Aquaculture 302 (2010) 19–25

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Infection status of zoonotic trematode metacercariae in Sutchi catfish (*Pangasianodon hypophthalmus*) in Vietnam: Associations with season, management and host age

Dinh Thi Thuy, Per Kania, Kurt Buchmann *

Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Stigbøjlen 7, DK-1870 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 6 December 2009
Received in revised form 21 January 2010
Accepted 1 February 2010

Keywords:
Sutchi catfish
Pangasianodon hypophthalmus
Trematodes
Metacercariae
Zoonosis
Control

ABSTRACT

The occurrence of four species of potentially zoonotic trematode metacercariae in Sutchi catfish *Pangasianodon hypophthalmus* from four provinces in the Mekong Delta of the Southern Vietnam is reported. A total of 1127 fish were collected from pond cultures comprising farm house systems (FHS) (279) and from farm household systems (FHHS) (848) and examined by classical and molecular methods. The trematode metacercariae *Haplorchis pumilio*, *H. taichui*, *Centrocestus formosanus* and *Procerovum* sp. were detected and the infections were analysed in relation to season, farm management type, host size and infection site in the host tissue. Generally, prevalences in the fish were higher during the rainy season from April to October when compared to the dry season. The infection rates and densities were lower in fishes from FHHS than from FHS. Fish at an age of 61 to 90 days post-hatch showed the highest parasite loads reflecting the presence of a continuous infection risk in the ponds following stocking. The parasites were found in the body musculature, head, fins and especially at the base of fins. The importance of Sutchi catfish farming is increasing both at the local and international markets and management practices in farms and industrial processing may reduce the infection levels and thereby improve the food hygienic standard of the products.

1. Introduction

Fish-borne trematodes belonging to the families Heterophyidae and Opisthorchiidae represent a public health problem throughout the world affecting the health of more than 18 million people, particularly in Asian countries. Members of the family Heterophyidae are minute intestinal trematodes using birds and mammals as final hosts and more than 22 heterophyid species are known to infect humans worldwide (Ito, 1964; Yu and Mott, 1994). Fish are in many cases the intermediate host carrying infective metacercariae which can be transmitted to humans ingesting raw or undercooked fish. Several studies have addressed the infection of various freshwater fishes in Southeast Asia (Kom et al.; 1999; Rim et al., 2008; Han et al., 2008; Skov et al., 2009) but despite the rapidly increasing importance of Sutchi catfish *P. hypophthalmus*, not only in Vietnam but also on the international export market, the knowledge on metacercarial infections in this host is still limited. The fact that Sutchi catfish *P. hypophthalmus* is the main freshwater fish species cultured in the Mekong Delta with an annual production of 1.3 million metric tonnes calls for further extensive analyses of parasites in this fish species. Investigations on the occurrence of monogeneans (Thuy and Buchmann, 2008a) and bucephaline trematodes (Thuy and Buchmann 2008b) are available. Preliminary studies by Thien et al. (2007, 2009) and Thu et al. (2007) reported very low prevalences of metacercariae in Sutchi catfish from the Mekong Delta. The only zoonotic species found were *Haplorchis pumilio* and *Centrocestus formosanus* but these authors also reported the occurrence of unidentified metacercariae which needs further investigations. Both food hygienic and fish pathological aspects should be addressed with regard to this host in order to develop control methods. It is particularly important to record the distribution of the parasites with regard to farm management, season and host size. The present study comprises an extensive survey on the occurrence of metacercariae in the Sutchi catfish reared in four provinces in the Mekong Delta. Diagnosis of parasites was based on both classical morphometric methods and molecular tools (PCR and sequencing of the ITS2 region). Further, the infections found were analysed with regard to production system, season and host size. Based on this analysis suggestions for future control of the infections are given.

2. Materials and methods

2.1. Collection sites and fish sampling

Farm house systems (FHS) and farm household systems (FHHS) in Can Tho, Ben Tre, Vinh Long and Dong Thap provinces in the Mekong Delta were selected as representative for the pond production types in the Mekong Delta. The FHS are relatively small and primitive systems with no strict drying and disinfection of ponds. The FHHS are characterized by larger units and higher investment compared to the FHS. Drying and disinfection of ponds between production cycles

* Corresponding author. Tel.: +45 35332700; fax: +45 35332755.
E-mail address: kub@life.ku.dk (K. Buchmann).

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doi:10.1016/j.aquaculture.2010.02.002

Table 1
Number of *P. hypophthalmus* collected in different seasons and locations. *Rainy season: from April to October; **Dry season: from November to March of the next year.

Cultured systems/season	Total number of fish collected	Number of fish collected in provinces			
		Can Tho	Vinh Long	Ben Tre	Dong Thap
Farm household	848	300	212	220	116
*Rainy 06	187	85	77		25
**Dry 07	186	50	60	47	29
*Rainy07	173	40	50	58	25
**Dry 08	155	60	15	60	20
*Rainy 08	147	65	10	55	17
Household	279	57	84	68	70
*Rainy 06	76	16	35		25
**Dry 07	109	27	32	35	15
*Rainy 07	94	14	17	33	30

are always performed. Feeding is exclusively based on commercial feed pellets and the water supply from the river is treated weekly by disinfectants (such as copper sulphate, formalin and/or oxidizing compounds) (Thuy and Buchmann, 2008b). Five communes were selected from each province; and ten ponds were chosen from every one of the communes. The fish samples (7–12 fish/sample) were collected randomly in the ponds and were taken in the different seasons from April 2006 to October 2008. Due to the varying climatic conditions in the Mekong Delta the year was divided into two seasons: the rainy season (April to October) and the dry season (November to March). Following sampling fish were transported to the laboratory (RIA2, Ho Chi Minh City) for examination. Fish age (days post-hatch) was recorded and the pond management system and type of farm were noted. Table 1 shows the number of catfish collected in different season and at different locations. Table 2 shows the distribution of sampled fish according to fish age.

2.2. Isolation of metacercariae

A total of 1127 Sutchi catfish samples were taken from April 2006 to October 2008. Fish age ranged from <30 days to more than 180 days (Table 2). The fish body was divided into 4 sub-sections (muscles, base of fins, fins and head). However, for fish with an age of >150 days, a subsample of the fish muscles (300 g) was used. Each part of the fish was ground separately and transferred into a beaker with an artificial pepsin solution (2% pepsin, at pH 2) (Buchmann, 2007). The digest was poured through a 1×1 mm mesh brass sieve, and washed with saline 7–8 times where after the metacercariae were sedimented. Some of the metacercariae were excysted by using a trypsin solution at slightly basic conditions (0.5% bile, 0.25% trypsin, 0.5% chymotrypsin, pH: 8.4), and placed in a 37 °C incubator for 5–10 min (Buchmann, 2007). The excysted metacercariae were collected and placed in separate vials with physiological saline. They were observed and identified live using a compound microscope (400–1000 magnification). In addition, excysted metacercariae were fixed in neutral formalin, stained with haematoxylin and mounted in glycerine jelly (Buchmann, 2007). Some specimens were fixed in hot neutral formalin (4%), a procedure which makes the worms stretch (Buchmann, 2007). Measurements were made by the use of a Leica DMLB.

2.3. Molecular analysis

A subsample comprising 69 of the recovered metacercariae was placed in clean physiological saline and preserved in 96% ethanol at 4 °C in order to supplement the morphometric analysis with a diagnosis based on molecular methods (PCR and subsequent sequencing of the ITS2 region).

2.3.1. DNA isolation

Parasites were lysed by protease treatment. Thus, parasite material preserved in 96% ethanol was dried and placed in 200 µl PCR tubes containing 30 µl lysis buffer (Tween 20 (0.45%), Proteinase K (60 µl ml^{-1}), 100 mM Tris and 1 mM EDTA) at 65 °C until complete digestion of tissue (confirmed by microscopy at 50× magnification). The protease was subsequently inactivated at 95 °C for 10 min.

2.3.2. Polymerase chain reaction (PCR)

The reaction was conducted in 60 µl reaction volumes using 1 µl lysate as template and 2 units of BioTaq DNA polymerase (Bioline no. BI O21040) (DNA Technology A/S, Risskov, Denmark) at 1.5 mM $MgCl_2$. A total of 36 cycles were run in a Biometra T3 Thermocycler with 96 °C for 30 s, annealing temperature at 60 °C for 30 s, elongation at 72 °C for 30 s. Post-elongation was performed for 7 min at 72 °C.

2.3.3. Primers

Based on the alignment of GenBank sequences from *H. pumilio*, *Centrocestus* sp. and *Procerovum* sp. a primer set was constructed in order to amplify the ITS2 sequence from the recovered digenean metacercariae. The sequence of the forward primer was CTCGGCTCGTGTGTCGATGA and the sequence for the reverse primer was GCATGCTTAARTTCAGCGGGTA. Products of approximately 470 bp were run on 2% ethidium bromide stained agarose gels and subsequently PCR purification was performed by using Illustra GFX (GE-Healthcare, Brøndby, Denmark) and sequenced.

2.3.4. Sequencing of the ITS2 region

Sequencing was conducted with BigDye 1.1 (Applied Biosystem no. 4337450) and analysed in an ABI-310 automated sequencer (Applied Biosystems, Foster City, USA).

Table 2
Number of *P. hypophthalmus* in different age categories collected in different seasons. *Rainy season: from April to October; **Dry season: from November to March of the next year.

The cultured systems/season	Total number of collected fish	Number of fish in different age categories (days post-hatch)						
		<30	30–60	61–90	91–120	121–150	151–180	>180
Farm household	848	134	170	139	122	139	81	63
*Rainy 06	181	20	40	42	29	24	15	11
**Dry 07	176	23	30	25	33	37	19	9
*Rainy 07	172	27	23	20	26	32	24	20
**Dry 08	181	39	37	35	17	28	13	12
*Rainy 08	138	25	40	17	17	18	10	11
Household	279	48	45	46	33	48	33	26
*Rainy 06	90	15	13	12	13	13	13	11
**Dry 07	97	18	10	21	10	20	10	8
*Rainy 07	92	15	22	13	10	15	10	7