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Lepeophtheirus salmonis - A POSSIBLE VECTOR IN THE SPREAD OF DISEASES ON SALMONIDS

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Introduction

Infestation of commercially reared salmonids at marine fish farms by *Lepeophtheirus salmonis* can result in significant damage and mortalities (Tully 1989). Feeding of *L. salmonis* primarily affects the integument and the reasons for the mortality might be osmotic shock or secondary infections. A third possibility is that *L.salmonis* functions as a vector transmitting viruses or bacteria. This, however, has not yet been proved.

Bruno and Stone (1990) have shown that saithe, *Pollachius virens,* can function as a host transporting *L.salmonis* and that the parasite may be transferred between saithe and salmon. *L.salmonis* can also move from one salmon to another. When a salmon dies, for instance from Infectious Salmon Anaemia (ISA) or furunculosis, the lice leave the host shortly afterwards.

Serious implications may be drawn if it can be shown that *L.salmonis* can transmit bacteria or viruses from one host to another. This paper is a report on bacteria found in the gut of *L.salmonis* taken from *Salmo salar* in fish farms in the Bergen area, Norway.

Materials and Methods

Lepeophtheirus salmonis were collected from several fish-farms close to Bergen, Norway.

Preparation for electron microscopy

The lateral parts of the cephalothorax were cut off and the animals were cut in pieces of about 2 mm. The tissues were fixed in a modified Karnovsky fixative, where distilled water was replaced by a Ringers solution (Nylund 1985). The fixative contained 8% sucrose. The pieces were fixed for 15 h, washed in a phosphate buffered sucrose solution, and treated with a phosphate buffered 1% OsO_4 solution for 1.5 h. After washing, the pieces were dehydrated through a graded series of acetone and embedded in Epon 812. The ultrathin sections were stained for 1 h in 2% aqueous uranyl acetate solution and then stained with lead citrate.

Preparation for light microscopy

Whole specimens were fixed for 15 h in the same fixative as that used for electron-microscopy. They were dehydrated through a graded series of ethanol and embedded in Historesin. Sections, 1.5 μm thick, were cut on a Reichert-Jung Supercut and stained in Diff.quick., toluidine or Giemsa.

Preparation for scanning electronmicroscopy

Whole specimens, male and female, were fixed for 15 h, as described above. They were cut in two pieces; a cephalothoracic part and an abdominal part, dehydrated through a graded series of acetone and critical-point dried. The pieces were mounted in Conducting Carbon Cement on aluminium nails with the ventral side up, and covered with gold/palladium. A Jeol JSM-35 Scanning electronmicroscope was used.

Results and discussion

The alimentary canal in *L.salmonis* consists of; a short, tubular, foregut; a large midgut running from the anterior part of the cephalothorax and into the abdomen; and a short, tubular, hindgut. The midgut, a wide tube, forms the major part of the alimentary canal (Fig. 1). Its wall is fairly thin, and consists of a single layer of columnar epithelial cells resting on a basement membrane. At irregular intervals, large transverse folds, longitudinal ridges, and fingerlike projections extend far into the lumen of the midgut. The epithelium lining the midgut consists of three cell types, but the midgut cannot be devided into zones with respect to

Fig.1.A longitudinal section through part of the midgut in a female. Gut epithelium (GE), gut lumen (L). x 125

frequency of the different cells (Nylund *et al.* submitted.).

Bacteria, long slender rods, were found both in the lumen of the midgut and in all the different types of midgut cells in some specimens, -most frequently the females (Fig. 2 and 3). The bacteria were not found in other tissues. The diameter and the length of the

Fig.2.*A. Bacteria* (arrow heads) in the epithelial cells of the midgut in *L.salmonis*. Gut lumen (L), myelin figure (M), nucleus in the epithelial cell (N). x 8 000. *B. Bacteria* (arrow heads) in the midgut lumen. Microvilli (asterisks). x 20 000.

bacteria were 0.2 μm and more than 4 μm, respectively. In the midgut lumen, the bacteria were most frequently seen parallel to the microvilli or associated with debris. The epithelial cells that were infected differed in the number of bacteria found. Some cells contained large colonies of bacteria which occupied most of the cell-volume. The epithelial cells did not show any significant subcellular changes even when a high number of bacteria were found intracellularly. However, myelin figures were found in the epithelial cells close to the bacteria.

From our material we are not able to indicate whether or not these bacteria are fish pathogens, or are simply restricted to *L.salmonis*. They are obviously not common to the gut of the *L.salmonis*, as the majority of the lice investigated were devoid of bacteria. In our opinion it is not inconceivable that fish pathogenic bacteria could survive in the gut of *L.salmonis*. The alimentary canal in *L.salmonis* lacks a masticatory stomach and filters like those found in the malacostracans. The gut is a tube filled with partly digested epithelial tissue and blood from the host. This could be a site were bacteria survive, even multiply. It has been shown that fish pathogens like *Vibrio salmonicida, V.anguillarum* and *Aeromonas salmonicida* can survive independant of the host in sediments and sea water (Hoff 1989, Enger *et al.* 1989, 1990, in press, Novotny, 1978), -at least in a starvation/survival stage.

Scanning electronmicroscopy also revealed bacteria on the surface of *L.salmonis* (Fig. 4). These rod-shaped bacteria (about 1 μm long) were most often located close to the mouth and anus. Benediktsdottir and Helgason (1990) isolated *A. salmonicida* from the gill surface in salmonids. In their opinion *A.salmonicida* is often present on the gills of fish in farms with furunculosis, -also on the surface of less severely infected fish or healthy carriers. It is known that some fish pathogens, like *V. salmonicida, A. salmonicida* and *Renibacterium salmoninarum*, are highly hydrophobic (Bruno 1988, Enger *et al.* 1990), which suggests that there could be an affinity for these bacteria to adhere to the outer lipid layer of the cuticula of *L.salmonis*. If this proves to be the case, bacteria may be transported on the surface of *L.salmonis* from one host to the next. When the lice feeds on the host there is an increased chance for secondary infection by fish pathogenic bacteria. The mode of entry of bacteria into the gut of *L.salmonis* may be a result of feeding on the surface layer of the host, in conjunction with anal intake of water, or both. Anal drinking has been documented in a number of crustaceans (Fox 1952) and is primarily effected by antiperistaltic contractions of the rectal muscles.

Fig.3. Scanning micrograph of the area around the anus. Note the bacteria (arrow heads). x 900.

Future studies should concentrate on identification of the bacteria from the gut of *L.salmonis*. One should also try to isolate bacteria and viruses from the gut of lice parasitising salmonids with different diseases as it seems likely that *L.salmonis* could be a vector in the spread of these.

Summary

Bacteria were found in association with the midgut in *Lepeophtheirus salmonis from Norwegian salmon farms. It is suggested that L.salmonis may function as a vector in the spread of pathogenic diseases in salmonids.*

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