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# Transmission potential of infectious hematopoietic necrosis virus in APEX-IHN®-vaccinated Atlantic salmon

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ABSTRACT: Infectious hematopoietic necrosis virus (IHNV) outbreaks have had a significant negative impact on Atlantic salmon *Salmo salar* production in British Columbia, Canada, since the first outbreak was reported in 1992. In 2005, the APEX-IHN® vaccine was approved for use in Canada for prevention of IHN. The vaccine was proven to be safe and efficacious prior to approval; however, it is unknown as to whether APEX-IHN®-vaccinated Atlantic salmon infected with IHNV can support replication and virus shedding in sufficient quantities to provide an infectious dose to a nearby susceptible host. To determine whether vaccinated, infected fish are able to transmit an infectious dose of IHNV, vaccinated Atlantic salmon were injected with IHNV ($10^4$ plaque-forming units per fish) and cohabitated with either naïve Atlantic salmon or naïve sockeye salmon *Oncorhynchus nerka*. APEX-IHN®-vaccinated fish were significantly protected against IHNV with mortality occurring in only 2.6% of the population as opposed to 97% in unvaccinated controls. Vaccination in IHNV-infected Atlantic salmon completely abolished disease transmission to cohabitating naïve sockeye salmon and reduced virus spread among cohabitating naïve Atlantic salmon. At 7 mo post-vaccination, IHNV-neutralizing antibodies were detected in nearly all vaccinated fish (94%) with similar titer occurring between vaccinated, infected fish and vaccinated, uninfected fish, indicating APEX-IHN® vaccination induces a robust seroconversion response. Taken together, these results demonstrate that vaccination greatly reduces the infectious load and potential for IHNV transmission. As such, APEX-IHN® should be included in fish health management strategies when culturing Atlantic salmon in IHNV endemic areas.

KEY WORDS: APEX-IHN® vaccine · Infectious hematopoietic necrosis virus · IHNV · Atlantic salmon · Sockeye salmon

## INTRODUCTION

Infectious hematopoietic necrosis virus (IHNV) is a pathogen of wild and cultured salmon and trout species worldwide. The virus is endemic in freshwater systems in western North America where it is commonly isolated from Pacific salmon (Wolf 1988). Disease and mortality due to IHNV infection are commonly seen during the fry and juvenile life stages although the virus is frequently isolated from spawning adults without disease. The presence of IHNV in the marine environments of the Pacific Northwest has also been demonstrated with the occurrence of multiple outbreaks in Atlantic salmon *Salmo salar* net-pen aquaculture operations located in the coastal marine waters of British Columbia (BC), Canada, and Washington State, USA (Armstrong et al. 1993, Traxler et al. 1993, Kent et al. 1998, Saksida 2006, Purcell et al. 2013).

IHNV outbreaks in farmed Atlantic salmon populations, a species exotic to the Pacific Northwest, have

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resulted in catastrophic losses. The first outbreak in BC occurred off the east coast of Vancouver Island in 1992 and spanned 4 yr, encompassing 13 sites within an 11 nautical-mile radius of the index case with farm level mortalities reaching 78% (Armstrong et al. 1993, St-Hilaire et al. 2002). Subsequent outbreaks in 2001–2003 were equally severe with high mortality and widespread infection among farm sites (Saksida 2006). Mortality was generally highest in Atlantic salmon that had been in seawater for 1 yr or less, but fish were susceptible to IHN disease at all stages of the saltwater production cycle with some affected fish weighing more than 6 kg and held in saltwater for over 2 yr (St-Hilaire et al. 2002).

Economically, IHNV disease has had devastating impacts to the industry with the 2001–2003 outbreak alone costing approximately CAD $200 million. Consequently, there was a need for effective vaccines to prevent and control IHN disease to ensure industry sustainability. Research studies have demonstrated that a single inoculation of an IHNV DNA vaccine encoding the G protein provides protection against IHN disease in rainbow trout (Corbeil et al. 2000), numerous species of Pacific salmon (LaPatra et al. 1989, Corbeil et al. 1999, Garver et al. 2005b), and Atlantic salmon (Traxler et al. 1999). Moreover, the protective immunity afforded by the DNA vaccine is long-lived with limited plasmid persistence and biodistribution (Garver et al. 2005a, Kurath et al. 2006), making it a favorable candidate for commercial applications. In July 2005, Novartis received approval for an IHNV DNA (APEX-IHN®) vaccine from the Canadian Food Inspection Agency (Salonius et al. 2007).

Between 2005 and 2015, over 100 million doses of APEX-IHN® have been administered in Canada. Despite the vaccine's nearly universal use in the BC salmon aquaculture industry, questions remain regarding the vaccine's use and its capacity to safeguard Atlantic salmon net-pen stocks. Specifically, it is unknown as to whether APEX-IHN®-vaccinated Atlantic salmon exposed to IHNV support replication and virus shedding in sufficient quantities to provide an infectious dose to a nearby susceptible host. This is relevant as Atlantic salmon are susceptible to IHNV disease and are capable of shedding high levels of virus once infected (Garver et al. 2013). Consequently, the goal of this study was to determine the protection afforded by APEX-IHN in Atlantic salmon and also to determine if viral transmission occurred to either co-habitated Atlantic salmon or sockeye salmon *Oncorhynchus nerka*.

## MATERIALS AND METHODS

### Virus and cell culture

Challenge studies utilized IHNV isolate BC93-057 (U genogroup) cultured from a diseased farmed Atlantic salmon sampled in 1993 during an IHNV epidemic in the Discovery Islands, BC. Virus isolation, subsequent amplification, and quantification were carried out as previously described (Garver et al. 2013). Virus titer is reported as plaque-forming units (PFU) $ml^{-1}$.

### Sockeye salmon smolt susceptibility to IHNV

Two susceptibility trials with sockeye salmon smolts (Pitt River stock) were conducted to determine susceptibility for this stock. For both experiments, sockeye salmon were obtained from Inch Creek Hatchery, BC. Upon arrival at Pacific Biological Station (Nanaimo, BC), fish were reared in 5°C dechlorinated freshwater. The hatchery has no previous occurrence of IHNV, and 60 fish sampled prior to transport proved negative for IHNV using a cell culture virus assay (USFWS and AFS-FHS 2014). To mirror the natural life cycle of sockeye salmon, fish were smolted after 16 mo in freshwater. Fish were exposed to IHNV within 24 to 48 h of smolting.

In the first trial, triplicate groups of 26 sockeye salmon smolts (brood year 2010; average weight 5.5 g) were waterborne exposed to IHNV at 4 doses ($10^1$, $10^2$, $10^3$, and $10^4$ PFU $ml^{-1}$). Fish were immersed in 19 l aerated static seawater (9°C) for 1 h after which water flow to the tanks was resumed. Fish were monitored daily for mortality or visible signs of disease for 72 d post-challenge (dpc). From all dead fish, individual brain and viscera tissue pools were homogenized 1:10 (w/v) in 1× Hanks' balanced salt solution (HBSS) (Gibco) + 1× antibiotic/antimycotic (Gibco) and analyzed for virus titer via plaque assay.

In the second trial, smolts (brood year 2012; average weight 28 g) were waterborne exposed to 3 different doses of IHNV ($10^2$, $10^3$, and $10^4$ PFU $ml^{-1}$). Fish were immersed in a 100 l aerated static seawater (10°C) bath containing virus for 1 h after which water flow to the tanks was resumed. Fish were held in 375 l flow-through tanks for the duration of the experiment. The 2 lower doses, $10^2$ and $10^3$, were conducted in triplicate and duplicate tanks, respectively, while the $10^4$ dose was conducted in a single tank. Fish were monitored daily for mortality or visible signs of disease for 41 dpc. Head kidney was

sampled from a subset of challenge mortalities and homogenized 1:4 (w/v) in 1× HBSS (Gibco) + 1× antibiotic/antimycotic (Gibco) and analyzed for virus titer via plaque assay.

## APEX-IHN® vaccine trial

### Fish care and vaccination

Atlantic salmon (Mowi strain; average weight 50 g) were obtained from an industry hatchery with no previous history of IHN outbreaks located in BC. At the hatchery, fish were dip vaccinated with the bacterins Ermogen, Furogen, and Vibrogen (Novartis) followed by intraperitoneal (i.p.) administration of the multivalent Alphaject 4000 (Syndel), which collectively provide protection against *Aeromonas salmonicida*, *Vibrio anguillarum*, and *V. salmonicida*. Upon arrival at Pacific Biological Station, fish were maintained in 9°C dechlorinated freshwater for 14 d prior to vaccination with APEX-IHN®. The vaccine was administered to a subset of fish (n = 200) following manufacturer's guidelines. Briefly, 0.05 ml of vaccine was delivered via a single intramuscular injection halfway between the lateral and dorsal fin in the epaxial muscle. The unvaccinated control group remained unhandled. Both vaccinated and unvaccinated fish were held for an additional 166 d in ambient freshwater during which time the temperature ranged from 5 to 10°C totalling 1276.6 degree days post-vaccination. At the end of this period, fish (average weight 88 g) were transferred to 10°C saltwater (~30 ppt) and maintained under these conditions for 2 d prior to IHN virus challenge.

Sockeye salmon used in the vaccine trial (brood year, 2010; average weight 5.5 g) were reared as described in the previous section. Both sockeye and Atlantic salmon were reared under a natural photoperiod and fed dry pellets (EWOS Canada). All fish handling was performed under tricaine methanesulfonate (MS-222; Syndel) anesthesia.

### Virus challenge of APEX-IHN®-vaccinated fish

Injection and cohabitation challenge models were used to evaluate if APEX-IHN®-vaccinated Atlantic salmon were capable of shedding and transmitting virus to susceptible hosts. APEX-IHN®-vaccinated Atlantic salmon (quadruplicate tanks of 25 fish each) were i.p. injected with IHNV ($10^4$ PFU fish$^{-1}$) and held in 700 l flow-through tanks (10°C seawater) for 20 h prior to the addition of cohabitants to prevent exposure of recipients to IHNV that may have leaked from i.p.-injected individuals. Naïve Atlantic salmon recipients were added to 2 tanks, 25 fish per tank, and naïve sockeye salmon recipients were added to the remaining 2 tanks, 40 fish per tank. For the cohabitation challenge, unvaccinated Atlantic salmon were i.p. injected with IHNV and cohabitated with APEX-IHN®-vaccinated Atlantic salmon in duplicate tanks. Identical tanks as the injection challenge were established for the positive virus transmission control with the exception that the IHNV donor population consisted of unvaccinated Atlantic salmon rather than APEX-IHN®-vaccinated Atlantic salmon. For the mock challenge control, vaccinated Atlantic salmon were i.p. injected with 100 µl of HBSS.

Fish were monitored daily for mortality or visible signs of disease for 50 dpc, at which time all remaining fish were euthanized with a lethal overdose of MS-222. Blood was collected from individual fish by caudal vein puncture and allowed to clot at 4°C overnight. The next morning, samples were centrifuged at 1500 × *g* for 10 min. Sera were collected and stored at −80°C until assayed for IHNV neutralizing antibodies. Only 3 sockeye salmon were large enough to allow for blood collection (1 from Tank 103 and 2 from Tank 111).

Viral titers were determined for 100% of the APEX-IHN®-vaccinated mortalities by plaque assay using polyethylene glycol-treated epithelioma papulosum cyprini (EPC) monolayers (Fijan et al. 1983, Batts & Winton 1989). To assess presence or absence of IHNV in the rest of the population, at least 50% of the fish (mortalities and survivors) in each treatment group were screened by tissue culture assay. Tissue samples were processed as described above. For Atlantic salmon, kidney samples were taken from individual fish. For sockeye salmon, kidney, spleen, and pyloric caeca samples were taken from each fish and pooled in groups of 2 or 3 fish.

## Neutralizing antibody titers

Neutralizing antibodies to IHNV were measured in serum samples using a complement-dependent plaque neutralization assay as previously described (LaPatra et al. 1993). Neutralizing activity was tested against a U genogroup isolate (collected at Baker Lake in 1994), and antibody titer is reported as the reciprocal of the highest dilution that resulted in a 50% reduction in the number of plaques observed in the negative controls.

### Statistical analysis

Differences in cumulative percent mortality (CPM) and mean days to death (MDD) were evaluated for the sockeye salmon susceptibility experiments using a Kruskal-Wallis 1-way analysis of variance ($\alpha$ = 0.05). Relative percent survival (RPS) for the APEX-IHN® study was calculated using the following formula: $[1 - (CPM_{vaccinated}/CPM_{unvaccinated})] \times 100$. Differences in neutralizing antibody titers between vaccinated, i.p.-injected fish and vaccinated, mock-infected fish were evaluated for significance using a Mann-Whitney rank sum test. Statistical analysis was completed using SigmaPlot v. 10.0 (Systat).

## RESULTS

### Sockeye salmon smolt susceptibility to IHNV

In the first trial, mortality occurred in the $10^3$ and $10^4$ PFU ml$^{-1}$ treatments (Table 1) but not the $10^1$ or $10^2$ PFU ml$^{-1}$ treatment. Of the 27 mortalities worked up, 24 (89%) were positive based on tissue culture results. In the second trial, mortality occurred at $10^2$, $10^3$ and $10^4$ PFU ml$^{-1}$ (a $10^1$ PFU ml$^{-1}$ treatment was not included); however, at the lowest dose ($10^2$ PFU ml$^{-1}$), mortality was inconsistent and only occurred in 2 of the 3 tanks (Table 1). Thirty-five percent of the mortalities in this trial were screened for IHNV by tissue culture, and all were positive. In both trials, differences in CPM between treatments were not significant. This was also true for MDD data, indicating there was little difference in viral kinetics between doses.

### Vaccine efficacy

Survival of APEX-IHN®-vaccinated Atlantic salmon was nearly 100% post-exposure to a lethal dose of IHNV regardless of challenge route, i.e. injection or cohabitation. The RPS values for this challenge ranged from 95 to 100%. For the 100 APEX-IHN®-vaccinated Atlantic salmon i.p. injected with IHNV (designated as donors in Tanks 101–104; Table 2), the average CPM was 2%, while the average CPM of the unvaccinated donor Atlantic salmon was 97% (Tanks 105–109). Similarly, when APEX-IHN®-vaccinated Atlantic salmon were exposed to IHNV through cohabitation with diseased cohorts, the average CPM was 4% (Tanks 105 and 106) while the CPM of the corresponding unvaccinated controls was 80% (Tanks 107 and 108) (Table 2). Of the 4 total vaccinated Atlantic salmon mortalities that occurred in the study, IHNV was only isolated from 2 fish representing the injection exposure group, while IHNV was not recovered from the 2 APEX-IHN® mortalities occurring in the cohabitation exposure tanks. At the conclusion of the study, viral diagnostics on 41% (60/146) of the surviving APEX-IHN® Atlantic salmon revealed detectable levels of IHN virus in 1 fish taken from a cohabitation exposure tank.

Table 1. Sockeye salmon smolt susceptibility to infectious hematopoietic necrosis virus (IHNV). Trial 1 smolts from brood year 2010, average weight 5.5 g; Trial 2 smolts from brood year 2012, average weight 28 g. A waterborne challenge model was used to expose fish to the virus. Cumulative percent mortality (CPM), average CPM ± standard error of the mean (SEM), and mean days to death (MDD) are reported. nt: not tested; na: not applicable

| Virus dose (PFU ml$^{-1}$) | Trial 1 CPM | Trial 1 Avg. CPM ± SEM | Trial 1 MDD | Trial 2 CPM | Trial 2 Avg. CPM ± SEM | Trial 2 MDD |
|---|---|---|---|---|---|---|
| $10^1$ | 0 | 0 | 0 | nt | nt | nt |
| | 0 | | 0 | | | |
| | 0 | | 0 | | | |
| $10^2$ | 0 | 0 | 0 | 3 | 1.3 ± 0.88 | 32.3 |
| | 0 | | 0 | 0 | | 0 |
| | 0 | | 0 | 1 | | 29 |
| $10^3$ | 12 | 12 ± 2.3 | 52 | 12 | 8.5 ± 3.5 | 25.7 |
| | 16 | | 36 | 5 | | 16.7 |
| | 8 | | 23.7 | | | |
| $10^4$ | 16 | 20 ± 8.3 | 31.8 | 10 | na | 28.3 |
| | 8 | | 37.5 | | | |
| | 36 | | 33.9 | | | |

### Virus transmission potential of APEX-IHN® Atlantic salmon

APEX-IHN®-vaccinated Atlantic salmon i.p injected with IHNV were cohabitated with either naïve sockeye salmon or Atlantic salmon to measure the potential of APEX-IHN®-vaccinated Atlantic salmon to transmit an infectious dose of virus to a susceptible recipient population. Among the duplicate sockeye salmon recipient tanks (Tanks 103 and 104; Table 2), no IHNV-related mortality was observed in the recipient population despite exposure to an IHN donor with high IHNV tissue titer ($1.9 \times 10^7$ PFU g$^{-1}$). In contrast, when recip-

Table 2. APEX-IHN® vaccine study. Vac Atl: APEX-IHN®-vaccinated Atlantic salmon; Unvac Atl: unvaccinated Atlantic salmon. Donors in Tanks 101–109 were intraperitoneal (i.p.) injected with infectious hematopoietic necrosis virus (IHNV) at a concentration of $10^4$ plaque-forming units (PFU) fish$^{-1}$. Donors in Tanks 110 and 111 were i.p. injected with 100 µl of Hanks' buffered salt solution. Cumulative percent mortality (CPM), relative percent survival (RPS) of APEX-IHN®-vaccinated Atlantic salmon, and tissue culture results given for both donor and recipient groups. na: none available; ns: no sample

| IHNV exposure route | Tank population | | Tank no. | CPM | | RPS | No. of positives/No. of samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Donor | Recipient | | Donor | Recipient | | Survivors | | Mortalities | |
| | | | | | | | Donor | Recipient | Donor | Recipient |
| Injection | Vac Atl | Unvac Atl | 101 | 0 | 0 | 100 | 0/10 | 0/5 | na | na |
| | | Unvac Atl | 102 | 4 | 72 | 96 | 0/10 | 2/5 | 1/1 | 16/16 |
| | | Sockeye | 103 | 4 | 0 | 96 | 0/10 | 0/20 | 1/1 | na |
| | | Sockeye | 104 | 0 | 0 | 100 | 0/10 | 2/20[a] | na | na |
| Cohabitation | Unvac Atl | Vac Atl | 105 | 100 | 4 | 95 | 0/1 | 1/10 | 10/10 | 0/1 |
| | | Vac Atl | 106 | 92 | 4 | 95 | 0/3 | 0/10 | 4/4 | 0/1 |
| Injection (positive control) | Unvac Atl | Unvac Atl | 107 | 96 | 76 | na | ns | ns | ns | ns |
| | | Unvac Atl | 108 | 96 | 84 | na | ns | ns | ns | ns |
| | | Sockeye | 109 | 100 | 12.5 | na | 0 | 0/10[a] | 5/5 | 5/6[a] |
| Mock infected | Vac Atl | Unvac Atl | 110 | 4 | 0 | na | 0 | 0 | 0/1 | na |
| | | Sockeye | 111 | 0 | 0 | na | 0 | 0 | na | na |

[a]Sockeye samples assayed in pools of 2 or 3 fish

ient sockeye salmon were cohabitated with unvaccinated, IHNV-infected Atlantic salmon, IHN disease was spread to the recipient sockeye salmon population, resulting in a cumulative mortality of 12.5% (Table 2).

Survivors from the sockeye salmon recipient population in Tanks 103 and 104 were negative for IHNV by tissue culture with the exception of one pool of 2 fish from Tank 104. Cytopathic effect was observed for this pooled sample, indicating the presence of virus. As there were no mortalities nor the detection of IHNV in the vaccinated donor fish, we hypothesize the virus detection in sockeye may be due to contamination during simultaneous tissue processing and plate inoculation of highly positive specimens. No IHN virus was detected by tissue culture in the surviving donors from this tank.

When naïve Atlantic salmon were utilized as recipients (Tanks 101 and 102, Table 2), exposure to an infectious dose of IHNV from APEX-IHN®-vaccinated Atlantic salmon was inconsistent with IHNV mortality in the recipient population occurring in only one of the duplicate tanks (Tank 102). In this tank, IHNV-related mortality in the recipient population began 4 d after the death of an APEX-IHN®-vaccinated Atlantic salmon with a tissue titer of $1.2 \times 10^8$ PFU g$^{-1}$. The IHNV epizootic in the recipient population lasted for 34 d and resulted in a cumulative mortality of 72% (Fig. 1). For all mortalities and 40% (2/5) of the survivors in the recipient group in Tank 102, IHNV was confirmed based on the CPE observed, i.e.

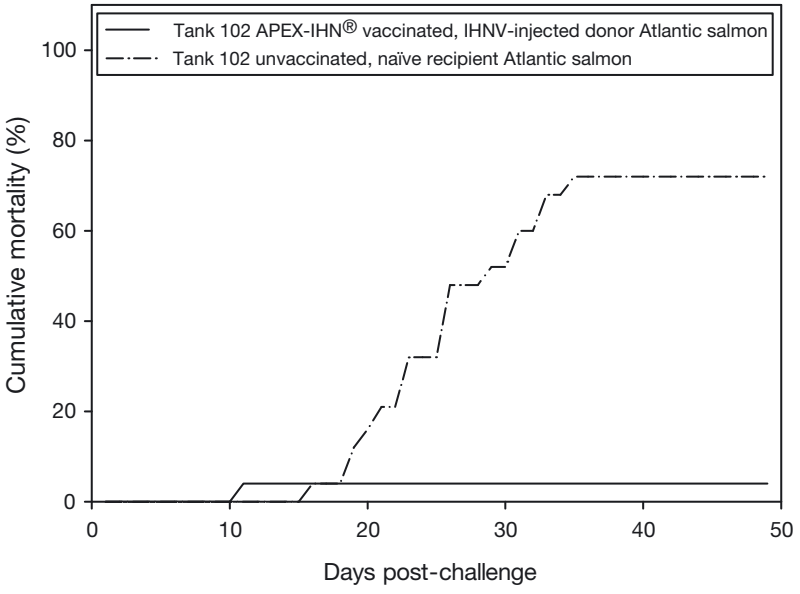


Fig. 1. Survival curves for APEX-IHN®-vaccinated Atlantic salmon intraperitoneally injected with infectious hematopoietic necrosis virus (IHNV) and cohabitated with unvaccinated, naïve Atlantic salmon. Tank 101 not included on this graph due to the lack of mortality in this tank

rounded cells and plaques (LaPatra 2012). In the parallel non-vaccinated treatment group where unvaccinated Atlantic salmon IHNV donors were cohabitated with naïve Atlantic salmon (Tanks 107 and 108), an infectious dose of IHNV was transmitted to the recipient Atlantic salmon population resulting in 76–84% mortality. The temporal nature of the disease outbreak in these tanks was similar to Tank 102, as IHNV-related mortality in the recipient populations began 5 (Tank 108) and 8 (Tank 107) d after the first donor fish died (data not shown).

### Neutralizing antibody titer

Neutralizing antibody titers for vaccine trial survivors are given in Table 3. Overall, APEX-IHN®-vaccinated fish had equally high neutralizing antibody titers irrespective of IHNV exposure route. Additionally, no significant differences in antibody titers were observed between APEX-IHN®-vaccinated survivors (Tanks 101–106) and APEX-IHN®-vaccinated, mock-infected fish (Tanks 110 and 111).

Neutralizing antibodies were not detected in surviving Atlantic salmon recipients from Tank 101, further confirming that the APEX-IHN®-vaccinated donor population was not transmitting an infectious dose. In duplicate Tank 102, where the recipient population was exposed to an infectious dose of IHNV, neutralizing antibodies were detected in 78% of the survivors tested. Although only one recipient from Tanks 103 and 104 was tested, neutralizing antibodies were not detected. The level of neutralizing antibodies in surviving donors in Tanks 105 and 106 was variable. Neutralizing antibody titers in surviving vaccinated recipients in these 2 tanks were high, and all fish had a measurable titer.

## DISCUSSION

Open net-pen farming of Atlantic salmon in IHNV endemic waters has resulted in catastrophic virus outbreaks and disease spread among neighboring farms (St-Hilaire et al. 2002, Saksida 2006). In BC, where farmed salmon is the province's largest agricultural export, use of the APEX-IHN® vaccine has steadily increased since its licensure in 2005. With the exception of the initial studies testing the safety and efficacy of the APEX-IHN® vaccine (Salonius et al. 2007), there has been no further work to evaluate vaccine effectiveness in salmon farms and its ability to thwart virus transmission and disease spread. Newly developed particle tracking models simulating previous outbreak events have illustrated that in the absence of disease mitigation measures, water-

Table 3. Neutralizing antibody titers for survivors from the APEX-IHN® vaccine study. Numbers in parentheses indicates number of fish with titer. Abbreviations as in Table 2

| Tank population | | Tank | No. of positive survivors/ No. of survivors sampled | | Neutralizing antibody titer | |
|---|---|---|---|---|---|---|
| Donor | Recipient | no. | Donor | Recipient | Donor | Recipient |
| VacAtl | UnvacAtl | 101 | 8/10 | 0/20 | (8) ≥160, (2) <20 | (20) <20 |
| | | 102 | 10/10 | 7/9 | (9) ≥160, (1) 20 | (4) ≥160, (3) 40, (2) <20 |
| VacAtl | Sockeye | 103 | 10/10 | 0/1 | (9) ≥160, (1) 80 | (1) <20 |
| | | 104 | 10/10 | ns | (9) ≥160, (1) 80 | ns |
| UnvacAtl | VacAtl | 105 | 0/1 | 10/10 | (1) <20 | (7) ≥160, (1) 80, (2) 20 |
| | | 106 | 3/3 | 10/10 | (2) ≥160, (1) 40 | (9) ≥160, (1) 80 |
| UnvacAtl | UnvacAtl | 107 | ns | 4/5 | ns | (2) ≥160, (1) 80, (1) 40, (1) <20 |
| | | 108 | ns | 6/7 | ns | (6) ≥160, (1) <20 |
| UnvacAtl | Sockeye | 109 | ns | ns | ns | ns |
| VacAtl | UnvacAtl | 110 | 7/10 | 0/20 | (5) ≥160, (2) 80, (3) <20 | (20) <20 |
| VacAtl | Sockeye | 111 | 10/10 | 0/1 | (9) ≥160, (1) 80 | (1)[a] <20 |

[a]Sample consisted of 2 pooled fish

borne transport of IHNV from diseased sites to downstream sites can occur and can account in part for disease dispersal among neighboring farms (Foreman et al. 2015). In addition, there is concern about potential viral spill-back events from farmed populations to wild salmonids, namely sockeye salmon, during their migration through an aquaculture occupied area. Given the widespread use of APEX-IHN® vaccine in BC Atlantic salmon aquaculture, this study was designed to evaluate the efficacy of the APEX-IHN® vaccine in eliminating IHNV transmission and disease spread from a vaccinated Atlantic salmon population to nearby susceptible hosts.

Our results demonstrate that regardless of whether Atlantic salmon were exposed to a lethal dose of IHNV via i.p. injection or cohabitation, significant protection (RPS 95–100%) was afforded by a single intramuscular injection of the APEX-IHN® vaccine. Despite the extremely high efficacy of the APEX-IHN® vaccine in Atlantic salmon, herd immunity did not appear to be extended to the naïve Atlantic salmon cohabitated with this group. The failed protection and resulting mortality of a single fish in the APEX-IHN® Atlantic salmon donor population (Tank 102) was sufficient to initiate an IHNV outbreak in the recipient Atlantic salmon population. Not only do these results corroborate the extreme susceptibility of Atlantic salmon to IHNV (Mulcahy & Wood 1986, Traxler et al. 1993, Garver et al. 2013), but they also demonstrate the need to vaccinate 100% of farmed Atlantic salmon in order to prevent the spread of IHN disease among aquaculture sites.

Conversely, the APEX-IHN® vaccine interrupted the spread of IHN disease from donor Atlantic salmon to recipient sockeye salmon smolts, as vaccine failure also occurred in the APEX-IHN® Atlantic salmon donor population in Tank 103. However, IHN disease was restricted to the one fish, and there was not an outbreak in the naïve Sockeye salmon population in this tank. We hypothesize that the virus transmission potential of the donor Atlantic salmon in this tank was limited due to the APEX-IHN® vaccine, thereby keeping the IHNV infectious dose below the threshold needed to cause a disease outbreak in sockeye salmon smolts.

Information regarding the susceptibility of sockeye salmon to IHNV at the smolt life stage is limited although a report of mortality in a freshwater sockeye salmon smolt population suggests older life stage sockeye are susceptible to IHN disease (Follett & Burton 1995). The results of the sockeye salmon susceptibility trials done herein reveal sockeye salmon smolts in seawater are susceptible to IHN disease, as a 1 h exposure of IHNV at $10^2$ PFU $ml^{-1}$ was sufficient to initiate IHN disease in this stock of sockeye salmon smolts. In comparison, Atlantic salmon smolts experienced IHN disease after a 1 h immersion with only 10 PFU $ml^{-1}$ (Garver et al. 2013). Taken together, this data suggests that sockeye salmon at the smolt life stage are approximately 10-fold less susceptible than Atlantic salmon smolts. As such, to initiate IHN disease in a population, sockeye salmon smolts likely require IHNV exposure doses at least 10 to 100 times higher than that of Atlantic salmon smolts. These differential susceptibilities explain why the APEX-IHN® vaccine is sufficient at abolishing disease spread among sockeye salmon but not among Atlantic salmon.

Serum collected from vaccinated, mock-infected Atlantic salmon smolts at 7 mo post-vaccination revealed that the APEX-IHN® vaccine elicited a robust and long-lasting seroconversion response in 85% of the population. Previous APEX-IHN® efficacy studies suggested similar (50%) seroconversion rates up to 12 mo, but by 17 mo post-vaccination, no neutralizing antibody titers were detected (Salonius et al. 2007). While it is unclear whether such reduced antibody titers would equate to reduced vaccine efficacy, declines in vaccine efficacy post-vaccination have been demonstrated for similar IHNV and related aquatic rhabdoviral DNA vaccines (Corbeil et al. 1999, Lorenzen et al. 2000, McLauchlan et al. 2003, Kurath et al. 2006). The typical production cycle of farmed Atlantic salmon in BC is 18 mo, and although IHNV outbreaks at farms predominantly affected fish within the first 12 mo, outbreaks in older harvest size fish suggests their susceptibility to disease. A longer-term test of APEX-IHN® efficacy in adult fish is needed to evaluate if the reductions in viral transmission potential as observed in our study would be sustained 7 mo post vaccination. It should be noted that a similar IHNV vaccine administered at 0.1 µg afforded significant protection to rainbow trout 24 mo post-vaccination (Kurath et al. 2006).

In the current study, there was not a significant difference in neutralizing antibody titers between vaccinated, mock-infected fish and vaccinated, IHNV-injected fish. This suggests that at 7 mo there was little waning of the immune response elicited solely by the APEX-IHN® vaccine. While there have been reports of anamnestic responses in fish vaccinated with the IHNV DNA vaccine (Corbeil et al. 1999, Traxler et al. 1999), such a response was not detected in the current study. The high seroprevalence and persistence of neutralizing antibodies in this study is

likely a result of the formulation of the commercial APEX-IHN® vaccine. Furthermore, the plasmid concentration in the APEX-IHN® vaccine is 10 µg, while efficacy studies investigating a similar IHNV DNA in rainbow trout (LaPatra et al. 2000, Kurath et al. 2006), Pacific salmon (Garver et al. 2005b), and Atlantic salmon (Traxler et al. 1999) typically tested concentrations of 0.1 or 1.0 µg. Finally, the upper measurement limit for the neutralizing antibody titer assay employed herein was 160. Given that the majority of the vaccinated, IHNV-injected survivors had a titer value of ≥160, it is possible there would have been a more pronounced difference in neutralizing antibody titer values between these fish and vaccinated, mock-infected fish if a greater range of measure was used.

In our study, the detection of IHNV in surviving vaccinated and unvaccinated Atlantic salmon at 52 dpc suggests a prolonged presence of virus in the population. The long-term presence of IHNV in asymptomatic hosts has been demonstrated through the use of controlled laboratory studies in various Pacific salmon species (Drolet et al. 1995, Kim et al. 1999, St-Hilaire et al. 2001, Müller et al. 2015) as well as in Atlantic salmon (Kurath et al. 2016, A. Long unpubl. data). From our results it is unclear as to whether the detections in the survivors truly represent viral persistence or merely individuals that recently became infected from a donor that succumbed to IHN disease near the conclusion of the study. Nevertheless, only 5% (1/20) of the APEX-IHN®-vaccinated survivors showed detectable virus while 20% (2/10) of the unvaccinated survivors remained infected at the conclusion of the study. Further research will be required to better understand IHNV persistence and epidemiological consequences, if any, in Atlantic and Pacific salmonids.

In summary, the results of this study demonstrate that APEX-IHN®-vaccinated Atlantic salmon are protected against a lethal exposure to IHNV. Moreover, APEX-IHN® vaccination of highly susceptible Atlantic salmon greatly reduces the infectious load and potential for IHNV transmission between both Atlantic and sockeye salmon. For these reasons, APEX-IHN® should be included in fish health management strategies when culturing Atlantic salmon in IHNV endemic areas.

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