COMPARATIVE EVALUATION OF THE SUSCEPTIBILITY OF CULTIVATED FISHES TO THE NATURAL INFECTION WITH MYXOSPOREAN PARASITES AND TISSUE CHANGES IN THE HOST

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ABSTRACT

The purpose of the study was to evaluate the susceptibility of 4 important cultivated fishes to sporozoan parasites. Fishes were collected bimonthly from a pond for a period of 1 year. *Myxobolus colossomatis* and *Henneguya piaractus* were found in the internal organs and gills, respectively. The combined incidence of parasitism by both myxozoa was 97.3% in pacu (*Piaractus mesopotamicus*), 33.3% in hybrid tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*), 5.6% in tambaqui (*Colossoma macropomum*) and 0% in carp (*Cyprinus carpio)*. Pacu was the most susceptible fish and was parasitized 79.2% in the gills, 66.7% in the kidney and 50% in the spleen. Histopathological evaluation of the gills showed hemorrhages, inflammatory reaction with mononuclear cells and fibroblasts and hyperplasia of basal and goblet cells.

Key words: Myxozoa, susceptibility, histopathology, cultivated fish.

RESUMO

Avaliação comparativa da susceptibilidade de peixes cultivados à infecção natural com parasitos mixosporídeos e alterações teciduais no hospedeiro

O objetivo deste estudo foi avaliar a susceptibilidade de 4 importantes peixes cultivados a parasitos esporozoários. Os peixes foram coletados bimestralmente de um tanque de cultivo, durante 1 ano. *Myxobolus colossomatis* e *Henneguya piaractus* foram encontrados nos órgãos internos e brânquias, respectivamente. A incidência de ambos os parasitos foi de 97,3% em pacu (*Piaractus mesopotamicus*), 33,3% no híbrido tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*), 5,6% em tambaqui (*Colossoma macropomum*) e 0% em carpa (*Cyprinus carpio)*. Pacu foi o peixe mais susceptível, encontrando-se parasitado 79,2% nas brânquias, 66,7% nos rins e 50% no baço. A análise histopatológica das brânquias mostrou hemorragias, reação inflamatória com células mononucleares, fibroblastos e hiperplasia das células basais e mucosas.

Palavras-chave: Myxozoa, susceptibilidade, histopatologia, peixes cultivados.

INTRODUCTION

Myxosporidiosis is an infectious disease caused by myxosporean of the Phylum Myxozoa, Class Myxosporea and Order Bivalvulida. Poor water quality and handling stress predisposes cultivated fishes to sporozoan infection in Brazil and in the other countries (Lom & Noble, 1984; Martins *et al.,* 1997).

Several species of *Henneguya* infecting fish have been described in Brazil (Pinto, 1928; Guimarães & Bergamin, 1933; Cordeiro *et al.,* 1983/1984; Gioia *et al.,* 1986; Azevedo & Matos, 1989; Rocha *et al.,* 1992; Torres *et al.,* 1994; Azevedo & Matos, 1995, 1996; Gioia & Cordeiro, 1996; Martins & Souza, 1997; Casal *et al.,* 1997).

Myxobolus spp from the gills and internal organs have also been described by Pinto (1928), Walliker (1969), Molnár & Békési (1993) and Casal *et al.* (1996).

The majority of the published works in Brazil are taxonomic studies but no information exists on the susceptibility of the host species or on the pathology associated with these sporozoan. Ichthyopathological studies in 6 fishfarms situated in the Ceará State, were related by Békési (1992), in a period of 1987 to 1990. The author reported the occurrence of *Henneguya* and *Myxobolus,* among other parasites.

In the present work the authors studied the natural susceptibility of pacu (*Piaractus mesopotamicus* Holmberg, 1887), tambaqui (*Colossoma macropomum* Cuvier, 1818), tambacu (a hybrid between *P. mesopotamicus* female and *C. macropomum* male) and carp (*Cyprinus carpio* Linnaeus, 1758) to the myxosporean in natural infections. Determination of incidence and histopathological studies were performed.

MATERIAL AND METHODS

Fishes

Pacu (*P. mesopotamicus* Holmberg, 1887), tambaqui (*C. macropomum* Cuvier, 1818), carp (*C. carpio* Linnaeus, 1758) and tambacu (hibrid of *P. mesopotamicus* female with *C. macropomum* male), were collected bimonthly, from a 3,500 m^2 pond. Fish were maintained in 1,000 L aquarium until samples.

Histopathological analysis and myxosporidian spores observation

Small pieces of liver, kidney, spleen and gills were squeezed between a glass microscope slide and a coverslip to obtain a smear. Wet mounts of the smear were examined microscopically and when parasites were found, the smears were air dried at room temperature, fixed in methylic alcohol, stained in 1:9 Giemsa's solution for 10 min. (Meyers *et al*., 1977, slightly modified) and mounted in synthetic Canadian balsam.

These slides were then used to identify parasites following the recomendations of Lom & Arthur (1989). Parasitized tissue were fixed in 10% buffered formalin and embedded in paraffin-block, sectioned (6 μm) and stained in haematoxylin-eosin, periodic acid of Shiff or Masson trichrome.

Water quality

Water quality was monthly measured according to Golterman *et al.* (1978) with the aid of Van Dorn bottle in a 0.3 m depth. Dissolved oxygen (Winkler method), pH (Quimis phmeter), temperature, electric conductivity (conductimeter Methrom-Herisau E–527) and total alkalinity, were measured. The samples were collected and measured at 9:00 hours.

RESULTS

Parasitological analysis

Parasitological analysis revealed the presence of *Myxobolus colossomatis* Molnár & Békési, 1993 in the kidney, liver, spleen, muscle and gall bladder of Pacu. The same species was observed in the kidney of *C. macropomum. Henneguya piaractus* Martins & Souza, 1997 was observed in the gills of pacu, tambaqui and *t*ambacu. In carp the presence of myxosporean protozoan was not observed. Specific identification of the parasites was performed according to Lom & Arthur (1989) and Martins & Souza (1997).

Susceptibility of fishes to myxosporean

Thirty 7 pacu (average standard length 22.6 cm), 8 tambaqui (average standard length 31.2 cm), 6 tambacu (average standard length 33.5 cm) and 36 carp (average standard length 23.4 cm), were necropsied. Table 1 presents incidence of parasitism in the different host and samples collected.

TABLE 1

Prevalence of myxosporean in the different hosts during February, 1995 to February, 1996.

Month	Fish	Number of fishes necropsied	Infected fishes	Prevalence (%)
February	*C. carpio*	–	–	–
	P. mesopotamicus	12	12	100
	Tambacu	–	–	–
	C. macropomum	2	0	0
April	*C. carpio*	11	0	0
	P. mesopotamicus	10	10	100
	Tambacu	2	2	100
	C. macropomum	–	–	–
June	*C. carpio*	5	0	0
	P. mesopotamicus	2	2	100
	Tambacu	–	–	–
	C. macropomum	8	1	12.5
August	*C. carpio*	11	0	0
	P. mesopotamicus	5	5	100
	Tambacu	–	–	–
	C. macropomum	–	–	–
October	*C. carpio*	2	0	0
	P. mesopotamicus	2	2	100
	Tambacu	–	–	–
	C. macropomum	2	0	0
December	*C. carpio*	3	0	0
	P. mesopotamicus	4	3	75
	Tambacu	4	0	0
	C. macropomum	4	0	0
February	*C. carpio*	4	0	0
	P. mesopotamicus	2	2	100
	Tambacu	–	–	–
	C. macropomum	2	0	0
Total	*C. carpio*	36	0	0
	P. mesopotamicus	37	36	97.3
	Tambacu	6	2	33.3
	C. macropomum	18	1	5.6

Pacu were 100% infected for all months, except in December when they were only observed 75% of the fishes.

Thus, a total of 97.3% of pacu were infected by *H. piaractus* and *M. colossomatis* followed by 33.3% of tambacu hybrid, 5.7% of tambaqui and 0.0% of carp.

The incidence of parasitized tissues in the pacu was: gills (79.2%), kidney (66.7%), spleen (50%), heart (20.8%) and muscle (16.7%). Liver and gonads showed 12.5% infection, eyes (8.3%) and swim bladder and brain (4.2%) (Fig. 1a). One hundred percent of the tambacu had parasitized gills and 50% had parasitized kidneys and spleens (Fig. 1b). In tambaqui, *H. piaractus* spores were present in all the gills (Fig. 1c). Grossly, cysts measuring 0.1 to 0.5 mm diameter throughout the gill filament were observed. Sometimes, cysts and associated hemorrhage were observed throughout the entire length of the primary lamellae. Six out of 37 pacu had pale livers, spleens and kidneys.

Histopathological analysis

Hemorrhages were observed in the most intense infections. Cysts were surrounded by 2 layers of elongate fibroblast-like cells and an inflammatory mononuclear infiltrate.

Larger cysts displaced the adjacent lamellae. Hyperplasia of the basal cells and displacement of the respiratory epithelium was observed. Sometimes sub-epithelial edema with displacement of the gill epithelium and congestion of the sinusoid capillary, was observed.

Hyperplasia of the goblet cells at the end of the secondary lamellae, was frequently observed as well.

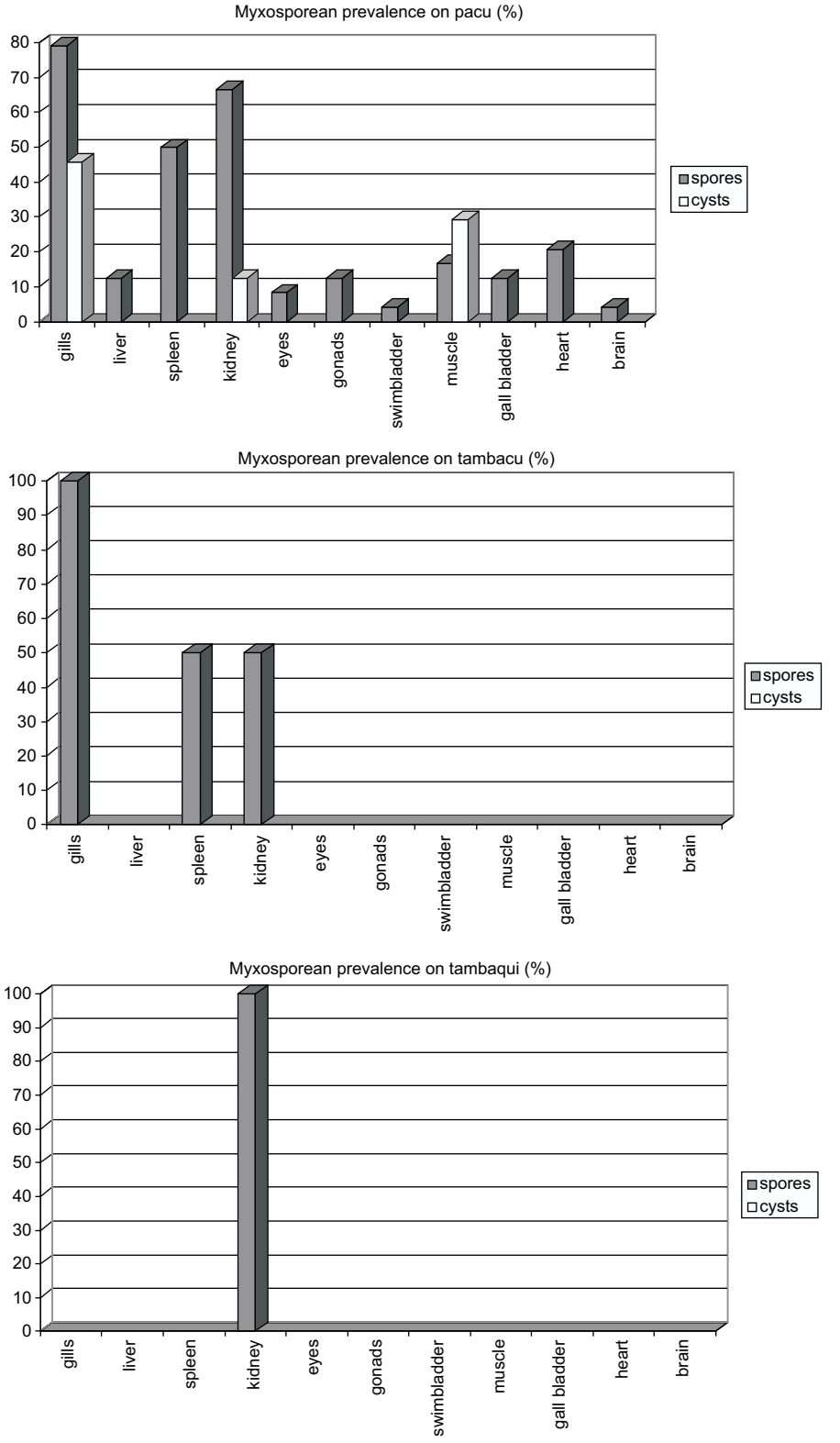

Fig. 1 — Prevalence of myxosporeans found in the tissues of infected fish.

In the liver of pacu an accumulation of spores with evident polar capsules identified as *M. colossomatis* were observed. In these cases it was not possible to detect the presence of the cystic membrane and the host response was slight with rare thrombocytes surrounding the spores.

Water quality

Water quality (Table 2) showed pH slightly acid, without significant changes, except in the day 26th of January. Throughout the course of the analysis, dissolved oxygen was 2.8 to 6.5 mg/l. Temperature values in May through October were 20 to 21.5°C. For the period November-February temperature were 25 ± 2°C. Conductivity was 31.6 to 60.0 $mS.cm^{-1}$ and alkalinity 17.6 to 24.6 mg/l.

DISCUSSION

Present results showed that carp were not susceptible to myxosporean parasites. In the studied fishes, pacu was the most susceptible and the myxosporean were observed in 97.3% of them. It must be emphasized that 6 out of 7 collected samples presented 100% of infection with the *H. piaractus* and *M. colossomatis*. In this kind of fish, gills, kidney and spleen were the most parasitized organs. Tambaqui and tambacu presented intermediate susceptibility 5.6% and 33.3%, respectively. The interesting result is the intermediate prevalence of tambacu compared to their ancestry pacu and tambaqui. Moreover, the frequency of parasitized organs was always higher in pacu than in the others. Tambacu presented 16.7% prevalence in the spleen and kidney. In tambaqui the spores were present in 5.6% of examined kidney. Comparative studies of the fish susceptibility to myxosporean are rare in the literature and the common finding is prevalence of parasites in the fish. Moser & Love (1975) related 50% of prevalence of *H. sebasta* in *Sebastes serranoides*. In carp 95% of the old fishes were infected (Molnár & Kovács-Gayer, 1985).

TABLE 2

Water analysis of fish pond situated at Aquaculture Center, UNESP, Jaboticabal, SP.

	PH	O_2 (mg/L)	T (°C)	Cond. ($\mu S.cm^{-1}$)	Alcal. (mg/L)
31/05/95	6.8	2.8	21.5	50	21.1
23/06/95	6.8	3.4	20	60	22
31/07/95	6.7	5.8	20	41	20.5
31/08/95	6.7	6.5	21	34	17.6
28/09/95	6.6	4.4	21	31.6	18.5
23/10/95	6.4	3	21	43.6	17.6
30/11/95	6.8	3.3	25	37.6	18.5
29/12/95	6.7	3.1	24.5	43.6	24.6
26/01/96	7.3	4.1	27	43.6	17.6
28/02/96	6.4	4.6	26	46.5	17.6

The infection percentage of *Henneguya* in *Pomatomus saltatrix* was 24% to 57%, for a period of 3 years (Meyers *et al.,* 1977).

In lambari (*Hyphessobrycon anisitsi*) myxosporean prevalence was 20% (Cordeiro *et al.,* 1983/84) and 6.7% in traíra (*Hoplias malabaricus*) (Azevedo & Matos, 1996). Dana (1990) observed myxosporean prevalence in common carp reared at different temperatures. At 25°C the prevalence was higher (70%) than at 31°C (53.3%). In the North of Brazil, Molnár & Békési (1993) related 100% of infection in tambaqui with 2 to 14 cm length. The authors identified the parasites as *M. colossomatis* in the different organs. By the way, Békési (1992) related the occurrence of *Ichthyophthirius, Henneguya* and *Myxobolus*, during the period of 1987 to 1990. The author commented that myxosporean infection provoked no mortality, but *Ichthyophthirius, Chilodonella* and monogenean usually caused mortality. The

effects of the parasitism in Brazilian fishes from several fishfarms from Jaboticabal (SP), were analyzed in the Aquaculture Center, UNESP, where Martins & Romero (1996) observed 15.3% *Henneguya* infection of pacu and 2.2% *Henneguya* infection of tambacu. Martins *et al*. (1997) reported a severe outbreak of *Henneguya* sp infection in cultivated pacu. All (100%) of the fishes in a pond died.

Histopathological study of the gills showed that the lesions caused were related to the infection degree. A few number of cysts and parasites in the gills with a slight hyperplasia of the basal cells and goblet cells of the epithelium associated or not to congestion was observed. Severe infection caused hyperplasia of the basal and goblet cells and increase in the mucus production. In these cases, the inflammatory infiltrate was severe with the presence of fibroblast-like cells, mononuclear cells, congestion and subepithelial edema. The inter and intralamellar presence of cysts, associated to hyperplasia and inflammation increased the adherence between secondary lamellae. This fact and the hyperplasia of the goblet cells associated with an increase in the mucus production caused respiratory distress syndrome and suffocation of fishes (Dyková & Lom, 1978; Current & Janovy, 1978; Shariff, 1982; Bowser & Conroy, 1985; Kalavati & Narasimhamurti, 1985; Martins *et al.,* 1997). The lesions were similar to those observed in *H. psorospermica* infections in perch, *Perca fluviatilis* (Dyková & Lom, 1978).

In the present work, the accumulation of the *Myxobolus* spores in the liver of the *P. mesopotamicus* cause no changes in the host, except a discreet presence of thrombocytes surrounding the spores. Some authors described severe lesions caused by *Myxobolus* in carp, involving necrosis and gut degeneration (Molnár & Kovács-Gayer, 1985; Dyková & Lom, 1988). However, the presence of *Myxobolus* in the internal organs may cause or not disease. Probably this variation is related to the host response and parasite species involved.

According to the analyzed data the water quality does not interfere with the prevalence of the studied parasites. It is necessary to emphasize that fish ponds are a dynamic system with constant flow of water with a direct effect to the biotic and abiotic factors of the pond (Sipaúba-Tavares & Colus, 1995). In the artificial system of little depth, blow wind is sufficient to provoke a complete mix in the water, preventing water stratification (Oliveira *et al.,* 1992). Nevertheless, ground, morphometry, evaporation and precipitation processes, were mechanisms that affect the systems dynamics (Durigan *et al.,* 1992; Sipaúba-Tavares & Moreno, 1994; Sipaúba-Tavares & Colus, 1995). In the present work, the values were in line with those observed by Sipaúba-Tavares *et al.* (1995) in a pond situated at Aquaculture Center, UNESP, Jaboticabal, SP.

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