

Case Report: Suspected Piscine *Chlamydia* like infection in Tanzania

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SUMMARY

Ten moribund fish were received at pathology laboratory to establish the cause of spontaneous mortalities of farmed tilapia recorded in Kilosa District, Morogoro region. Post-mortem examination revealed macroscopic mucous bands connecting gills and operculum; and oval to round grey-white cysts on the gill lamella. Gill samples were collected for microscopic and bacteriological investigation. Histopathological investigation revealed gill epithelial hyperplasia and characteristic enlargement of epithelial cells infected with pale and basophilic cytoplasmic inclusions. The gross pathological changes coupled with histopathological findings recorded in this case are typical features of epitheliocystis. Furthermore, fish mortalities ceased in the farm after water replacement and reconnection of all fish ponds with direct supply of fresh water from the source. This is the first report in Tanzania describing characteristic epitheliocystis lesions. Furthermore, this report re-affirms previous findings that epitheliocystis can be managed through routine management of water quality.

Key words: *Chlamydia*, Epitheliocystis, Tilapia, *Oreochromys niloticus*, Tanzania

INTRODUCTION

Chlamydia like organisms belong to the order Chlamydiales, which consists of several genera known to infect terrestrial and aquatic animals (Pawlikowska *et al.*, 2016) and has been reported in different countries including Uganda, South Africa and other parts of the world (Steigen *et al.*, 2018). Among this group, more than 11 species which are specific pathogens in aquatic fish have been described (Pawlikowska *et al.*, 2016). The disease caused by the pathogens is called epitheliocystis and affects mainly the skin and respiratory system of fish (Toenshoff *et al.*, 2012). The disease is characterized by grey to white cystic lesion on the gills and skin of the affected fish and microscopic membrane-enclosed basophilic inclusions in host cells resulting

in hyperplasia and hypertrophy of the affected gills (Sood *et al.*, 2018; Steigen *et al.*, 2018).

Clinical history

Ten moribund fish aged approximately 8-9 months were submitted from Kilosa District, Morogoro region for laboratory investigation. The fish were collected from one of the two fish ponds each with surface area of 150 square meters and 1.5-1.6 meter deep were received in the pathology laboratory for diagnostic procedures. Based on information contained in the submission form, the two fish ponds were arranged in series and contained approximately a total of 1000 fish in each pond translating into 4-5 fish per cubic meter. The first pond received water directly from water inlet source and the second pond received water indirectly through the first pond and the pond was

reported to develop a layer of algal bloom during afternoon, when the sun shine was strong. The farmer regularly applied cattle manure to enrich the ponds, and provide protective cover against predators.

The farm experienced mortalities only in the second pond which did not have direct inflow of fresh water, with average mortality of 3-5 fish per day. There was no any mortality recorded in the pond which had direct water supply from the water source. The colour of water in the two ponds was described as greenish in appearance and more intense in the pond which was not directly connected to the water source.

Physical-chemical parameters of water including pH, ammonia levels, conductivity and dissolved oxygen were not reported. Measures taken after recording fish mortalities include replacement of water in the two ponds and change water supply system by connecting the two ponds directly to the fresh water source. Post intervention mortality decreased from an average of 3-5 fish per day to 1-2 fish per day and ceased completely within three days.

Post-mortem laboratory results

Moribund fish submitted for post-mortem examinations were received in 20 litter buckets and were observed to lose balance and swim at an angle of 45 degrees. The fish were euthanized for detailed examination. All fish had fairly good body condition relative to their size based on visual examination. The average standard fish lengths were 14 centimetres, and all fish showed focal areas of hyperaemia on the tail fin and gills. The affected gills showed mucous bands between the gills and operculum as well as oval to round grey-white cysts on the gill lamella (Figure 1A-C). Histologically, the gills showed cellularity between primary lamellae and characteristic enlargement of

epithelial cells which were infected with pale to basophilic cytoplasmic inclusions (Figure 1D-F). Bacteriological culture using MacConkey agar did not reveal presence of pathogenic bacteria in all fish samples examined. In addition, impression smears from gills were stained with periodic acid and schiff reagent (PAS) to investigate for presence of fungal infection and no fungal structures were detected.

DISCUSSION

In this report, the pathological lesions suggestive of the presence of *Chlamydia* like infection, the causative agent of epitheliocystis are presented (Figure 1A-F). There is no other pathogen known to cause similar histopathological lesions in fish. The term epitheliocystis is derived from the appearance of the microscopic lesions that characterized by the enlargement of infected epithelial cells filled with basophilic granular contents. This is the first report describing fish epitheliocystis lesions in Tanzania. Main risk factors for the development of epitheliocystis are known to include stress and poor water quality (Nowak and LaPatra, 2006) which pre-disposes the gills and skin which are in constant contact with the external environment to infection. Although histopathological lesions are known to be specific to *Chlamydia* like microorganisms, gross lesions are not specific to the organism and can be found in fish infected with parasites and other infectious organisms capable of inducing irritation to the gills. However, bacteriological, histopathological and cytological investigations did not reveal presence of other pathogenic microorganisms including bacterial, fungal or parasitic infection.

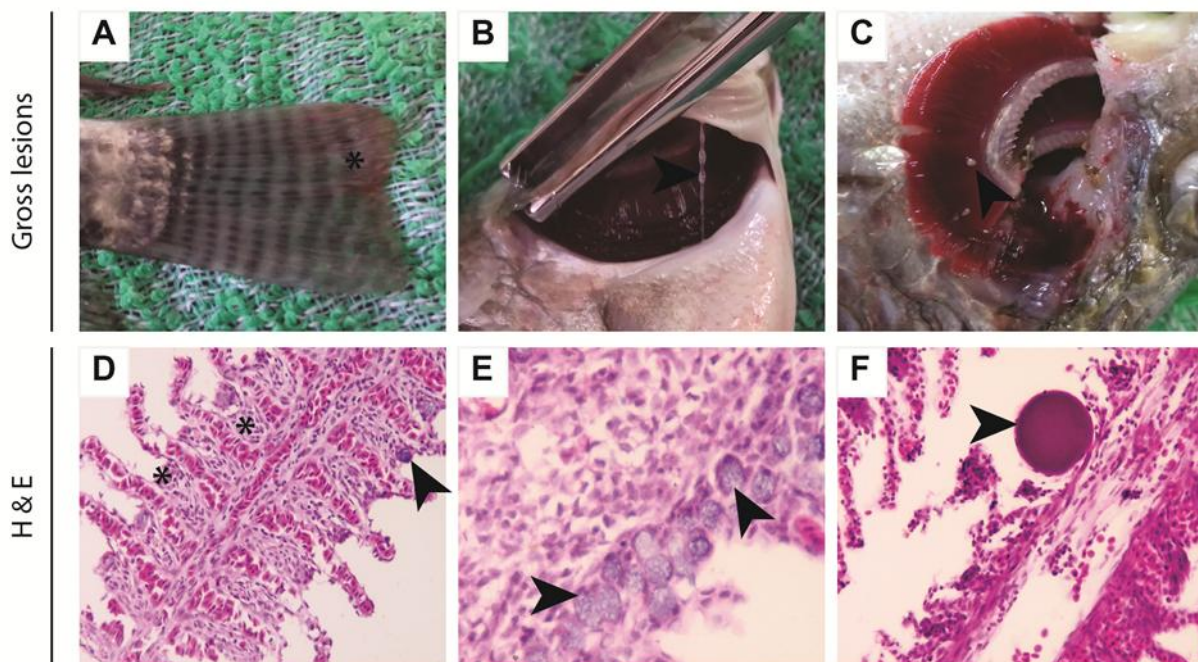


Figure 1. Gross lesions showing moderate tail fin hyperaemia indicated with an asterisk (A), mucoid band connecting the operculum and gills (B) and grey cystic lesions (C) indicated with arrow heads. Hematoxylin and Eosin stained gill tissues showing increased cellularity between the primary lamellae as shown with an asterisk (D) and pale to intense basophilic intracellular inclusions indicated with arrow heads (E & F)

The relationship between environmental stress and fish diseases is not clearly understood. However, because the gills are severely affected in most epitheliocystis cases and pre-mortality clinical signs are usually associated with hypoxia including congregating near the air-water interface (Sood *et al.*, 2018). Decreased water quality associated with accumulation of organic matter might have facilitated growth and multiplication of *Chlamydia* like organisms and or increased oxidative stress predisposing the fish to microbial infections during contact between polluted water and the gills. Whether hypoxia occur secondary to epitheliocystis damage or poor water quality remains to be investigated.

Different treatment approaches have been applied successfully for treatment of epitheliocystis including salt, formaldehyde and antibacterial bath as well as water replacement (Somridhivej *et al.*, 2009). However, based on the case history and progression of fish mortalities before and after intervention, water replacements were found to be sufficient to reduce and eventually prevent fish mortalities. The control of fish mortality after water replacement suggests that water quality management is essential in preventing mortalities associated with epitheliocystis in farmed tilapia.

The *Chlamydia* like organisms which are known to cause epitheliocystis are difficult to isolate in culture but the presence of these obligate intracellular parasites can be

confirmed by genomic analysis of DNA through polymerase chain reaction (PCR) (Steigen *et al.*, 2018). Genomic analysis was not one of the laboratory test conducted during the course of investigation for this report. However, because mortalities associated with water quality deterioration are not uncommon, and gross lesions are similar in many fish diseases affecting gills, future investigations of similar cases should include genomic analysis for confirmation or ruling out the presence of Chlamydia like microorganism. Furthermore, more study involving more samples and different fish habitats is recommended in order to provide more understanding on the status of the disease condition in different regions of Tanzania.

Finally, based on the findings in this case, minimal chemical interventions can be used in cases of epitheliocystis related mortalities and maintenance of water quality should be taken as the basic routine management activity in fish farms to prevent diseases related losses

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