Histopathological study on parasites in freshwater ornamental fishes in Iran

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Received: 15 March 2014 / Accepted: 8 September 2014 / Published online: 5 October 2014
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Abstract During March 2012 through February 2013, 100 freshwater ornamental fishes in 22 species from some aquarium fish shops were examined. Specimens were dissected and tissue samples consisted of liver, kidney, spleen, heart, intestine, ovary, brain and eye were fixed in 10 % buffered formalin and sections were provided and stained with hematoxylin and eosin, Periodic Acid-Schiff, Giemsa and acid-fast staining (Ziehl-Neelsen). At present study six species of protozoans consisting of *Eimeria* spp., *Cryptosporidium* spp., *Tetrahymena corlissi*, *Thecamoeba* spp., *Giardia* spp., *Myxobolus* spp. and two metazoan parasite consisting of *Nematoda* spp. and *Benedenia monticelli* were identified. *Thecamoeba*, *B. monticelli* and *Cryptosporidium* spp. were not reported in previous Iranian studies and it is the first report of infestation to this parasite in ornamental fish in Iran.

Keywords Histopathologic method · Iran · Ornamental fish · Parasite

Introduction

Ornamental fish farming is one of the most valuable industries in recent years. Ornamental fish are often referred as living jewels due to their color, shape and behavior. They are peaceful, generally tiny, attractively colored and could be accommodated in confined spaces.

In the aquatic environment, ornamental fish are in intimate contact with a wide range of pathogenic and non-pathogenic microorganism, which can result in serious economic losses (Aly et al. 2008). Parasitism is one of the most impacting problems with predominant signs of weight loss, disruption of reproduction or impotency, blindness, abnormal behavior, epithelial lesions, deformities of gills and other symptoms that result in economic loss in ornamental fish industry.

Although the ornamental fish industry in Iran has tremendous potential for development, one of the weaknesses observed is the lack of understanding of different disease situation. However, there are some studies on the identification of external parasites in some ornamental fish (Mehdizadeh et al. 2009; Raissy et al. 2010; Pazooki et al. 2007), there is a lack of information on the ornamental fish parasites especially endoparasites. Therefore, present study was undertaken to identify the ornamental fish parasites in 100 fish samples from some aquarium fish shops by histopathological method.

Materials and methods

During the period of March 2012 through February 2013, one-hundred freshwater ornamental fishes in 22 species (Table 1) from some aquarium fish shops were examined. Collected fish showed the signs of diseases such as

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Table 1 The species and number of ornamental fish examined

Fish species	Common name	Number examined
<i>Poecilia latipinna</i>	Sailfin molly	16
<i>Botia almothae</i>	Yo-Yo loach	2
<i>Puntius conchoni</i>	Rosy barb	9
<i>Poecilia reyculata</i>	Guppy	4
<i>Aulonocara baenschi</i>	Peacock cichlid	4
<i>Xiphophorus</i> spp.	Platy	5
<i>Pterophyllum scalare</i>	Angelfish	6
<i>Aequidens rivulatus</i>	Green terror	2
<i>Carassius auratus</i>	Goldfish	13
<i>Balantiocheilos melanopterus</i>	Silver shark	2
<i>Trichogaster leeri</i>	Pearl gourami	10
<i>Corydoras</i> spp.	Cory	2
<i>Astronotus ocellatus</i>	Oscar	9
<i>Betta splendens</i>	Betta	2
<i>Cichlasoma hualorhan</i>	Flower horn	2
<i>Heros severus</i>	Severum	2
<i>Cichlasoma parrot</i>	Parrot	1
<i>Xiphophorus helleri</i>	Swordtail	2
<i>Epalzeorhynchus frenatum</i>	Rainbow	2
<i>Symphysodon aequifasciatus</i>	Discus	1
<i>Puntius tetrazona</i>	Green barb	2
<i>Labidochromis caeruleus</i>	Macro	2

anorexia, listlessness, blindness, epithelial lesions, distended abdomen, and deformity of gills.

At the laboratory, small fish less than 2 cm in length were preserved in 10 % buffered formalin as a chemical fixative. Larger specimens were dissected and tissue samples such as liver, kidney, spleen, heart, intestine, ovary, brain and eye were collected and preserved in 10 % buffered formalin prior to staining. All sections were stained with hematoxylin and eosin (H&E), Periodic Acid-Schiff (PAS), Giemsa and acid-fast staining (Ziehl-Neelsen).

Results

A list of parasites identified from different body locations in different fish species has been presented in Table 2. Six species of protozoans, one round worm and one monogenean parasites were observed at this study.

Specimens of *Cryptosporidium* were identified by PAS and H&E staining methods. The identification of *Eimeria* spp., *Tetrahymena corlissi*, *Thecamoeba*, *Giardia*, *Myxobolus* and *Benedenia monticelli* was confirmed by Giemsa and H&E staining methods.

Table 2 Parasites recovered from different body locations of ornamental fish

Parasite	Host fish species	Body location	Percent of infected fish
Protozoa	<i>Eimeria</i> spp.	Oscar	11
		Goldfish	
		Rosy barb	
	<i>Cryptosporidium</i> spp.	Molly	17
		Angel fish	
	<i>Tetrahymena corlissi</i>	Angel fish	1
Metazoa	<i>Thecamoeba</i> spp.	Guppy	2
		Goldfish	
	<i>Giardia</i> spp.	Oscar	1
	<i>Myxobolus</i> spp.	Oscar	2
		Goldfish	
	<i>Nematoda</i> spp.	Goldfish	5
		Molly	
		Oscar	
	<i>Benedenia monticelli</i>	Rosy barb	2
		Guppy	

Discussion

Although a wider spectrum of parasites in ornamental fishes were expected, the failure to find certain groups of parasites might be due to a number of reasons. Firstly, the fish holders perform some chemotherapy in ornamental fishes when they encounter some mortality in their aquarium fishes which result in fewer spectrums of parasites. Secondly, not complete parasitological examinations were made for all collected fishes even though in previous studies just wet smears were prepared to determine the parasitic infections in ornamental fishes. In general, infected fishes were found from 12 of the 100 ornamental fish species. The majority of fish species were infected with protozoan parasites especially *Cryptosporidium* spp. and *Eimeria* spp.

Many species of ‘true’ coccidian (e.g. *Calyptospora*, *Cryptosporidium*, *Eimeria*, *Epieimeria*, *Goussia* and *Isospora*) have been identified in fishes, with infection occurring in both freshwater and marine fishes species. ‘True’ coccidian are exclusively intracellular tissue parasites, although many coccidian species develop in intestinal epithelial and sub-epithelial cells of fish host. This is also very important problem in the carp and goldfish culture. For example, two of the most known *Eimeria* are *Eimeria subepithelialis* and *E. carpelli* which cause serious diseases in carp culture. Various development stages of coccidian can be observed in histological sections (Bruno et al. 2006). In this study microscopic

characteristics of coccidiosis namely trophozoites, schizonts and gamonts, and even oocyst of *Cryptosporidium* spp. was observed in prepared sections from intestine of 11 and 17 fishes, respectively. The other microscopic characteristics of these parasites were congestion and mild inflammation in lamina propria and submucosa and catarrhal enteritis in location sites in intestinal epithelium (Steinhagen and Hespeler 1997). Infection with eimeriidae has been reported from the intestine of marine and freshwater fishes in Vietnam, Alaska and Canada (Gibson et al. 2011; Moles 2007; Molnar and Fernando 1974). Zanguee et al. (2010) have investigated the prevalence of *Cryptosporidium* species in 200 aquarium fishes of 39 different species in Western Australia by PCR amplification at the 18S rRNA locus. A total of 21 positives were detected by PCR (10.5 % prevalence) from 13 different species of fish. This is the first report of cryptosporidiosis in fishes that is identified by molecular methods. No previous studies have observed the presence of fish cryptosporidiosis in Iran. In our study, 17 out of 100 unhealthy fishes were infected with *Cryptosporidium* spp. Since some species of this protozoan are zoonotic, more studies on these species of cryptosporidium are needed.

In this study, *B. monticelli* was observed from rosy barb and guppy gills. This ectoparasite is a member of Capsalidae principally on marine fishes. No special fish host has been observed for this parasite in Middle East, Mexico and Australia (Roberts 2001; Kohn et al. 2006; Byrnes 1986). This is normally found attached to the oral integument, but in heavy infections the parasite may spread to other parts of the body. In our study, this parasite was determined in fish gill region which is the first report in Iran.

In the current study, *T. corlissi* was observed in angle fish liver. This ciliated protozoan has been reported from a variety of fresh water ornamental fishes in USA and South Korea (Hoffman et al. 1975; Kim et al. 2002). In an experimental challenge on *Tetrahymena pyriformis*, Ponpornpisit et al. (2000) proved that guppy, *Pristella* and barb species are sensitive to this parasite. (Shafiei et al. 2009) also proved the presence of *T. corlissi* in Iran and explained that this parasite might enter by imported aquarium fishes to Iran.

In this study, *Thecamoeba* spp. was observed in blood vessel and gill lamella of in guppy and goldfish. This is an ectoparasite on the gill of marine and fresh water fishes. Microscopic lesions associated to this parasite were necrosis and hyperplasia of bronchial epithelium around the invading amoeba (Bruno et al. 2006). *Thecamoeba* was not reported in previous studies and it is the first report of infestation to this parasite in ornamental fishes in Iran.

In Oscar and goldfish gills, was also observed *Myxobolus* spp. In Iran, Mehdizadeh et al. (2009) recorded this parasite in goldfish. (Sanaullah and Ahmed 1980) reported heavy losses of major carp in Bangladesh caused by gill infection with *Myxobolus* spp. Although the vegetative

stages and spores are found in a variety of organs, the parasites often concentrate in the gill, causing destruction of much of the tissue, leading to hemorrhage, anemia and death Sanaullah and Ahmed (1980).

Although in this study *Nematoda* spp. were identified in intestine and gill of 5 specimen consist Goldfish, Molly and Oscar fish, but in pathologic sections diagnosis of their genus was not possible. Because classical keys are limit for parasite identification of fish, stained tissue sections are useful for recognizing morphological details and determining the life stages of parasite in the fish.

Acknowledgments The authors thank the University of Tabriz for financial support.

References

- Aly SM, Ahmed YAG, Ghareeb AAA, Mohamed MF (2008) Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunol 25:128–136
- Bruno DW, Novak B, Elliott DG (2006) Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. Dis Aquat Org 70:1–36
- Byrnes T (1986) Five species of monogenea from Australian bream. Aust J Zool 34:65–86
- Gibson S, Thuy NTN, Elliot A, Jones JB, Nicholls PK, Thompson RCA (2011) An intestinal *Eimeria* infection in juvenile asian seabass culture in Vietnam. Vet Parasitol 181:106–112
- Hoffman GL, Landolt M, Camper JE, Coats DW, Stookey JL, Burekf JD (1975) A disease of freshwater fishes caused by *Tetrahymena corlissi* and a key for identification of holotrich ciliates of freshwater fishes. J Parasitol 61:217–223
- Kim J, Hayward CJ, Joh SJ, Heo GJ (2002) Parasitic infections in live freshwater tropical fishes imported to Korea. Dis Aquat Org 52:169–173
- Kohn A, Cohen SC, Salgado-maldonado G (2006) Checklist of monogenea parasites of freshwater and marine fishes, amphibians and reptiles from Mexico. Zootaxa 1289:1–114
- Mehdizadeh S, Mousavi H, Masoumian M, Omrani B (2009) Report of *Myxobolus* in pearlscale goldfish gill imported in Iran. In: Aquaculture Europe 2009 conference, Trondheim, Norway
- Moles A (2007) Parasites of the fishes of Alaska and surrounding waters. Alsk Fish Res Bull 12:243–247
- Molnar K, Fernando CH (1974) Some new *Eimeria* from freshwater fishes in Ontario, Canada. Can J Zool 52:413–419
- Pazooki J, Masoumian M, Yahyazade HM, Abbasi K (2007) Metazoan parasites from freshwater fishes of northeast Iran. Iran J Fish Sci 9:25–33
- Ponpornpisit A, Endo M, Murata H (2000) Experimental infection of a ciliata *Tetrahymena pyriformis* on ornamental fish. Fish Sci 66:1026–1031
- Raissy M, Ansari M, Jalali B (2010) Occurrence of parasites in selected fish species in Gandoman Lagoon. Iran J Fish Sci 9:464–471
- Roberts RJ (2001) Fish pathology. W.B. Saunders, London
- Sanaullah M, Ahmed ATA (1980) Gill myxoboliasis of major carps in Bangladesh. J Fish Dis 3:349–354
- Shafiei A, Ghazi SM, Antikchi M, Nabavi R (2009) Parasite of Guppy fish in Isfahan province. In First International congress of aquatic

- animal health management and disease, Iranian Veterinary Council, Tehran
- Steinhagen D, Hespe K (1997) Carp coccidiosis: activity of phagocytic cells common carp infected with *Goussia carPELLI*. Dis Aquat Org 31:155–159
- Zanguee NN, Lymbery J A, Lau JJ (2010) Identification of novel *Cryptosporidium* species in aquarium fish. Vet Parasitol 174:43–48