Trypanosomiasis in cultured *Epinephelus areolatus*

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Abstract
Parasitaemia with large numbers (approximately 24 organisms per 1000x field) of haemoflagellates was observed in *Epinephelus areolatus*. The trypomastigote of approximately 30 µm total length showed a single flagellum, a well developed undulating membrane, a kinetoplast and a nucleus. These features are consistent with *Trypanosoma sp.*. Trypomastigotes were observed in blood vessels, kidney, eye, gills, swim bladder, brain, liver, thymus and spleen.

Trypanosomiasis is uncommon in cultured fish but relatively common in wild species, especially for cold freshwater species eg. European carp and tench. Disease causing trypanosomes include *Trypanosoma carasii* (Woo, 1987) infecting goldfish and common carp and *T. murmanensis*, a well known marine species in the North Atlantic affecting cod, plaice and eels (Khan, 1985). The major differential diagnoses are trypanoplasms and *Cryptobia*, which have 2 flagellae. Trypanosomes have a single flagellum (Noga, 1996). The life cycle of trypanosomes begins in the digestive tract of leech as sphaeromastigotes, which presumably produce epimastigotes that migrate to the proboscis of the leech. There they transform to infective metatrypanosomes, which are inoculated into fish when the leech feeds (Khan, 1976). For *T. murmanensis*, development takes 62 days at 0-1 °C or 42 days at 4-6 °C to complete (Woo, 1995). At 3 days after infection, small trypomastigotes appear in the blood of cod. They become large trypomastigotes within 29-55 days. As the parasitaemia subsides, pleomorphic trypomastigotes are more apparent (Khan, 1976). Trypomastigotes in fish blood can be small (in acute infections) or large (in chronic infections). No multiplication of the parasite occurs in fish. Marine fish trypomastigotes can be up to 100 µm while freshwater species are no more than 50 µm. When ingested by a leech, the trypomastigotes form amastigotes, which have no flagellum. Trypanosomes can cause anaemia, haematopoietic damage and death (Noga, 1996). Infected fish are anaemic, lethargic, with emaciation and in some fish, splenomegaly (Khan, 1985). The parasite has caused mortality as high as 65% in...
experimentally infected 0+ year cod and 17-56% in immature winter flounders \textit{(Pseudopleuronectes americanus)}. Adult or larger flounders however did not die from the infection (Khan, 1985).

The first farm reported that thirty thousand areolated grouper \textit{(Epinephelus areolatus)}, (Figure 1) imported from Myanmar in March 2002 suffered a cumulative mortality of 25% in October 2002, some 7 months post stocking. A second farm that imported areolated grouper from Thailand in 2003 were also infected with similar trypanosomes. Each farmer only submitted a single fish for laboratory diagnosis. This case report is based on two areolated grouper specimens. Histology of formalin fixed tissues was conducted using haematoxylin & eosin staining. The spleen section was examined after Perl’s Prussian Blue staining; blood and kidney smears were examined after Giemsa staining. Identification of the haemoflagellate as \textit{Trypanosoma sp.} was assisted by Professor PTK Woo from Giemsa stained blood smears sent to him.

Large numbers (estimated at 24 per 1000x field) of \textit{Trypanosoma sp.} were observed in wet mounts prepared from the peritoneum, kidney and gills as well as in the Giemsa stained blood smear (Figures 2 and 3). The trypomastigotes were very active. Each trypomastigote possessed a single flagellum; a well developed undulating membrane, a kinetoplast and a nucleus, which were consistent with \textit{Trypanosoma sp.} (Stoskopf, 1993). The trypomastigotes measured approximately 30 mm (including the flagellum) in length. The trypomastigotes appeared monomorphic and were of a size suggestive of recent infection. They were distributed in most host tissues including the liver (Figure 4), spleen (Figure 5), brain, eye, kidney, swim bladder, and thymus. Thus a parasitaemia with trypomastigotes was observed. Tissue damage occurred in the spleen, swim bladder, choroid gland of the eye and renal glomeruli. The spleen section contained erythrocytes with extensive necrosis and abundant haemosiderin, which stained blue using Perl’s Prussian Blue. This

\textbf{Figure 2.} \textit{Trypanosoma sp.} trypomastigotes in blood smear of \textit{Epinephelus areolatus}. Scale bar ~10 µm. Giemsa stained peripheral blood smear x1000.

\textbf{Figure 3.} \textit{Trypanosoma sp.} trypomastigotes of \textit{Epinephelus areolatus}. Morphological features: A single flagellum (F), well developed undulating membrane (M), a kinetoplast (KN) and a nucleus (N). Scale bar ~ 10 µm. Giemsa stained peripheral blood smear ~X 3500.
is consistent with a haemolytic anaemia caused by *Trypanosoma sp.* There was necrosis and inflammation of the swim bladder and swelling oedema of the choroid gland and distension of renal glomeruli.

The severity of the anaemia is related to the heavy load of trypanosomes in the blood. Haemolysis in trypanosomal infection results from both haemolysin and haemodilution. Haemolysin is secreted by living trypomastigotes in the absence of specific antibodies (Woo, 1995). A leech vector (*Piscicola sp.*) is required to complete the life cycle of *Trypanosoma sp.*, and although not found on the submitted fish, is known to infect *Epinephelus areolatus* locally. Leeches are uncommon in cultured fish and are sometimes seen in wild or pond-raised fish. Their life cycle is direct with immatures being hatched from cocoons laid by hermaphrodite adults. Some species have quite a wide host range, while others are more species specific. Heavily infested fish often develop a chronic anaemia as well as transmit microbes and haemoparasites during feeding. The small size of the trypomastigotes indicated recent infection as marine trypanosomes grow up to 100 mm in length the longer they remain in the fish host blood (Noga, 1996). The uniform morphology and size also indicate a single infection by a leech vector. The source of the infection is unknown. It may have existed in wild fish as trypanosomiasis occurs in 13 marine fish species (Noga, 1996) and *Trypanosoma murmanensis* is not host specific (Khan, 1977). Treatment recommended to the farmer was control of leeches with Trichlorphon 2 ppm bath for 60 minutes in seawater, however, the outcome of the treatment, if it was applied, is not known. Isometamidium chloride (a trypanocidal drug) used at 1.0 mg/kg intramuscularly is reportedly effective against a related haemoflagellate *Cryptobia salmositica* in chinook salmon (Woo, 2001). An antimalarial drug - pyrimethamine is reportedly effective against *Cryptobia spp.* (Herwig, 1979).

There is currently no known public health risk with piscine trypanosomiasis (Stoskopf, 1993). Due to the small size of the trypomastigotes and the delayed onset of mortalities some months after importation of...
the fish, it can be suggested that the source of the parasite is local in Hong Kong. However survey studies of imported and indigenous marine fish in Hong Kong are needed to provide necessary information on the type and distribution of tropical and sub-tropical piscine trypanosome species. This will enable a risk assessment on the health impact of these haemoparasites on cultured marine fishes.

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References


