The Epidemiology of Hemorrhagic Kidney Syndrome - Infectious Salmon Anemia in Atlantic Salmon in Atlantic Canada

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ABSTRACT

Hemorrhagic Kidney Syndrome (HKS) is characterized by high mortality rates in Atlantic salmon seawater sites with unique histological kidney lesions. Virus isolation results were positive for the orthomyxovirus responsible for Infectious Salmon Anemia (ISA) experienced by Norway and also a new virus tentatively classified in the *Togaviridae* family was isolated in some, but not all outbreaks. This study examined the associations between husbandry and environmental factors and the risk of high mortality rates in Atlantic salmon transferred to seawater in the spring of 1996. The study population consisted of over 2.2 million fish in 218 cages in three bays in the Bay of Fundy. The mean duration of an outbreak was 37 days and ranged from seven days to 125 days. Median total mortality during an outbreak period was 6,600 per 100,000 fish, while ten percent of case cages lost more than 29,000 per 100,000 fish. Cages containing larger fish populations, cages which had high mortality rates in the 1996 season, and cages at sites which had one or more positive cages, had increased risk of experiencing an outbreak of HKS/ISA in 1997. Collection of data in this type of aquaculture field study presented some unique challenges, such as adequacy of farm records, maintaining farm confidentiality, and relating mortality rates to diagnostic surveillance results. Although the orthomyxovirus is believed to be the etiologic agent, there remain many questions about the interaction of farm factors and the presence of other infectious agents which may alter the presentation and severity of the disease.

Considerations for Investigation of Emerging Diseases in Atlantic Canada

Identification

An emerging disease is difficult to identify unless the disease patterns from multiple farms are available for examination on a regular basis. This is especially true in the salmon farming conditions of New Brunswick where sites are sufficiently close to other sites that the transmission from one site to another may be as great a risk as transmission from one cage to another at the same site. Due to the fact that salmon farming is still relatively new to this area, the success of individual farmers can be attributed to their innovation and creativity when faced with any major deterrents. This attitude contributes to the farmer secrecy that is inherent in discussions about mortality and disease rates occurring on individual farms. It has also been a deterrent to establishing a confidential multi-site, performance monitoring system that would include mortality rates. Patterns of disease

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transmission among multiple farms remains difficult to describe without a standard method of data collection.

Regulatory environment

The fish health regulations in the province of New Brunswick have included some endemic agents, such as *A. salmonicida*, as a reportable finding with the usual control method being quarantine at freshwater hatcheries. Although the production aspects, such as total production levels and stocking density, have been regulated for the net pen component of the industry, disease regulations have, for the most part, been inactive at the seawater sites. However, the disease regulations do specify that identification of any agent causing cytopathic effects in fish viral cell lines is considered a potential pathogen and must be reported. Due to a general mistrust of government disease regulations and lack of defined response by the regulatory agencies (e.g. action taken following detection of a reportable agent), the farmers have not been very open about when they may have mortalities due to unexplained causes.

Viral surveillance

The fish health regulations require that any viral agents identified in fish farms or hatcheries be reported to the authorities. However, there is no mandatory viral surveillance or inclusion of viral diagnostic screening in situations of increased mortality rates. Surveillance for the presence of viruses in apparently healthy populations has not been done due to the regulatory environment and lack of defined response if a pathogen is detected. As a result, there is very little information regarding the presence, or prevalence, of viruses in salmon farming in Atlantic Canada. It is probable that many viruses are prevalent in farmed and feral fish in Atlantic Canada but do not cause disease. These orphan viruses are quite well documented in many species of animals.

Emergence of Haemorrhagic Kidney Syndrome (HKS)

The first pen of Atlantic salmon to experience increased mortality rates (greater than 1% per week) that remained unexplained by routine diagnostic tests was first affected in July or August, 1996. The group of fish had been transferred to seawater in May, 1995, and were approaching market weight (i.e. 3.5 kilograms) when the problem occurred. The diagnostic investigation has been reported previously [1] and can be summarized with the following description. Gross lesions associated with HKS included one or several of the following: swelling and/or reddening of the kidney, pale gills, exophthalmos, serosanguinous ascites, darkening of posterior intestine, and splenomegaly. The primary histologic lesion was renal interstitial haemorrhage and acute tubular necrosis and tubular casting, hence the reason for calling the problem Haemorrhagic Kidney Syndrome [1]. The attending veterinarian performed numerous diagnostic tests which all gave negative results, including histopathology, bacterial isolation, viral isolation, and selected toxicological analyses. Immuno-diagnostic tests, such as fluorescent antibody tests, were performed against Infectious Salmon Anemia virus (ISAV), a prime suspect in the case, but these too were negative.

Early Surveillance for HKS

In the fall of 1996, there did not exist a definitive diagnostic test for HKS beyond the presence of histological lesions. However, only 20% to 50% of moribund fish showed the typical histologic lesion of HKS. For this reason, the only method of diagnosing HKS was through examination of a sample of moribund fish and diagnosing the pen as HKS positive based on the presence of any fish with the appropriate lesion. At the request of the salmon growers' association, the veterinarians organized a confidential survey in November, 1996, to determine the number of farms with HKS lesions in any moribund fish, regardless of mortality rates. The results indicated that there were four sites with one fish or more which had the HKS lesion, three sites had elevated mortality rates. A second survey in February, 1997, discovered the presence of five sites with HKS lesions, three of which were newly detected as positive and two were part of the first four sites detected in the previous November. A final survey in April, 1997, revealed eight sites with HKS lesions. All of the positive sites were located within two adjoining bays containing 18 sites in total (one site was in a distant area unrelated to these two bays). Many of the farmers immediately slaughtered the entire group of fish which tested HKS positive since they were all at or approaching market weight. This likely slowed the spread of the disease in the early stages.

Viral Isolation

Several researchers began investigating the possibility of viral etiology by sampling moribund fish from net pens experiencing an increased mortality rate associated with HKS (defined by histology) and using the standard viral cell lines and the recently developed Salmon Head Kidney (SHK) cell line [2]. By the spring of 1997, several laboratories began to isolate agents causing cytopathic effects (CPE) on various cell lines, including Chinook Salmon Embryo (CHSE-213) and SHK. It was later determined that one agent was the orthomyxovirus responsible for ISA in Norway, and one was a previously unidentified virus whose genome structure and size resembled the *Togaviridae* (Kibenge, unpublished results). The ISAV is now considered the agent responsible for HKS and the togavirus is likely an orphan virus that is present in many populations of fish in Atlantic Canada. The lack of information about viruses present in farmed or feral fish created confusion about the role that any newly identified virus might play in a disease outbreak.

HKS - ISAV Epidemiology Investigation

Introduction

The objectives of this study were to describe the mortality patterns within affected cages and affected sites of the HKS positive area of Lime Kiln Bay, Bliss Harbour and Seal Cove, New Brunswick. The ultimate objective of the entire investigation was to identify factors that were associated with increased mortality rates among cages within the affected sites and among sites. The definition of mortality due to HKS was difficult to establish when this investigation was initiated due to the fact that the etiological agent remained unknown. Through the period of investigation, the intensity of disease surveillance and the types of diagnostic tests employed changed so dramatically that it was not possible to include diagnostic results as part of the definition of an affected cage except to exclude any with obvious alternative diagnoses.

Methods

Mortality Data Collection

All sites in the three affected areas of Lime Kiln Bay, Bliss Harbour and Seal Cove, were included in a survey of mortality records. Only records concerning fish that were transferred to seawater in April to June of 1996 were examined. Stocking numbers, fish transfers, and mortality data were collected during three site visits through examination of farm records retrospectively back to the time of transfer from the freshwater hatchery in the spring of 1996. Fish groups were followed with repeat communications until the end of November, 1997. The sea cage group of fish was the unit of concern in this study.

Definition of Cases

A group of fish (i.e. cage) was defined as a case which experienced an outbreak of unexplained mortality, when it met either of the two following criteria: 1) the group experienced seven or more high mortality days where a high mortality day was defined as a day with a mortality rate greater than one per 1000 fish, or 2) a cumulative mortality rate greater than five percent of the initial number of fish in that cage. Data were excluded from the data set used for all subsequent analyses if they met one or more of the following criteria: 1) suspect mortalities occurred during the first 30 days after transfer to seawater, 2) fish groups which had less than three months worth of data, 3) cages with less than 1000 fish, and 4) cages which did not contain fish on April 1, 1997.

Questionnaire

A standardized questionnaire was prepared to facilitate data collection. All site managers were interviewed to obtain a basic understanding of the farm data collection and recording systems prior to initiating data collection into the standardized format. For all sites, information was recorded about the initial stocking dates and numbers, any movements of fish into or out of cages, the fish group identification (i.e. the cage identification), the harvest dates and numbers, and the mortality numbers by date (including fish removed due to morbidity). Disease diagnostic results were also recorded where applicable.

Statistical Analysis

The cage was used as the statistical unit of analysis, with the presence of an outbreak of unexplained mortality (as defined previously) used to compare case and non-case fish groups. Descriptive statistics were generated using STATA (Stata Corporation, College Station, Texas) and APHIN graphing software (Animal Productivity and Health Information Network, University of Prince Edward Island, Charlottetown, Canada).

Results

Outbreak frequency

Outbreaks occurred in 49% of the 218 cages of Atlantic salmon which were investigated in the three affected areas. The total number of fish in the affected areas belonging to this year class was approximately 2.2 million, representing about 25% to 40% of the New Brunswick industry.

Outbreak pattern

Many of the case cages demonstrated a pattern of increasing mortality rates which were similar to propagated epidemic patterns. However, the pattern of mortality experienced by groups of fish was highly variable in terms of duration and severity. The mean loss of fish during an outbreak was 12,200 per 100,000 fish, while the median loss was 6,600 per 100,000 fish. Approximately 10% of cages lost more than 29,000 per 100,000 fish of their initial stocking numbers during an outbreak.

Maximum mortality rate

The median maximum mortality rate experienced by case cages was 492 per 100,000 fish per day, compared to the rate of 29 per 100,000 fish per day for non-case cages. However, peak mortality rates did exceed 5,200 per 100,000 fish per day for about 10% of case cages. As water temperatures decreased in October and November of 1997, the mortality rates of cases did not decrease despite the decrease in the number of new cases occurring.

Date of first onset

More than 54% of the cases had onset dates in June or July, 1997 (see Fig. 1). The date of onset was defined as the first day when the cage had a daily mortality rate greater than 100 per 100,000 fish at risk, or the date at which cumulative mortality exceeded 5,000 per 100,000 fish.

Duration of outbreaks

The mean duration of an outbreak was 37 days. However, 25% of cases had outbreaks that extended for 56 days or longer. Many of the cages had insufficient data to define the total duration due to the fact that there was early harvests or other manipulations of the group which precluded analysis to define the end date of an outbreak.

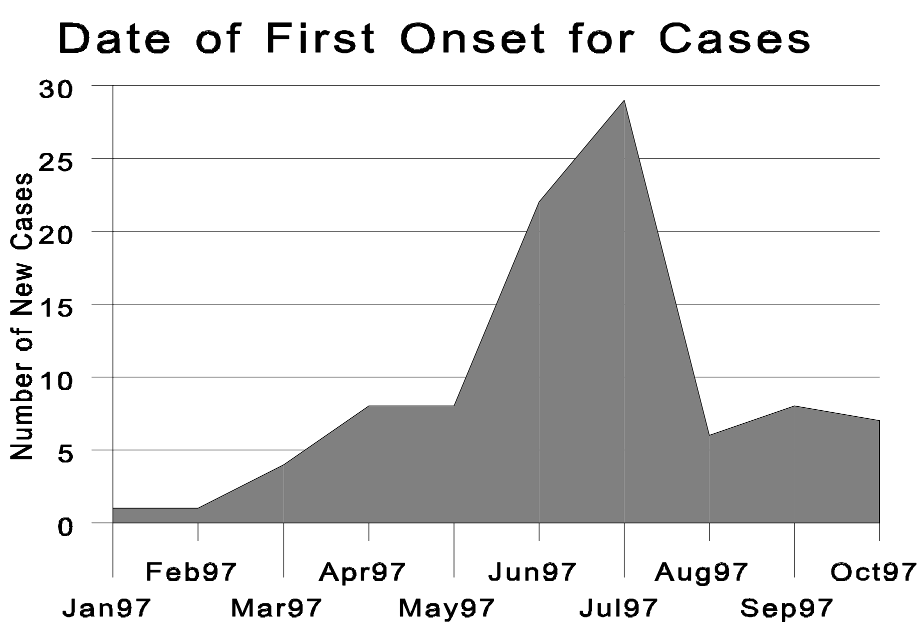

Fig. 1. Chronological distribution of the onset of outbreaks of Hemorrhagic Kidney Syndrome – Infectious Salmon Anemia in farmed salmon in Atlantic Canada. The date of onset was the first day when a cage had daily mortality rate greater than 100 per 100,000 fish at risk, or the date at which cumulative mortality exceeded 5,000 per 100,000 fish.

Discussion

The mortality patterns of Haemorrhagic Kidney Syndrome were investigated to describe the outbreak and to determine the outcome measures that would eventually be used in a study of factors associated with the risk of a cage having unexplained mortality problems. Although at the initiation of this study the etiology was still very much debated, it is now generally accepted that the causal agent is the orthomyxovirus responsible for causing Infectious Salmon Anemia in Norway [2]. It is impossible for a retrospective study such as this one to differentiate the causes of mortality except to exclude known causes of mortality. Even this is susceptible to bias in that there are different diagnostic intensities practiced by farmers and their veterinarians which are difficult to standardize.

It was concluded based on this study that HKS-ISA followed a propagated epidemic pattern of disease transmission within cage groups consistent with a contagious viral agent. One of the most difficult aspects of performing field studies in the salmon farming industry, retrospective or prospective, is the lack of standardized record keeping systems and the different cage identification practices. It is common for fish farmers to designate the group of fish by the identification of the actual physical cage in which the group resides. This can lead to difficulty in evaluating when a group ends and another group begins residence in that particular cage. The data is not valid when the characteristics of one group are attributed to another group on the basis of the cage of residence. In this particular study, it required a substantial effort to identify when this confusion may have occurred.

The risk factors that are part of the subsequent investigation using these mortality patterns as the outcome measure include, among other factors, the feed type, site diving patterns, the presence of another, already positive, cage at the site, the cage physical characteristics and dimensions, the size of the population contained within a cage, the health status during the first summer growing season, lice control methods, and environment around the site (e.g. depth under cages). The most likely portal for transmission demonstrated under laboratory conditions was the gills and a waterborne infection through the transfer of mucus between fish is probable [5]. This would suggest that the outbreak experienced in New Brunswick would have an increased risk of transmission at the site if there was a positive group of fish already present at the site. This appears to be true in the data that we have analyzed thus far. However, more analyses that control for other possible confounding factors are required before this can be stated with assurance. Salmon lice are suspected as possible vectors for transmission of ISA [4]. The numbers of lice were not counted during this period which makes it impossible to measure associations with mortality rates. However, the number of lice treatments will be used as surrogate measures of the intensity of lice infestation.

Other studies into the risk factors associated with ISA infections have elucidated several husbandry factors which affect transmission of the virus between sites, with discharge of biological material from a positive site or a processing plant as the most important factor [3, 6]. In each instance the site was used as the unit of concern. In our study, the sites affected were clustered within a very small geographical area and the number of positive sites was too small to have a meaningful evaluation of site-level factors of positive sites compared to negative sites. We chose instead to investigate the mortality patterns within the affected areas and compare severity of mortality at the cage level. Although the farmers could use the information about cage-level risk factors to mitigate an existing infection at the site, or to reduce the probability of transmission to their site from close neighbors, the detail required to investigate such factors requires a more complete and standardized data collection and recording system than is currently available. The transmission of viruses between sites remains an uncontrolled risk when farming in close proximity to other farm sites.

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