VETERINARSKI ARHIV 77 (4), 355-363, 2007

Changes in blood parameters of hybrid tambacu fish parasitized by *Dolops carvalhoi* (Crustacea, Branchiura), a fish louse

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TAVARES-DIAS, M., F. RUAS DE MORAES, E. M. ONAKA, P. C. B. REZENDE: Changes in blood parameters of hybrid tambacu fish parasitized by *Dolops carvalhoi* (Crustacea, Branchiura), a fish louse. Vet. arhiv 77, 355-363, 2007.

ABSTRACT

Measurement of blood parameters has been used for many years as a tool for monitoring the health of fish. The present study investigated the effects of a natural infestation of *Dolops carvalhoi* Lemos de Castro, 1949, on red blood cells, thrombocytes and white blood cell counts, as well as plasma glucose and serum electrolyte levels in hybrid tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). Parasitized fish showed low haematocrit and magnesium levels and increases in MCHC, plasma glucose levels, serum protein, sodium and chloride levels, number of monocytes and PAS-positive granular leukocytes (PAS-GL), when compared with values in control fish. This study is the first to report changes in fish physiology caused by *D. carvalhoi* infestation, and the results obtained indicate that a mild infection can lead to important osmoregulatory disturbances in hosts.

Key words: haematological, *Dolops carvalhoi*, blood cells, freshwater fish, infestation, parasites

Introduction

The hybrid tambacu (male *Piaractus mesopotamicus* × female *Colossoma macropomum*) is a fish of great economic importance to Brazilian aquaculture. It is widely appreciated for sport and pleasure fishing and for fish farming, due to its quick growth and quick weight

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ISSN 0372-5480
Printed in Croatia

gain. Furthermore, the hybrid tambacu is more resistant to stress and parasitic diseases than *P. mesopotamicus* and *C. macropomum* (TAVARES-DIAS et al., 2001; MARTINS et al., 2002).

Branchiuran fish lice belonging to the genus *Argulus* are the most common parasites infecting Brazilian fish farms (TAVARES-DIAS et al., 1999; TAVARES-DIAS et al., 2001; MARTINS et al., 2002). These parasites are important pathogens of both farmed and natural *Oncorhynchus mykiss* populations (RUANE et al., 1999; HAOND et al., 2003) causing great losses in fish stocks (RUANE et al., 1999) in several countries. Parasitized fish experience different challenges which depend on the species of parasite involved, the parasites' feeding habits and their location on the host. *Lepeophtheirus salmonis* causes a local inflammatory response and blood loss at the site of attachment and severe infestations may lead to the host's death (NOLAN et al., 2000; BOWERS et al., 2000; MUSTAFA et al., 2000; WAGNER and MCKINLEY, 2004). However, this parasite did not appear to suppress hosts' defence mechanisms during the earlier stages of infestation (MUSTAFA et al., 2000). The *Argulus foliaceus* usually attaches to the host's skin and feeds on epithelial cells and blood, causing skin lesions and disruption of epithelium (RANZANI-PAIVA et al., 1987; TAVARES-DIAS et al., 1999; RUANE et al., 1999; VAN DER SALM et al., 2000). Parasites of genus *Dolops* possess a dorsal-ventral flattened shape and an oral apparatus shaped for suction with hook-shaped appendages, used for attachment to the host (THATCHER, 1991; GOMES and MALTA, 2002). When feeding on blood, these ectoparasites may cause punctiform lesions on the host's skin (THATCHER, 1991). Although *D. carvalhoi* is widely distributed in South America (GOMES and MALTA, 2002), its effects on fish physiology are not known.

Infestations with *Argulus foliaceus* (SHIMURA et al., 1983; RANZANI-PAIVA, 1987; RUANE et al., 1999; TAVARES-DIAS et al., 1999; HAOND et al., 2003), *Lepeophtheirus salmonis* (BOWERS et al., 2000; MUSTAFA et al., 2000; RUANE et al., 2000; WAGNER and McKINLEY, 2004) or *Lernaea cyprinacea* (SILVA-SOUZA et al., 2000) can influence blood parameters in different host fish. Glucose levels may be a primary indicator of chronic stress, while haematocrit and electrolyte levels are secondary indicators (RUANE et al., 1999; BOWERS et al., 2000; MUSTAFA et al., 2000; WAGNER and McKINLEY, 2004; TAVARES-DIAS and MORAES, 2004; JONES and GRUTTER, 2005), indicating the osmoregulatory status of the fish. If parasites act as stress inducers, haematocrit values could be increased by splenic release of stored blood cells, loss of plasma, or by swelling of red blood cells (BOWERS et al., 2000; WAGNER and McKINLEY, 2004; JONES and GRUTTER, 2005). However, depending on their number, parasites could also cause a reduction in the host's haematocrit by blood ingestion (WAGNER and McKINLEY, 2004; JONES and GRUTTER, 2005) or by osmoregulatory failure, caused by exposed lesions (JONES and GRUTTER, 2005). In addition, leukocyte counts may indicate activation of cellular immune

mechanism (TAVARES-DIAS et al., 1999; TAVARES-DIAS and MORAES, 2004) in the host. The present study set out to evaluate the haematological parameters of hybrid tambacu naturally parasitized with *D. carvalhoi* and thus determine the physiological impact of the parasite.

Materials and methods

Fish and experimental procedure. Specimens used were hybrid tambacu (22.0 to 29.0 cm in length) from cultivation tanks of a commercial fish farm that were transported to the Laboratory of Ichthyopathology at the Research Center of Animal Health, CPPAR/UNESP, Jaboticabal, São Paulo State, Brazil. Fish were randomly distributed into three 500-l. tanks with running water and examined beforehand for any visible signs of parasite infestation. Seven days after arrival, the presence of crustacean ectoparasites on fish in one tank was observed. Fish in the other two tanks appeared normal no treatment measures were performed until haematological examinations had been performed on parasitized fish (n = 15) and unparasitized (n = 20) controls.

Biochemical and haematological measurements. Two blood samples were collected by caudal venipuncture. One sample was collected with a syringe containing anticoagulant 10%-EDTA and the other without it. These procedures were carried out within 0.8-1.2 minutes to minimise stress. The blood samples containing EDTA were divided into two aliquots. One aliquot was used to measure plasma glucose by glucose oxidase and the other for determination of red blood cell count (RBC), haematocrit (HCT), and haemoglobin concentration (HB). RBC determination was performed using an automatic blood cell counter (Celm, Model CC510). HCT was measured by microhaematocrit determination and Hb by cyanomethaemoglobin determination. Secondary Wintrobe indices, such as mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were derived from the primary indices. Blood smears stained by May Grünval-Giemsa-Wright (TAVARES-DIAS and MORAES, 2003) were used for total white blood cell and thrombocyte counts, according to the method described by TAVARES-DIAS and MATAQUEIRO (2004). Differential leukocyte counts were performed by identifying 200 leukocytes in each slide which were classified according to the method described by TAVARES-DIAS et al. (2000).

The second blood sample, without anticoagulant, was left for 30 min. at room temperature and then centrifuged for 10 min. at 750 g. The separated serum was frozen at -80 ^{o}C until analysis. Total serum protein concentration was determined by biuret reaction. Serum sodium and potassium concentrations were measured by flame photometry (Zeiss M4Q2). Calcium and magnesium levels were measured by atomic emission and spectrometryomic absorption (IL200, Thermo Electron). Serum chloride concentration was measured using a commercial kit (Sigma 461).

Parasite identification and quantification. Following blood sampling, all crustacean parasites found were removed from hosts, counted, fixed (TAVARES-DIAS et al., 2001) and identified (THATCHER, 1991). In addition, the tank was submitted to treatment with 4% sodium chloride (MARTINS et al., 2002).

Statistical analysis. Blood values of control and parasitized fish were analyzed by Student's *t*-test. Differences were considered to be significant at $P<0.05$. The presence of correlations between the number of parasites and blood variables was evaluated by lineal regression and analyzed by *t*-test ($P<0.05$).

Results

Three to 55 *D. carvalhoi* Lemos de Castro, 1949, were found attached to skin of parasitized fish, averaging 19.0 ± 14.6 (mild infection). However, as parasite removal was performed after blood withdrawal, some crustaceans from the hosts' bodies could have been lost. *D. carvalhoi* were found attached only to the tegument of the fish host and visual examination revealed no macroscopic lesions. All gills were free of parasites and without apparent lesions. No parasites were observed on fish from the control group.

Table 1. Mean values ± standard deviation of red blood cells, plasma glucose and serum electrolyte levels in hybrid tambacu both control and parasitized with *Dolops carvalhoi*

Parameters	Control	Parasitized	*t*-test
RBC (x 10^6/µL)	2.815 ± 0.71	2.587 ± 0.40	0.50
Haemoglobin (g/dL)	9.8 ± 2.1	9.9 ± 1.4	0.02
Haematocrit (%)	43.3 ± 6.8	31.9 ± 2.6	30.72**
MCV (fL)	163.8 ± 49.7	125.5 ± 18.2	2.77
MCHC (g/dL)	23.2 ± 6.0	31.1 ± 4.2	16.23**
Glucose (mg/dL)	88.9 ± 24.7	122.0 ± 20.3	15.31**
Total protein (g/dL)	3.3 ± 0.2	3.5 ± 0.3	7.12*
Sodium (mmol/L)	151.3 ± 7.8	158.6 ± 0.3	6.75*
Potassium (mmol/L)	3.4 ± 1.6	3.8 ± 0.9	0.57
Calcium (mg/dL)	10.2 ± 1.0	10.3 ± 0.7	0.15
Magnesium (mg/dL)	3.2 ± 0.5	2.6 ± 0.3	13.46**
Chloride (Meq/L)	115.1 ± 13.2	128.0 ± 7.3	9.54**

*= significant ($P<0.05$); **= significant ($P<0.01$)

Compared to fish in the control group, parasitized fish had lower haematocrit and serum magnesium levels, while MCHC was higher than in controls. Increased plasma glucose ($P<0.01$), total serum protein ($P<0.05$), sodium ($P<0.05$) and serum chloride levels ($P<0.01$) were also observed in the parasitized fish (Table 1). Red blood cell counts, MCV, haemoglobin, and potassium and calcium levels were not significantly different compared to control fish.

In blood smears from parasitized and non-parasitized fish, thrombocytes, lymphocytes, neutrophils, monocytes, PAS-positive granular leukocyte (PAS-GL) and eosinophils were observed. These observations were similar to those described by TAVARES-DIAS et al. (2000) for the hybrid tambacu, and did not differ significantly between groups ($P>0.05$). The exceptions were a significant increase in monocytes and PAS-GL in the parasitized fish (Table 2). No correlation was found between the number of parasites and any of the blood variables.

Table 2. Mean values ± standard deviation of thrombocyte counts and white blood cell counts in hybrid tambacu both control and parasitized with *Dolops carvalhoi*

Parameters	Control	Parasitized	*t*-test
Thrombocytes (μL)	50955.0 ± 27749.0	60425.0 ± 29380.0	0.84
WBC (μL)	28375.0 ± 17656.0	35875.0 ± 17585.0	1.36
Lymphocytes (μL)	112740.0 ± 12877.0	9386.1 ± 10425.2	0.18
Neutrophils (μL)	15351.0 ± 9483.0	17127.9 ± 8613.9	0.28
Monocytes (μL)	451.0 ± 520.0	1929.2 ± 1712.3	13.15**
PAS-GL (μL)	1860.0 ± 275.0	6632.0 ± 10099.3	8.2**
Eosinophils (μL)	1112.0 ± 2247.0	799.8 ± 759.5	0.21

*=significant ($P<0.05$); **= significant ($P<0.01$)

Discussion

Blood parameters can be useful for the measurement of physiological disturbances in parasitized fish and thus provide information about the level of damage in the host and the prognosis for the diseases. In the present study, the haematocrit values of control hybrid tambacu was similar to values previously reported for this fish (TAVARES-DIAS et al., 2000), yet, both were higher when compared to fish with a mild infestation by *D. carvalhoi*. This lower haematocrit value was caused by parasite feeding on the host's blood. The gills of hybrid tambacu were found to be free of *D. carvalhoi*, which parasitized only the skin.

This parasite has been shown to have a preference for the host's skin (THATCHER, 1991; GOMES and MALTA, 2002).

Crustacean parasite infestation elicits a stress reaction in fish, with the occurrences of a series of compensatory and/or adaptive behavioural and physiological responses (WENDELAAR BONGA, 1997; HAOND et al., 2003). In hybrid tambacu parasitized with *D. carvalhoi,* significant hyperglycaemia was observed compared with control fish. A similar finding was reported for *Oncorhynchus mykiss* parasitized by *A. japonicus* (RUANE et al., 1999) and *L. salmonis* (RUANE et al., 2000). Glucose levels can increase in response to elevated epinephrine and cortisol activity (BOWERS et al., 2000; HAOND et al., 2003).

Serum and plasma from fish possess several proteins (trypsin, lysozyme, antibodies, C-protein, complement factors and other lytic factors) acting as antimicrobial agents, and which are a first line of defence (DALMO et al., 1997; JONES, 2001), providing a primary barrier against invasion (DALMO et al., 1997; JONES, 2001) and proliferation of pathogens, including crustacean ectoparasites (JONES, 2001). Thus, hybrid tambacu parasitized by *D. carvalhoi* had an increase in serum protein, but no lysis of red blood cell. In contrast, in *O. masou*, a reduction in plasma protein levels after *A. coregoni* infestation was reported (SHIMURA et al., 1983). Lyses of red blood cells, a common finding in stressed fish, would be responsible for the release of intracellular protein and electrolytes as a mitigating function for maintenance of osmotic pressure when there is an egress of sodium and chloride (HARMS et al., 1996).

When hybrid tambacu were parasitized by *D. carvalhoi,* no changes in calcium or potassium serum levels were observed. Only sodium and chloride serum levels increased, while that of magnesium decreased. Changes in ion balance were also reported for other fish when parasitized with crustacean parasites and other parasites. The decrease in magnesium levels in blood has also been reported in *C. carpio* with ichthyophthiriasis (HINES and SPIRA, 1974) and in *Oreochromis niloticus* (L.) with *Edwardsiella tarda* (BENLI and YILDIZ, 2004). In *O. masou,* an infestation of A. *coregoni* decreased serum calcium levels (SHIMURA et al., 1983), whereas in *Salmo salar,* infection with *L. samonis* increased plasma sodium and chloride levels (BOWERS et al., 2000). Atlantic salmon parasitized with pre-adult and adult sea lice, *L. salmonis,* showed a high turnover of chloride cells associated with an increase in gill Na^+/K^+-ATPase activity and serum chloride levels. A decreased sodium/chloride ratio in serum was observed in these fish, indicating a stress response induced by infection (NOLAN et al., 1999). Hence, these results lend support to the suggestion that an infestation with crustacean parasites such as *D. carvalhoi* may elicit an osmoregulatory response in the host, which needs to maintain homeostasis, since the maintenance of a consistent internal environment is essential for normal cell function in pluricellular organisms.

It is well known that defence mechanisms in fish play an important role in all stages of parasitic infestation. In *O. mykiss*, infestation by *A. japonicus* resulted in the migration of lymphocytes out of the blood and into the epidermis (HAOND et al., 2003), while in *Schizodon intermedius* infestation by *L. cyprinacea* triggered blood lymphocytopenia, neutrophilia and monocytosis (SILVA-SOUZA et al., 2000). In hybrid tambacu, infestation with *D. carvalhoi* caused an increase in number of monocytes and PAS-GL, although thrombocyte, lymphocyte, neutrophil and eosinophil counts remained unaffected. In contrast, in *P. mesopotamicus*, infestation by *Argulus* sp. induced a decrease in thrombocyte percentage, whereas the percentage of monocytes and PAS-GL increased and total white blood cell counts remained unaffected (TAVARES-DIAS et al., 1999). These blood cells may represent the third line of defence in hybrid tambacu and *P. mesopotamicus* when parasitized by branchiuran ectoparasites (DALMO et al., 1997). Blood leukocytes, especially granulocytes and monocytes, could destroy pathogenic organisms.

In conclusion, ectoparasite infestations represent an important challenge to fish. The studied case demonstrated that a mild infestation of hybrid tambacu fish by *D. carvalhoi* lead to osmoregulatory disturbances and to an activation of the cellular defence mechanism. Even a mild infestation by this parasite is undesirable in farmed fish for two reasons: a) stocking densities during rearing facilitate a fast parasite spread and a severe disease outbreak can occur; b) detected haematological alterations indicate a partially compromised health status that may lead to reduced resistance to other challenges.

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Received: 3 May 2006
Accepted: 2 July 2007

TAVARES-DIAS, M., F. RUAS DE MORAES, E. M. ONAKA, P. C. B. REZENDE: Promjene pokazatelja krvne slike hibridnih riba tambacu invadiranih ribljom uši *Dolops carvalhoi* (Crustacea, Branchiura). Vet. arhiv 77, 355-363, 2007.

SAŽETAK

Poznato je da se mjerenje vrijednosti različitih pokazatelja u krvi riba upotrebljava za praćenje njihova zdravlja. U ovom istraživanju promatran je učinak prirodne invazije ribljom uši *Dolops carvalhoi*, Lemos de Castro, 1949., na broj eritrocita, trombocita, leukocita te na razinu glukoze u plazmi i elektrolita u serumu u hibridne ribe tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). U invadiranih riba zabilježene su niže vrijednosti hematokrita i magnezija te povećane vrijednosti srednje koncentracije hemoglobina, glukoze u plazmi, serumskih proteina, natrija i klorida, broja monocita te PAS pozitivnih granuliranih leukocita u odnosu na kontrolne ribe. Ovo je prvo izvješće o fiziološkim promjenama uzrokovanima ribljom uši *Dolops carvalhoi*. Rezultati naznačuju da blaga invazija može dovesti do znatnih osmozoregulacijskih poremećaja u domaćina.

Ključne riječi: hematologija, *Dolops carvalhoi*, krvne stanice, slatkovodna riba, invazija, paraziti