J. Comp. Path. 2014, Vol. 150, 316–324

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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Comparative Study of Infection with *Tetrahymena* of Different Ornamental Fish Species

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Summary

Tetrahymena is a ciliated protozoan that can infect a wide range of fish species, although it is most commonly reported in guppies (*Poecilia reticulata*). The aim of this study was to compare the susceptibility to infection with *Tetrahymena* of five different ornamental fish species from two different super orders. The species examined were platy (*Xiphophorus*), molly (*Poecilia sphenops*) and angelfish (*Pterophyllum scalare*) of the Acanthopterygii super order (which also includes guppies) and goldfish (*Carassius auratus auratus*) and koi carp (*Cyprinus carpio*) of the Ostariophysi super order. These two super orders are phylogenetically distant from each other. Infection with *Tetrahymena* resulted in parasite invasion of internal organs, skin and muscle in all fish species. A relatively strong inflammatory response was observed in infected goldfish and koi, with negligible response in fish species of the Acanthopterygii super order. Guppies were the most susceptible to *Tetrahymena* infection, exhibiting a mortality rate of 87% and 100% in two separate experiments. A high mortality rate was also observed in platy (77%), while that of molly and angelfish was significantly lower (23% and 33%, respectively). Goldfish and koi carp were less susceptible to infection compared with guppies (24% and 59% mortality, respectively). Immunization studies revealed that the *Tetrahymena* are immunogenic, since infection of koi carp increased their *Tetrahymena* immobilization response by approximately three-fold at 3 weeks post infection, while immunization with *Tetrahymena* plus adjuvant increased their immobilization response by approximately 30-fold.

Keywords: fish; histopathology; protozoa; *Tetrahymena*

Introduction

Tetrahymena spp. is a ciliated protozoan of the phylum ciliophora (Corliss, 1952). It is a saprozoic ciliate that feeds on organic matter and bacteria in natural habitats (Ponpornpisit *et al.*, 2000). This protozoan is common in nature and appears to have no geographical limits (Hoffman et al., 1975). *Tetrahymena* spp. is the causative agent of tetrahymeniosis or 'tet disease', also known as 'guppy killer disease' in tropical aquarium fish, which causes severe economic losses in commercial guppy farms worldwide. *Tetrahymena* is an invasive pathogen, which has predilection for guppies for reasons that are not clear, but infections have been reported in other species of ornamental fish, edible fish and even freshwater leeches (*Nephelopsis obscura*). The latter are themselves parasites of trout (*Salmo* spp.) and can therefore transfer the protozoa to their host (Saglam and Sarieyyupoglu, 2002). Ornamental fish species reported to be infected with *Tetrahymena* spp. include zebrafish (*Danio rerio*; Astrofsky *et al.*, 2002), angelfish (*Pterophyllum scalare*), platy (*Xiphophorus variatus*), neon tetra (*Paracheirodon innesi*) (Ponpornpisit *et al.*, 2000; Pimenta Leibowitz *et al.*, 2005) and the bristle-nosed catfish (*Ancistrus* spp.; unpublished).

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2013.08.005

Susceptibility to *Tetrahymena* increases in fish that are wounded and/or weakened by stress conditions, such as high ammonia level, high organic load, extreme water temperature, non-optimal shipment conditions (e.g. high fish density) or a disease (Ferguson *et al*., 1987; Imai *et al*., 2000; Hatai *et al*., 2001; Pimenta Leibowitz *et al*., 2005).

In the present work we tested parameters related to *Tetrahymena* infection of different ornamental fish species. Species selected for this study are popular ornamental fish that are often cultured in close proximity on individual farms. Our aim was to study the threat that this infection, common in guppies, poses on other fish species that are cultured in the same system.

Guppy (*Poecilia reticulata*) is native to the Trinidad Island and the northern part of South America, where it lives in natural water at 24–30°C and exhibits tolerance to a wide range of salt concentrations. There are ~300 subspecies of guppies and they are among the most popular ornamental fish species, widely traded across the world. Molly (*Poecilia sphenops*) is native to Central and South America (Mexico to Colombia) where it lives in tropical water at a temperature range of 18–28°C. Platy is a common name for two related freshwater species of the *Xiphophorus* genus, the Southern platy (*Xiphophorus maculatus*) and the Variatus platy (*X. variatus*), both native to the Eastern coast of Central America and Southern Mexico. Most ornamental platy are hybrids of both species, which have been interbred to the point that they are difficult to distinguish. Platy lives in temperatures ranging between 18 and 25°C. The origin of angelfish is the Amazon, Orinoco and Essequibo rivers in the tropical South America. Its Latin name, *Pterophyllum*, means 'winged leaf' and it is among the most common aquarium species, prized for its unique shape, colour and behaviour. Angelfish live in tropical freshwater at temperatures ranging between 24 and 30°C and reach a maximal length of 7.5 cm. The guppy, molly, platy and angelfish belong to the super order Acanthopterygii (spiny finned fish).

Goldfish (*Carassius auratus auratus*) was one of the first fish species to be domesticated and is the most commonly kept aquarium fish. It is closely related to the less colourful carp, *C. auratus*, which is native to Eastern Asia and was domesticated in China more than a thousand years ago. The freshwater subtropical goldfish inhabits rivers, lakes, ponds and ditches with stagnant or slow-flowing water. It lives better in coldwater. The koi (meaning 'carp' in Japanese), or more specifically nishikigoi, represents ornamental varieties of domesticated common carp (*Cyprinus carpio*) that are kept for decorative purposes in outdoor ponds or water gardens. Koi carp and goldfish belong to the family Cyprinidae, super order Ostariophysi.

The relative susceptibility of the different fish species to infection with *Tetrahymena* was analyzed and a comparative histopathological analysis carried out.

Materials and Methods

Fish

Naive guppy, platy, molly, angelfish, koi and goldfish were obtained from commercial aquaculture farms in Israel and acclimated for a minimum of 2 weeks before experimentation. Fish were maintained at a water temperature of 25 ± 1°C. Feeding occurred daily at 2% of the body weight (Tropical Orange, Tzemah, Israel, for the tropical ornamental species and Hazorea Food, Ranan Marketing, Israel, for the coldwater species, koi and goldfish).

To maintain adequate water quality, submerged biological filters were used and 40% of the water was exchanged every other day. Water quality parameters were monitored weekly and ammonia, nitrite and nitrate were measured by visocolour kits (Macherey–Nagel, Düren, Germany). Ammonia and nitrite levels were maintained at >0.5 ppm and nitrate levels were maintained at 5–10 ppm. The water pH was kept constant at 7.6 (pH meter, Eutech Instruments, Singapore) and dissolved oxygen was maintained at >80% saturation (YSI 52-dissolved oxygen meter, YSC incorporated, Yellow spring, Ohio, USA). Fish were treated in compliance with the principles for biomedical research involving animals. The experimental protocol was approved by the Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments (http://in.bgu.ac.il/fohs/AnimalFacility/Pages/default.aspx), authorization numbers IL-67-11-2002 and IL-51-8-2008.

Tetrahymena *Maintenance*

The *Tetrahymena* spp. (Tet-NI) used in this study was originally isolated at our laboratory in 2005 from guppies imported to Israel by a commercial fish farm. The fish were found to be infected with *Tetrahymena* sp. during the quarantine stage, brought to our laboratory and stocked in 10 litre aquaria. Comparative DNA barcode analysis indicated that the parasite Tet-NI, was a new species of *Tetrahymena* (Chantangsi *et al*., 2007).

The disease-causing parasite was maintained *in vivo* and *in vitro*, as described by Pimenta Leibowitz and Zilberg (2009). Briefly, in-vivo infection was maintained in two separate containers of 100 litres each by the regular addition of naïve fish to replace