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Note

Experimental infection of *Heterobranchus longifilis* (Teleostei, Clariidae) with *Trichodina maritinkae* (Ciliophora, Peritrichida)

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*Infection expérimentale d'*Heterobranchus longifilis *(Teleostei, Clariidae) avec* Trichodina maritinkae *(Ciliophora, Peritrichida).*

INTRODUCTION

Trichodinid ciliates are frequent parasites on the skin and gills of a wide range of fishes. Although considerable information is available on their taxonomy and distribution, reports on their pathology are rather limited (Frank, 1962; Khan, 1975). The majority are believed to be non-pathogenic, whereas a few species are now known to be primary pathogens of fishes causing considerable mortality particularly among juveniles (Ahmed, 1976; Takeda *et al.*, 1969).

Catfishes of the family Clariidae are increasingly being used for freshwater aquaculture in Africa owing to several favourable cultural characteristics. An emerging candidate in this family is *Heterobranchus longifilis* Val. which in some West African countries has been hybridized with *Clarias gariepinus* to produce viable and culturable offspring. Despite the relative ease of fry production through established induced breeding practices, inadequacies in the supply of fingerlings for stocking remain a major handicap to the expansion of African catfish aquaculture (Hogendoorn, 1980). Pathogens may contribute to significant losses during early life stages and several cases of mortality in hatchery-reared fry have been reported due to epizootic infections with protozoa (Bragg, 1988) and monogenea (Obiekezie and Taege, 1991).

Trichodina maritinkae which was first described from cultured *Clarias gariepinus* in South Africa by Basson and Van As (1991) occurs frequently at high intensities on juveniles of *Clarias* and *Heterobranchus* in all the hatcheries sampled in Southern Nigeria. The parasite is apparently distributed throughout Africa with a remarkable affinity for the Clariidae (Van As and Basson, 1992). In view of the varying host-relations among species of *Trichodina* and apparent differences in their pathogenicities, the objective of this study was to determine the pathogenic potentials of *T. maritinkae* infections to African catfish. This paper presents observations made during experimental infections of *Heterobranchus longifilis* early life stages with regard to histopathological alterations of affected organs and mortality patterns in infected populations.

MATERIALS AND METHODS

Fry of the African catfish, *Heterobranchus longifilis* at different development stages were obtained through

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induced breeding in an indoor hatchery and maintained on a diet of *Artemia* nauplii under pathogen-free conditions using a bore-hole water source. They were grouped in three batches of 6, 14, and 21 days post-hatching. Seventy juveniles from each batch were selected at random for experimental infection and held in separate 5 litre aquaria at a density of 30/l while 50 were maintained simultaneously as uninfected controls under identical conditions. Each experimental run was replicated three times. *T. maritinkae* was successfully transmitted by placing 8 excised gill arches of naturally infected *H. longifilis* which harboured a total of approx. 1 000 ciliates into the respective aquaria. The silver impregnation technique of Klein (1958) was used for specific identification of the ciliates. As could be determined under microscopy, no other parasites were present.

Water quality parameters in both infected and control tanks were maintained at optimal levels for catfish, as follows: temperature, 25 ± 2°C; DO, 6.5 mg/l; pH, 7 ± 0.5; and alkalinity, 20 mg/l. Adequate water exchange was maintained. Both infected and control batches were fed *ad libitum* with constant removal of food remnants. Response and behaviour of the fish were observed and daily mortalities recorded.

For histopathological studies, pieces of heavily infected gills, or whole larvae (for *in situ* sections), were fixed in phosphate buffered formalin or Bouin's fluid. Sections were cut at 5 μm after processing according to standard histological methods and stained in haematoxylin and eosin.

RESULTS

Ciliates recovered from moribund and recently dead specimens (*fig.* 1*a*) agreed in morphology and measurements with the description of Basson and Van As (1991) for *Trichodina maritinkae*. Infection was concentrated on the gills and occasionally occurred on skin and fins.

Gross observations

Infection was transmitted within 2 hours of initial exposure and attained 100% prevalence after 10 days in all the infected tanks. Build up of ciliate population was greatest in the 21-day-old batch reaching peak abundances about 14 days post-infection. Infected fish were generally darker in coloration, and exhibited listlessness and lack of appetite. Their gills and body surfaces were covered in a thick layer of mucus in which were contained massive numbers of ciliates. At advanced stages of the infection, they adopted a vertical hanging position near the water surface with continuous lethargic swimming motions.

No other parasitic infections were detected on the fry examined.

Histopathology

Disintegration of secondary lamellae leading to exposure of the erythrocytes in the vicinity of *T. maritinkae* is shown in *figure* 1*b*. The adoral cilia of the parasite and parts of the denticular ring are quite visible. In wet mounts, epithelial cells were continually being dislodged resulting in the erosion of some gill filaments. Host response was in the form of excessive mucus secretion and hyperplasia of gill epithelium (*fig.* 1*c*). Release of large numbers of blood cells into the branchial chamber consequent on the disintegration and disorganization of the lamellae is demonstrated in *figure* 1*d* where sections of the ciliate could be recognised among haemorrhaged blood cells. This was observed particularly in heavily infected 14-day old juveniles.

Cumulative mortality

Cumulative mortality curves in the different batches infected at 6, and 14 days post-hatching in relation to simultaneously maintained uninfected controls are shown in *figure* 2. Low levels of mortality were observed until after 11 days post-infection, when it increased sharply to 100% within 48 hours. Survival in the control tanks was 70 and 95% for the 6- and 14-day-old groups respectively at the end of 28 days.

For fry infected at 21 days post-hatching, the pattern of cumulative mortality in both infected and control tanks is shown in *figure* 2*c*. After an extended period of 41 days 70% of infected fish had succumbed to the infection compared to only 17% in the controls.

In all the cases, infection with *T. maritinkae* led to significantly enhanced mortality when compared to uninfected controls. In the absence of treatment, survival from the infection was observed only in fry infected at 21 days post-hatching (30%) and none in those exposed at earlier stages.

DISCUSSION

The observed pathological effects in the gills of *Heterobranchus longifilis* can only be attributed to *Trichodina maritinkae* which was the only parasite present and closely associated with the lesions. There is similarity in the pathogenicity of *T. maritinkae* with that of *T. domerguei* in goldfish, *Carassius auratus* in terms of disorganization of gill epithelia and penetration of hyperplastic tissue. However, the severe haemorrhage in branchial chambers consequent on disruption of capillaries which was suspected by Frank (1962) could be demonstrated in this study. It is concluded that *T. maritinkae* is a definite pathogen of the Clariidae with a potential to cause severe histopathological changes on the gills. Apparently not all trichodinids found on Clariidae have this potential

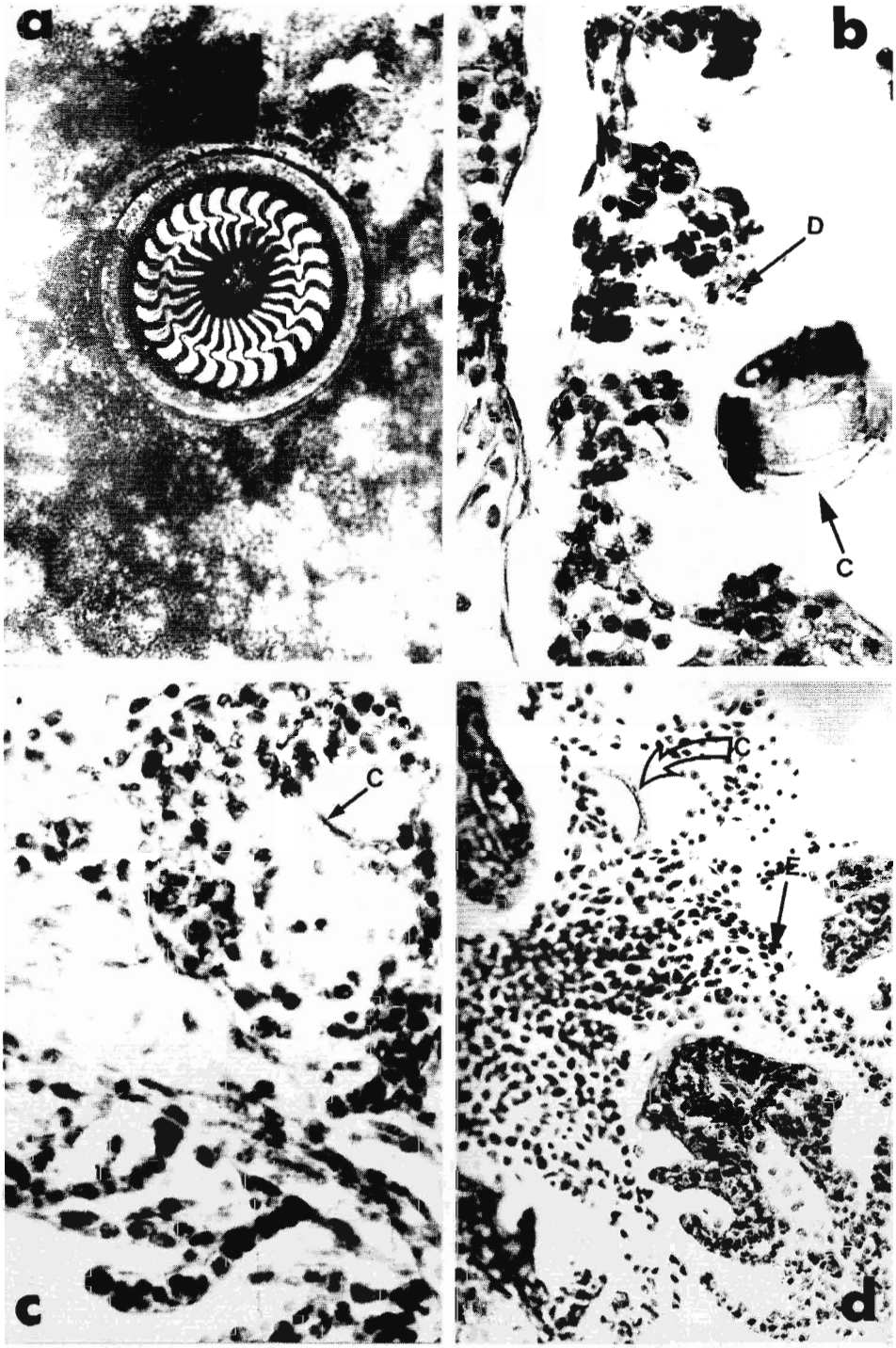

Figure 1. – a: Silver impregnated *T. maritinkae* from moribund *Heterobranchus* fry (× 1 250). b: Disintegrated secondary lamellae in the vicinity of *T. maritinkae* (c). Note epithelial cell debris (d) at the adoral end of the ciliate. c: Hyperplasia of gill epithelium. Note section of the ciliate (c) within hyperplastic tissue (× 1 000). d: Haemorrhage within the gill chamber of infected *H. longifilis*. Note section of *T. maritinkae* (arrow c) among haemorrhaged blood cells (e) (× 200).

since *Trichodina diaptomi* failed to establish viable infections on *Clarias gariepinus* fry (Basson and Van As, 1991).

The significant differences in mortality between infected and control batches could also be attributed to the parasite and is possibly related to the severe

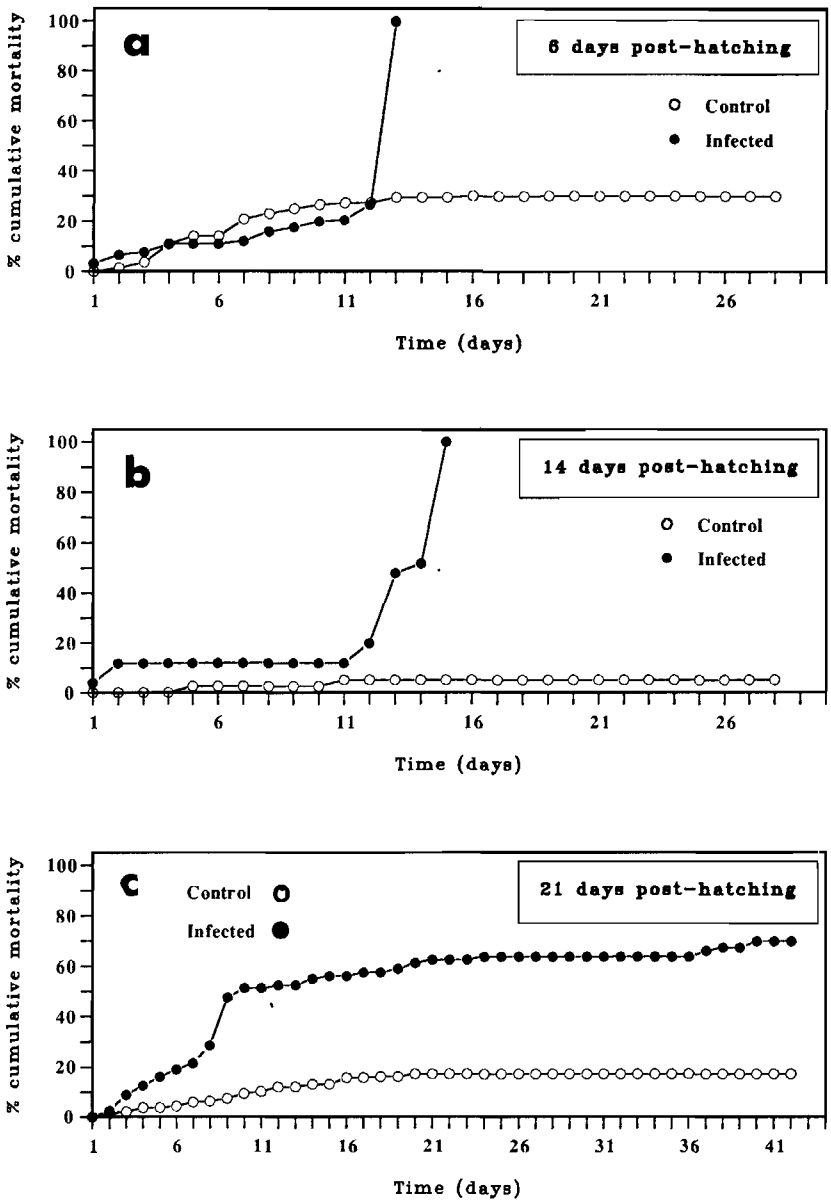

Figure 2. – Cumulative mortality (%) of *Heterobranchus longifilis* infected with *T. maritinkae*.

respiratory impairment consequent upon the destruction of the branchial organs. This was particularly pronounced in larvae infected at 6 days post-hatching where further gill development was forestalled by the infection. The mortalities recorded in the control batches are attributed to natural causes and lie within the ranges observed by Hecht and Appelbaum (1987) during normal rearing of African catfish fry.

T. maritinkae seems to be a particular problem of juvenile Clariidae. Whether mortalities in ponds would be as severe as under the present experimental conditions is not known. Although *Trichodina*-induced mortalities are usually enhanced by confined conditions such as aquaria and hatchery tanks (Moskness *et al.*, 1989), severe cases have also been reported in ponds (Frank, 1962; Ahmed, 1976) and even in natural populations (Schäperclaus, 1926). There is reason to believe that this might also be the case for catfish exposed to infection in outdoor nursery ponds during the early larval stages where there is a lack of facilities for intensive rearing under controlled conditions as in many African countries. The results show that the severity of mortality is related to the age at initial exposure. As a widespread pathogen of the Clariidae, *Trichodina maritinkae* is a likely factor in the nursery mortality of their juveniles.

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