Nodular cap disease in the red Oranda and red cap breeds of goldfish (*Carassius auratus*) associated with *Dermocystidium* species

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Abstract

Ornamental fish constitute an expanding sector of fish industry in Egypt. Several native, naturalized and exotic fish species are known to be raised by fish hobbyists. Among freshwater aquarium fish, goldfish (*Carassius auratus*) is considered among the most popular and expensive fish species (70 LE per adult fish retail prize). Apart from its importance as an aquarium fish, goldfish presents a problem due to its likelihood role in disease transmission for native and naturalized cyprinid fish in Egypt. This has invigorated the interest to examine imported fish species and study their role in transmission of diseases to other fish species in Egypt. Most recently, a disease with a substantial morbidity and low mortality rate has been identified among imported capped breeds of goldfish. The affected fish showed whitish nodules in the cap region of the head. Upon examination, the nodule oozed milky fluid, leaving hemorrhagic lesions that were refilled with milky fluid 1-2 weeks later. Erosion and ulceration have been reported in some fish during late stages of the disease. Dermocystidium spp. has been associated with the disease and detected in the nodules and milky fluid. The clinical signs of the disease, some epizootiological data, parasite morphology and the histopathological criteria of the lesions are detailed in the present study.

Introduction

Goldfish (*Carassius auratus*) is considered among the most important ornamental fish in Egypt. Currently, several fish farms raise goldfish and other ornamental fish and form a significant proportion of the fish industry in Egypt. However, continuing regular import of some exotic breeds of ornamental fish could present a real threat of disease transmission to native and naturalized fish species in Egypt.

Dermocystidium, a pathogen of unclear taxonomy, is associated with diseases in different fish (Hatai, 1989). Because of the presence of spores and, in some cases hyphae, it has been believed to be a fungal infection (Allen *et al*., 1968; Cervinka *et al*., 1974; Wildgoose, 1995), however, others have described it as haplosporidian sporozoan (Reichenbach-Klinke, 1950; van Duijn, 1973). A recent breakthrough into phylogenetic affinities revealed

that Dermocystidium belongs to a clade Mesomycetozoa, a group of microorganisms near animal-fungal divergence which include (Dermocystidium, Rosset agent, Ichthyophonus, Psorospermium and Rhinosporidium)(Herr, *et al*., 1999).

In fish, dermocystidiosis is characterized clinically by formation of white cysts (0.1 - 4.0 mm) filled with white milky fluid on skin and gills. The cyst is enclosed in hyaline capsule and contains a 3 - 10 mm spherical spore with characteristic central refractile vacuole and peripherally located cytoplasm and nucleus (Noga, 1996). Various species of dermocystidium have been reported in fish. *Dermocyctidium koi* (*D.* koi) was detected in the skin of Koi carp (*Cyprinus carpio*) (Wildgoose, 1995), while *D. salmonis* was isolated from the gills of salmon (Davis, 1947). Other species have been reported from pike, perch and eel (Hatai, 1989). However, a complete overview of the disease in fish is lacking, since no detailed description of the disease clinical signs, host susceptibility, and mortality rate have been reported.

In the present study, dermocystidium spp. was detected in nodular lesions of goldfish (*Carassius auratus*) for the first time in Egypt. The fish susceptibility, clinical, parasitological and histopathological findings of the disease were thoroughly studied for a complete overview of such fish disease.

Materials and Methods:

Case History:

A total of 262 Goldfish (*Carassius auaratus*) of different breeds, size and age were imported from Singapore in August 2000, among ornamental fish shipped to aquarium fish dealers in Egypt. In addition to goldfish, the shipment included other fish species such as, Angelfish (*Pterophyllum scalare*), Siamese Fighter (*Betta splendens*), Dwarf Gourami (*Colisa lalia*), Tiger barb (*Barbus tetrazona*), Platy (*Xiphophorus maculatus*), Guppies (*Poecilia reticulata*), and Koi carp (*Cyprinus carpio*).

Goldfish and koi carp of varying size were kept in the same glass aquaria (120 X 50 X 50 cm) with a density of 50 fish/aquarium. Small fish species (guppies, platy, angelfish, tiger barb and dwarf gourami) were stocked with a density of 200 fish/aquarium. Males of Siamese fighter were kept in separate containers (one male/ drinking disposable plastic cup). The aquaria were supplied with dechlorinated tap water and Resun aerator, AC9908 (Guandong Risheng Group Corp. Ltd., China) at 27 ±1 °C. The fish were fed at a rate of 3% of their body weight daily on Takara floating pellet (Buatan Malaysia Kian Weng Trading Co., Malaysia).

Parasitological Examination

Simple wet mount preparations from the milky fluid extracted from the nodules at the head region of affected fish were examined. Preparations were fixed and stained with Giemsa according to Krus and Britchard (1982) for further parasitological identification according to Lom and Dykova (1992).

Histopathological Examination

Tissue specimens from the head of affected fish were prepared and fixed in 10% buffered neutral formalin, dehydrated in alcohol and cleared in xylene. Paraffin embedded sections (4-6 um) were stained with hematoxylin and eosin (H&E) according to Carleton (1976).

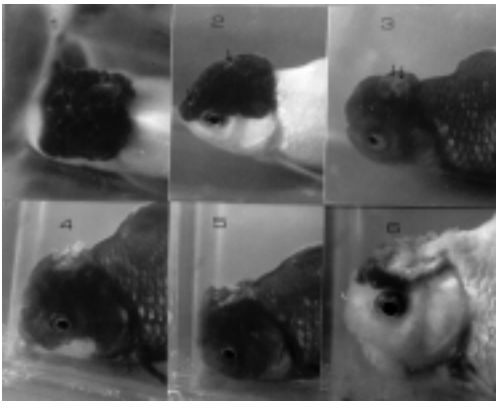
Fish species	Number	Mean length (cm)	No. infected fish	Stage
Redcap	60	7±1	0	
Redcap	21	13±2	8	3 stage I, 2 stage II, 2 stage III, 1 stage IV
Red Oranda (Hi Cap)	34	8±1	0	
Red Oranda (Hi Cap)	73	13±2	45	36 stage I, 3 stage II, 2 stage III, 4 stage IV
Calico	18	13±1	0	
Bubble-Eye	56	5±1.5	0	
Total	262		53	

Table 1. Number and length of different breeds of goldfish (Carassius auratus) naturally infected with *Dermocystidium* spp.

Results

Two weeks after arrival, goldfish started suffering from whitish nodules or cysts oozing milky fluid on the head. Erosion and ulceration in the place of the nodules were noticed in some fish. Fish behavior did not change during the early stage (nodules) of the disease. However, during late stage of the disease (erosion and ulceration), fish became lethargic, stopped feeding and stayed at the bottom of aquarium most of time. Out of 262 imported goldfish (*Carassius auaratus*), 53 fish (20.2%) showed whitish nodules on the head region (Table 1). Gross examination of affected fish revealed raised white nodular cysts measured 0.5 - 3 mm in diameter and restricted to the raised cap area of the head of the capped breeds of goldfish (Red Oranda and Red cap) (Fig 1&2). No similar lesions were found in other non-capped goldfish breeds (ordinary goldfish, blackmoor, bubble eye and calico), small goldfish of all breeds or contacted koi carp.

The cysts usually oozed whitish milky fluid on examination leaving reddish inflamed spots that refilled with milky fluid 1- 2 weeks later (Stage I) (Figs. 1 & 2). Cysts in some affected fish were diffused to the surrounding tissue forming whitish patch on the head (Stage II) (Fig 3). The formed patch became softer in consistency than the surrounding head cap tissue. Some fish showed erosion of the nodules leaving shallow ulcer with mac-



Figures 1 & 2. Red cap breed of goldfish (*Carassius auratus*) showing whitish nodules (arrows) on the head region (Stage I). Figure 3. Red Oranda breed of goldfish (*Carassius auratus*) showing diffuses nodules to the surrounded tissue (arrows) of the caped area of the head (Stage II). Figures 4 & 5. Red Oranda breed of goldfish (*Carassius auratus*) showing erosion and shallow ulceration of the capped area of the head with macerated and shredded tissues (Stage III). Figure 6. Red cap breed of goldfish (*Carassius auratus*) showing severe ulceration of the cap area of the head with exposure of the skull bone (StageIV).

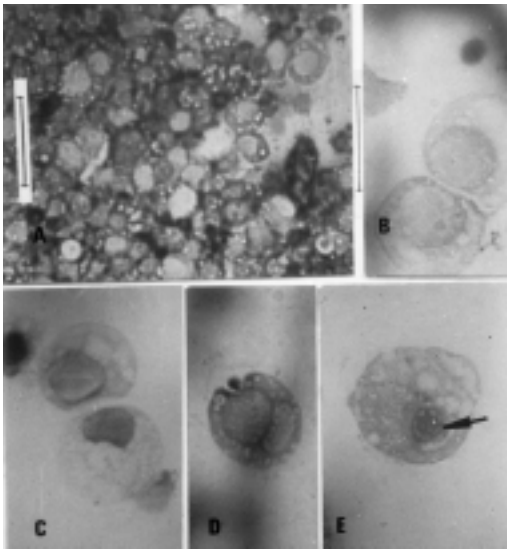


Figure 7. A) Spores of Dermocystidium species detected in milky fluid oozed from whitish cyst and skin scrapping from macerated head region of redcap and red oranda breeds of goldfish (*Carassius auratus*). (Geimsa stain. Scale bar = 0.02 mm) B, C) Spores of Dermocystidium showing variable sized vacuoles, peripherally located nucleus and different stages of divisions (Geimsa stain. Scale bar = 0.02 mm). D) Spores of Dermocystidium showing variable sized vacuoles, peripherally located nucleus (Geimsa stain. Scale bar = 0.02 mm). E) Spores of Dermocystidium showing prominent inclusion body (arrow) (Geimsa stain. Scale bar = 0.02 mm).

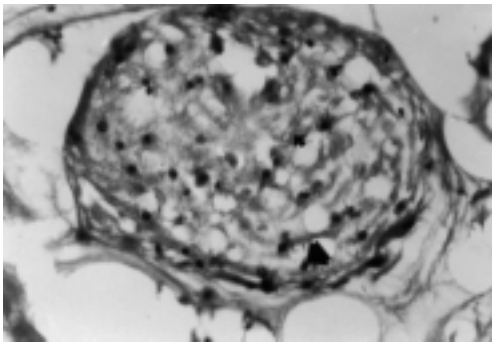


Figure 8. Head region of goldfish affected by Dermocystidium species cyst. The cyst showed the Dermocystidium species spores (Large arrow) and remnant of necrotic tissues (small arrow), with scattered mononuclear cells. (H&E stain. X 100)

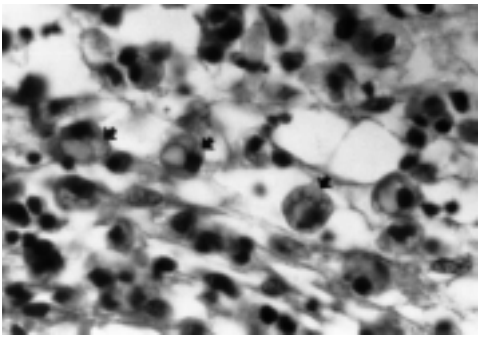


Figure 9. Spores of Dermocystidium species showed vacuoles of different sizes and peripherally located nucleus (Arrows) (H&E stain. X1000)

erated and shredded tissues (Stage III) (Figs. 4 & 5). In severe cases, maceration and ulceration progressed and the cap area completely sloughed with subsequent exposure of the skull bone (Stage IV) (Fig 6). Five fish died after the clinical signs progressed to stage IV. No cysts or whitish nodules were detected in internal organs upon inspection of affected fish.

Examination of wet mount and stained smears prepared from milky fluid oozed from the cyst as well as skin scrapping of ulcerated

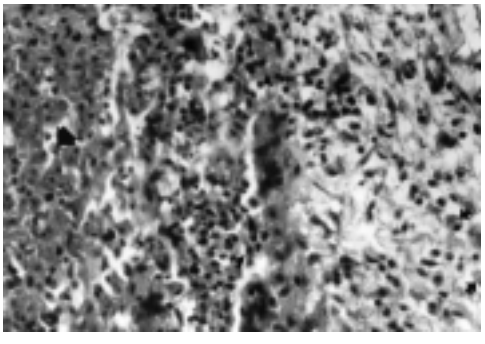


Figure 10. Histopathological findings of the head region of the goldfish affected by Dermocystidium cyst. The cyst showed large number of spores (small arrows). Area of hemorrhages was detected (Large arrow) with mononuclear cells infiltration (head arrow). (H&E stain. X 400).

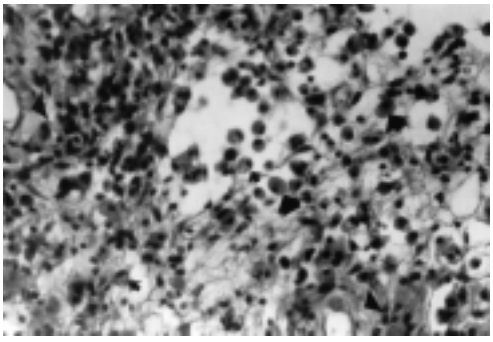


Figure 11. Histopathological findings of the head region of the goldfish affected by Dermocystidium cyst showed multiple Dermocystidium spores (small arrows), large number of melanoblast cells (large arrows) and perivascular edema (head arrow) (H&E stain. X400)

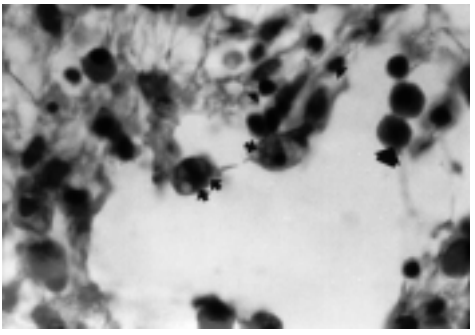


Figure 12. Spores of Dermocystidium species (small arrow), surrounded with melanoblasts (large arrow). Some spores showed division with enlarged cell size and elongated nucleus (2 small arrows) (H&E stain. X1000).

and macerated head regions revealed presence of large numbers of round to oval spores of variable size (9 - 36 µm X 6 – 33 µm range) (Fig 7A). However, stained spores were smaller in size than those seen in fresh preparations. The spores possess a highly vacuolated cytoplasm, with a large nucleus (1.5 – 6 µm X 3 – 22 µm range) that mainly located peripherally (Fig 7 B, C and D). Different stages of divisions were also observed in the examined spores (Fig 7 B and C). Inclusion body in its initial stage of constriction was also observed in some spores (6 - 21 µm Ø) (Fig 7 E).

Histopathological investigations for sections from nodular lesions revealed round to oval cysts. Each cyst contained large numbers of spores aggregated together by a very thin layer of fibrous connective tissue capsule and contained remnant of necrotic tissues and scattered mononuclear cells (Fig 8). The spores showed highly vacuolated body with peripherally located cytoplasm and nucleus. The eccentric nucleus appeared voluminous with condensed chromatin (Fig 9). Focal area of hemorrhages with perivascular edema was also observed. Moreover, large number of mononuclear and melanoblast cells infiltration with lysis of host tissue have also been noticed (Fig 10 -12).

Discussion

Examination of goldfish revealed the presence of whitish cysts on the head region. The whitish cysts detected in the present study measured 0.5 - 3 mm and were restricted only to cap area of the head of capped goldfish breeds (Fig 1 &2). Similar whitish cysts have been reported in the skin, fins and gills of various fish species infected with Dermocystidium spp (Reichenbach-Klinke and Marsha, 1973). Likewise, site variation of nodules on the skin is a property of different dermocystidium spp. in various fish species. *Dermocystidium koi* (*D. koi*) has been isolated from the skin and fins of *Cyprinus carpio* (Wildgoose, 1995), while *D. salmonis* and *D. anguillae* have been reported on the gills of Chinook salmon (*Onchorhynchus tshawyscha*) and eel (*Anguilla anguilla*)(Hatai, 1989).

	Host	Habitat	Cyst	Spore	Origin	Reference
D. branchialis	Brown trout	Gills	Round	Round 7-8μm Ø* 6.7μm IB**	Ireland Switzerland Former USSR	Leger, 1914
D. veydovskyi	Pike	Gills	Round (0.1 mm)	Round or oval 3.5-4 μm x 3-4 μm	Danube Volga basin	Jirovic, 1939
D. salmonis	King salmon	Gills	Round (1 mm)	Round 8-12μm Ø	Pacific America Former USSR	Davis, 1947
D. truttae	Trout	Gills	Round (1 mm)	Round 8-12 μm Ø		Weiser, 1949
D. percae	Perch	Fins and skin	elongated (1mm x 0.4-0.8mm)	Round 6-7.7μm Ø 5-6.25 μm IB	Danube Volga basin	Reichenbach-Klinke, 1950
Dermocystidium sp.	Eel	Gills	Spherical (1-2mm)	Round 10-12 μm Ø 5-6 μm IB	Baltic sea	Timoleev, 1962
Dermocystidium sp.	Ussurian white fish	Gills	Spherical (0.1-1,5 mm)	Round 5.7-7.7 μm Ø 2.2-4.4 μm IB 2μm Ønucleus	Amur river basin	Timoleev, 1962
D. anguillae	Eel	Skin	Dumbbell shaped 0.8-1.0mm x 3.8mm	Round 4.8-8.5μm Ø 2.4-7.3 μm IB	Europe	Spangenberg, 1975
D. koi	*Cyprinus carpio*	Fins and skin	Round (upto 10mm)	Round 8.89-14.6 μm Ø	London, UK	Hoshina & Sahara, 1950
Dermocystidium sp.	Carassius auratus (Capped breeds)	Head	Round (0.5-3 mm)	Round-oval 9-36μm x 6-33 μm 6-21 μm IB 1.5-6μm x 2-22 μm nucleus	Egypt	Present study

Table 2. Comparison between Dermocystidium cysts and spores detected in previous studies compared to those of the present study

Round non-motile spores were recovered from milky fluid squeezed from white cysts upon parasitological examination of wet mount and stained smears. The refractile central vacuolated cytoplasm and eccentric nucleus, characteristic of dermocystidium spores, were identified in the present samples (Fig 7 A and B)(Table 2). However, the present spores were larger than spores identified in the previous studies, suggesting that a different species of dermocystidium may be responsible for the present disease condition.

The whitish nodules were restricted only to the head region of exotic capped breeds of goldfish namely, red cap and red Oranda. The reported morbidity rate was 20.2% of the total number of goldfish examined. Thorough investigation of infected fish revealed that the infection was limited only to certain age and breeds. Adult red cap and red Oranda showed morbidity rate of 38.1 and 61.6% respectively. While small fish of all goldfish breeds showed no lesions. This fact could be attributed to the susceptibility of certain age and breed of goldfish to Dermocystidium infection and/or the long incubation period needed to produce the lesion after infection. Wildgoose (1995) reported similar finding concerning restriction

of dermocystidium infection to a particular age group. Dermocystidium cysts were reported in older koi carp (*Cyprinus carpio*) (1-4 years old). Similarly, Dermocystidium infections reported by Davis (1947) and Pauley (1967) were limited only to adult Chinook salmon (*Onchorhynchus tshawysch*).

Koi carp maintained with diseased goldfish did not develop similar skin lesions and could be as a result of host susceptibility to Dermocystidium infection. Comparable findings have been reported by Wildgoose (1995) who found that cohabiting goldfish did not develop the clinical skin lesions identified in koi carp.

Five fish died after the clinical signs progressed to stage IV, comprising a mortality rate of 9.4% of adult susceptible capped breeds. Similar signs, yet higher mortalities (16-20%) were recorded by Hoskins *et al*., (1976) during a Dermocystidium epizootic in sockeye salmon (*Onchorhynchus nerka*). Nevertheless, the extent of responsibility of Dermocystidium infection for such mortality would be difficult to evaluate in the present study.

Noticeable tissue damage and ulceration was noted in the late stages of the infection among affected goldfish. This ulceration was coincident with mononuclear cell infiltration and severe tissue lysis. This tissue damage could be attributed to certain factors released by Dermocystidium spores that cause damage to surrounding tissues. Studies on *Perkinsus (P.) marinus* (a closely related parasite and formerly named *Dermocystidium marinus*) have been able to identify extracellular proteases (LaPeyre and Faisal, 1995). The reported proteases were presumed to be the factor resposible for the tissue damage caused by *P. marinus* in bivalves (LaPeyre and Faisal, 1995).

In conclusion, the clinical signs in goldfish concurrent with the characteristic spores and histopathological data suggest that the parasite belongs to Dermocystidium spp. The host susceptibility and the available epizootiological data suggest that the identified Dermocystium is a new species. However, ultrastructural studies, isolation, and molecular identification are required to confirm this suggestion.

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