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Epizootic mortalities in tilapia *Oreochromis mossambicus*

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ABSTRACT: Periodic mortalities reaching 100% over a period of 60 d were observed in fry of tilapia *Oreochromis mossambicus*. Moribund fish exhibited rapid corkscrew-like swimming patterns ('spinning'), and the syndrome was successfully transmitted via cannibalism to naive populations of tilapia fry. Histology revealed no evidence of bacteria or parasites. Bohle iridovirus (BIV) infected tilapia and those succumbing to 'spinning tilapia' ('ST') syndrome shared similarities in histopathological lesions of kidney and muscle. Virus isolation *in vitro* from affected fish was unsuccessful. The barramundi *Lates calcarifer* bioassay indicated that the aetiological agent of the epizootic was BIV.

KEY WORDS: Epizootic · Tilapia *Oreochromis mossambicus* · Bioassay

INTRODUCTION

The role of iridoviruses as agents of systemic infections of fish has emerged in the past decade (Hedrick et al. 1992). Locally, Bohle iridovirus (BIV) has shown the ability to infect both native and introduced species across classes from amphibians to fish (Cullen et al. 1995, Moody & Owens 1994, Ariel & Owens in press).

Epizootic mortalities approaching 100% affected a population of tilapia held in an aquatic disease laboratory. Aetiology of the outbreak was tentatively attributed to Bohle iridovirus (BIV), a virus originally isolated from the ornate borrowing frog *Lymnodynastes ornatus* (Speare & Smith 1992). The following year, tilapia fry were collected from mouthbrooding female survivors of that epizootic. Recurrent mortalities in the fry population were noticed in association with erratic swimming patterns exhibited by moribund fish. This article describes the characteristics of 'spinning tilapia' ('ST') syndrome, and addresses the possibility of 'ST' syndrome being a systemic BIV infection with abnormal behavioural signs.

MATERIALS AND METHODS

Husbandry. Approximately 200 fry (5 to 22 mm fork-length), obtained from previously exposed females in captivity, and 200 fry caught from the wild (Curralea Lake, north Queensland, Australia) were held in separate closed freshwater systems in 200 l tanks. Two corner-filters (KiS Aquarium) fitted with activated carbon and filterwool filtered the water. Thermostatic water-heaters maintained the water at 24°C. The fish were fed a commercial diet consisting of Wardley's Tropical Food Flake (Wardley's, Australia) once a day.

Video recording. During periods of mortality, fish showing abnormal swimming behaviour (spinning) were placed in an aquarium (50 cm × 20 cm × 19 cm) that was illuminated from the top with fluorescent light (Sylvania, GTE Gro-Lux, F18W/GRO, Germany). A National Panasonic NVM 7 video camera was placed on a tripod at a distance of 2 m from the tank with the focus fixed at a manual setting. TEAC E-180HX video cassettes were used for recording. Swimming patterns were described during slow motion re-play.

Transmission of 'ST' syndrome. A fish with 'ST' syndrome from the original fry population (group A) was transferred to a 20 l tank with 12 fry (group B) that were assumed to have no history of exposure to BIV. Twelve days after consuming the dead 'spinner', some of the newly exposed fry from group B started to exhibit 'ST' syndrome. A 'spinner' from group B was then transferred to another tank with another 12 presumably unexposed fish (group C) which were subsequently monitored for signs of abnormality. The transfer experiment was carried out in duplicate.

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Histology. Moribund fry were euthanized in 2-phenoxy ethanol before fixing in Bouin's fixative. Tissues were trimmed and placed in formalin, before standard processing and embedding in paraffin wax. Sections were cut at a thickness of 5 µm, stained with haematoxylin and eosin and mounted using routine methods (Culling et al. 1985).

Viral isolation. Of fish exhibiting 'ST' syndrome, 150 were sampled for viral isolation. Processing of samples and attempts at viral isolation were performed according to Moody & Owens (1994) on Bluegill fry (BF2) cells (Wolf et al. 1966), subculture #132-138, and the cultures were checked for wet cytopathic effect of Bohle iridovirus (Speare & Smith 1992) every day for 2 wk.

Barramundi bioassay and transmission electron microscopy. Live tilapia with the 'ST' syndrome were fed to barramundi fingerlings *Lates calcarifer*, a species known to be highly susceptible to BIV (Moody & Owens 1994) and to display classical signs when infected with either enveloped or chemically un-enveloped virus (Ariel et al. 1995). These qualities render barramundi suitable as a bioassay for BIV when it cannot be isolated from an infected species such as tilapia (Ariel & Owens in press). Barramundi which died following consumption of tilapia with the 'ST' syndrome were sampled for virus isolation and histological examination. Diced kidney tissue was collected from moribund barramundi immediately upon euthanasia. Samples were processed for transmission electron microscopy examination according to Owens et al. (1992).

RESULTS

Clinical signs

Fish affected by the 'ST' syndrome would periodically race through the water in a spiral path at a rapid speed. Between intervals of this behaviour, a fish would generally sink motionless to the bottom, seemingly exhausted, and then, after a short 'recovery' stage, it would rise to hang at an angle of 45° immediately under the surface, gasping for air. Affected fish did not respond to physical stimuli and did not feed. They had a dark coloration and displayed 'fin clamping' Tilapia exhibiting the 'ST' syndrome died within 24 h of onset of behavioural signs.

Transmission of the syndrome

The 'ST' syndrome was successfully transmitted with a 'ST' fish from the population (group A) originally infected, to a naive population (group B). Fry from group B cannibalised the dead 'ST' fish and subsequently acquired the specific signs. An 'ST' fish from the second population (group B) was then transferred to a third population (group C) which likewise acquired the 'ST' syndrome and experienced the associated mortality after ingesting the carcass. In the duplication of the experiment, the 'ST' fish was not consumed after death by the other fry and the 'ST' syndrome and mortalities were not transferred to the next population. Mortality in each of the 3 infected populations was periodic and recurrent (Fig. 1). Excluding enzootic mortalities occurring after 60 d (Fig. 1), the mean percentage of available population dying within episodes was 22.89%, with a range of 8 to 51%. The mean frequency of episodes in the epizootic was 13.3 d with a range of 6 to 22 d between periods of mortality.

Histopathology

Kidney and muscle tissue were the only 2 organs affected by the 'ST' syndrome in tilapia. Shrinkage of renal tubules, haemorrhaging and infiltration of eosinophilic granular cells characterised the state of the kidney (Figs. 2 & 3). Most muscle groups were afflicted by focal myolysis (Figs. 4 & 5).

Viral isolation

Viral isolation was not successful, neither directly from tilapia fry, nor after passage through barramundi.

Barramundi bioassay

Barramundi that consumed the 'ST' fish exhibited classical BIV pathology (Moody & Owens 1994) with focal necrosis of spleen and kidney. From Day 10 post-

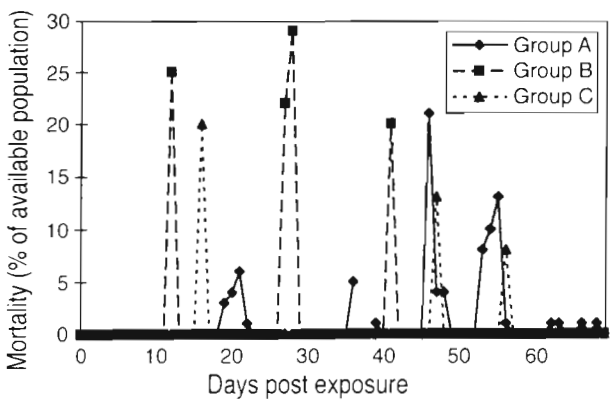

Fig. 1. Percent mortality of available population through time, of 3 groups of tilapia exposed to the pathogen causing 'ST' syndrome

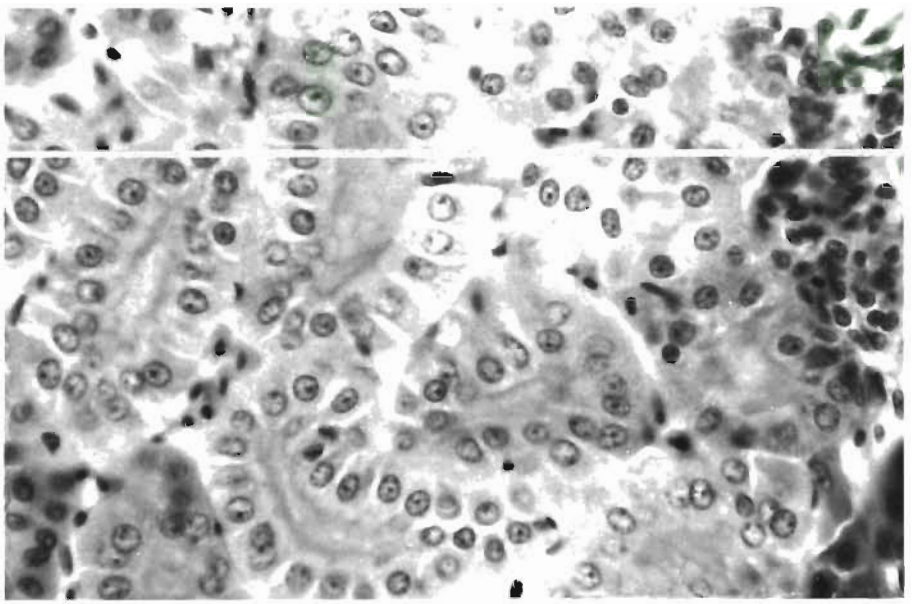

Fig. 2. *Oreochromis mossambicus*. Healthy tilapia kidney (H&E, ×640)

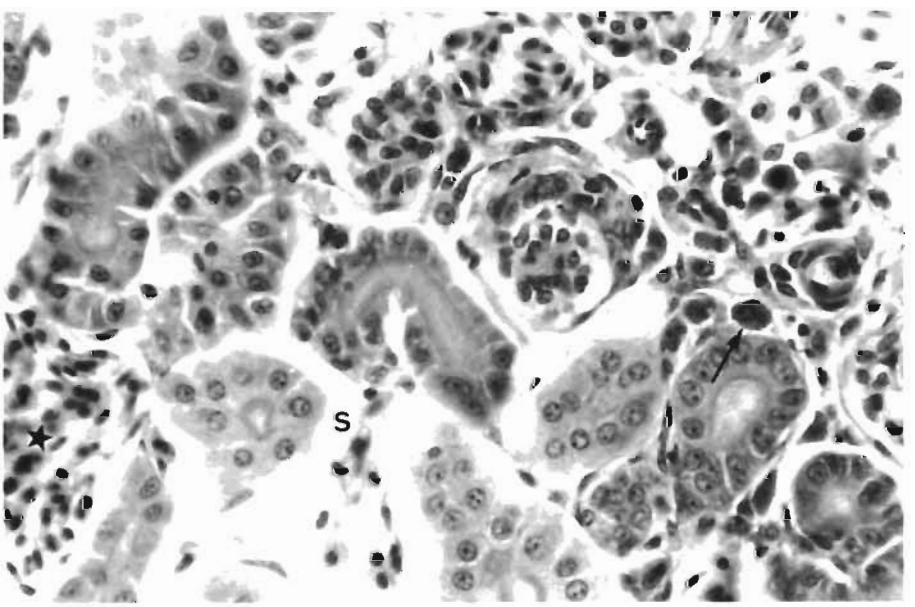

Fig. 3. *Oreochromis mossambicus*. Kidney from tilapia with 'ST' syndrome. Renal tubules are shrunken (S), haemorrhaging (★) and infiltration of eosinophilic granular cells (arrow) can be observed (H&E ×500)

feeding they exhibited headstanding, circling motions and unresponsiveness. Thirteen days after consuming the 'ST' fish, the barramundi died. Control barramundi that had been fed apparently healthy tilapia survived and displayed no abnormal behaviour. No histological changes were detected in their spleen or kidney. Transmission electron microscopy (TEM) revealed electron-dense particles associated with the smooth endoplasmatic reticulum (Fig. 6). Some of the particles had an icosahedral shape and an electron-dense core indicative of iridovirus morphology. The size range (core: ~110 nm; outer shell: ~140 nm) was similar to that recorded for Bohle iridovirus grown in cell culture (Speare & Smith 1992).

DISCUSSION

The successful transfer of 'ST' syndrome from an affected population to a naive population indicates that an infectious agent is responsible for the disease. Systemic iridovirus-like agents can infect fry of redfin perch *Perca fluviatilis*, sheatfish *Siluris glanis*, turbot *Scophthalmus maximus*, and fingerlings of barramundi *Lates calcarifer*, all with signs of atypical swimming patterns in the terminal stages of the infection (Langdon 1989, Ogawa et al. 1990, Bloch & Larsen 1993, Moody & Owens 1994). The aetiological agent of epizootics in fry of several species of tilapia (*Oreochromis aureus*, *O. niloticus*, *Sarotherodon galilaeus*

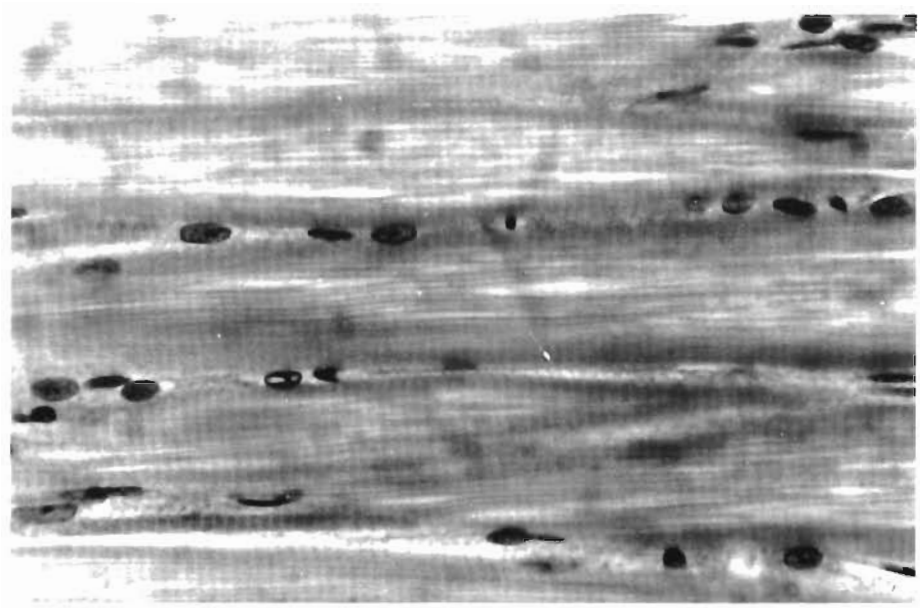

Fig. 4. *Oreochromis mossambicus*. Healthy muscle tissue from tilapia (H&E, ×640)

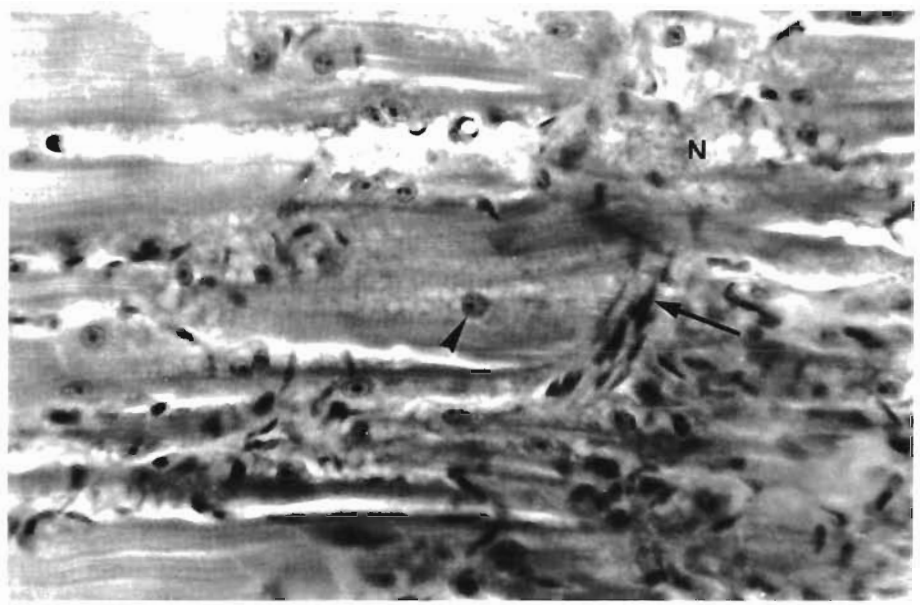

Fig. 5. *Oreochromis mossambicus*. Muscle tissue from tilapia exhibiting 'spiralling disease' Sarcolemma nuclei (arrowhead) are migrating through the necrotic area (N); fibrocyte-like cells (arrow) with elongated nuclei can be seen (H&E, ×500)

and red tilapia) which were accompanied by whirling is thought to be an iridovirus of maternal origin. The virus was observed by transmission electron microscopy (TEM) in brain tissue (Avtalion & Shlapobersky 1994).

The unifying character in the histopathology caused by systemic iridoviruses is the constant and serious involvement of the kidney and spleen. Pathological alterations observed in fry with 'ST' syndrome are similar to those caused by a BIV infection in fingerling tilapia, which in addition is accompanied by haemosiderin and melanin deposition in the spleen (Ariel & Owens in press).

Some of the iridovirus-like agents that cause systemic infections in fish have been isolated *in vitro* (Langdon et al. 1986, Ogawa et al. 1990, Inouye et al. 1992, Pozet et al. 1992, Speare & Smith 1992, Nakajima & Sorimachi 1994), whereas others, although visualised by TEM, have not been isolated on a cell line (Leibovitz & Riis 1980, Armstrong & Ferguson 1989, Bloch & Larsen 1993, Chua et al. 1994). Bohle iridovirus can successfully be recovered *in vitro* from infected barramundi (Moody & Owens 1994), but not after passage through tilapia (Ariel & Owens in press). Thus, if the infectious agent responsible for 'ST' syndrome is BIV, it is to be expected that it can-

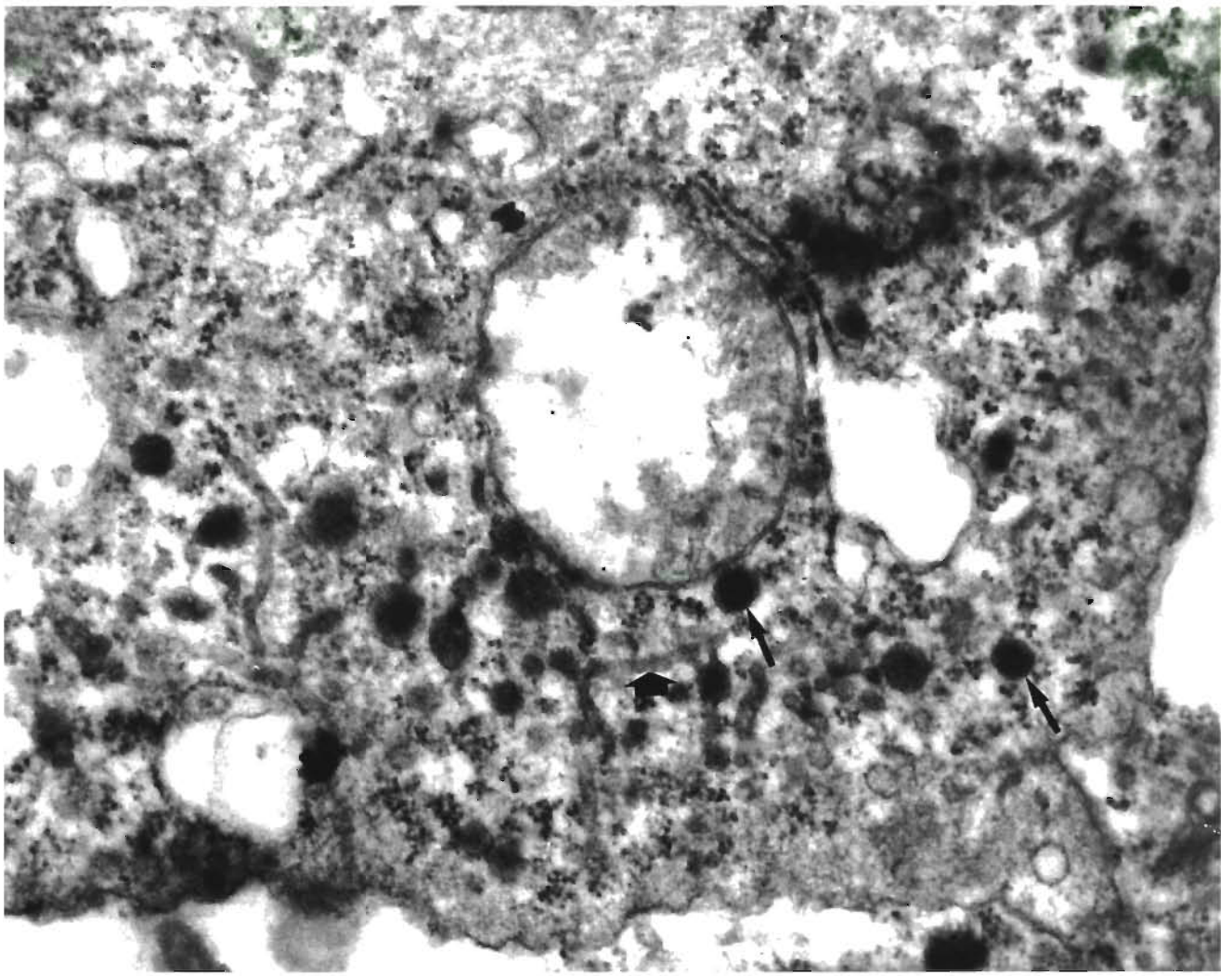

Fig. 6. *Lates calcarifer*. Electron micrograph from kidney of a barramundi which has ingested tilapia with 'ST' syndrome. Electron-dense particles (arrows) with iridovirus-like morphology was observed in association with the smooth endoplasmatic reticulum (arrowhead) (EM, ×46 000)

not be isolated from affected tilapia, which in fact was the case.

Barramundi are susceptible to chemically unenveloped BIV as well as entire enveloped BIV, and have been used as a bioassay for detection of Bohle iridovirus refractory to cell culture (Ariel et al. 1995). Histopathological similarities between the barramundi inoculated with BIV and those fed spinners, together with TEM evidence of iridovirus-like particles in the cytoplasm of the latter, suggest that 'ST' syndrome could possibly be caused by Bohle iridovirus.

The agent of this epizootic may have tremendous impact on tilapia culture internationally, as it appears to be a primary pathogen able to inflict mass mortalities in a population of tilapia fry. Tilapia is hardy and opportunistic in the wild, thriving in both fresh and saltwater and may be able to harbour either an acute BIV infection, as described here, or a chronic BIV infection (Ariel & Owens in press). These are all attributes which enable tilapia to carry BIV into naive populations of poikilotherms along waterways of northern Australia.

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