The effects of changing demographics on the distribution of marine anemia in farmed salmon in British Columbia

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Abstract

The changing geographic distribution of marine anemia (plasmacytoid leukemia) was compared with the evolving demographics of the chinook farming industry in British Columbia to explore the hypothesis that the disease had spread throughout the province between 1987 and 1992. Through retrospective and prospective methods, it was shown that the apparent spread of the disease was likely an artifact, resulting from changes in the distribution of fish farms throughout the province and corresponding changes in the intensity of regional disease surveillance. When viewed over a 5-year period, there were no statistically significant differences in the prevalence of the disease amongst fish sampled from each of the major salmon farming regions of British Columbia. By increasing the intensity of surveillance for the disease in apparently negative regions or chinook farms, one could routinely find cases of the disease. The results suggest that marine anemia is an endemic problem for farmed chinook salmon in British Columbia and is not a spreading epidemic.

Résumé

Les effets du changement démographique sur la répartition de l’anémie marine chez le saumon de pisciculture en Colombie-Britannique

Le changement de la distribution géographique de l’anémie marine a été comparé au développement démographique de l’industrie de pisciculture du chinook, en Colombie-Britannique. L’hypothèse émise est que la maladie s’est propagée à travers la province entre 1987 et 1992. Les auteurs ont démontré par des méthodes rétrospectives et prospectives que la propagation de la maladie était vraisemblablement un artefact provenant du changement de la distribution des fermes de pisciculture dans la province et de l’intensification des moyens de surveillance régionale des maladies. Sur une période de 5 ans, il n’y avait pas de différence dans la prévalence de la maladie entre les échantillons prélevés des fermes de pisciculture des principales régions de la Colombie-Britannique. En intensifiant les moyens de surveillance de cette maladie, des cas pourraient être identifiés de façon routinière même dans des régions ou des fermes apparentement sans maladie. Les résultats suggèrent que l’anémie marine est un problème endémique en Colombie-Britannique rencontré chez le saumon de culture chinook et qui ne se répand pas de façon épidémique.

(Traduit par docteure Thérèse Lanthier)


Introduction

The changing geographic pattern of an evolving disease outbreak can reveal much about the genesis and maintenance of the disease in nature. This is particularly so in the epidemiologic investigation of neoplastic disorders (1). The search for spatial clusters or changes in the geographic distribution of specific cancers has provided important data for the generation and testing of causal hypotheses (1). Biogeographic studies of diseases are, however, faced with the challenge of separating true nonrandom spatial patterns of disease from spurious patterns that are only a reflection of the distribution and the method of sampling of the population at risk (2).

Marine anemia, also known as plasmacytoid leukemia, is a recently described disease affecting farmed chinook salmon (Oncorhynchus tshawytscha) in British Columbia. The disease has been implicated as the cause of massive mortalities in salmon farms in British Columbia (3). Because of its association with a newly described retrovirus (4), it has been suggested that, like retroviral leukemias of other animals, marine anemia could spread throughout the population at risk (5).

Evidence supporting the hypothesis that marine anemia is a spreading, infectious, neoplastic disease could have profound regulatory effects on the salmon farming industry.

Although the aquaculture industry in British Columbia dates back to the early 1900s, salmon farming itself is new to the province. Since its inception in the 1970s, salmon farming has rapidly expanded to become one of the largest animal agriculture systems in British Columbia (6). Salmon farming experienced a period of rapid growth in the province from 1985 to 1992, when it entered a stage of transition and rationalization (7). In a search for better water quality, and fewer conflicts with upland owners and other resources, the industry expanded from its origins in the Sechelt Inlet on the mainland of British Columbia to a variety of regions along the coastline of the southern mainland and Vancouver Island (7).

In this paper, the changing geographic patterns of diagnoses of marine anemia are compared with the changing demographics of the salmon farming industry in British Columbia to examine the hypothesis that...
marine anemia is a disease that spread from its original site of detection to all of the major fish farming regions of the province.

Materials and methods

The 5 major regions of farmed salmon production in British Columbia are illustrated in Figure 1. Areas other than those shown were listed under the category “outer coast.” This latter region was principally represented by the Prince Rupert area of northern British Columbia, but also included sporadic submissions from mainland locations northeast of Vancouver Island.

The distribution of cases of marine anemia between 1987 and 1992 was established retrospectively by reviewing 3 sources of information. First, the clinical records of 5 aquaculture veterinarians were collected. From 1987 to 1992, veterinary services were principally supplied to salmon farmers in British Columbia by veterinarians employed by 4 companies providing feed or pharmaceuticals to the industry. The records reviewed came from 2 of these companies. The area of practice for the veterinarians involved included all of the regions shown in Figure 1. The location, species, and clinical diagnosis of each submission were recorded. A “submission” was not equivalent to a single fish but was, instead, the record of a single farm visit. Veterinarians usually collected clinical and postmortem information from more than 1 fish during a farm visit. To be classified as a positive submission, the clinical record had to specify marine anemia as the final diagnosis, with supporting clinical and pathological evidence.

The 2nd source of data was a review of the diagnostic records of aquaculture submissions to 2 pathology laboratories: a federal fish health laboratory (Department of Fisheries and Oceans, Nanaimo, British Columbia) and the provincial Animal Health Centre (British Columbia Ministry of Agriculture, Fisheries and Food, Abbotsford, British Columbia). These 2 laboratories received submissions from all of the major fish farming regions in the province. They were responsible for most of the diagnostic support available to the aquaculture industry in British Columbia during the 6 y covered by the retrospective portion of this study. Information identical to that collected in the review of veterinary clinical records was recorded for the laboratories. Historical information was often lacking with the laboratory submissions, so the laboratory diagnosis of marine anemia depended almost exclusively on histological criteria. To be included in this study, the laboratory reports had to specify marine anemia as the final diagnosis. Cases including only a histological description consistent with marine anemia, but failing to specify the disease in the report, were not included. Over 745 veterinary records and 492 laboratory reports were available for review.

The final sources of retrospective data were 5 independent disease surveys that had been conducted under the sponsorship of federal and provincial government agencies between 1988 and 1992 (5,8–11) and a 1991–1992 survey conducted as part of this study. All of these surveys relied on convenience sampling methods that were based upon accessibility to farms and the cooperation of salmon farmers. As for the previous sources of information, the location, species involved, and date of diagnosis were recorded for all clinically and pathologically supported diagnoses of marine anemia that were identified during a review of these surveys.

The changing geographic distribution of the chinook salmon industry was documented by examining the operating licenses granted to seacage rearing sites from 1987 to 1992. From 1987 to 1990, issuing such licenses was the responsibility of the Canadian Department of Fisheries and Oceans. In 1991–1992, the British Columbia Ministry of Agriculture, Fisheries and Food became the licensing agency. Therefore, both federal and provincial government licenses were collected. The license data were supplemented by referring to a 1992 report on salmon farming in British Columbia prepared by industry sources (7).

A prospective, case-finding exercise was conducted between April 1991 and December 1992 to intensively survey regions and individual farms that had not previously been diagnosed positive for marine anemia, and to subsequently determine the geographic origin of affected stock and their parents. Clinical cases of the disease were sought on licensed chinook farms in a wide variety of locations along the coast of British Columbia by convenience sampling methods. Generally, farms were visited at 2- to 4-week intervals until the disease was diagnosed at the site. Over 30% (n = 20) of all active chinook sites, representing approximately 25% of all operating companies, were visited. Because of inaccessibility, the northern and outer coasts were not included in this portion of the study. As in the retrospective survey, a region or farm was considered positive if 1 affected fish was found. To be considered a positive case, affected fish were required to display gross pathological features consistent with those reported by Kent et al (3). In addition, positive cases were required to demonstrate a proliferation of immature lymphocytes in the caudal kidney and at least 1 organ other than the spleen in routinely prepared, hematoxylin and eosin-stained, tissue sections (12). Because virtually all of the diagnoses of marine anemia identified in the retrospective portion of the study had been made in moribund, surface catchable salmon, the source of samples in the prospective survey also focused on this subpopulation of the seacages. Also recorded for each farm was the hatchery from which the stock originated, the strain of fish used, and the source of broodstock.
Table 1. The changing geographic distribution of marine anemia in British Columbia between 1987 and 1992

<table>
<thead>
<tr>
<th>Year</th>
<th>South coast</th>
<th>Central coast</th>
<th>West coast</th>
<th>Northwest coast</th>
<th>North coast</th>
<th>Outer coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1988</td>
<td>V L S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1989</td>
<td>V L S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1990</td>
<td>V L S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1991</td>
<td>V S</td>
<td>V L S</td>
<td>V L S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1992</td>
<td>V L S</td>
<td>V S</td>
<td>V S</td>
<td>V S</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = region declared negative for marine anemia
V = region declared positive using veterinary records
L = region declared positive using diagnostic laboratory records
S = region declared positive in disease surveys

The data were subjected to 2 principal methods of statistical analysis. The Pearson correlation coefficient (r) was used to explore the relationships among the regional proportion of licenses, the proportion of diagnostic submissions, and the proportion of diagnoses of marine anemia per geographic area (13). Inferences regarding the statistical significance of calculated r values were based on table values (13). Differences in the total 5-year regional proportions of marine anemia were compared by conducting a chi-squared test for homogeneity (14). All statistical procedures were conducted on Statistix 4.0 software (Analytical Software, 1992, St. Paul, Minnesota, USA).

None of the sources of information in this study represented exhaustive surveys of the total susceptible population, and thus estimates of disease prevalence were subject to sampling error. Confidence intervals can be used to describe a range of possible values for the true population prevalence that is consistent with the observed data, given the variability within the sample (15). The upper 95% confidence limit for an estimated prevalence of 0% marine anemia in a region was derived from values provided by Sackett et al (16). These values were used to describe the highest prevalence of marine anemia possible, based on the number of submissions examined, for regions declared negative for the disease.

Results

Table 1 demonstrates the evolving geographic pattern of diagnoses subsequent to the original reports of marine anemia in 1988. Initially restricted to isolated farms in the Sechelt Inlet in the southern portion of the coast, marine anemia had been diagnosed at over 20 sites in the southern region of the province by 1989. Over the course of the next 3 years, the Marine salmon farming regions in British Columbia had been classified as positive. The apparent spread of the disease outside the southern region was first detected during government-sponsored disease surveys in 1990.

The southern region of the coast held the majority of licensed chinook sites during each of the 6 years of this study. Generally, in terms of abundance of licensed chinook seaises, the south coast was followed, in order, by the central region, the west coast, the northwest coast, and the north coast (Figure 2). Over time, there was a progressive shift in farm locations away from the southern region to the north, west, and northwest coasts. However, the total proportion of chinook licenses per year for the entire industry remained relatively stable: from 1987 to 1992, 46.3% ± 2.1% of all seacage licenses issued per year were to chinook sites.

During 1988–1989, disease surveys for marine anemia concentrated exclusively on the southern region of the coast. The 1st surveys that collected samples outside this region did not take place until 1990, coincident with the 1st report of the disease on the central coast. From 1990 to 1992, surveyors expanded their investigations to include all regions except the outer coast. By 1992, only the north and outer coasts failed to be classified as positive regions.

Table 2 shows the changing pattern of diagnostic submissions from chinook salmon submitted to veterinarians. There was a high positive correlation between the proportion of chinook farm licenses per region and the proportion of chinook submissions per region in

Figure 2. Annual number of farm licenses for chinook salmon (Oncorhynchus tshawytscha) issued per region in British Columbia.
the veterinary records ($r = 0.89, P < 0.01$). A similar relationship was seen for laboratory submissions ($r = 0.82, P < 0.01$). There was also a high positive correlation between the proportion of chinook samples submitted and the proportion of diagnoses of marine anemia in a region in both veterinary and laboratory diagnostic records ($r = 0.71$ and $0.69$, respectively, $P < 0.01$ in both cases).

Upon collapsing the yearly distributions of marine anemia diagnoses between 1987 and 1992 into a 5-year regional summary (Table 3), we found no statistically significant differences among the proportions of marine anemia diagnosed per region ($P = 0.94$). Between 1987 and 1992, the mean regional prevalence was $2.5\% \pm 0.4\%$ of veterinary submissions.

Table 4 provides the highest possible value for the prevalence of marine anemia in regions where no disease was found, given the number of samples collected from each region. None of the regions classified as negative were subjected to sufficiently intense sampling for us to be $95\%$ confident that the disease was not present at or below the $4\%$ level (16).

The prospective case-finding study revealed cases of marine anemia in all regions sampled. In addition, all of the 20 farms visited were declared positive for the disease. In some cases, the disease was diagnosed on the 1st visit to the farm, whereas others required biweekly visits for 3 mo before a case of marine anemia was diagnosed.

The results of the prospective portion of this study failed to demonstrate a consistent pattern for the origins of salmon affected with marine anemia. No single hatchery or strain of chinook could be identified as the exclusive source of affected fish. The broodstock of positive progeny came from commercial and federal government hatcheries throughout Vancouver Island and the southern coast of British Columbia. Wild stocks from the northern part of Vancouver Island and the Yukon also provided progeny that were later diagnosed with marine anemia.

**Discussion**

A study of the evolving geographic distribution of a disease can provide important clues to the epidemiology and etiology of the disease (2). For this reason, descriptive epidemiological studies are often used in attempts to define the changing geographic distribution of a disease (2). However, descriptive data can sometimes lead to biased impressions of the nature of the disorder being studied (17). Unless investigators pay due attention to the potential biases affecting studies of the distribution of marine anemia, they can easily be misled.

The review of diagnostic submissions appeared, at first, to support the hypothesis that marine anemia was originally limited geographically to the south coast and then spread to other fish farming regions. However, this interpretation failed to consider the underlying changes in the demographics of the susceptible population. Once the spatial pattern of a disease has been established, the geographic characteristics of the susceptible population should be studied and considered before epidemiologic hypotheses are generated (2,18). Failure to do this generates the risk of encountering a "demographic bias," which can occur when the specification and selection of a study sample fails to take into account the demographic characteristics of the general population at risk. This failure was an important source of bias in the biogeographic study of marine anemia.

The principal question of concern in this investigation was, “Can it be confidently stated that marine anemia spread throughout the major salmon farming regions of

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**Table 2. Proportion of total chinook submissions by region and by year according to veterinary clinical records from 1987 to 1992**

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>South coast</th>
<th>Central coast</th>
<th>West coast</th>
<th>Northwest coast</th>
<th>North coast</th>
<th>Outer coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>32</td>
<td>0.53</td>
<td>0.38</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.0</td>
</tr>
<tr>
<td>1988</td>
<td>201</td>
<td>0.55</td>
<td>0.30</td>
<td>0.10</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>1989</td>
<td>196</td>
<td>0.42</td>
<td>0.37</td>
<td>0.11</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>1990</td>
<td>77</td>
<td>0.31</td>
<td>0.29</td>
<td>0.27</td>
<td>0.07</td>
<td>0.07</td>
<td>0.0</td>
</tr>
<tr>
<td>1991</td>
<td>133</td>
<td>0.24</td>
<td>0.41</td>
<td>0.27</td>
<td>0.08</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1992</td>
<td>137</td>
<td>0.28</td>
<td>0.23</td>
<td>0.40</td>
<td>0.12</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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**Table 3. Marine anemia status of all diagnostic submissions according to veterinary records from 1987–1992**

<table>
<thead>
<tr>
<th>Marine anemia</th>
<th>South coast</th>
<th>Central coast</th>
<th>West coast</th>
<th>Northwest coast</th>
<th>North coast</th>
<th>Outer coast</th>
</tr>
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<tbody>
<tr>
<td>Positive</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>278</td>
<td>234</td>
<td>149</td>
<td>43</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>240</td>
<td>152</td>
<td>44</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Overall</td>
<td>3.1%</td>
<td>2.5%</td>
<td>2.0%</td>
<td>2.3%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
British Columbia?" To answer this question, one must correctly classify a region, on a yearly basis, as positive or negative with respect to the disease. Too few diagnostic submissions were received from most of the regions to confidently rule out the presence of marine anemia. To be confident, with 95% certainty, that the prevalence of marine anemia in a region was less than 2%, one would need to find no positive cases in no less than 150 submissions (16). None of the regions classified as negative exceeded 73 submissions per year. Since prevalence is a fraction, a larger number of cases will tend to be identified as more fish are examined. For a sample to identify a specified number of cases, a certain proportion of the population must be sampled. Since the prevalence of the disease in submissions from each region appeared to be low (2.5%), there existed the possibility that all regions classified in previous years as negative might have been misclassified. When dealing with a disease of low prevalence, it is invalid to declare a region as unaffected when no cases are found, especially if unequal sampling protocols have been applied or if too small a proportion of the population has been sampled (19). The prospective portion of this study demonstrated that, unless positive farms were subjected to intense, ongoing disease surveillance, they could easily be misclassified as negative. The results of the retrospective study suggested that a similar risk of misclassification also existed at the regional level. Unbiased samples, collected by a formal random sampling scheme, are generally recommended for investigations designed to determine the prevalence of a specific disease in a population (20). None of the retrospective data used in this study were derived from any form of random sampling. Indeed, the disease surveys and the veterinary and laboratory submissions were all biased by the knowledge of the disease status of a region or farm. Areas considered positive for the disease were generally sampled more intensively than areas not declared positive. This was likely a reflection of regional differences in the concern of fish farmers, and the emphasis of early researchers to study the disease at the individual fish or pen level and not at the regional level. A sampling scheme that has a random component and samples only a portion of the population at frequent intervals is a more effective means of monitoring a disease than one that expends all resources on a single, large-scale census (21). The regional and farm level misclassifications of marine anemia status encountered in this study emphasize the potential danger of relying on single, opportunistic visits to determine the disease status of a larger demographic group.

The results of this study suggest that the apparent spread of marine anemia was due to changing regional disease surveillance efforts that arose from the shifting distribution of the susceptible population. The ubiquitous nature of marine anemia, as seen in the prospective portion of the study, and the lack of a documented spread of the disease suggest that marine anemia is an endemic problem in farmed chinook salmon in British Columbia and not a spreading epidemic. Identifying marine anemia as an endemic instead of an epidemic disease is an important distinction. The 3 commonly recognized patterns of disease occurrence (sporadic, endemic, and epidemic) are the result of different critical ecological and pathological factors (2,17). Whereas endemic diseases are usually the result of a predictable, long-term balance among the host, environment, and pathogenic agent, epidemic diseases reflect a major imbalance that favors the agent (2). A clear understanding of the pattern of occurrence of marine anemia is, therefore, a critical 1st step in the development of a rational basis for disease management recommendations.

References


Table 4. Highest possible true prevalence of marine anemia for regional samples detecting 0% marine anemia (based on 95% confidence levels for estimates of prevalence (16))

<table>
<thead>
<tr>
<th>Region</th>
<th>South coast</th>
<th>Central coast</th>
<th>West coast</th>
<th>Northwest coast</th>
<th>North coast</th>
<th>Outer coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1987</td>
<td>18%</td>
<td>26%</td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>1988</td>
<td>+</td>
<td>5%</td>
<td>14%</td>
<td>53%</td>
<td>53%</td>
<td>78%</td>
</tr>
<tr>
<td>1989</td>
<td>+</td>
<td>4%</td>
<td>14%</td>
<td>31%</td>
<td>31%</td>
<td>53%</td>
</tr>
<tr>
<td>1990</td>
<td>+</td>
<td>14%</td>
<td>14%</td>
<td>45%</td>
<td>45%</td>
<td>100%</td>
</tr>
<tr>
<td>1991</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>26%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>1992</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

+: samples demonstrating >0% marine anemia
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