



Study on Streptococcus Infection in Aquarium Fishes

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ABSTRACT

The present article reports on the streptococcus infections in aquarium fishes. Smears from the abdominal cavity showed the presence of small Gram-positive cocci in pairs or chains. Sections revealed the presence of a severe acute necrotizing reaction in many organs, but especially the spleen and kidney. Gram-stained sections showed that the changes corresponded with massive numbers of Gram-positive cocci; large numbers of bacteria were also present within blood vessels throughout the fish. Axenic cultures of pinpoint non-hemolytic off-white colonies (1 to 2 mm in diameter) were recovered from all fish on TSA and blood agars, but not on MacConkey's agar. These isolates were catalase and oxidase negative, non-motile Gram-positive cocci, which occurred in pairs or short chains.

KEYWORDS : Aquarium fish; Gram-positive cocci; Axenic cultures; blood vessels.

1. Introduction

Streptococcosis is considered to be a serious economic problem in India and Japan. In North America, non-hemolytic Group B *Streptococci* were isolated from diseased golden shiners in freshwater, and from several species of feral marine fishes from the Gulf of Mexico [1-4]. *Streptococcus* sp. was also recovered from water and from tissue slices that included the gastro-intestinal tract, of a small number of aquarium fish imported into India from Southeast Asia [5-6]. To the best of our knowledge, however, there have been no more reports of streptococcal infections associated with disease in aquarium fish in India.

Streptococcus is a septicemic disease that affects freshwater and marine fish, in both farmed and feral populations. In marine fish it has been reported worldwide in a variety of commercially important species including yellowtail *Seriola* spp. [7-9], eels *Anguilla japonica*, menhaden *Brevoortia patronus*, striped mullet *Mugil cephalus*, bluefish *Pomatomus saltatrix*, and striped bass *Morone saxatilis* [10]. Similarly in freshwater fish, *Streptococcus* sp. has been isolated from rainbow trout *Oncorhynchus mykiss*

Materials and methods:

Species used for the experimental studies were Zebra danios, Gourami and Gold fish. The temperature requirements for the bacterium (best at 25 °C) dictated the temperature at which the fish were held, even though this was high for the rainbow trout. Accordingly, all fish were maintained at room temperature (25 ± 1 °C) in individual 30 liter glass aquaria; the water from each tank was aerated and recirculated through aquarium corner filters.

Several colonies of the chichlid fishes isolate were inoculated into 1 liter of brain-heart infusion broth (BHIB, Difco) and grown at 25 °C for 48 h on a rotary shaker (200 rpm). A 50 ml portion of the broth was then added to 450 ml of sterile phosphate-buffered saline (PBS, pH 7.4) and each group of fish was netted out and placed in a separate freshly prepared bacterial suspension (3 x 10⁷ CFU ml⁻¹) for 5 min. Following exposure, both bath-challenged and control (uninoculated BHIB) groups were rinsed in 500 ml of sterile PBS for 20 s before being returned to their respective aquaria.

Results and Discussion:

The mortality and bacteriology data for each species are summarized in [Table 1]. Nine out of 16 exposed rainbow trout died, with mortality starting 3 days post-exposure. *Streptococcus*-like bacteria were recovered from all 8 fish, in pure culture from 7 fish. External lesions were seen in only 2 fishes. Both fish exhibited exophthalmos, while 1 also had intra-ocular and periorbital hemorrhage. Internally, all fish had splenomegaly to varying degrees, while the liver was yellow and had sub-capsular petechial hemorrhages. Histologically, the overall response was primarily one of hemorrhage and neutrophil exudation, plus bacteremia and occasional thrombosis; large numbers of coccoid bacteria were easily seen in most blood vessels, especially in the capillary beds of the gills, cranial meninges, retina, and choroidal rete.

Tissue changes targeted the spleen, which showed congestion and fibrinoid necrosis of the ellipsoidal sheaths with loss of reticular macrophages. Bacteria were widely scattered throughout the parenchyma of the spleen, but were also present within macrophages. Bacteria were present within the lamina propria of the intestinal tract, but the mucosa itself remained largely unaffected, [Plate A and B].

External lesions were visible in most fish, and were identical to those seen in the naturally occurring disease; they comprised pronounced congestion and hemorrhage around the base of the pectoral fins and over the heart. Seven of these fish were examined bacteriologically, and *Streptococcus*-like bacteria were recovered from them all; in 3 fish they were in pure culture. Histopathological lesions were similar to those described for the cichlid except that in addition to the splenic lesions, there was severe congestion of the renal parenchyma, and pronounced degeneration and necrosis of many tubules. Large numbers of bacteria were present in the pericardial area, especially within the hemorrhagic musculature associated with the pectoral girdle, also liver shows schirrosis

Table 1: Mortality and bacterial recovery data for fish exposed for 5 minutes to streptococcus

Species	Challenged	Days to Death						Mortality	Bacterial Recovery
		1	2	3	4	5	6		
Brachydanio rerio	Yes 32	0	20	8	2	0	0	30/32	14/14
	No 32	0	0	0	0	0	0	0/32	0/14
Cichlids	Yes 10	0	2	5	3	0	0	10/10	6/6
	No 10	0	0	0	0	0	0	0/10	0/6
Gourami [<i>Trichogaster gourami</i>]	Yes 16	0	0	4	3	2	0	9/16	8/8
	No 8	4	1	0	1	0	0	6/8	0/8

Although the results are clearly preliminary, it seems reasonable to suggest that the streptococcus like bacteria were the cause of mortality in the original outbreak. The appearance of the fish in the subsequent experimental infection was strikingly similar to the naturally occurring outbreak. Some of the interesting aspects of the disease include the ease with which the fish became infected and the speed with which they subsequently died (2 to 4 days). In most of the previous reports on streptococcus sp. infections in a variety of species of fish, including rainbow trout, disease was produced only following injection (either i.p or i.m).

The taxonomic relationship among fish pathogenic streptococci is poorly understood especially their relationship to other well-defined species within the genus. Physiological and DNA-rRNA hybridization studies have confirmed that the traditional streptococcal groups can be subdivided into 4 distinct groups with *enterococci*, *lactococci*, and anaerobic *streptococci* apparently showing no specific genetic relationship to any of the streptococcal species [11]. This taxonomic distinction has been confirmed among a group of fish-pathogenic 'streptococci' recovered from diseased yellowtail and eel in Japan,

which warranted their reclassification as a new species of *Enterococcus* [12]. A comprehensive taxonomic study of the fish-pathogenic *streptococci* is badly needed, especially genomic relationship which would help to identify taxonomically significant phenotypic markers, as well as help to define standardized laboratory methodologies, so that diagnosticians and researchers can identify their streptococcal isolates.

In the controlled groups, none of the zebra danios and none of the chichlid died, but streptococci were not isolated from these fish, and there were no pathological lesions suggesting a reason for the deaths. The water temperature was fairly high for these fish how-ever, and it was felt that inadequate acclimation at this relatively high temperature for chichlid was the most probable reason for the mortality.



Plate (A) : Growth of *Streptococcus* spp on agar plate

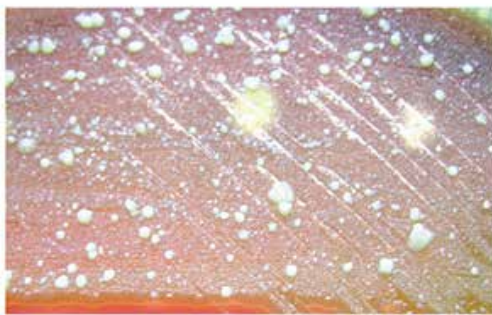


Plate (B) : : Growth of *Streptococcus* spp on blood agar plate

Conclusions:

In the present study, the pathological changes in the experimental fish closely paralleled those seen in the naturally occurring outbreak, and they were similar to those previously reported in other species for the septicemia, non-granulomatous form of the disease. The presence of small thrombi in a few fish suggests the possibility of death due to disseminated intravascular coagulation, although much more work needs to be done to confirm this. The source of the infection must also remain a matter for speculation. It is interesting to note that there are no disease control regulations that apply when importing aquarium fish into India, even though, as we have demonstrated, indigenous species such as the economically important fishes may also be susceptible to pathogens that can affect both.

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