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HISTOLOGICAL EXAMINATION OF THE GILLS AS A METHOD OF DETECTING ASYMPTOMATIC CARRIERS OF *A. SALMONICIDA* IN ATLANTIC SALMON *(Salmo salar)*

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One of the main problems in the control of furunculosis in salmonids has been the difficulty in detecting asymptomatic carriers of the causal organism *Aeromonas salmonicida*. Also, little is known concerning the tissue localisation of the bacterium in infected fish (Tatner et al, 1984). Normal bacteriological culture methods are generally recognised as being inadequate and experience at this laboratory bears this out. Stress testing of fish using Prednisolone Acetate (P/A) would appear to give greater sensitivity (McCarthy, 1977), however this is not always a practical procedure for the busy diagnostic laboratory. Epidemiological, histological and serological evidence obtained at this laboratory has indicated that the gills are an important site of localisation of the causal organism in carrier fish and that histological examination of gills may be a useful, relatively quick technique for detection of carriers in this disease. Three clinical histories out of a number of similar cases are cited to support this view and serological evidence has confirmed these findings.

CASE 1

In 1984 salmon smolts from a supply hatchery in the west of Ireland were transferred to a number of commercial cage salmon farms in the region. Although clinical furunculosis had been known to occur at the hatchery, at the time of transfer there was no evidence of clinical disease, and routine bacteriological examination of kidneys of fish just prior to transfer yielded no evidence of *A. salmonicida* on culture. Following the transfer, outbreaks of clinical furunculosis occurred at virtually all farms supplied from this source with *A. salmonicida* being readily isolated from kidneys of affected fish. The outbreaks were successfully controlled using either oxolinic acid or flumequine. Histological examination of material obtained from fish prior to transfer showed no obvious signs of disease in all internal organs examined such as kidney, spleen, liver or heart. However the gills in some fish showed the presence of occasional bacterial colonies, the full significance of which was not realised at the time of the transfer.

CASE 2

A routine examination of salmon parr at a hatchery was carried out in December 1984. The hatchery had no prior history of furunculosis and no disease problems were being experienced at this time. Bacteriological examination of the kidneys of about 30 fish yielded sterile cultures and

histological examination of a variety of internal organs showed no obvious abnormalities. However, histological examination of the gills showed occasional bacterial colonies similar to the previous case. Because of the experience described in that case it was considered possible that an outbreak of furunculosis might occur at this hatchery. The fish remained healthy until the following April when disease broke out in the fish with *A. salmonicida* being isolated from affected fish. The gills yielded *A. salmonicida* on culture in this case.

CASE 3

Approximately 100,000 salmon parr were transferred from a hatchery with a known history of furunculosis to a cage site on a lake late in 1985. No mortalities were occurring at the time of transfer and the fish remained apparently healthy following transfer. In February 1986 about 40 fish were examined bacteriologically and histologically. Bacteriological examination of the kidneys yielded negative results and the only significant histological finding was again the presence of occasional bacterial colonies on the gills of a very few fish. In this instance P/A stress testing of the fish was also carried out. 150 fish were stress tested in the usual way and after 15 days one fish died with *Aeromonas salmonicida* being isolated from the kidney indicating a low carrier rate in these fish.

Histologically the bacterial colonies, which stain Gram – negatively are focal and usually lie on or between secondary lamellae. They are usually enclosed in a thin membrane which appears to be continuous with the basement membrane of the lamellar epithelium (Fig. 1). The size of the individual bacterial cells appears to be considerably shorter than those observed in clinical cases of furunculosis.

To identify the organisms observed an indirect fluorescent antibody test (FAT) was performed. Paraffin sections of gills showing the presence of typical bacterial colonies were cut, placed on a slide and rehydrated. The slides were washed for 20 minutes in normal saline and flooded with *A. salmonicida* antiserum (1 : 10 dilution) prepared in rabbits. Slides flooded with normal rabbit serum were used as controls. After a further washing the slides were flooded with fluorescent labelled sheep anti-rabbit serum (Wellcome). Using this test the bacterial colonies showed specific fluorescence using the *A. salmonicida* antiserum but not using the non immune rabbit serum (Fig. 2).

The clinical, epidemiological and serological evidence presented indicates that the Gram-negative bacterial colonies observed on the gills of fish examined in these instances are *A. salmonicida*. These findings could have significant implications for our understanding of the carrier state in this disease, for methods of detection of carrier fish and also for methods of prevention, particularly vaccination. If, as our results indicate, the gills are a critical site of carriage of *A. salmonicida*, then the use of dip vaccines may provide a more effective method of protecting fish against furunculosis than parenteral administration. The administration of antibiotics by bath treatment could also prove to be more effective in eliminating carrier fish than either the oral or parenteral routes.

Summary

Details of 3 clinical histories of furunculosis in *Salmo salar* indicate that *Aeromonas salmonicida* can be present on the gills but be undetectable in visceral organs of freshwater parr and smolts showing no sign of disease. The data suggest that microcolonies of the bacterium on the gills may represent the site of carriage in carrier fish and the potential for improved diagnosis and therapy by bathing are discussed.

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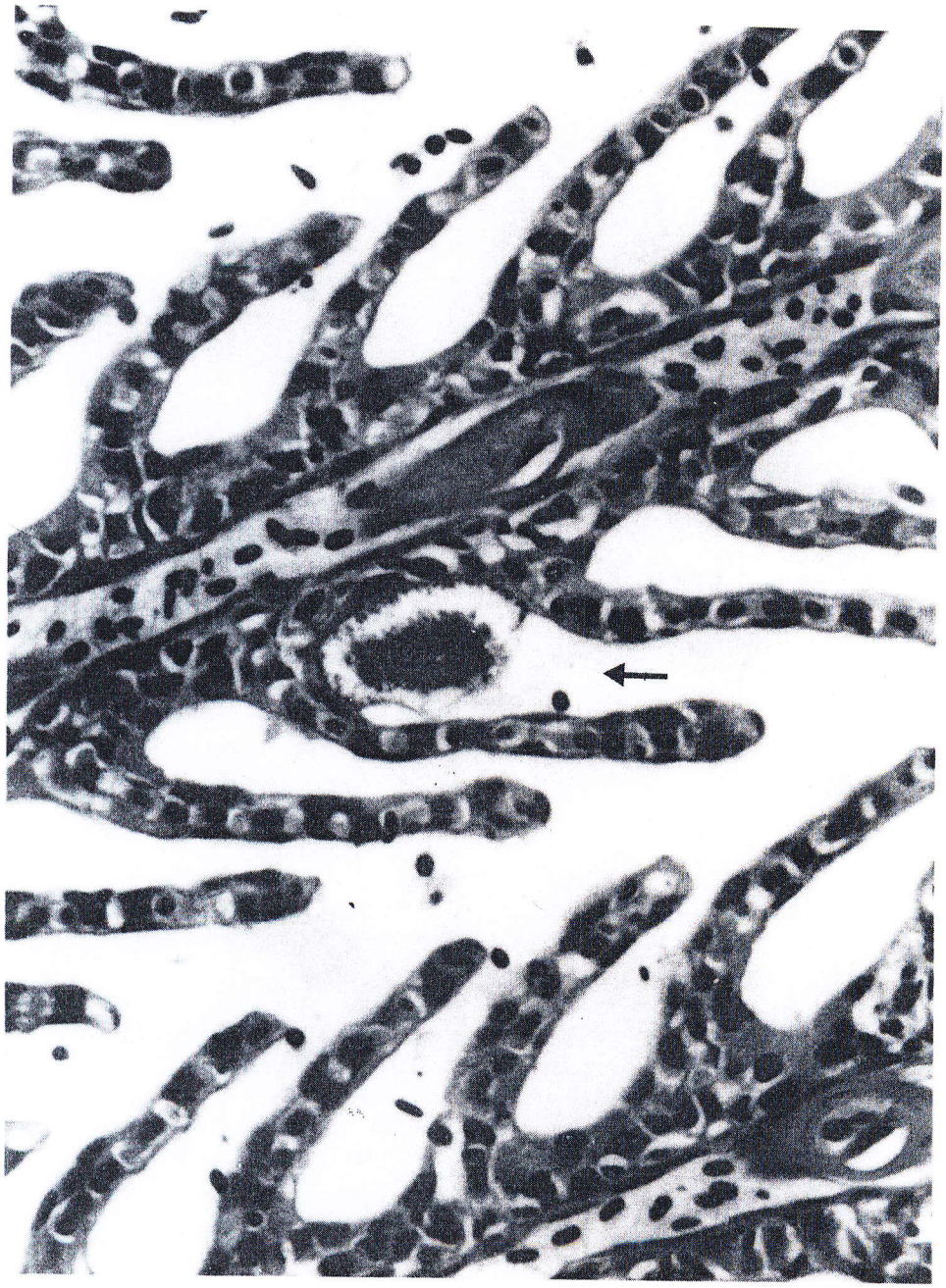

Fig. 1: Showing single bacterial colony lying between adjacent secondary lamellae. Note the thin membrance surrounding the colony.

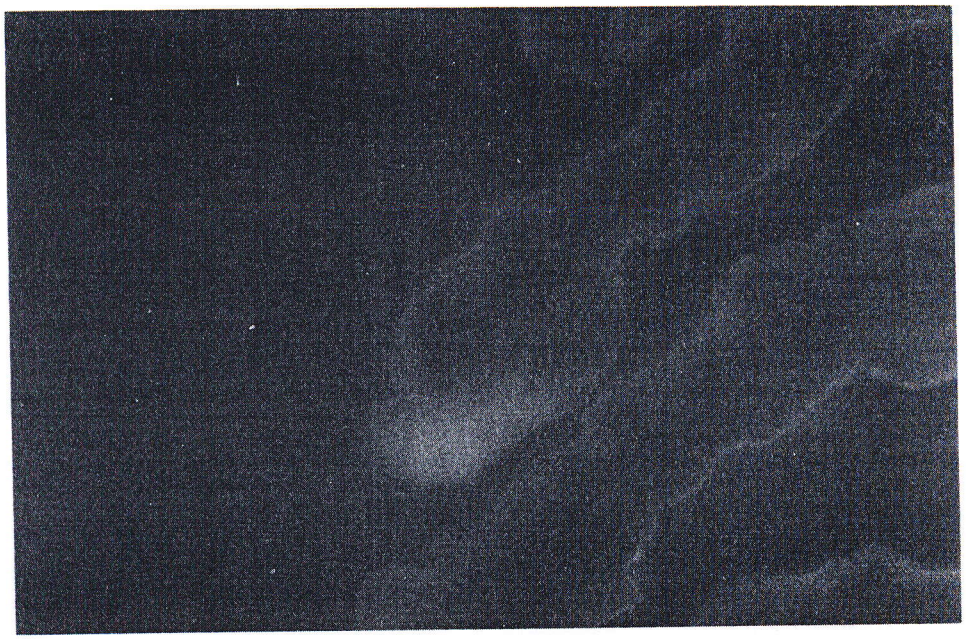

Fig. 2: Bacterial colony at tip of secondary lamella showing specific fluorescence.