

Brief communication

VIBRIOSIS (VIBRIO ANGUILLARUM) AS AN EPIZOOTIC DISEASE IN RAINBOW TROUT (SALMO GAIRDNERI)

Since the first description of *Vibrio anguillarum* (Bergman 1909) this organism has been reported to affect a variety of salt-water fish species. In Norway *V. anguillarum* has never been isolated in connection with diseases in fish until 1964 when this vibriion was proved to cause mortality in rainbow trout reared in sea water. In the past 6 years epizootic outbreaks of vibriosis in rainbow trout have caused heavy losses in several salt-water farms in Norway.

Description of the disease

The disease has been recorded during the period June until December, but most of the outbreaks have occurred in September and October. Rainbow trouts of all ages have been affected. In most of the outbreaks sudden and often greatly increased mortality has been the only initial symptom. The mortality rate has varied, but has not exceeded 30 %. Necropsy of a great number of fish from different outbreaks showed that the pathological findings varied even within the same outbreaks. The most common finding was a slightly enlarged spleen with no other pathological changes. In some cases swollen kidneys, congestion in the caudal part of the intestine and haemorrhages around the anus were observed. In each outbreak at least some fishes showed characteristic haemorrhagic and necrotic changes in the superficial and profound layers of the musculature. In some cases large, unbroken blisters containing haemorrhagic purulent material similar to those found in furunculosis were observed. Ulcerative lesions with necrosis of the dermis and superficial layers of the musculature were also seen. Some of these ulcerations may have originated from broken blisters.

Bacteriological examinations

By microscopic examination of material from spleen, kidney, blood and haemorrhagic lesions in most of the cases a rod shaped, motile bacterium could be demonstrated. The organism could easily be cultivated on ordinary media. Very often pure cultures were obtained even when the inoculum was derived from decayed material. Fourteen strains isolated from different outbreaks were submitted to morphological, cultural and biochemical examinations. Morphologically the organism was a Gram negative rod $0.5\text{--}1.0 \times 1.5\text{--}2.8 \mu$, motile by a single polar flagellum, non capsulated and asporogenous. On blood agar (5 % goat blood and 0.5 % NaCl) the colonies were round, mucoid, raised, translucent and measured approximately 2 mm in diameter. A slight haemolysis under the colonies was present after 24 hrs. incubation, and a distinct zone of haemolysis occurred around the colonies after 48 hrs. All the strains grew slowly at 7°C, showed optimum growth at 18—23°C, and no growth at 37°C. Little or no growth was apparent in media without NaCl. Abundant growth occurred in media containing 0.9—2.0 % NaCl with an optimum of about 1.5 %. In their biochemical reactions, all strains produced acid, but no gas from arabinose, fructose, galactose, glucose, mannose, cellobiose, maltose, sucrose, dextrin, mannitol and sorbitol and neither acid nor gas from rhamnose, xylose, lactose, inulin, raffinose, adonitol, dulcitol, inositol and salicin. They were negative on urea and aesculin. Only 2 strains grew slightly on citrate medium. Except for 3 strains, the rest slowly reduced nitrate to nitrite. They did not produce indole. Acetyl-methylcarbinol was produced only when the Clark Lub medium contained added NaCl (0.9—2.0 %). All strains were methyl red negative and they did not produce hydrogen sulphide. On gelatine stab the strains showed a slowly napiform or stratiform liquefaction. Except for 2 strains, the organisms in litmus milk produced a clot followed by proteolysis after 48 hrs. A light reduction of methylene blue occurred after 24 hrs. The strains were resistant to penicillin but sensitive to chloramphenicol, streptomycin, tetracyclin, nitrofurantoin and sulfamethizole. They were also sensitive to the vibriostatic agent 0/129 (2,4-diamino-6,7-diisopropylpteridine). Pathogenicity was tested by experimental inoculations of the organisms in rainbow trout and brown trout (*Salmo trutta*) held in fresh water. The fishes died

within 18—24 hrs. when injected intramuscularly or intraperitoneally. Necropsies showed enlarged spleen and haemorrhages at the site of inoculation. The causative organism could be re-isolated from blood, internal organs and musculature.

Comments

The outbreaks of the disease described in this report occurred chiefly in the late summer and autumn. This is in accordance with the occurrence of vibriosis in different species of salt-water fish reported by *Nybelin* (1935), *Bagge & Bagge* (1956) and in finnock described by *Smith* (1961). The vibriosis in fresh-water reared rainbow trout reported by *Hoshina* (1957) and *Ross et al.* (1968) occurred in the late autumn to spring. The pathological findings varied greatly which is in accordance with most of the reports on vibriosis in fish. Except for the 2 strains that grew on citrate medium and the 3 strains that failed to reduce nitrate to nitrite, the strains were equal in biochemical reaction and fit into the general description of *Vibrio anguillarum*. From different literature it is seen that the biochemical properties of *V. anguillarum* vary considerably. *Nybelin* classified this vibriosis into biotypes A and B. Type A was indole positive and produced acid without gas from sucrose and mannitol. Type B was indole negative, and produced neither acid nor gas from sucrose and mannitol. *Smith* proposed a third type C which was indole negative and produced acid without gas from sucrose and mannitol. According to that, the examined strains can be classified as type C. They differed from the strains described by *Smith* of which none produced acid from arabinose. Later investigations by *Håstein & Holt* (in press) have revealed that vibriosis occurs quite frequently among different fish species in the coastal waters of Norway. They isolated different biotypes of *V. anguillarum* of which 10 out of 20 examined strains could be classified as type C. From this it may be concluded that the rearing of rainbow trout in sea water together with feeding of marine fish species implies a permanent risk of vibriosis.

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