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Occurrence of *Ichthyophthirius multifiliis* within the peritoneal cavities of infected channel catfish *Ictalurus punctatus*

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ABSTRACT: *Ichthyophthirius multifiliis* is a ciliated protozoan parasite that infects the skin and gills of freshwater fish. This report describes the unusual finding of *I. multifiliis* within the peritoneal cavities of experimentally infected channel catfish *Ictalurus punctatus*. Twenty catfish fingerlings were exposed to *I. multifiliis* theronts using a standardized protocol. Five infected fish and 2 control fish were killed at various time points after infection and their tissues examined. Formalin-fixed, paraffin embedded sections were processed for light microscopy and immunohistochemical detection of *I. multifiliis* immobilization antigen. Trophonts were observed in skin and gill sections of all exposed fish. Parasites were associated with epithelial hyperplasia, focal areas of cellular disruption and necrosis. In addition to these usual sites of infection, individual trophonts were unexpectedly found within the peritoneal cavities of 4 fish. Staining for parasite antigen facilitated their detection within abdominal adipose tissue or adjacent to intestines. This discovery is interesting as it suggests *I. multifiliis* may be found in tissues other than the skin and gills during the course of a normal infection.

KEY WORDS: *Ichthyophthirius multifiliis* · Ich · Protozoa · Ciliate · *Ictalurus punctatus* · Channel catfish · Pathology · Immunohistochemistry

INTRODUCTION

The ciliated protozoan parasite *Ichthyophthirius multifiliis* infects freshwater fish worldwide causing an economically important disease referred to as 'ich' or 'white spot'. Infected fish are diagnosed by macroscopic observation of trophonts within the host's skin and gills. Fish may overcome mild infections and develop a parasite-specific immunity; however, high morbidity and mortality often occur in facilities such as aquaria or ponds, where fish are maintained at high stocking densities.

Ichthyophthirius multifiliis is considered a pathogen of mucosal surfaces and has not been shown to penetrate into the deeper tissues of its host. Pathological lesions associated with *I. multifiliis* have been well characterized. In the skin and gills, infections cause localized lymphocytic infiltration, focal necrosis and varying degrees of epithelial proliferation. In severe cases, sloughing of the epidermis has been observed (Hines & Spira 1974, Cross & Matthews 1993). Other than at these common sites, *I. multifiliis* has been reported within the cerebral cavity, circumorbital clefts and nasal pits of a naturally infected carp hybrid (Ventura & Paperna 1985). Additionally, under artificial conditions, *I. multifiliis* can survive and grow within the peritoneal cavities of channel catfish. This observation was made during experiments in which fish were injected with live parasites by the intraperitoneal route (Dickerson et al. 1985).

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In this study, 20 catfish were exposed to infective *Ichthyophthirius multifiliis* theronts. Histological and immunohistochemical staining of various tissues led to the unexpected discovery of trophonts within the peritoneal cavities of 4 fish. This report describes these atypically located parasites and discusses plausible routes of entry.

MATERIALS AND METHODS

Twenty catfish fingerlings (12 to 18 g each) were infected using a standard challenge protocol (Dickerson et al. 1981). Fish were exposed to 5000 to 7000 infective theronts/fish in glass jars containing 500 ml of aerated, charcoal-filtered water for 1 h. The fish, parasites and water were placed in a 76 l aquarium and maintained at 20 to 25°C for 11 d. Unexposed control fish were maintained under similar environmental conditions. Water quality (pH and NO_2) was monitored daily.

Five infected fish and 2 control fish were killed and examined at 1, 5, and 10 d after *Ichthyophthirius multifiliis* exposure. Tissues (gill, skin, head kidney, renal kidney, spleen, liver and intestines) were fixed in 10% neutral buffered formalin for 15 to 20 h and embedded in paraffin following standard protocols. Replicate 3 µm sections were cut and mounted on glass slides. One set was stained with hematoxylin and eosin, and a second set was processed for immunohistochemical detection of *I. multifiliis* surface immobilization antigen (i-Ag) as follows.

Sections were deparaffinized and covered with citrate antigen retrieval buffer (Vector Labs, Burlingame, CA, USA). Antigenic sites were exposed by heating the sections in a 1.1 kW microwave set on full power for 5 min. Nonspecific antibody binding sites were blocked with 2% normal goat serum diluted in phosphate buffered saline (137 mM NaCl, 12 mM NaH_2PO_4, 2 mM KH_2PO_4 and 0.2 mM KCl) plus 0.005% Tween 20 (PBST) (Sigma, MO, USA). The slides were drained and sections incubated for 2 h at 37°C with a Protein A-purified anti-*Ichthyophthirius multifiliis* i-Ag rabbit polyclonal antibody (1:3000 in PBST) (prepared by X. Wang, Dept of Medical Microbiology and Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA). Following two 5 min washes in PBST (0.2 to 0.5 ml per slide), sections were incubated for 1 h at 37°C with a biotinylated goat anti-rabbit antibody (Vector Labs) diluted 1:250 in PBST. Slides were drained, washed twice with PBST and the reactive sites detected with an enzyme complex of avidin-biotin-alkaline phosphatase (ABC Elite, Vector Labs) and substrate DAB (3'3-diamino-benzidine, Vector Labs). Slides were incubated with the enzyme complex for 1 h at 37°C, rinsed with PBST as described above and the substrate applied. Color development was evident within 8 to 20 min and the reaction stopped by immersing the slides in water. Sections were dried overnight at 22 to 25°C, counter-stained with hematoxylin and mounted with glass coverslips.

RESULTS

Ichthyophthirius multifiliis infection was confirmed in 100% of exposed fish within 5 d of exposure. By 7 d, the fish were anorectic and depressed. At 10 d, they were euthanized due to a severe, debilitating second-round infection. Gross morphological lesions observed in these fish included pale gills, pale livers and empty gastrointestinal tracts. Control fish remained free of parasites and collected tissues were normal.

Ichthyophthirius multifiliis trophonts were easily identified in skin and gill sections collected 5 and 10 d post-exposure. In the skin, they primarily occurred as single parasites; however, multiple trophonts were occasionally observed in close proximity to each other (Fig. 1A). Infected fish skin had areas of epidermal hyperplasia, disrupted cellular integrity and necrotic foci as well as areas that appeared normal. Alarm cells, a common feature of normal catfish skin, were disrupted, distorted or contained cytoplasmic vacuoles. Skin collected from fish 10 d after infection showed the greatest degree of pathology. In the gills, trophonts were observed at the base of primary filaments as well as within gill epithelia (Fig. 1B). By 10 d, primary filaments were severely blunted and epithelial hyperplasia occluded the majority of secondary filaments. Immunohistochemical staining for parasite antigen allowed visualization of parasite external membranes (Fig. 1A,B). Histopathological changes were not observed in the head kidney, renal kidney, spleen or livers of infected fish.

Four of 20 *Ichthyophthirius multifiliis*-infected fish intestines were found to contain trophonts. One fish examined 5 d after infection and 3 fish examined 10 d after infection, had single parasites embedded within abdominal cavity adipose tissue (n = 1), associated with intestinal serosa (n = 1) or adjacent to mesenteric blood vessels (n = 2). One parasite was found in a hematoxylin and eosin-stained section (Fig. 2A) while the other 3 were found in sections stained for parasite antigen. Other than their location, these parasites appeared similar in morphology and antigen staining pattern to those found in the skin and gills. Histopathological evidence of a host response to *I. multifiliis* within the peritoneal cavities was limited. Cells of host origin were observed in contact with a trophont found

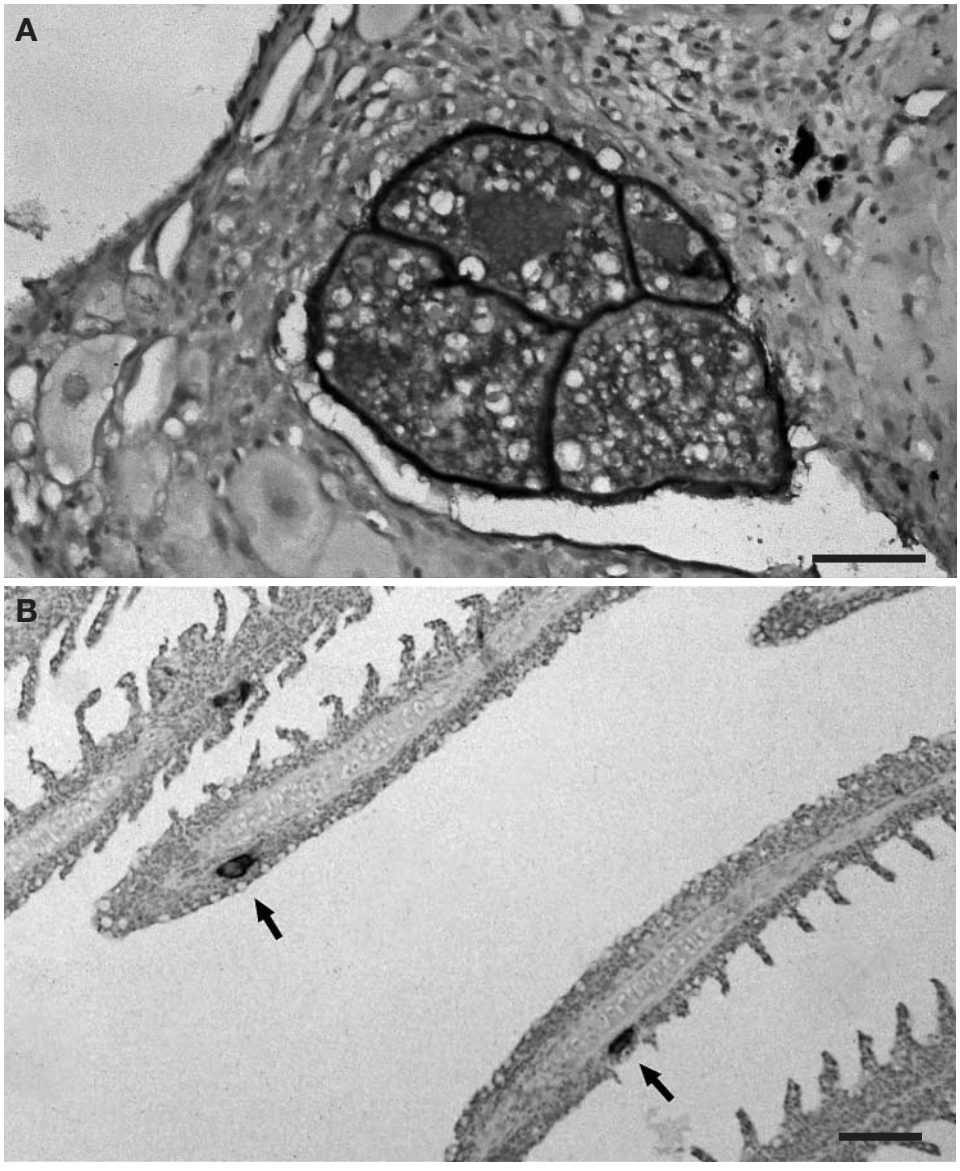

Fig. 1. Light photomicrographs of *Ichthyophthirius multifiliis* trophonts within the epidermis and gills of channel catfish stained for parasite immobilization antigen (i-Ag). Sections were blocked with normal goat antisera and stained with a rabbit anti-i-Ag antibody (1:3000) (X. Wang). A goat anti-rabbit biotin secondary antibody (1:250) was applied followed by an avidin-biotin-alkaline phosphatase complex (ABC Elite, Vector Labs) reagent, DAB (3′3-diaminobenzidine, Vector Labs) and counter-stained with hematoxylin. (A) Multiple trophonts within the epidermal layer of the skin (750×) (scale bar = 20 µm). (B) Individual trophonts in gill epithelium (arrows) (220×) (scale bar = 50 µm)

between 2 loops of intestine, suggesting a mild host inflammatory response (Fig. 2B).

DISCUSSION

Finding *Ichthyophthirius multifiliis* trophonts within the peritoneal cavities of recently infected channel catfish suggests that this ciliated protozoan parasite may occur in tissues other than skin and gills during the course of a normal infection. Our findings add to the single previous report of *I. multifiliis* within the cerebral space of a heavily infected carp hybrid. In this study, host-origin cells were observed in association with one trophont located between loops of intestine. The presence of a mild inflammatory response supports these results as not being a processing or sectioning artifact. Even so, additional histological observations are necessary to determine the frequency of this phenomenon.

Ichthyophthirius multifiliis trophonts are typically large enough to observe in fish skin and gills without immunohistochemical enhancement. In this experiment, infected catfish tissues were stained to determine if i-Ag is secreted during tissue migration. Unexpectedly, these methods also facilitated the discovery of trophonts in the peritoneal cavity, a previously unknown site of parasite entry. In retrospect, it is likely that 3 of these atypically located parasites would not have been found without parasite antigen-specific staining.

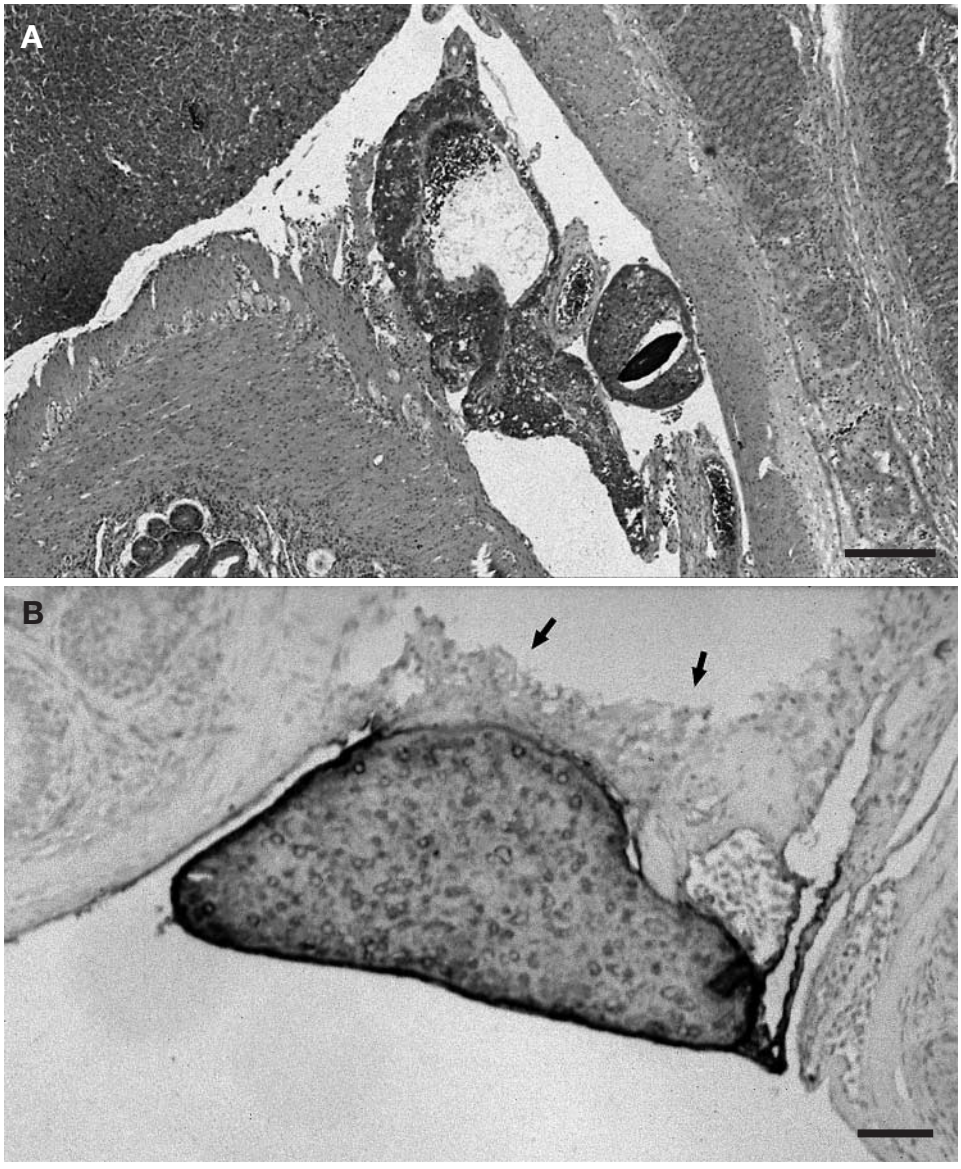

Fig. 2. Light photomicrographs of *Ichthyophthirius multifiliis* trophonts within the peritoneal cavity of 2 channel catfish. (A) A trophont is located adjacent to intestines and near mesenteric blood vessels (H&E) (226×) (scale bar = 53 µm). (B) Immunohistochemical staining of a trophont between 2 loops of intestine. Host-origin cells, suggestive of an inflammatory response, are adjacent to the parasite (arrows) (475×) (scale bar = 21 µm)

Demonstrating *Ichthyophthirius multifiliis* trophonts within the peritoneal cavities of catfish does not reveal their method of entry. Three possible routes are postulated. One would be through the esophageal wall, another would require penetration of the pneumatic duct, a thin-walled structure connecting the esophagus to the swim bladder (Grizzle & Rogers 1976). A third route would be a retrograde migration from the anus into the rectum and through the intestinal wall. Penetration of any of these structures would conceivably result in a trophont entering the peritoneal cavity. To date, however, *I. multifiliis* has not been shown to penetrate submucosa or tissues other than skin or gill epithelia.

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LITERATURE CITED

Cross ML, Matthews RA (1993) Localized leucocyte response to *Ichthyophthirius* establishment in immune carp *Cyprinus carpio*. Vet Immunol Immunopathol 38:341–358

Dickerson HW, Dawe DL, Gratzek JB, Brown J, Pyle SW (1981) Induction of *Ichthyophthirius multifiliis* Fouquet infections in channel catfish, *Ictalurus punctatus* Rafinesque: standardization of the procedure. Fish biologics:

serodiagnositics and vaccines. Dev Biol Stand 49:331–336
Dickerson HW, Lohr AL, Gratzek JB (1985) Experimental intraperitoneal infection of channel catfish, *Ictalurus punctatus* (Rafinesque), with *Ichthyophthirius multifiliis* (Fouquet). J Fish Dis 8:139–142
Grizzle JM, Rogers WA (1976) The digestive system. In: Anatomy and histology of the channel catfish. Agriculture Experimental Station, Auburn University, Auburn, AL, p 27–28
Hines RS, Spira DT (1974) *Ichthyophthirius multifiliis* (Fouquet) in the mirror carp *Cyprinus carpio* L., III. Pathology. J Fish Biol 6:189–196
Ventura MT, Paperna I, (1985) Histopathology of *Ichthyophthirius multifiliis* infections in fishes. J Fish Biol 27:185–203

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