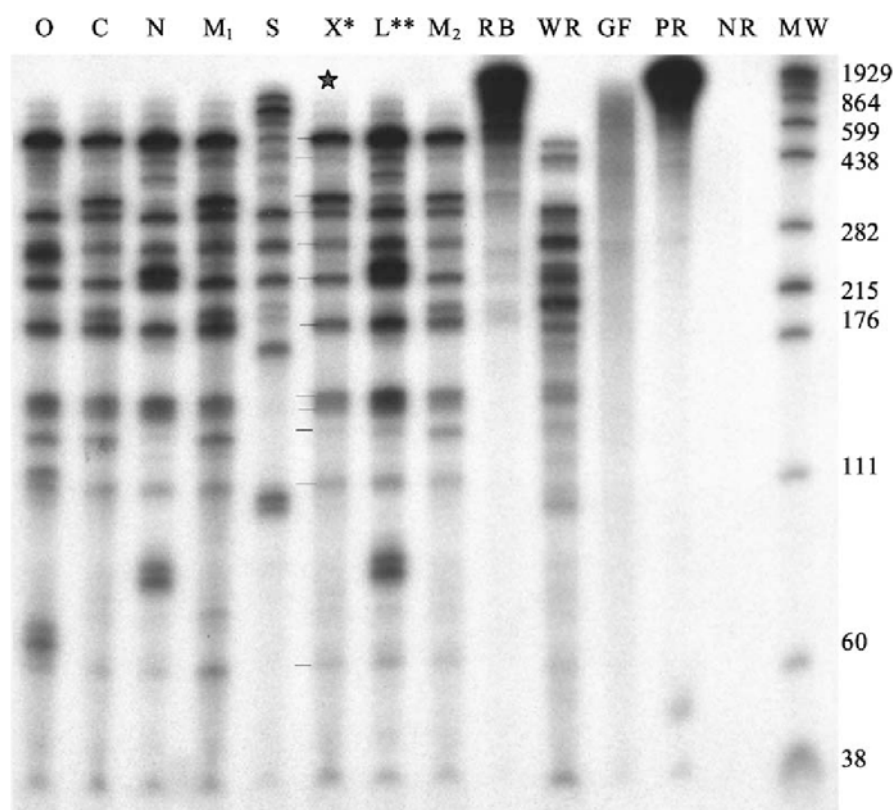
3.4.2. Virology

Sixty-three site visits were made to 11 sites over a 20-month period, and 883 viral tests were performed for IHNV (Table 2).[3] The only site with a history of IHN where the viral disease was diagnosed in the new year class of fish was site C. The diagnosis was made 10 months after the fish were introduced onto the site, and within 2 months of the introduction of two large barges to the site. These barges originated from site M, which was undergoing

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[3] This summary includes the results of the first 6 months of viral testing on sites with a history of the viral disease (sites A–G) published in St-Hilaire et al. (2001a).

a second outbreak of IHN at the time of the transfer. By the time the outbreak was detected on site C the weekly farm mortality rate was approximately 0.2% and virus was confirmed in all pens within two site visits; however, individual pen mortality records indicated that the first pens to have elevated mortality were those located adjacent to the barges.

The only other site where the viral disease was diagnosed during the course of the surveillance program was site O, 17 months after the fish were transferred to the site. This site had no history of IHN and no history of any fish or equipment transfers within 2 months of the outbreak. Records indicated that mortality associated with the virus first started to increase in one pen in the center of the net-pen system. Within 1 month of the first signs of IHN, before the weekly farm mortality rate was above 0.1%, the virus was confirmed in all pens.

★   Index case isolate

* This was also the fragment patter of an IHNV isolate from site B.

** This was also the fragment patter of IHNV isolates from sites H and K.

Fig. 4. Autoradiograph of ribonuclease protection assay cleavage fragment patterns from infectious hematopoietic necrosis virus collected from Atlantic salmon and a post-spawned sockeye salmon in British Columbia, Canada, between 1992 and 1997. Band patterns from the Atlantic salmon are labeled with the letter that identifies their site of origin (sites O, C, N, M, X, H, K, and L) and the wild sockeye salmon isolate is labeled as S. Controls include: the isolate used to develop the probe (labeled RB), the RNA transcript derived from the same plasmid used to make the probe (labeled GF), the probe treated with ribonuclease enzymes A and T1 (labeled NR), the probe alone (labeled PR), and a known virus isolate collected from rainbow trout in Hagerman, Idaho, in 1982 (labeled WR). A molecular weight marker was also included (labeled MW).

Table 5
Percentage of shared bands in the ribonuclease protection assay patterns between pairs of Atlantic salmon IHNV isolates and between an isolate from a wild post-spawned sockeye salmon found 6 nautical miles from a fish farm in October 1997. (Number of similar bands/total number of bands minus the number shared.)

Site of origin	X (index case), B	L, K, H	M_2, C	N	O	M_1
L, K, H	70.6 (12/17)					
M_2, C	92.3 (12/13)	66.7 (12/18)				
N	68.8 (11/16)	88.2 (15/17)	64.7 (11/17)			
O	73.3 (11/15)	55 (11/20)	68.8 (11/16)	61.1 (11/18)		
M_1	86 (12/14)	72.2 (13/18)	92.9 (13/14)	61.1 (11/18)	75 (12/16)	
Wild sockeye	23.8 (5/21)	29.2 (7/24)	28.6 (6/21)	26.1 (6/23)	21.7 (5/23)	27.3 (6/22)

The majority of dead fish examined during the IHN outbreaks on sites C and O had pathological lesions consistent with the viral disease (Table 3). Once virus was isolated on site O a high percentage of fish with signs of IHN tested positive for the virus (20/23) (Table 4). Petechial hemorrhage on the visceral organs of fish on site O was statistically associated with virus isolation ($v^2 = 7.70$, p = 0.02) (Table 4).

The viral disease was never diagnosed in any of the fish from the other three sites (P, Q, E) with no history of IHN, nor in the fish from the other six sites with a history of the disease (A, B, D, F, G, and H) (Fig. 1). Overall, there was a sudden drop in the 3-month incidence rate of IHN outbreaks after the implementation of the area-based management plan (Fig. 3).

3.4.3. Ribonuclease protection assay

There were six slightly different fragment patterns generated from 14 IHNV isolates collected from nine separate outbreaks of the viral disease in Atlantic salmon between 1992 and 1997 (Fig. 4). The fragment patterns from virus isolates collected on sites K in 1995, H in 1995, and L in 1996 were identical to one another. The fragment pattern from an isolate collected during the index outbreak was identical to an isolate collected during the outbreak on site B in 1996. One isolate collected from site M in the summer of 1997 was identical to isolates collected from three fish on site C during the outbreak that occurred in the fall of 1997. Another isolate, collected in 1996 from site M had a slightly different pattern (one band difference) (Fig. 4 and Table 5). All three isolates from site O were identical to one another, but slightly different from the fragment patterns generated from the other Atlantic salmon isolates.

Overall, the percentage of shared bands between RPA patterns of IHNV isolates collected from Atlantic salmon ranged from 55% to 100% (Table 5). In comparison, the percentage of shared bands between theses isolates and the wild sockeye salmon isolate was always below 29.2% (Table 5).

4. Discussion

Findings from the retrospective epidemiological investigation of outbreaks and IHN surveillance program suggested that the most probable sources of IHNV for Atlantic

salmon held in salt water net-pens in British Columbia, between 1992 and 1997, were other farmed Atlantic salmon. The duration, events preceding, and the spatial and temporal distribution of outbreaks, as well as the genetic similarity between IHNV isolated from different farms, and the sudden reversal of a 4-year rising incidence rate of IHN outbreaks once an area management plan was put into effect, all supported this hypothesis.

Data collected from individual farms affected by IHN indicated that once fish were diagnosed with the disease the virus was present in that population of fish for a long period of time. The duration of an IHN outbreak in 13 of 14 incidents, between 1992 and 1996, lasted 5 months or more. Moreover, even after an outbreak has subsided some Atlantic salmon may remain carriers of IHNV (St-Hilaire et al., 2001a). This may not have been known to producers in 1992, and may explain why, on two occasions, apparently "naïve" groups of fish (a new year class) introduced to site A containing known survivors of IHN developed the disease within a few months of transfer. Mixing year classes of animals on a farm has been associated with perpetuating infectious diseases in many animal production systems, including infectious salmon anaemia virus in salt water net-pens in Norway (Vågsholm et al., 1994).

Producers may also not have known their fish had a viral disease at the onset of the outbreaks. This was apparent from interviews with producers and the fact that some moved fish from one site to another during the initial stages of the outbreak. Once IHNV is confirmed on a site there is a high probability that all pens of fish have been exposed to the virus. On both sites where IHNV was detected during the surveillance program, the virus was isolated from dead fish in all pens before the total farm mortality rate was greater than 0.2% per week (within a month). Three producers reported moving fish from their farms just prior to, or at the onset of, an outbreak (Fig. 2B and C). In two of these cases (site C in 1994 and site D in 1995) there were already fish of a similar age on the recipient site. These transfers may explain why the fish on the receiving sites developed IHN.

Besides providing information on the plausibility of virus transfer, the descriptive information collected from different farm outbreaks is also useful for the management of this viral disease. If the virus is isolated on a farm it is likely that the mortality rate will be high. Although the cumulative mortality rate associated with IHNV in Atlantic salmon varied from one farm to another, the average rate was 47%. Even fish that had been in salt water for over 1 year had, on average, a cumulative mortality rate of 33%.

The wide range of cumulative mortalities observed for outbreaks of IHN (Table 1) may have been due to a number of factors, such as mortality due to other causes, early harvest of fish, stocking density, other management factors, and general fish health. The method of obtaining and interpreting data for calculating cumulative mortality may also account for some of the variation observed between sites, as it relied on individual farm data collection systems, which may have lacked consistency. Nonetheless, the lowest mortality rate associated with IHN was still quite high, so producers may consider early harvest of their fish to salvage those not clinically affected by the virus at the onset of the disease process. Because we obtained the data after the IHN outbreaks were over, it was not possible to confirm the starting date of IHN mortalities. Nor was it possible to determine the proportion of the mortality that was directly due to the virus. However, based on data collected from both outbreaks that occurred during the surveillance program, the majority of fish that died during IHN outbreaks may have been infected with the virus. A large percentage of dead

fish during the IHN outbreak on sites O and C had pathological lesions consistent with IHN (Table 3). These lesions were found to be good indicators of IHN viremia once the virus was detected on the sites. Fish on site O, with petechial haemorrahage in the visceral cavity, tested positive for IHNV 87% of the time (Table 4). Furthermore, lack of these signs did not exclude infection with the virus, as a number of fish with no pathological lesions or signs of other diseases also tested positive for the virus (Table 4). Whether this happened in other outbreaks of IHN prior to 1997 could not be verified.

Another observation with disease management implications was the occurrence of a second IHN outbreak on two of three sites where fish had recovered from the initial disease. The re-appearance of IHNV in survivors of IHN has been observed in populations of rainbow trout (Drolet et al., 1995) and sockeye salmon (Amend, 1975; Kent et al., 1988; Elson et al., 1989). In these other species, isolation of the virus after mortality associated with IHNV had subsided often occurred at the time of sexual maturation (Amend, 1975; Bootland and Leong, 1999). The second disease outbreaks in Atlantic salmon did not coincide with sexual maturation. The reason for the second outbreak of IHN on sites M and N could not be determined in this study. Nonetheless, it may be beneficial to harvest fish with a history of IHN. Besides preventing losses from a second outbreak, this practice may also reduce the risk of farm-to-farm transfer of the virus.

The spatial and temporal distribution of outbreaks within the affected area supported the plausibility of a farm-to-farm spread of IHNV. There was a systematic outward expansion of IHN outbreaks in the study area (Fig. 2A–D), indicative of a propagative disease outbreak, where initial cases served as sources of infectious agents for others. Once IHNV was detected in a channel there was always at least one site in that channel with infected fish, or fish that had survived IHN, to serve as a source of virus for other sites.

Unfortunately, we were not able to verify that all outbreaks of IHN were detected between 1992 and 1996. Some groups of fish in the area had elevated mortalities but were not tested for IHNV. Also, if the virus did not result in elevated mortalities it may not have been detected as routine testing was not done. Determining whether fish on all farms in the area were exposed to IHNV or suffered from disease associated with the virus would have required frequent viral testing of fish on sites, and this was not done between 1992 and 1996.

The surveillance program in 1997 was designed to better assess the exposure and disease status of fish on farms in the area. This program was initiated after the implementation of an area-based management plan aimed at reducing virus exposure from farmed fish. The program assessed two things: whether fallowing farms was effective at reducing the viral load on or around individual farms (St-Hilaire et al., 2001a), and whether removing a large number of infected farmed fish from the area was effective at reducing the incidence (number of new outbreaks) of the disease.

Findings from this surveillance program indicated there was a sudden reversal in the rising incidence rate of IHN outbreaks after the implementation of the management plan (Fig. 3). The changes put into place during the summer of 1996 included an increased awareness among local producers about potential modes of viral transmission between farms, simultaneous fallowing of as many infected sites as possible (9 out of 14) for a minimum period of 2 months prior to re-stocking, and fewer farms re-stocked with Atlantic salmon. All aspects of the strategy were aimed at reducing the risk of virus exposure from infected farms. If IHN outbreaks were not associated with farm-to-farm spread of the virus,

then these management strategies should have had little effect on the incidence rate of outbreaks. In addition, if IHNV was continually present in the study area (i.e., in wild fish), then fallowing sites should not have had an effect on the incidence of disease. Instead, we observed a large decrease in the incidence of IHN, and the low incidence of disease persisted over the entire surveillance period.

All fish in the area were vaccinated with an autogenous killed vaccine prior to salt water entry. It is possible that the vaccine reduced the magnitude of the outbreak on farms, but it is unlikely that it completely prevented disease. The general use of the autogenous killed vaccine for IHNV was started in the summer of 1995, before the decline in the incidence of IHN. In fact, all outbreaks of IHN after January 1996 occurred in vaccinated fish. In a laboratory experiment designed to determine the antibody profile of Atlantic salmon exposed to IHNV, vaccinated Atlantic salmon had an overall mortality rate of 37% (St-Hilaire et al., 2001b). Although this was less than the group of unvaccinated fish, it was still high enough that our surveillance program would have detected the disease.

The reduction in IHN outbreaks after the summer of 1996 appeared to be due to a lack of exposure to the virus. The two serological surveys done on fish in the area suggested that IHNV was not continuously present in the study area (St-Hilaire et al., 2001a). Also, IHN was not observed on 10 sites with newly transferred smolts, providing further evidence that IHNV was not constantly present in the area. These fish should have been good sentinels for IHNV, given the susceptibility of Atlantic salmon to the virus (Traxler et al., 1993) and the stress of smoltification and salt water transfer (Maule et al., 1987). Furthermore, on the two sites where IHN was detected during the surveillance period the fish had been on the farms for over 10 months. Fish on both sites did not have IHNV-specific antibodies 1 month prior to disease onset, which suggested they were exposed to the virus only shortly prior to the outbreaks (St-Hilaire et al., 2001a).[4]

The second serological component of the program suggested that IHN had not gone undetected during the period of surveillance (St-Hilaire et al., 2001a). Of the four farms that harvested their fish and were tested for IHNV-specific antibodies, only one site had seropositive fish at harvest, and disease was detected on this site (St-Hilaire et al., 2001a). Fish on the other sites did not have detectable antibodies and no disease was detected on these sites.

All data collected during the surveillance program indicated that the virus was not consistently present in the area, and the incidence of IHN was reduced after an area management plan was implemented. Furthermore, new outbreaks detected during the course of the surveillance program appeared to be related to other farm outbreaks. In one case, there was a direct link to one of the sites that did not participate in the fallowing plan. Site C, which appeared to be free of IHN for 10 months, had two large barges transferred to the site two months before IHN was diagnosed on the site. These barges originated from site M, which was undergoing a second outbreak of IHN at the time of the transfer. The two barges on site C were located at the end of the net-pen system where mortality associated with IHN was first observed and mortality rates were highest.

In addition, the fragment pattern generated from the RPA analysis of three virus isolates collected from site C were identical to the pattern generated from an isolate collected 5

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[4] Data from site O are published in St-Hilaire (2000).

months earlier from site M (prior to disease on site C) (Fig. 4), indicating the two isolates were genetically similar. It has been shown for RNA viruses that RPA pattern similarity correlates with genetic relatedness as determined by nucleotide sequencing (Dopazo et al., 1993; Garcia et al., 1994; Emmenegger et al., 2000; Troyer et al., 2000). This information, in addition to the history preceding the onset of disease, suggested that the source of IHNV for site C was most likely site M.

The other site (O) that developed IHN during the course of the surveillance program did not have a history that linked it directly to either of the two sites (M and N) holding survivors at the time of the outbreak on site O. Despite this, the RPA data indicated that the virus isolates from site O were of similar origin to the isolates collected from other Atlantic salmon in the study area (Table 5).

All six RPA patterns generated by the IHNV isolates collected from farmed fish between 1992 and 1997 were more similar to one another than to the isolate collected from the wild sockeye salmon included in this study, and a number of isolates from Atlantic salmon from different outbreaks had identical RPA patterns (Fig. 4). In general, IHNV isolates collected from fish on the same farm were identical (data from sites C and O); however, there was one fragment difference between two isolates collected from site M (Fig. 4), which suggests there may be some genetic variation in isolates over time. This finding could be explained by the fact that IHNV is an RNA virus and, therefore, would not have a proof-reading mechanism during virus replication and would be expected to have an error frequency rate at 10^{-4} to 10^{-5} per base site (Morse, 1993). The similarity in RPA fragment patterns between IHNV isolates from Atlantic salmon is not inconsistent with farm-to-farm spread of the virus.

Given that outbreaks of IHN last for a long time, and that fish may be infective for an even longer period of time, it is possible that the virus could be inadvertently spread from one farm to another via management practices. There was evidence to support that the movement of equipment and fish from infected sites to clean sites were the sources of IHNV for some outbreaks. Farm-to-farm spread of IHNV via other routes, such as waterborne transfer and wild or feral fish movements between sites could not be assessed in this study, but should be further investigated given the virus can survive for up to 2 weeks in salt water (Toranzo and Hetrick, 1982) and wild fish are occasionally observed in and around net-pens (Haegele et al., 1991; Kent et al., 1998). The outward spread of the viral disease, the genetic similarity between the farm fish IHNV isolates, and the removal of a large number of clinically diseased fish and survivors of the disease from the study area coinciding with the reversal in a rising incidence rate of IHN outbreaks suggested that many farm outbreaks of IHN were related. If many outbreaks of IHN in the Atlantic salmon industry were due to farm-to-farm spread of the virus, as the evidence suggested, then this disease may be largely preventable in the future.

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