Gill Diseases of Cultured Salmonids in Ontario

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ABSTRACT

Between 1977 and 1981, the Fish Pathology Laboratory of the Ontario Veterinary College received 239 cases from trout farms of southern Ontario, 51 (21.3%) of which had diseased gills. Branchial lesions in 86.3% of these 51 cases were characterized by marked lamellar epithelial hyperplasia with epithelial hypertrophy and lamellar fusion. Filamentous bacteria were seen on the surface of the branchial filaments and lamellae in 68.6% of the cases. Our observations highlight the importance of gill diseases as a production problem of farmed salmonids in southern Ontario.

Key words: Gill diseases, trout, aquaculture.

RÉSUMÉ

De 1977 à 1981, le laboratoire d'ichtyopathologie du collège vétérinaire de l'Ontario reçut 239 envois de truites qui provenaient de pisciculturesdu sud de cette province. On constata la présence de lésions branchiales dans 51 ou 21, 3% de ces envois. Dans 86,3% des cas, les lésions se caractérisaient surtout par une hyperplasie marquée de l'épithélium lamellaire, laquelle s'accompagnait d'une hypertrophie des cellules épithéliales et d'une fusion des lamelles. Dans 68,6% des cas, des bactéries filamenteuses étaient présentes à la surface des filaments et lamelles. De telles constatations illustrent l'importance des maladies branchiales, comme problème inhérent à l'élevage artificiel des truites, dans le sud de l'Ontario.

Mots clefs: maladies des branchies, truite, pisciculture.

INTRODUCTION

High densities of fish associated with intensive aquaculture favour the occurrence and spread of infectious diseases in a manner comparable to other domestic livestock. Water quality problems, in the form of toxic pollutants, suspended solids or accumulation of metabolic waste products, are additional factors that may adversely affect the health of farmed fish, either directly or by increasing the susceptibility of these fish to infectious diseases.

According to Roberts (1), the gills are among the most delicate structures of the teleost body. Because of this and of the vulnerable external location of the gills, one might expect these organs to be frequently involved in disease problems. Gill lesions characterized by epithelial hypertrophy and hyperplasia are common in intensively raised trout and are often associated with the presence of filamentous bacteria on the respiratory surfaces. This condition, known as "bacterial gill disease" (BGD), is thought to result primarily from poor water quality, with bacteria proliferating on already damaged gill tissue (2,3,4). Although the association of poor water quality and filamentous bacteria with gill lesions of cultured salmonids is recognized, the actual frequency of their involvement in spontaneous cases of gill disease has rarely been reported (5).

This article is based on a retrospective study of cases of gill disease received at the Ontario Veterinary College (OVC) from fish farms of southern Ontario during the period 1977 to 1981. The objectives of this study were to estimate the frequency of gill diseases in cultured trout of southern Ontario and to examine in detail the microscopic lesions occurring in affected gills in an attempt to determine their pathogenesis and etiology.

MATERIALS AND METHODS

Between 1977 and 1981, 239 submissions of salmonids [primarily rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*)] from artificially raised populations were received for diagnostic purposes by the Fish Pathology Laboratory at OVC. Most of these cases came from commercial fish farms; others were from holding facilities of research institutions. Routinely prepared H & E stained histological sections of gills and other major organs from 61 of these cases were reexamined. These 61 cases were selected on the basis of comments in the final diagnosis suggesting gill lesions. The age of the animals involved varied between two months and two years. For each case, several parameters of pathomorphological changes involving the gill filaments and lamellae were rated on a scale of 0 to 5 (0 = normal, 1 = slight or questionable,

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2 = clearly present but not conspicuously so, 3 = marked, 4 = severe, 5 = extreme). Since most cases involved more than one fish, the value ascribed to each parameter in a given case represented a subjective average of the intensity of the change for all gill arches of all fish examined in that case.

RESULTS

In ten of the 61 cases examined, gill lesions were considered to be minimal (1+) or absent, and the morphological appearance of the filaments and lamellae was, in general, similar to previous descriptions of normal fish (6,7) (Figs. 1 and 2). The filaments were covered by a thick stratified epithelium with many superficially located mucous cells; the lamellae comprised leaf-like structures composed of a pillar cell system delimiting blood spaces and covered by flattened or slightly enlarged epithelial cells. Mucous cells and chloride cells, the latter characterized by an abundant acidophilic cytoplasm, were occasionally seen among lamellar epithelial cells.

In 51 cases (21.3% of the 239 cases), the gill lesions were considered severe enough (≥2+) to have contributed to the death or poor health of the animals. Marked (≥3+) lamellar epithelial hyperplasia, with various degrees of epithelial hypertrophy and lamellar fusion, was by far the most common type of lesion encountered (44, or 86.3%, of 51 cases) (Table I, Figs. 3 and 4). Occasionally, the hyperplastic process was severe enough to have resulted in fusion between filaments (Fig. 5). No particular pattern was noted with respect to the primary site of epithelial hyperplasia. The lesion was either diffuse in the more severe cases or randomly multifocal. Undifferentiated epithelial cells, characterized by a large vesicular nucleus and abundant cytoplasm, were those most commonly involved in the proliferative process (Figs. 4 and 7). Groups of hyperplastic mucous cells were prominent (≥3+) in 12 cases. In three of these cases, the final report included a reference to an excess amount of mucus observed in the gills at the time of necropsy. Such comment was in fact made in a total of 15 cases, but in only eight of these was there any obvious (≥2+) hyperplasia of mucous cells in the gill lamellae. The presence of cellular debris was not a prominent feature of branchial lesions, being obvious (≥2+) in 25 cases but in substantial amount (≥3+) in only five of these. Marked (≥3+) thrombosis of the pillar cell system of gill lamellae was present in only three cases, and these had been submitted on separate occasions from one fish farm with a nitrite toxicity problem; thrombosis was not seen in organs other than the gills in these three cases.

In 35 (68.6%) of the 51 cases with gill lesions, filamentous bacteria

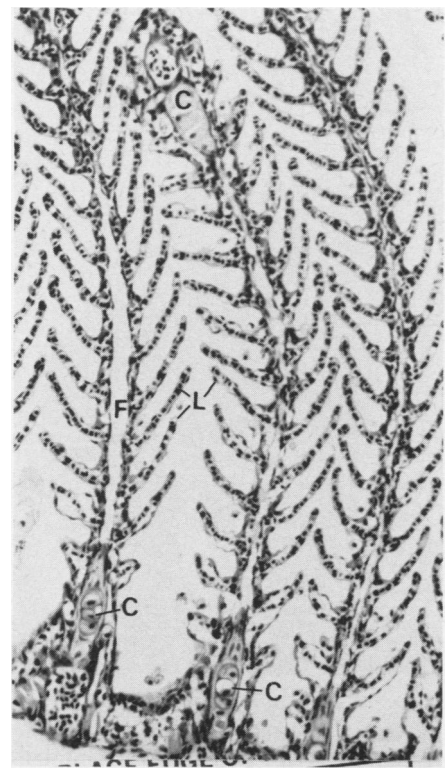

Fig. 1. Normal gill filaments (F) and lamellae (L) of a rainbow trout. Portions of the supporting cartilaginous rods (C) are seen in each filament. H & E. X172.

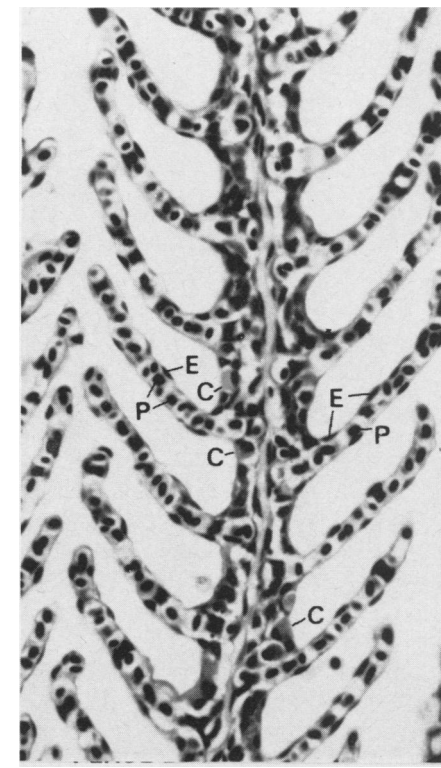

Fig. 2. Higher magnification of normal filament and lamellae. Each lamella consists of a system of pillar cells (P) delimiting blood spaces and covered by flattened epithelial cells (E). Chloride cells (C), characterized by an abundant acidophilic cytoplasm, are found mostly in interlamellar regions. H & E. X381.

TABLE I. Distribution of Lesions in 51 Cases of Gill Disease According to the Degree of Branchial Lamellar Damage and the Type of Microorganisms Present on the Branchial Lamellar Surfaces

	Lamellar Epithelial Damage[b]			
Microorganisms[a]	Marked	Moderate	Mild	Total
Bacteria	29	4	2	35
Protozoa	3			3
No microorganism	12	1		13
Total	44	5	2	51

[a] ≥2+ microorganisms on the surface of the branchial lamellae
[b] Marked: lamellar epithelial hyperplasia ≥3+; lamellar epithelial hypertrophy and/or lamellar fusion ≥3+
Moderate: lamellar epithelial hyperplasia =2+; lamellar epithelial hypertrophy or lamellar fusion ≥3+
Mild: lamellar epithelial hyperplasia and hypertrophy and lamellar fusion ≤2+

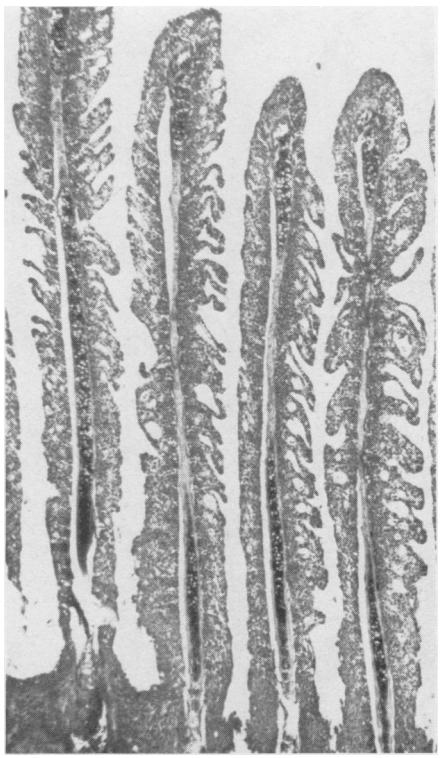

Fig. 3. Gill filaments from a brook trout with severe branchial proliferative lesions. Epithelial hyperplasia has resulted in extensive lamellar fusion. H & E. X38.

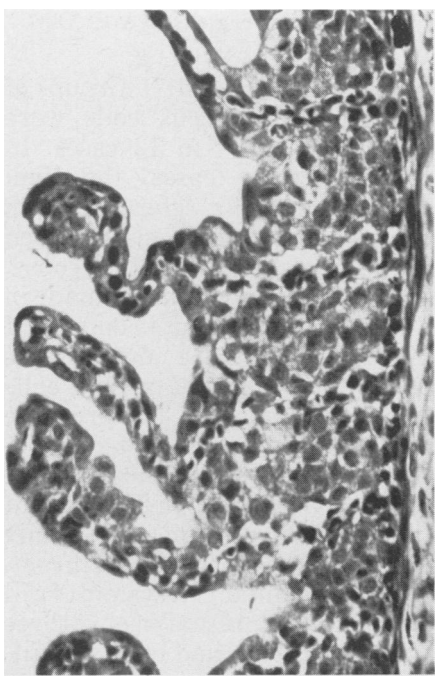

Fig. 4. Higher magnification of a portion of a filament from the previous figure. There is marked hypertrophy of the epithelium of lamellae and interlamellar regions. Epithelial hyperplasia is most evident in the interlamellar regions. H & E. X305.

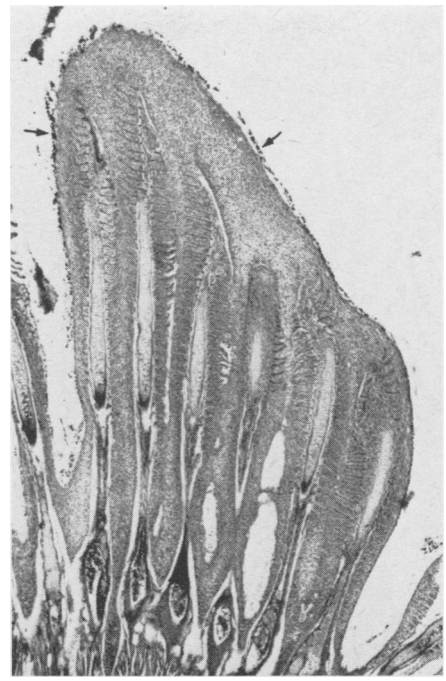

Fig. 5. Massive proliferation of the branchial epithelium in this rainbow trout has resulted in almost complete fusion of six filaments. Large numbers of protozoa (arrows) are associated with the lesion, which is shown at a higher magnification in Fig. 9. H & E. X25.

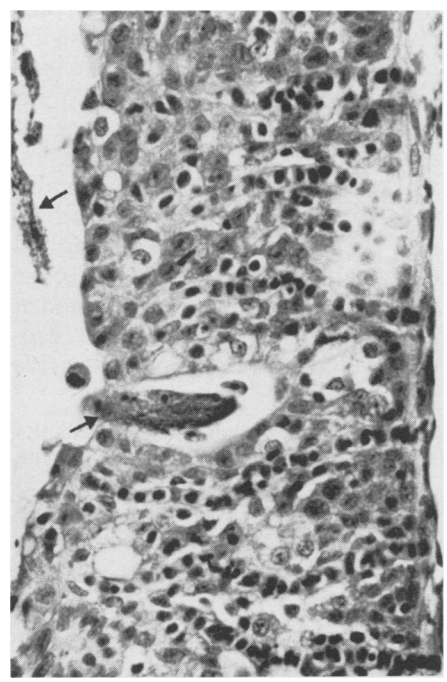

Fig. 6. Severe gill lamellar epithelial hypertrophy and hyperplasia with complete lamellar fusion in a brook trout. Colonies of filamentous bacteria are above and among the lamellae (arrows). H & E. X295.

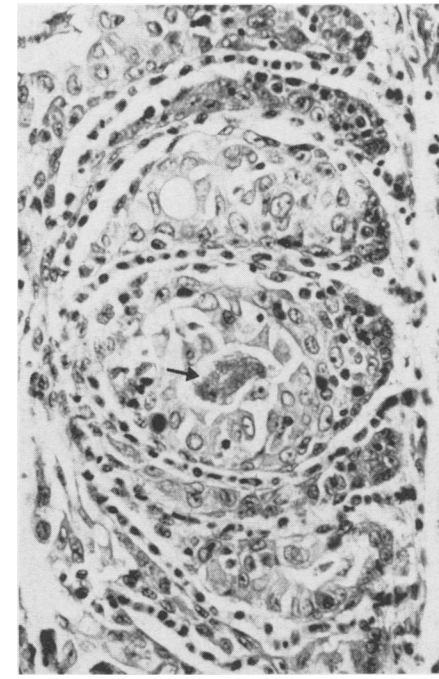

Fig. 7. Branchial lamellar lesions similar to those in the previous figure. A colony of filamentous bacteria (arrow) is surrounded by the proliferating epithelium. The filament is on the right. H & E. X257.

were clearly present (≥2+) among the gill lamellae and filaments (Table I, Figs. 6 and 7). The relationship between the severity of lamellar lesions and the abundance of bacteria was not always clear since, in some cases, the bacteria were more numerous in less severely affected areas of the gills while, in others, they were confined to the spaces between filaments. In two cases, the presence of filamentous bacteria was the major abnormal finding, these bacteria lying against a relatively normal lamellar epithelium (Fig. 8). Some degree (≥2+) of infiltration of mononuclear leukocytes among lamellar epithelial cells (mostly in interlamellar regions) was seen in only 11 of the 35 cases involving filamentous bacteria. In 15 of these 35 cases, the report included a reference to some environmental problem such as crowding or exposure to toxic substances; in three other cases, the fish had recently been handled.

In three cases, large numbers of protozoa were associated with the gill lesions. One of these cases

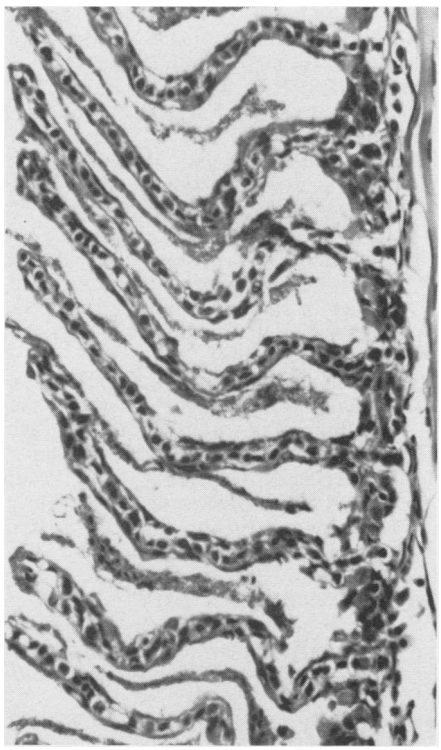

Fig. 8. Large numbers of filamentous bacteria are among the gill lamellae of this rainbow trout, although the lamellae themselves have no obvious lesion. H & E. X285.

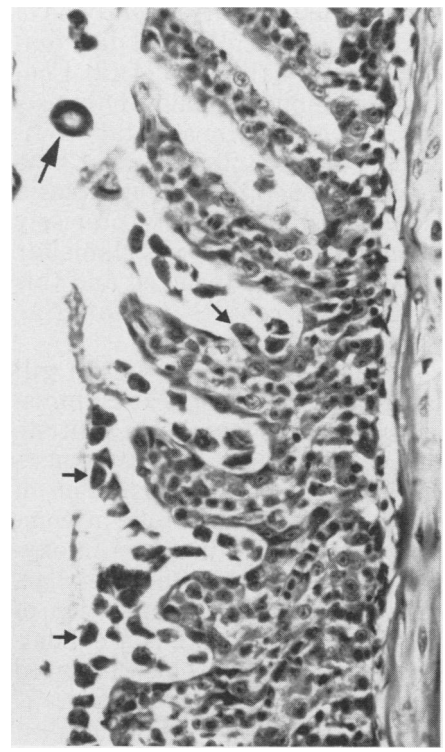

Fig. 9. Gill lamellar epithelial hypertrophy and hyperplasia in a rainbow trout, associated with the presence of many protozoa (small arrows). Examination at higher magnification of one of the protozoa sectioned through its flat plane (large arrow) revealed a ciliated ring typical of a *Trichodia* species. H & E. X293.

involved a *Trichodina* species (Fig. 9) and followed recent transport and handling of the fish. The protozoa involved in the two other cases were not identified. In 13 cases, no microorganism could be seen microscopically, and, in six of these cases, the history suggested an environmental problem such as high levels of ammonia or of suspended solids in water as the most likely cause of the gill lesions.

DISCUSSION

This study emphasizes the importance of gill diseases in trout farms of southern Ontario. Our estimate of their frequency relative to other diseases (21.3%) is actually a minimal figure since it does not take into account the subclinical cases, i.e. those in which gill lesions did not kill the fish but may have been severe enough to affect their growth rate or predispose them to other diseases.

The present study also illustrates the frequency with which gill tissue responds to injury by epithelial hyperplasia. This type of response has been described in fish chronically exposed to a variety of noxious agents, including pollutants such as heavy metals, pesticides and suspended solids (1,3) and microorganisms such as protozoa and trematodes (3,8). Marked gill lamellar epithelial hyperplasia also occurred within two days of exposure of trout and killifish (*Fundulus heteroclitus*) to acutely toxic concentrations of heavy metals (9,10,11). Unspecialized epithelial cells are likely the main type of cell involved in the hyperplastic process, although our observations suggest that proliferation of mucous cells also occurs to a lesser extent. According to Conte and Lin (12), undifferentiated replacement cells of the gill epithelium of coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon are "basally located, primarily in the interlamellar region of the filament". However, the unspecialized ultrastructural appearance of lamellar epithelial cells (6) suggests that these may retain the potential to proliferate, either in the normal process of cell renewal or following injury. This is substantiated by the observation of epithelial hyperplasia initially affecting the distal portion of lamellae in certain gill diseases (see below). The fate of chloride cells in the face of lamellar epithelial hyperplasia is not known. These cells have a central role in osmoregulation, ammonia excretion (13,14) and acid-base balance (15). Therefore, impairment of their function, possibly caused by the proliferation of other cells, could result in serious metabolic upset. The paucity of inflammatory reaction in affected gills likely paralleled the limited production of mediators of inflammation in these organs since there was little epithelial necrosis, and microorganisms, when present, had not invaded the tissue.

Water quality problems related to overcrowding and characterized by increased levels of nitrogenous waste products and decreased levels of dissolved oxygen are capable by themselves of causing severe gill lesions in the form of extensive proliferation of lamellar epithelium. Ammonia in the unionized form is usually considered to be the metabolic product responsible for the gill damage (16,17). However, experiments in which fish were exposed to sublethal aqueous concentrations of salts of ammonia failed to produce gill lesions characterized by epithelial hyperplasia (2,18).

Although water quality problems may often be the primary cause of gill lesions, this study demonstrates that bacterial colonization of branchial lamellar surfaces is the most common manifestation of gill disease in trout farms of southern Ontario. Bacterial gill disease is recognized as a major cause of mortality among artificially raised salmonids (4). The bacteria usually involved belong to the genera *Flexibacter* and *Cyto-*

phaga, which are part of the normal flora of water and of fish gills (2). However, most authors agree that BGD is primarily an environmental disease related to water quality problems that is secondarily complicated by bacterial proliferation on the surface of gill lamellae (2,3,4). Therefore, the actual contribution of these bacteria to the production of gill lesions is not clear. Alteration of branchial electrolyte transport could be as detrimental to the fish as the production or exacerbation of epithelial hyperplasia. As in our study, Wood and Yasutake (19) observed rather limited hyperplasia of gill lamellar epithelium in some moribund fish with heavy concentrations of filamentous bacteria on the surface of their gill lamellae. The close association of such bacteria with the gill epithelium is reminiscent of the behavior of enterotoxigenic strains of *Escherichia coli* in calves (20). Bullock (2) observed an increased resistance to BGD in older trout and suggested that this may correlate with levels of specific antibodies in the plasma of these fish; such immunity, however, can be overcome. For example, branchial lesions resulting from poor water quality may affect quantitatively or qualitatively the branchial mucus and thus favour bacterial colonization. According to Roberts (1) and Richards and Roberts (4), excess amounts of mucus and cellular debris resulting from gill damage provide a substrate for growth of microorganisms such as bacteria and protozoa.

The distribution of gill lesions may vary according to the type of insult. Smith and Piper (17) observed that in trout raised under crowded conditions, fused lamellae occurred more commonly at the tip of the filaments than at their base. Lesions of BGD appear to be characterized by epithelial hyperplasia at the distal third of the lamellae and they frequently have an irregular multifocal distribution along the filament (4,19). Conversely, in nutritional gill disease, which is caused by a dietary deficiency of pantothenic acid, the initial site of epithelial hyperplasia is in the filament, more precisely its distal end; the interlamellar regions are also involved and this eventually results in lamellar fusion (19,21).

Marked thrombosis within gill lamellae in three separate submissions from one farm with a nitrite toxicity problem suggests a cause-effect relationship. Formation of methemoglobin, possibly in combination with an increase in oxygen tension within the lamellae, may have caused deformation of the erythrocytes and, consequently, sludging of the blood followed by intravascular coagulation.

ACKNOWLEDGMENTS

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