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Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(3): 267-271
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doi:10.3906/vet-0903-26

The effects of ichthyophthiriasis on some haematological parameters in common carp

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Received: 18.03.2009

Abstract: The present study examined the haematological effects of ichthyophthiriasis in common carp. Blood was collected from 4 groups of juvenile carp: 1. healthy; 2. with minor symptoms of ichthyophthiriasis; 3. with severe symptoms of ichthyophthiriasis (moribund); survivors of ichthyophthiriasis after a 3-week recovery period. Blood was subjected to a standard haematological procedure and the following parameters were evaluated: haematocrit (Ht), haemoglobin concentration (Hb), erythrocyte (RBC) and leukocyte (WBC) counts, mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC). The differential leukocyte count and thrombocyte count were calculated using stained blood smears.

No change in Ht or Hb, but a significant decrease in RBC compensated by an increase in MCV and MCH were observed. Fish with minor symptoms of disease had a significant increase in WBC (167.4×10^3/μL) without an alteration in the differential count, as compared to the controls (93.3×10^3/μL), while those with severe symptoms had a significant decrease in WBC (48.4×10^3/μL) accompanied by lymphopenia, and an increase in neutrophil and monocyte contribution. After 3 weeks of recovery WBC returned to a level similar to that in the controls (89.5×10^3/μL). In all the parasite-infested fish metabolic activity of phagocytes was significantly reduced, as compared to the controls. In the fish with severe symptoms of disease thrombocytosis and hypercoagulability were observed.

Key words: Blood, *Cyprinus carpio*, fish, *Ichthyophthirius multifiliis*

Introduction

Ichthyophthirius multifiliis Fouquet (commonly referred to as ich) is a widespread ectoparasitic ciliate that occurs in temperate, subtropical, and tropical zones, and may cause considerable loss of fish, particularly under farm or hatchery conditions (1,2). According to Hines and Spira (3), ichthyophthiriasis is probably the most devastating parasitic disease of cultured fish. The parasite invades surface epithelia of the gills and skin, causing significant damage and resulting in impaired osmotic balance (1,4). Various chemicals are used to control the disease, including formaldehyde, NaCl, $KMnO_4$, and $CuSO_4$ solutions, though none is effective in a single dose (2,5-8).

Crowding stress increases the susceptibility of fish to infestation with ich due to suppression of innate defences (9). Transportation from natural to indoor conditions may result in rapid disease outbreak and considerable fish mortality (10). In addition, ich renders fish more susceptible to bacterial infections (11).

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It is known that fish develop immune responses against ich, including innate mechanisms (NCC cells) and antibody responses (3,12). According to Sin et al. (13), ich-infested fish exhibit heavy infiltration of mononuclear leukocytes into the skin, and both humoral- and cell-mediated immune responses. The presence of antibody-producing B cells in the skin of channel catfish and a considerable increase during ich invasion was reported by Zhao et al. (14). Cross and Matthews (15) reported eosinophils and basophils infiltrating the infection site, and localised phagocytosis by neutrophils and macrophages.

However, few data are available on the haematological effects of ichthyophthiriasis. According to Hines and Spira (16), carp invaded by *Ichthyophthirius multifiliis* developed lymphopenia and neutrophilia, accompanied by rejuvenation of the leukocyte pattern, but without alterations in the total leukocyte count. Tavares-Dias et al. (17) reported anaemia and a shift towards phagocytes in *Oreochromis niloticus* with gill ichthyophthiriasis and saprolegniosis. The present study was undertaken to evaluate the haematological changes in common carp during *Ichthyophthirius multifiliis* invasion.

Materials and methods

The study included 5-month-old common carps (*Cyprinus carpio* L.) with a body mass of 60.7 ± 27.9 g that were harvested in mid October 2008 from the rearing ponds of Warsaw University of Life Sciences at the Łąki Jaktorowskie Pond Station. The fish were transported to the laboratory of the Department of Animal Physiology in Siedlce, where they were stocked in a continually aerated flow-through system at the density of about 40 g/L. Water temperature was 18-19 °C, oxygen saturation was 60%-80%, pH was 6.9, and the total ammonia level was 3.5-5 mg/L. The fish were fed commercial carp starter twice a day.

After 3 weeks some of the fish were transferred to aerated aquaria (density about 7 g/L) in which water was changed daily without disturbing the fish (about 75% of the water was quickly siphoned out and then water flow was applied until complete water renewal occurred). After about 1 week most of the fish developed ichthyophthiriasis. Parasites were present in the gills and skin, although typical white spots on the skin were not noted. Blood was collected 3 days after disease outbreak from 3 groups (10 fish in each): healthy, parasite-free fish (control), fish with minor symptoms (appetite loss, abundant mucus on skin, and small skin haemorrhages [Ich-1]), and fish with severe symptoms (extensive skin haemorrhages and necrosis, fin and gill lesions, poor locomotor activity, and anorexia [Ich-2]). All Ich-2 fish died within 4 days of infection outbreak, versus only 30% of Ich-1 fish. Survivors were subjected to a 30-min bath in 0.2 mL/L of formaldehyde solution (5) and placed in 16 °C. These fish very soon regained their appetite and apparently recovered (skin and gill lesions healed, but some parasites were still present in the gills). After 3 weeks of recovery blood was sampled from these fish (Ich-3).

Blood (about 300 µL from each fish) was collected into chilled heparinised Eppendorf tubes via heart puncture, using heparinised needles, and was subjected to standard haematological procedures. Blood smears were also made, stained with May-Grunwald and Giemsa solutions, for evaluation of the differential leukocyte count. Haematological analyses included red blood cell parameters (haematocrit [Ht], haemoglobin concentration [Hb], and erythrocyte count [RBC], as well as the following calculations: mean cell volume [MCV = Ht × 10/RBC], mean cell haemoglobin [MCH = Hb/RBC], and mean cell haemoglobin concentration [MCHC = Hb × 100/Ht]). White blood cell parameters included leukocyte count (WBC), metabolic activity of phagocytes (NBT), and the differential leukocyte count (percentage of lymphocytes, neutrophils, and monocytes).

The thrombocyte count was estimated from WBC and the number of thrombocytes accompanying 100 leukocytes in each smear. Haematocrit was measured using the microhaematocrit method, after centrifugation of capillaries with blood at 12,000 rpm for 5 min. Red and white blood cells were counted in blood diluted 100 times with Hayem's solution in a Burker chamber. Haemoglobin concentration was measured using the spectrophotometric cyanmethaemoglobin method and Drabkin's solution at 540 nm. Metabolic activity of phagocytes was measured using the spectrophotometric method of nitrotetrazolium blue (NBT) reduction to formazan,

modified for use in fish (18). The differential leukocyte count was calculated from analysis of the stained blood smears in which various types of leukocytes were counted per 100 cells. The results were subjected to one-way ANOVA and Duncan's post-hoc test, assuming a significance level of $P \leq 0.05$.

Results

Haematocrit and haemoglobin concentration did not significantly differ between the experimental groups, but the erythrocyte count decreased during the disease and after 3 weeks of recovery (Ich-3) was significantly lower than that in the control group (Table). This was accompanied by an increase in the size of erythrocytes (MCV) and their haemoglobin load (MCH).

More dramatic changes were observed in the white blood cell parameters (Table). In the Ich-1 group (minor symptoms of disease) the leukocyte count increased to a very high level without alteration in the differential leukocyte count, while in Ich-2 (severe symptoms) it dramatically decreased, as compared to the control group. The observed WBC decrease in Ich-2 was primarily related to lymphopenia, and was accompanied by an increase in the percentage of neutrophils and monocytes. After 3 weeks of recovery (Ich-3) WBC and the percentages of various leukocyte types returned to levels similar to those observed in the control group. Phagocyte activity (NBT) was significantly reduced in all groups of parasite-infested fish, as compared to the control group.

The thrombocyte count increased in the infested fish, and reached the maximum in the Ich-2 group

Table. Changes of haematological parameters in common carp during ectoparasite, ich, *Ichthyophthirius multifilii*. Invasion and recovery (in each group n = 10, mean ± S.D., different letter denominators indicate significant differences, Duncan's test, $P < 0.05$).

Parameter	Groups of fish			
	Control	Ich-1	Ich-2	Ich-3
Ht [%]	23.8 ± 3.7	26.4 ± 3.0	27.3 ± 4.2	27.0 ± 4.6
Hb [g/L]	80.8 ± 36.4	87.9 ± 11.9	101.1 ± 18.8	88.9 ± 13.2
RBC [10^6/μL]	2.04 ± 0.32 ^{a}	1.87 ± 0.29 ^{ab}	1.83 ± 0.24 ^{ab}	1.69 ± 0.34 ^{b}
MCV [fL]	117 ± 18 ^{a}	143 ± 18 ^{ab}	154 ± 19 ^{b}	166 ± 48 ^{b}
MCH [pg]	37.5 ± 14.8 ^{a}	48.1 ± 10.3 ^{ab}	55.8 ± 11.2 ^{b}	54.3 ± 11.8 ^{b}
MCHC [g/L]	325 ± 86	335 ± 43	375 ± 49	332 ± 43
WBC [10^3/μL]	93.3 ± 3.7 ^{a}	167.4 ± 57.5 ^{b}	48.4 ± 22.6 ^{c}	89.5 ± 34.8 ^{a}
NBT [g/L]	1.51 ± 0.67 ^{a}	0.41 ± 0.16 ^{b}	0.59 ± 0.27 ^{b}	0.51 ± 0.16 ^{b}
Lymphocytes [%]	91.8 ± 5.9 ^{a}	95.5 ± 2.4 ^{a}	61.4 ± 16.3 ^{b}	96.3 ± 2.1 ^{a}
Neutrophils [%]	5.8 ± 4.4 ^{a}	3.5 ± 2.3 ^{a}	33.2 ± 17.7 ^{b}	2.6 ± 1.9 ^{a}
Monocytes [%]	1.9 ± 1.6 ^{a}	0.9 ± 1.3 ^{a}	5.6 ± 5.4 ^{b}	0.8 ± 0.8 ^{a}
Thrombocytes [10^3/μL]	20.8 ± 11.3 ^{a}	27.5 ± 17.2 ^{ab}	33.5 ± 17.6 ^{b}	19.1 ± 9.2 ^{a}

Control – healthy fish, Ich-1 – fish with light ichthyophthiriasis symptoms, Ich-2 – fish with severe ichthyophthiriasis symptoms (moribund), Ich-3 – survivors after a 3-week recovery period.

(Table), which was accompanied by high coagulability of blood (to obtain blood from 10 fish, 16 individuals were sampled and blood from 6 of them coagulated immediately and could not be used for analyses). After 3 weeks of recovery the thrombocyte count returned to a level similar to that observed in the control group.

Discussion

A decrease in the erythrocyte count indicates a slightly anaemic condition; however, a gradual increase in MCV was observed, indicating erythrocyte swelling, probably due to stress-related catecholamine secretion (19). Swelling is a low-cost mechanism of increasing oxygen transport capacity, as diluted haemoglobin exhibits higher oxygen binding affinity (20,21). In the present study erythrocyte swelling was accompanied by an increase in MCH, which may suggest haemoglobin synthesis via circulating erythrocytes; this possibility was reported by Speckner et al. (22). MCV and MCH levels remained elevated until the end of the experiment. Similar results were obtained by Kurovskaya and Osadchaya (23), who did not report anaemia in common carp infested with *I. multifiliis*. On the other hand, Tavares-Dias et al. (17) reported a significant decrease in RBC, Hb, Ht, and MCHC accompanied by an increase in MCV in *Oreochromis niloticus* with gill ichthyophthiriasis and saprolegniosis.

In the present study the observed drop in the leukocyte count in the fish with severe symptoms of ichthyophthiriasis differs from the results obtained by Hines and Spira (16), who did not report any alterations in WBC and showed that the leukocyte count in the course of the disease is dynamic and probably related to its severity. The decrease in the leukocyte count and phagocyte activity explains why ichthyophthiriasis renders fish very susceptible to other pathogens (11). Lymphopenia and the increase in the percentage of phagocytes observed in the Ich-3 group are in accordance with the results obtained by Hines and Spira (16) for common carp, and by Tavares-Dias et al. (17) for Nile tilapia. Metabolic activity of phagocytes in all the infested fish in the present study was significantly reduced during the entire experimental period, including recovery. According to Alvarez-Pellitero (24), ciliates may modulate inflammatory reactions involving phagocytosis, oxidative activity of phagocytes, and complement activity.

Among the neutrophils in the present study, primarily juvenile stages (myelocytes and metamyelocytes) were noted, while mature cells (band and segmented) were very scarce, as was reported by Hines and Spira (16); however, only single blast cells occurred. Probably, phagocytes quickly migrated to the infested tissues where they reached functional maturation, and their elevated contribution in blood indicates a high rate of their release from haemopoietic tissue. Infiltration of various types of leukocytes into the skin, and both local non-specific and specific immune responses were observed in ich-infested fish by various authors (13-15).

Increases in the thrombocyte count and hypercoagulability are frequently observed symptoms of stress in fish (25). Another potential function of thrombocytes in fish is their participation in immune functions and their blood level may increase after stimulation using various antigens (26). They are also involved in phagocytosis (27,28). Thus, the increases in the thrombocyte count observed in the present study might have been related to defence against the parasite.

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