ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XV Fasc. 2 Szczecin 1985

Antonina SOPIŃSKA

Hematology

EFFECTS OF PHYSIOLOGICAL FACTORS, STRESS, AND DISEASE ON HEMATOLOGIC PARAMETERS OF CARP, WITH A PARTICULAR REFERENCE TO THE LEUKOCYTE PATTERNS. III. CHANGES IN BLOOD ACCOMPANYING BRANCHIONECROSIS AND BOTHRIOCEPHALOSIS

WPŁYW CZYNNIKÓW FIZJOLOGICZNYCH, STRESSOWYCH I CHOROBOWYCH NA PARAMETRY HEMATOLOGICZNE KARPIA ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OBRAZU BIAŁOKRWINKOWEGO. III. ZMIANY KRWI WYSTĘPUJĄCE PRZY BRANCHIONEKROZIE I BOTRIOCEFALOZIE

Institute of Infectious and Invasive Diseases, Department of Fish Diseases Academy of Agriculture, Lublin

The work presented treats hematologic assays as complementary procedures to aid in the determination of fish health.

Changes in the carp blood, occurring in two diseases: branchionecrosis and bothriocephalosis (a disease induced by a cestode, *Bothriocephalus acheilognathi*), are described and treated statistically.

INTRODUCTION

Hematologic tests are helpful in assessing the health state of an organism as all physiological disturbances affect quantitative and qualitative blood parameters (Blaxhall, 1972; Houston and De Wildae, 1972). On the one hand, hematologic assays allow to estimate the force of a disease-causing agent (Einszporn-Orecka and Wierzbicka, 1974; Grabda et al., 1974); on the other, they can be very helpful in identifying the disease and in diagnosing conditions of unclear etiology (Lejman, 1955; Spoljanskaja, 1966; Blaxhall and Daisley, 1973).

There are a few papers only dealing with carp blood changes in contagious and invasive diseases and they are of a limited value due to their purely fragmentary nature and non-uniform nomenclature following four different systems now in existence (Puczkow, 1962; Jakowska, 1956; Weinreb, 1963; Ivanowa, 1970).

Some studies consider quantitative changes in erythrocytes only. Svobodova and Tesarcik (1973) observed such changes in Spring viremia of carp, swimming bladder inflammation, and erythrodermatitis. Changes in abundance of erythrocytes and leukocytes were described in carp suffering from swimming bladder inflammation, branchionecrosis, ichthyophthiriasis, dactylogyrosis, and ligulosis (Špoljanskaja, 1953; Sadovskaja, 1958; Uspenskaja, 1961; Rošetnikova, 1962; Metelev, 1963; Čečina and Ekelčik, 1976; Pilarczyk, 1978).

More detailed hematologic studies involving not only quantitative changes, but also morphological ones in blood cells in carp diseases are very scarce indeed. Golovina et al. (1977) described changes in leukocytes, associated with swimming bladder inflammation, branchiomycosis, and ichthyophthiriasis, while Waluga and Flis (1971) observed such changes in ammonia intoxication. No other carp diseases were looked at from the standpoint of similar studies.

There are only a few papers on disease-related changes of blood cells of fish species other than carp, eg., in bream affected by leukemia (Einszporn-Orecka and Wierzbicka, 1974), and in tinch with ergasiliasis (Einszporn-Orecka, 1973).

The present work was eimed at following changes in carp blood, accompanying two disease: branchionecrosis, a condition caused by adverse environmental effects, and bothriocephalosis induced by a cestode, *Bothriocephalus acheilognathi*. The two conditions are common in carp cultures in Poland, the accompanying changes in blood being relatively poorly known.

MATERIALS AND METHODS

MATERIALS

A. Branchionecrosis

The diseased fish were divided into two experimental groups depending on the intensity of clinical changes:

– one group involved carp individuals aged 12, 14, 24, and 26 months with clear symptoms of acute branchionecrosis (swelling, hyperemia, ecchymoses, increased amount of mucus),

– the other group consisted of individuals aged 12 and 24 months with symptoms of chronic form of the disease (anemia in gills, necrotic lesions).

Control individuals, each time at the same age, showed unchanged gills; they were collected at a fish farm where neither branhionecrosis nor other disease had been recorded that year.

In each case blood samples were collected from 20 individuals.

Hematologic indices were compared between the experimental and control fish at the same age and in the same calendar months. Additionally, in the acute branchionecrosis the hematologic parameters were compared between fish of differing age. The tests were made in June and August.

B. Bothriocephalosis

The individuals to be examined were obtained from the Samoklęski Fish Farm. Blood samples were collected from specimens aged 9, 12, 14, 16, 24, 26, and 28 months in March, June, July, August, September, and October; each time 20 individuals were examined. After blood collection, the extent of infestation was assessed on dissected individuals. Subsequent blood analyses were made exclusively on samples obtained from cestode-infested carp. The mean invasion extent is presented in Table 1.

The experiment involved a double control. One control group consisted of carp aged 6, 12, 14, 16, 24, 26, and 28 month obtained from the Podlodowo Fish Farm (denoted by I). The other control comprised cestode-free fish obtained from the same fish farm as the infested carp (i.e., Samoklęski, the farm being denoted by II), aged 9, 12, 14, and 16 months.

Blood samples were taken each time in the same calendar month from the experimental group and two controls. On each occasion, the fish of the groups compared were of the same age.

The carp obtained from Farm I, in addition to having not experienced any *Bothriocephalus acheilognathi* infestation, showed no invasion of other intestinal parasites. Only their gills sometimes housed low numbers of parasitic protozoans and sporadic cases of a *Dactylogyrus sp.* invasion were found.

No disease symptoms were ever found in those fish.

The control fish from Farm II, free of *Bothriocephalus acheilognathi,* showed in some cases loosened and slightly hyperemic intestinal mucosa and were occasionally emaciated. No other intestinal parasites were revealed, few parasitic protozoans occurring on gills.

METHODS

Blood samples were collected as described by Klontz and Smith (1968). The details of the procedures applied are given in Part I of the work.

Table 1

Mean invasion extent of *Bothriocephalus acheilognathi* in the experimental carp.

Fish age (months)	9	12	14	15	16	24	26	28
Date of blood sampling	20.03.78	15.06.78	14.08.78	10.09.78	15.10.78	15.06.78	16.08.78	18.10.78
Invasion incidence (%)	62.50	44.50	25.00	20.00	20.00	24.80	12.00	9.00
Invasion intensity (%)	12.30	10.50	6.20	3.00	4.20	4.50	3.40	2.90

Table 2

Peripheral blood hematologic parameters of control (K) and branchionerosis-affected carp showing acute (B_o) and chronic (B_c) forms of the disease

Age (months)		Data of blood sampling	Erythrocyte ($x10^6/mm^3$)		Hematocrit (%)		Hemoglobin (%)		Leukocyte count. ($x10^3/mm^3$)	
			SR	SD	SR	SD	SR	SD	SR	SD
12	K	20.06	1.60^{c}	0.19	37.30^{b}	4.00	8.17^{c}	1.26	47.04^{a}	6.13
	B_o	12.06	1.15^{a}	0.19	29.80^{a}	5.16	4.03^{a}	1.38	143.63^{b}	34.96
	B_c	14.06	1.35*	0.22	30.20^{a}	6.21	5.61^{b}	0.94	58.19^{c}	13.65
14	K	10.08	2.34^{b}	3.32	34.50^{b}	3.76	8.04^{b}	0.83	47.25^{a}	6.59
	B_o	22.08	0.99^{a}	0.18	27.40^{a}	5.32	3.89^{a}	1.02	117.45^{b}	24.27
24	K	20.6	1.50^{b}	0.12	34.10^{b}	2.99	7.68^{c}	0.99	50.25^{a}	5.68
	B_o	10.06	1.15^{a}	0.27	29.50^{a}	6.00	4.38^{a}	0.99	98.19^{b}	16.04
	B_c	04.07	1.44^{b}	0.18	34.10	4.60	5.79^{b}	0.82	56.38^{a}	16.25
26	K	18.08	1.53^{b}	0.11	34.90^{b}	2.70	7.68^{b}	0.81	46.14^{a}	5.89
	B_c	04.08	1.03^{a}	0.17	24.80$^{a}_{*}$	4.24	4.35$^{a}_{*}$	1.14	56.38$^{a}_{*}$	16.25

SR – arithmetic mean, SD – standard deviation, a, b, c – graduated significance of differences between means within an age group (a – the lowest significance), Bo – means significantly different (p 0.05) in relation to age, * – significance of differences between means for B_o carp 12 – and 14–mo–old, * – significance of difference between means for B_o carp 14 – and 26–mo–old, * – significance of differences between means for B_o – carp 24 – and 12–mo–old, * – significance of differences between means for B_o carp 26– and 24–mo–old, no * – non significat differences

The following hematologic indices were examined: erythrocyte and leukocyte counts, hematocrit, hemoglobin level, MCV, MCH, and MCHC. The leukogram was determined and morphology of erythrocytes and leukocytes observed both in the control and in disease-affected fish. Leukocytes were identified as indicated by Ivanova (1970).

All the data obtained were treated statistically, the results being compared by means of Student's test.

When determining the quantitative hematologic parameters, morphological changes in blood cells, induced by branchionecrosis and bothriocephaliasis, were revealed.

RESULTS

A. Branchionecrosis

The erythrocyte count is significantly lower only in the fish affected by the acute branchionecrosis (B_0) as compared to the controls (Table 2). The 12- and 14-mo-old individuals affected by this form of the disease differed significantly in their leukocyte counts (in June and August). The hematocrit was significantly different in the control and affected carp, being always lower in those individuals showing the acute branchionecrosis (B_0). The 24-and 26-mo-old B_0 carp showed also significant differences in their hematocrit (Table 2).

The hemoglobin levels differed significantly between the control fish (higher level) and those affected by both forms of the disease. Moreover, there was a significant difference between the values obtained for 14- and 26-mo-old B_0 carp examined in August (Table 2).

The fish affected by the acute branchionecrosis had their MCV, MCHC, and MCH values always higher than the controls.

Differences in leukocyte counts of the control and experimental fish were in most cases statistically significant (Table 2); noteworthy is a marked increase in those counts showed by the individuals affected by the acute disease. The B_0 carp of all age groups compared showed significant differences.

The myelocyte percentage is usually higher in the controls than in the affected fish, all differences being significant.

On the other hand, no clear-cut differences were revealed between the metamyelocyte percentages of the experimental and control fish.

The rod neutrophil percentage was always lower in the blood of the unaffected fish than in the B_0 ones, the lowest values being typical of those showing the chronic form of the disease. All the differences were significant.

The segmented neutrophil percentage varied in the fish examined, the observed differences being not always significant (Fig. 1).

The percentage of all neutrophils was significantly lower in older fish (24- and 25-mo-old) suffering from the acute form of the disease. In the chronic form, a

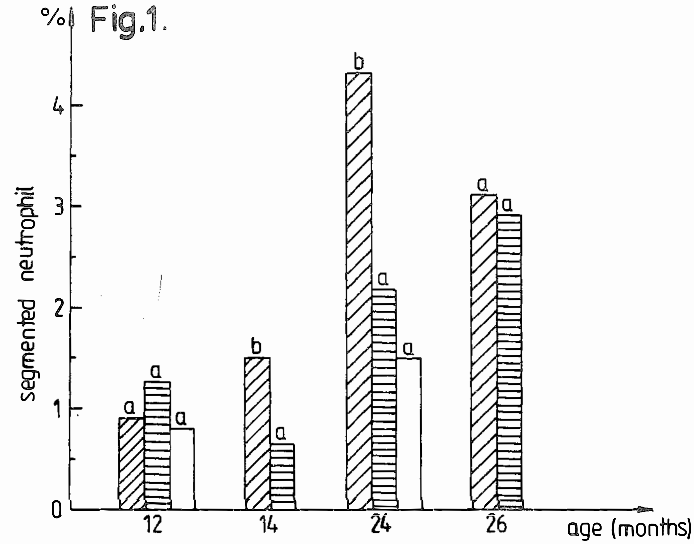


Fig. 1. Segmented neutrophil percentages in the peripheral blood of healthy and disease-affected (acute and branchionecrosis) carp

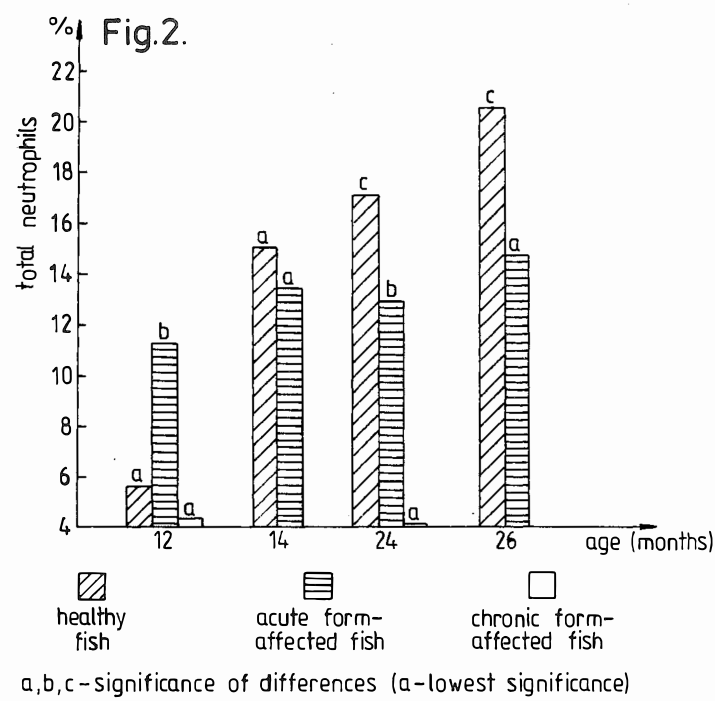


Fig. 2. Neutrophil percentages in the peripheral blood of healthy and disease-affected (acute and chronic branchionecrosis) carp.

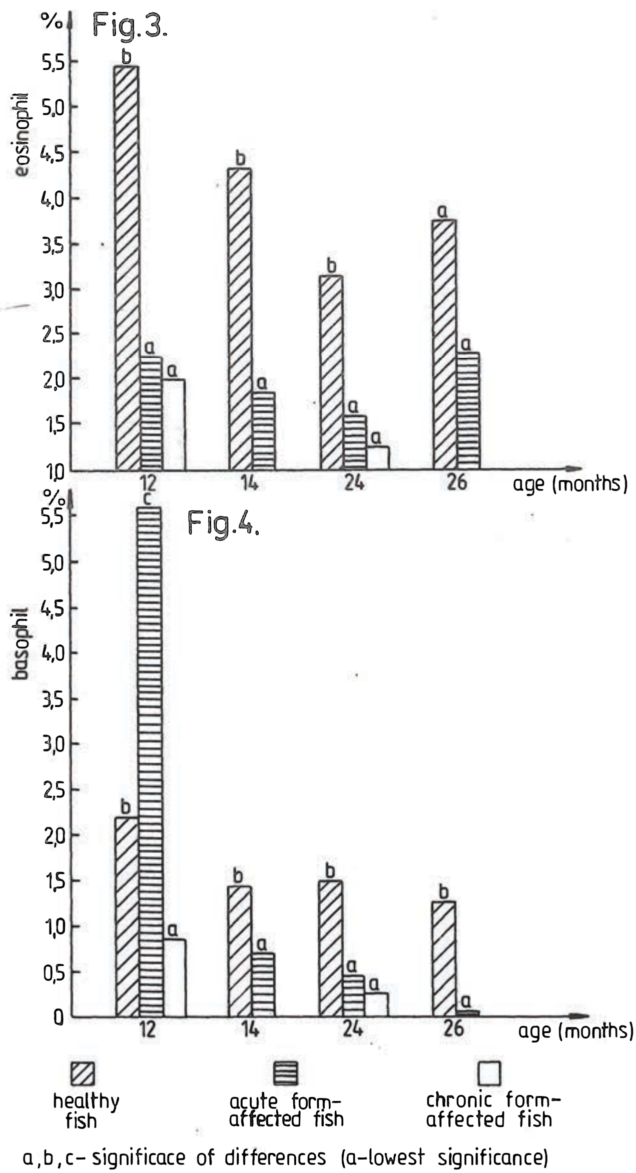


Fig. 3. Eosinophil percentages in the peripheral blood of healthy and disease-affected (acute and chronic branchionecrosis) carp.

Fig. 4. Basophil percentages in the peripheral blood of healthy and disease-affected (acute and chronic branchionecrosis) carp.

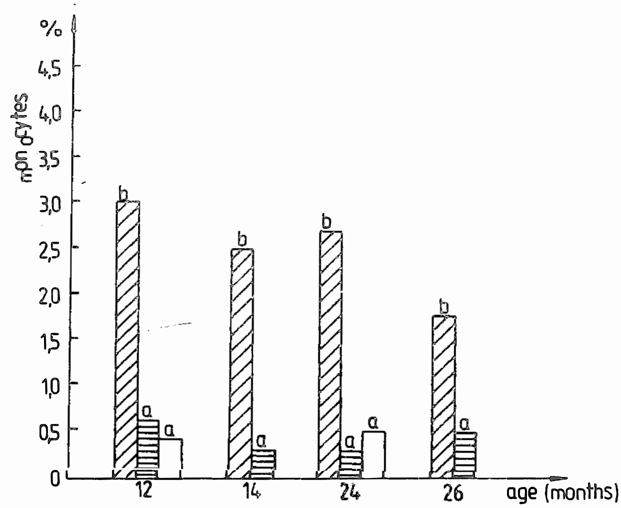


Fig. 5. Monocyte percentages in the peripheral blood of healthy and disease-affected (acute and chronic branchionecrosis) carp.

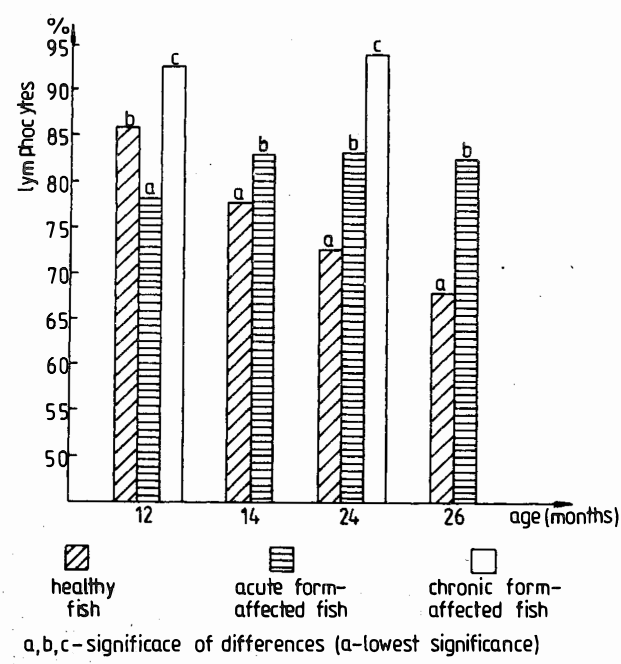


Fig. 6. Lymphocyte percentages in the peripheral blood of healthy and disease-affected (acute and chronic branchionecrosis) carp.

considerable reduction in the neutrophil percentage was observed both in younger and older carp. Noteworthy is a gradual increase in the neutrophil percentage with age of the control fish (Fig. 2).

The control fish eosinophil percentage was significantly higher than that in the diseased fish in all age groups (Fig. 3).

The basophil and monocyte percentages in the control 14-, 24-, and 26-mo-old carp were higher than those in the affected fish. Noteworthy is a particularly well-marked increase in the basophil percentage in the 12-mo-old B_0 carp as compared to the control (Figs. 4, 5).

The control and experimental specimens differed significantly in their lymphocyte percentages. The values were much higher in the carp showing symptoms of the chronic form than in the remaining groups. A significant difference was revealed for the 12- and 24-mo-old B_0 carp examined in June and August (Fig. 6).

B. Bothriocephalosis

The erythrocyte count in the infested fish was lower than that in the controls, the significant differences being found between the infested fish and both controls (Fig. 7), no significant difference in this parameter being found between the two controls.

The hematocrit differed significantly between the infested fish and both controls. The infested fish hematocrit was lower than that in the parasite-free individuals obtained from the same farm (Fig. 8).

The hemoglobin level was lower in the infested fish; the between-controls difference was not always significant (Fig. 9).

Erythrocyte indices (MCV, MCH, MCHC) showed, on occasions, significant differences between the infested fish and both controls, the values being usually lower in the parasite-affected individuals (Fig. 10).

The leukocyte counts always differed significantly between the infested and two control groups, the values being clearly higher in the first. No significant differences occurred usually between the two controls (Fig. 11).

The blastic leukocyte forms, i.e., myeloblasts and promyelocytes were more frequent in the infested fish blood. The myelocyte percentage showed usually significant differences between the parasite-infested and-free individuals, the values being higher than the controls in the infested 9- and 15-mo-old ones and lower in the 16- to 28-mo-old ones. Differences between the two controls were sometimes significant.

The infested and healthy fish differed significantly in their percentage of total neutrophiles, the percentage being always higher in the infested group than in the Samoklęski control one; on the other hand, it was sometimes lower and in other cases higher in the infested fish than in those obtained from Podlodowo (Fig. 12).

The eosinophil percentage was significantly higher in the infested fish compared to that in both controls. Also, most often the two controls differed significantly in this respect (Fig. 13).

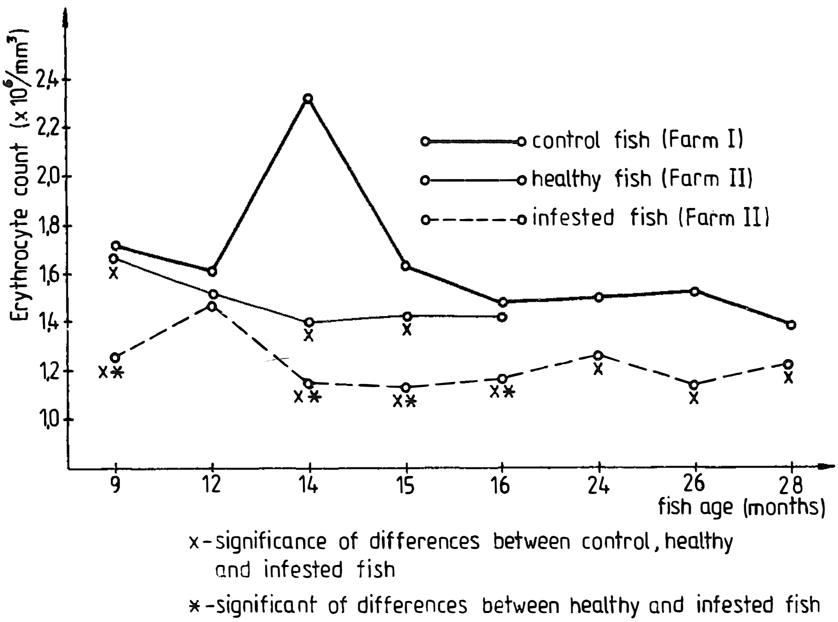


Fig. 7. Erythrocyte count in control (Farm I), healthy (Farm II), and *Bothriocephalus acheilognathi*-infested carp (Farm II)

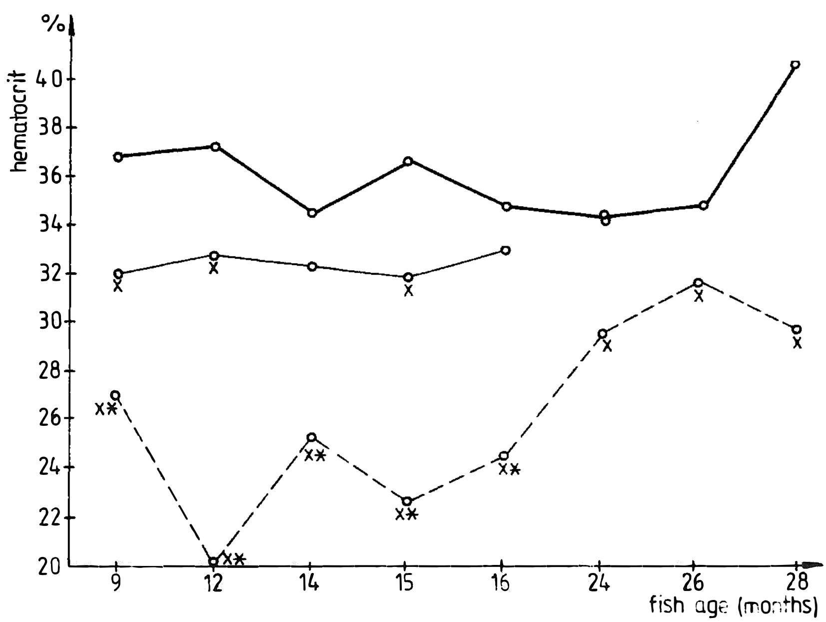


Fig. 8. Hematocrit in control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)

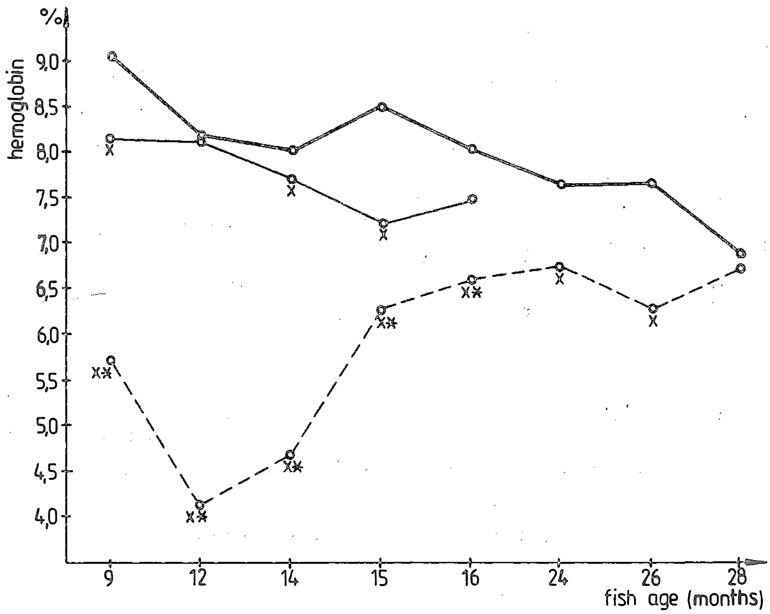


Fig. 9. Hemoglobin levels in control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)

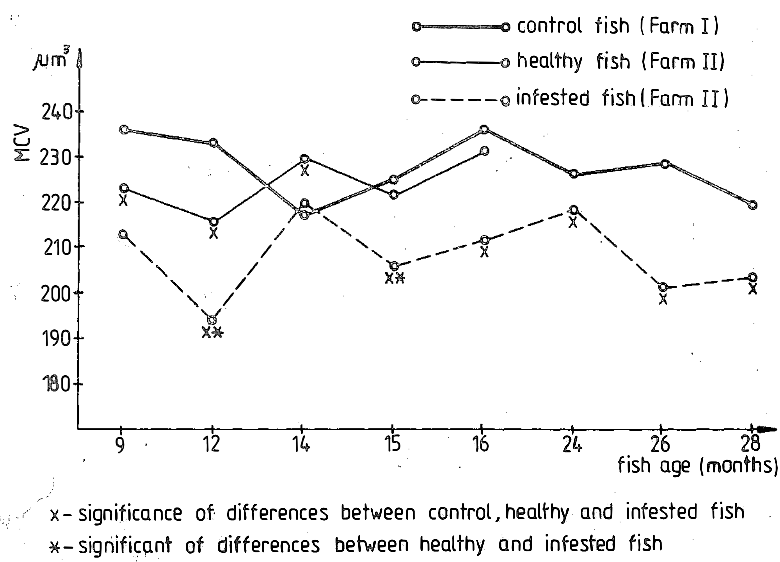


Fig. 10. Mean corpuscle volume (MCV) of erythrocytes in control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)

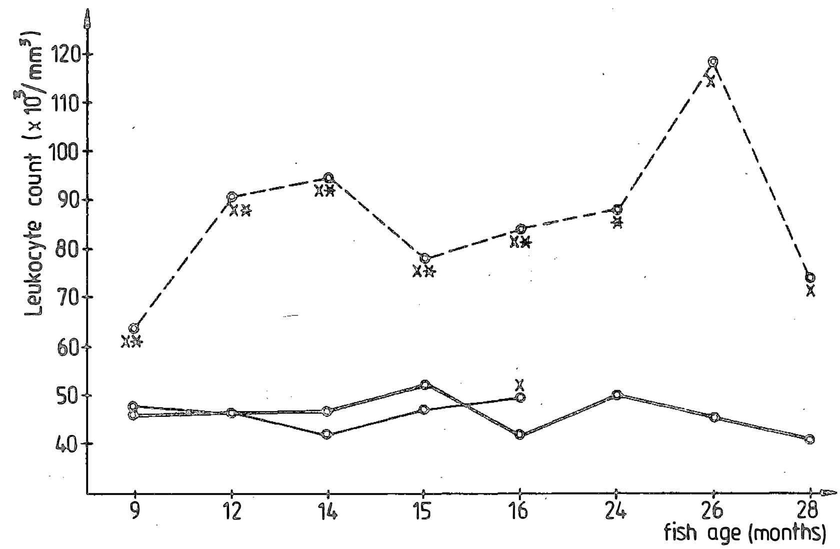
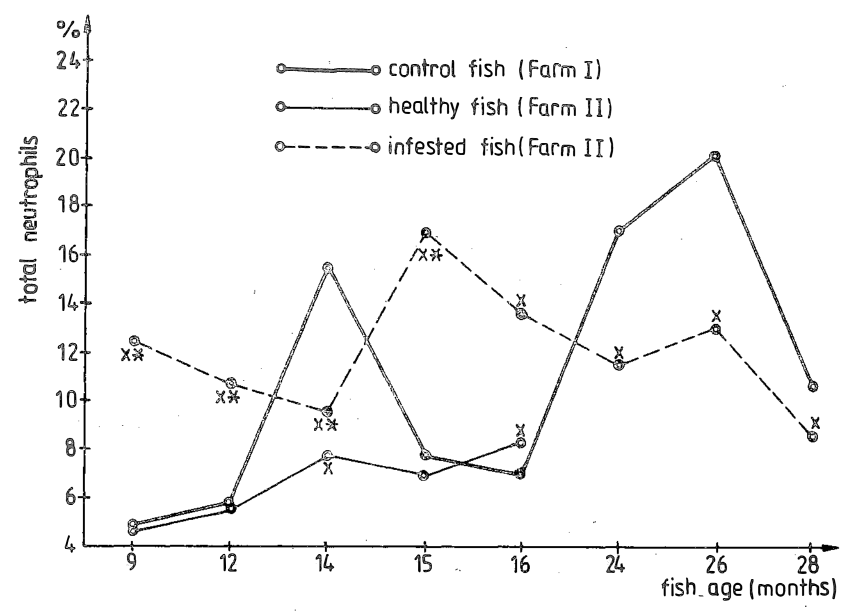


Fig. 11. Leukocyte count in control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)



x-significance of differences between control, healthy and infested fish

*-significant of differences between healthy and infested fish

Fig. 12. Neutrophil percentage in the peripheral blood of control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)

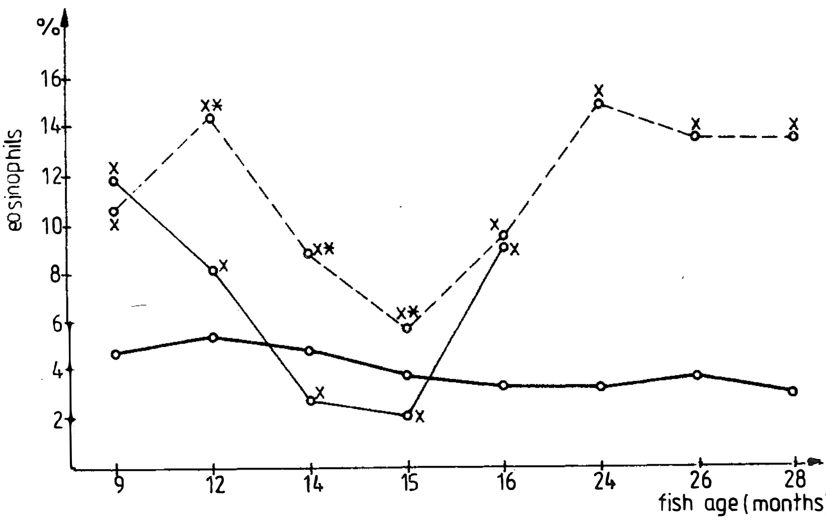
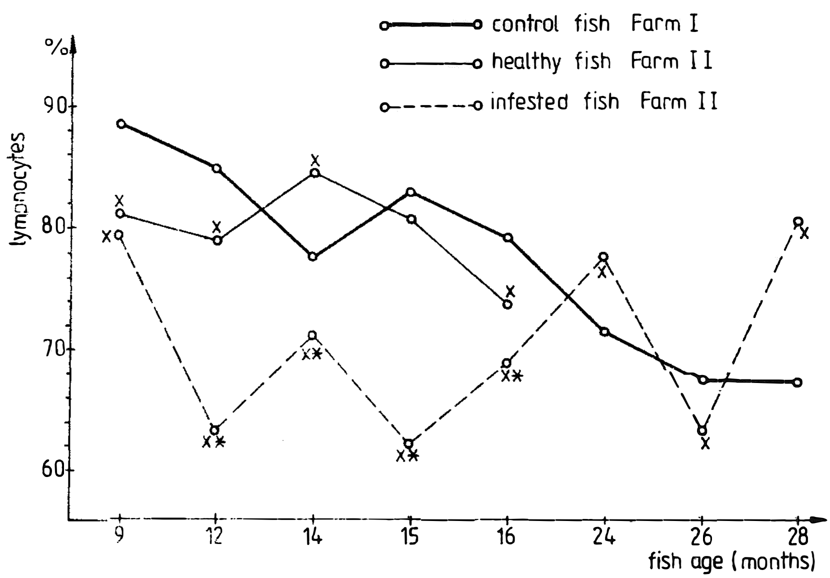


Fig. 13. Eosinophil percentage in the peripheral blood of control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)



x - significance of differences between control, healthy and infested fish
* - significant of differences between healthy and infested fish

Fig. 14. Lymphocyte percentage in the peripheral blood of control (Farm I), healthy (Farm II), and cestode-infested carp (Farm II)

The basophil percentage was on occasion significantly different between the infested and Podlodowo control fish, the significance of differences not always occurring between the first and the Samoklęski control. Significant differences, however, were revealed most often between the two controls.

The monocyte percentage showed more significant differences between the infested fish and Podlodowo control than between the first group and the Samoklęski control. It was not always that significant differences were being recorded between the two controls.

The lymphocyte percentage showed significant differences between the infested fish and the two controls, the percentage being usually lower in the first. The two controls differed significantly in this respect (Fig. 14).

Changes in bloos cells in the branchionecrosis-affected carp.

Two forms of branchionecrosis, acute and chronic, were revealed in the affected carp examined.

In the acute branchionecrosis, the peripheral blood cells showed enlarged nuclei and cytoplasm hypochromasia. Some erythrocytes were undergoing cytolysis (Plates 1, 2). Necrotic changes were found to have affected lymphocytes as well, the cells showing nuclear chromatin of increased thickness. The shrunk nuclei became irregular in shape (Plates 1, 2). No changes were seen in other blood cells.

In the chronic branchionecrosis, the peripheral blood erythrocytes showed aniso- and poikilocytosis; sometimes the regular pattern of the nuclear chromatin became obliterated. The cytoplasm surrounding the nucleus showed a clear less dense band (Plate 3a).

Pathologic changes involved granulocytes as well. Numerous neutrophils and eosinophils were considerably enlarged. In the cytoplasm of these cells which showed altered staining properties land obliterated structure, single vacuoles and pathologic granulations were observed (Plattes 3b, 3c, 3e).

Changes occurring in basophils involved irregular shaping of the cells.

Monocytes exhibited clear degenerative changes, i.e., vacuolar degeneration of the cytoplasm and nucleus (Plate 3d), obliteration of cytoplasm structure, and changed shape of the nucleus. Some monocytes showed altered, irregular shapes.

Typical of this form of the disease is a considerable lymphocytosis, affecting almost exclusively small lymphocytes, with a narrow band of cytoplasm which sometimes contains azurophilous granulations.

Degenerative changes in small lymphocytes and disintegration of those cells involve:

1) a clearly increased thickness of nuclear chromatin (karyopycnosis); the shrunken nucleus becomes irregular in shape, its obliterated structure showing sometimes brighter areas indicating the presence of vacuoles (Plate 3a);
2) chromatin break-down into granules and lumps (chromatorrhexis) (Plates 3b, 3c);
3) disappearance of the cellular membrane, pointing out to the disintegration.

No degenerative changes of blood cells were observed in the control fish.

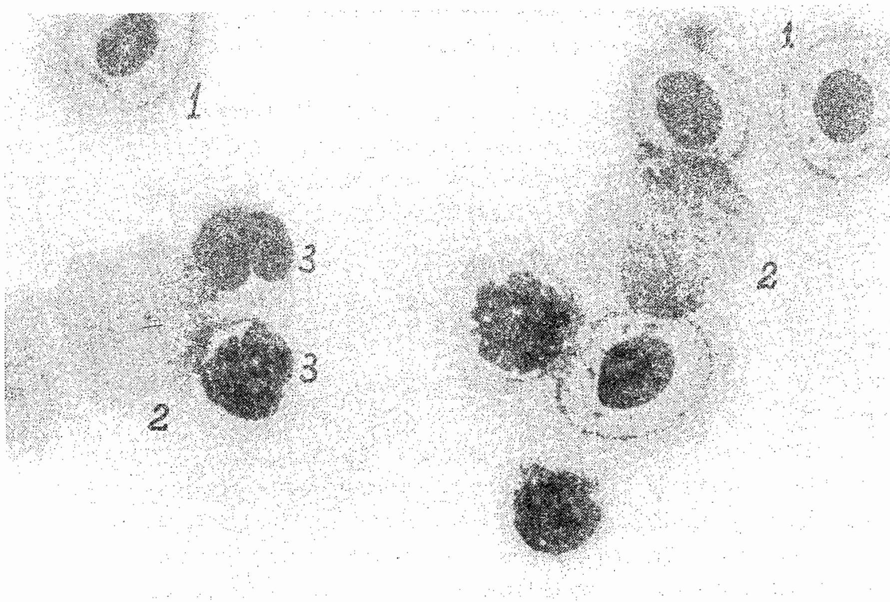


Plate 1. Degenerative changes in erythrocytes and lymphocytes in acute branchionecrosis (x 1800). Photo: J. Pacewicz: 1 – erythrocyte with basophil granulation, 2 – disintegrating erythrocyte, 3 – lymphocyte

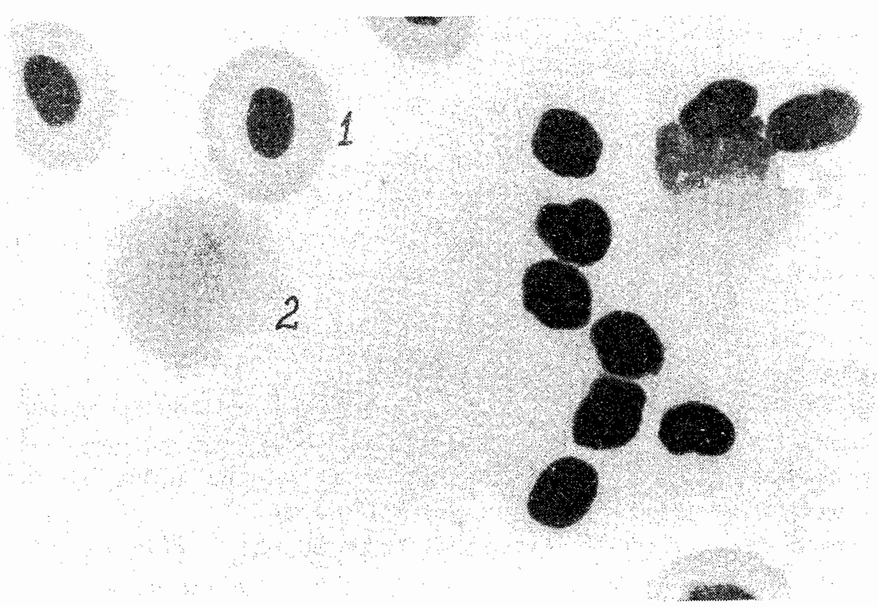
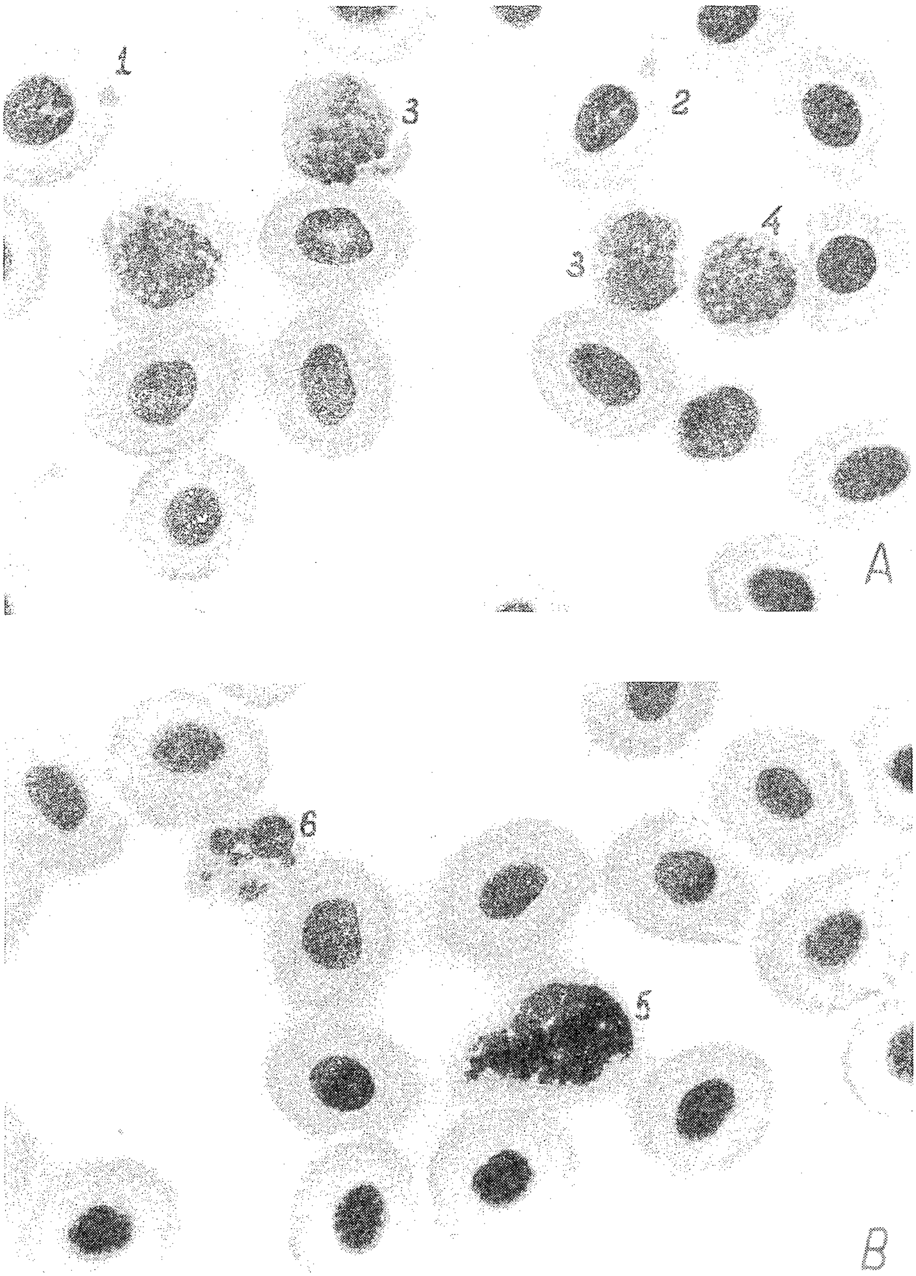


Plate 2. Erythrocyte hypochromasia (1) and cytolysis (2) in acute branchionecrosis (x 1800). Photo: J. Pacewicz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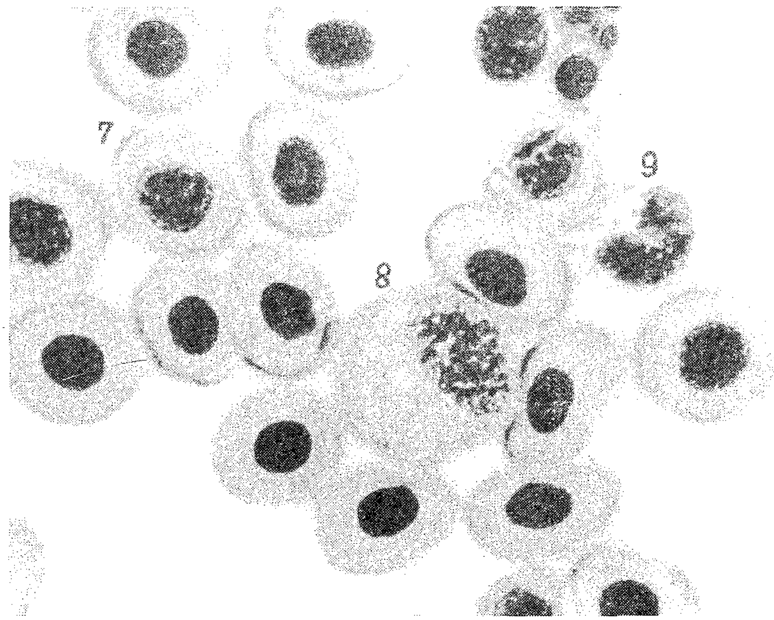

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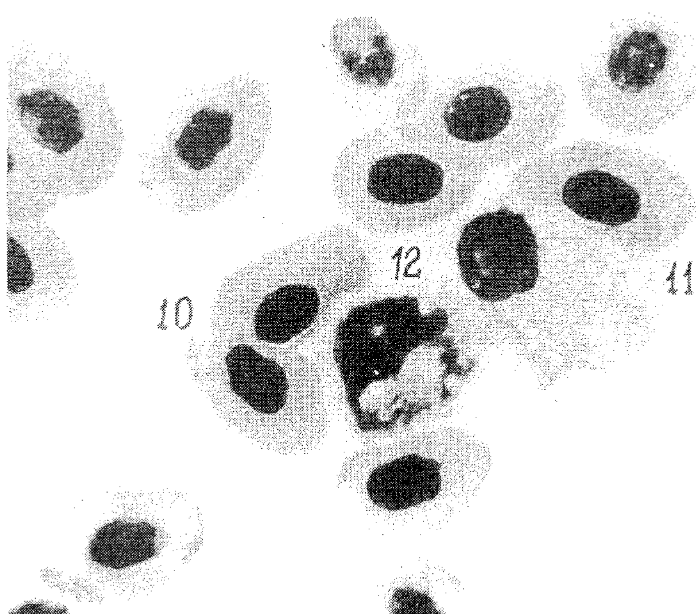

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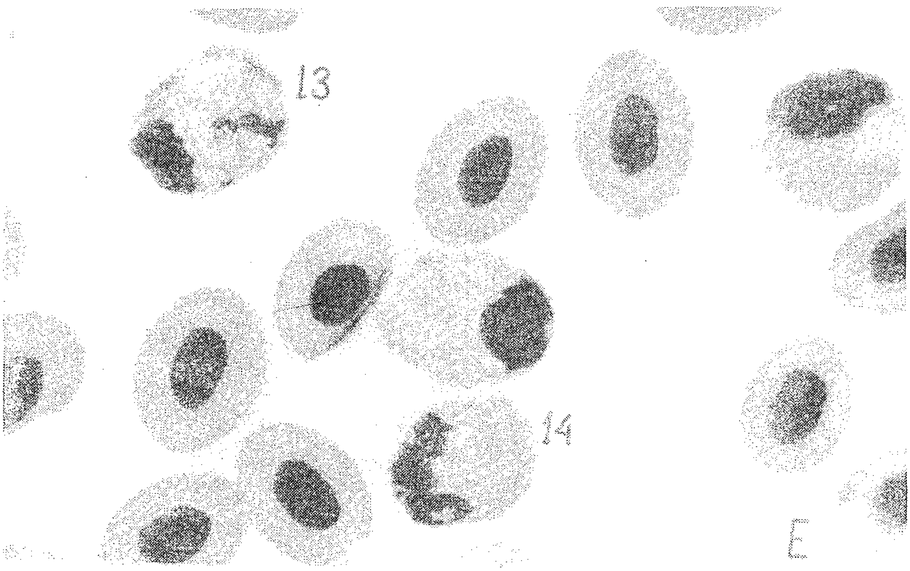


Plate 3. Degenerative changes in carp blood cells in chronic branchionecrosis (x 1800) Photo: J Pacewicz. A. 1 – polychromatic erythrocyte, 2 – erythrocyte with partial lysis of nuclear chromatin and brighter band around the nucleus, 3 – lymphocytes with degenerated chromatin, 4 – lymphocytes with vacuolar degeneration of cytoplasm. B. 5 – eosinophil: changed shape and pathologic granulation, 6 – lymphocyte with broken nuclear chromatin, C. 7 – polychromatophil erythrocytes, 8 – lymphocyte with vacuolar degeneration of cytoplasm. D. 10 – degenerative changes in erythrocytes, 11 – neutrophil, 12 – monocyte with vacuolar degeneration of nucleus and cytoplasm. E. 13 – nucleocyte with pathologic granulations, 14 – metamyelocytes with obliterated cytoplasm structure and degenerative changes in nuclei.

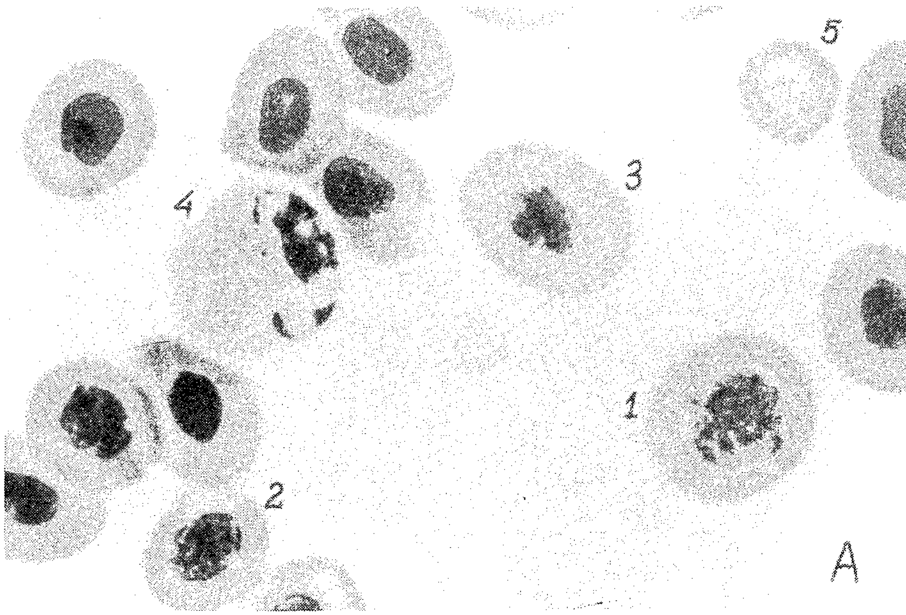
Changes in peripheral blood morphotic elements in the bothricephaliasis-affected fish.

The condition in carp results in hypochromatic anemia as well as in aniso- and poikilocytosis of erythrocytes (Plate 4a). The peripheral blood erythrocyte population is dominated by large cells, the so-called macrocytes. Disorders in the erythrocyte nucleus maturation are visible, the nuclear structure being loosened, with vacuoles. As a result of degenerative processes, numerous nuclei have altered shapes. The cytoplasm surrounding the nucleus shows a brigther band (Plates 4a, 4b. 4d).

Neutrophils are dominated by immature forms, sometimes growing to irregular dimensions. Their nuclei are spherical and enlarged. The cytoplasm structure is obliterated and foamy (Plate 4b).

No changes were observed in eosinophils.

Monocytes, particularly those in the fish affected by a stronger invasion, show sometimes a clear and highly advanced disintegration of nuclei and cytoplasm, the latter showing in places an obliterated structure (Plate 4a).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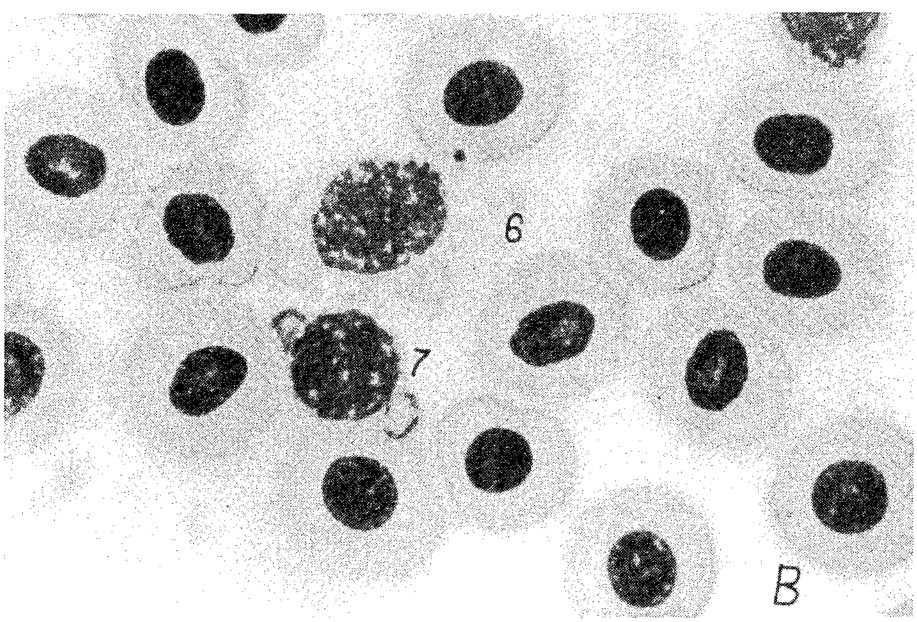
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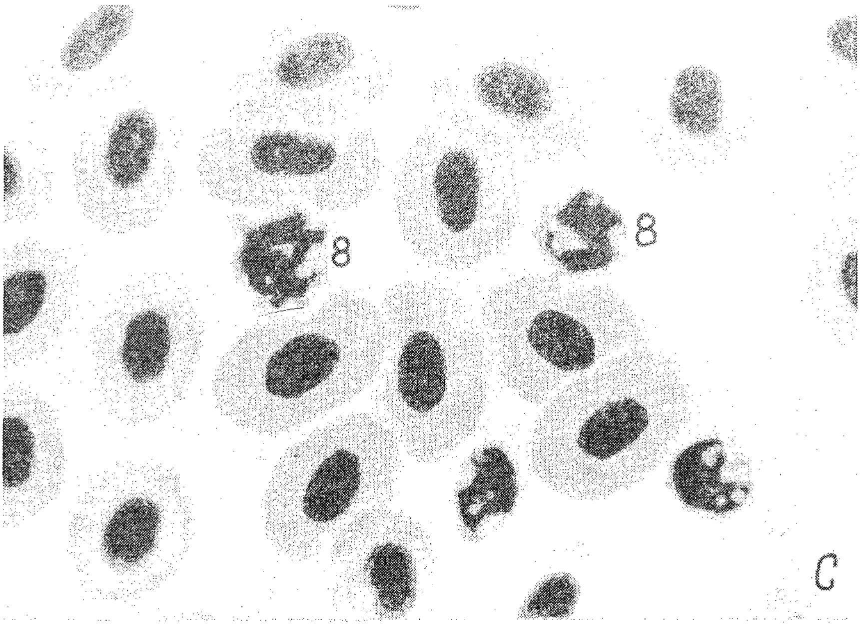
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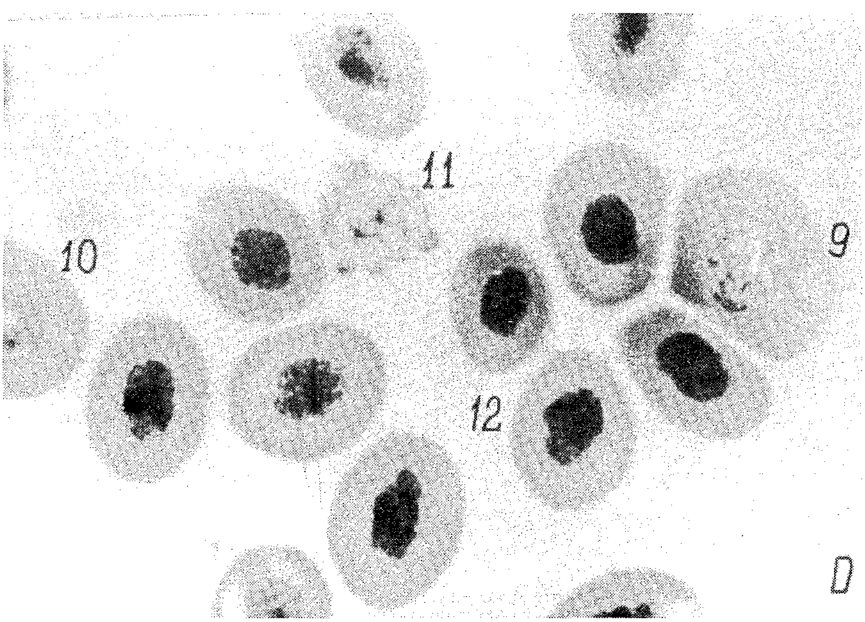
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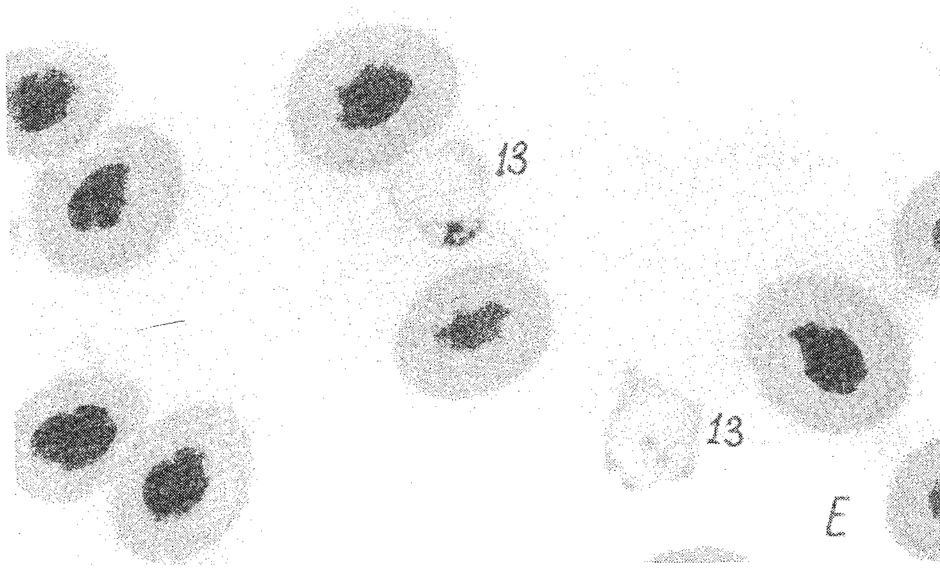

Plate 4. Degenerative changes in peripheral blood cells of bothriocephaliasis-affected carp (x 1800) Photo: J. Pacewicz: A. 1 – basophil erythroblast, 2 – polychromatic erythrocytes, 3 – macrocytes, 4 – monocyte with obliterated structure of nucleus and cytoplasm, 5 – lymphocyte with vacuolar degeneration of nucleus and cytoplasm, B. 6 – neutrophil with vacuolar degeneration, 7 – lymphocyte, C. 8 – lymphocytes with vacuolar degeneration of nuclear chromatin. D. 9 – macrocytes with vacuolar degeneration of nucleus, 10 – erythrocytes with hyperchromatic cytoplasm and vacuoles in cytoplasm and nucleus

Lymphocytes show highly advanced degenerative changes of their nuclei. The following symptoms of nuclei disintegration can be observed:

1) increased chromatin thickness and granulation, which makes the nuclei irregular in shape (karyopycnosis) (Plate 4c);
2) obliteration of nuclear outline in the cell, chromatin becoming lysed and poorly staining as it does not absorb basic stains (karyolysis);
3) total chromatin lysis; the nucleus is then invisible; on the cell margins there is a cytoplasm formed into a semicircular band; more advanced stages of disintegration show the cell affected as a circle of a diameter not larger than that of an unchanged lymphocyte (Plate 4e).

The lymphocyte plasm shows vacuolar degeneration.

No changes in the morphotic elements were revealed in the two control groups of fish.

DISCUSSION

A. Branchionecrosis

A considerable reduction in the erythrocyte count and erythrocyte cytolysis were found to accompany the acute form of the disease. Cytolysis has been known as one of the effects of toxic agents (Waluga and Flis, 1971; Grabda et al., 1974; Schreckenbach and Spangenberg, 1978). Recent findings on adverse effects of ammonia as a direct branchionecrosis-causing factor seem to be corroborated by such morphological changes in erythrocytes. Although the erythrocyte count in the chronically affected fish did not differ significantly from that in the control, morphological changes observed in the blood cells were clear-cut. Aniso- and poikilocytosis as well as the nuclear structure obliteration in those cells can also be taken as indicators of effects of a noxious factor. This case proves that both quantitative data and morphology of blood cells should be covered by hematologic assays performed to aid in the disease diagnosis.

Most observations on changes in erythrocytes of the branchionecrosis-affected fish made in the course of the present work agree well with those reported by Pilarczyk (1978). That author, however, was wrong in determining the two forms of the disease; consequently, his results make it impossible to ascribe the changes to an appropriate form. Conversely to Pilarczyk (1978), no immature erythrocytes were observed in the present study. On the other hand, a considerable decrease in the erythrocyte count was observed to accompany the acute branchionecrosis, which agrees with results reported by Kreutzmann (1976).

The hematocrit in the acute branchionecrosis was always lower than that in the control, the value being quite variable in the chronic form of the disease. A decreased hematocrit accompanying the acute branchionecrosis in carp was also observed by Kreutzmann (1976), while Pilarczyk (1978) recorded a decreased hematocrit in the carp exhibiting necrotic lesions in gills, i.e., in the chronic form.

The hemoglobin level can facilitate branchionecrosis diagnosing as the index drops significantly in the affected fish, particularly so in those suffering from the acute form. A decreased hemoglobin level associated with branchionecrosis was also found by Kreutzmann (1976).

The mean erythrocyte corpuscle volume (MCV) was much higher in the acute form-affected carp than in the healthy fish. Increase in the volume is reflected in the morphology as enlarged erythrocytes were observed. The above results do not agree with Kreutzmann (1976) who found a decrease in this index in the acute branchionecrosis-affected carp and observed erythrocyte microcytosis.

The mean hemoglobin content (MCH) and the mean cell hemoglobine content (MCHC) were significantly lower in the affected than in the healthy fish. Low hemoglobin contents evidence an impaired oxygen-binding faculty and, consequently, respiratory disorders. The erythrocyte cytoplasm hypochromasia observed on morphological examination is thus a consequence of a low hemoglobin content in those cells.

Leukocyte count

The fish affected by the acute branchionecrosis show a considerable, even several-fold, increase in the leukocyte count relative to the control, the response being most marked in the youngest fish (Fig. 1). The defense mechanism activity, stronger in younger individuals, seems to be involved and triggered by the disease-causing factor.

The organismal response manifest as an increased leukocyte count occurs usually as a consequence of contagious diseases. Etiology of branchionecrosis cannot rule out some infectious factor and perhaps it was this factor that affected the results obtained. Waluga and Flis (1971) found a decrease in the leukocyte count in the blood of carp affected by ammonia. Their data, differing from those of the present study, point out to a possibility that the etiology of branchionecrosis may involve, appart from ammonia, some contagious factor.

As shown by the present study, the acute branchionecrosis-affected carp had in their blood blastic forms of leukocytes (myeloblasts and promyelocytes), which indicates an intensified activity of hemopoietic organs in those fish.

Among the granulocytes, the clearly lower eosinophil percentage in the affected than in the healthy fish (Fig. 4) may justify concluding on the effect of a disease on this parameter, which is also substantiated by changes in the morphology of these blood cells.

The basophil and monocyte percentages dropped markedly in the fish affected by both the acute and chronic branchionecrosis. This result can be regarded as caused by the disease since the parameter underwent almost no change in the control. Fairly extensive degenerative changes in monocytes (Plate 3d) may lead to the conclusion that the low percentage of the cells found in the peripheral blood was partly caused by their disintegration.

The increased lymphocyte percentages in the fish affected by both forms of the disease relative to the controls evidences the cellular defense system being activated by the condition. Clear degenerative changes in the cells (Plates 3a, 3b, 3c) also suggest this factor to have been operative. Pilarczyk (1978) described vacuolisation of lymphocyte nuclei and cytoplasm in the course of branchionecrosis. On the other hand, particularly noteworthy in the present study are regressive changes in the nuclei of the cells discussed.

Bothriocephaliasis

The erythrocyte count in the infested fish was usually significantly lower than that in the two controls (Fig. 7). Since the parameter remained at a similar level in the latter, its decrease in the infested fish seems to have resulted from the presence of the parasites, harmful for the fish.

Siginificant differences between the two control groups may suggest that the invasion-free individuals obtained from the some farm as the affected one could have been affected before, that invasion subsequently disappearing.

That the infested fish were suffering from anemia was evidenced not only by the decreased erythrocyte count and hematocrit, but also by morphological changes in the red blood cells (Plates 4a, 4b, 4d).

The appearance of macrocytes and the disturbed erythrocyte nuclei maturation point out to an intensified activity of hemopoietic organs compensating for erythrocyte deficits in anemia. Changed shapes and structures of the nuclei, vacuoles occurring in them, and brighter bands around them indicate toxic effects of the cestode presence on the blood cells in circulation. The decrease in the erythrocyte count and hematocrit have been observed in a number of parasite-induced discases: Golodiec (1954) and Sadovskaja (1958) found such decrease to accompany invasion of *Dyctylogyrus* in carp, while Einsporn-Orecka (1970, 1973), Golovina et al. (1977), and Lowe-Finde (1979) reported this phenomenon as associated with the ergasilosis-affected tinch.

As shown by the present study, also the hemoglobin level in the infested fish can be more than twice as low as that in the parasite-free individuals (Fig. 9), particularly in the 12-, 14-mo-old individuals. The higher values of the parameter in the older fish, approaching even the control levels, could have resulted from a less intensive invasion. Decreased hemoglobin levels accompanying various parasitic invasions were recorded by Golodiec (1954), Einszporn-Orecka (1970), Koševa (1956), Sadovskaja (1958), Golovina et al. (1977), and Dubinina (1980).

The values of erythrocyte indices (MCV, MCH, MCHC) in the affected fish do not allow any conclusions to be drawn about the disease effects.

The highly increased leukocyte count in the affected fish (Fig. 5) points out to the mobilisation of defense mechanisms of the body. This situation seems to be brought about by the invasion rather than by the fish age, the conclusion being supported by a uniform leukocyte counts in all the control fish. Data on the dynamics of the parameter in bothriocephalosis are reported only by Syrow (1968) who observed progressing leukocytosis. Rošetnikova (1962) and Golovina et al. (1977) observed leukopoenia and leukocytosis, respectively, to accompany bothriocephalosis.

Among the neutrophils, increased percentages of myelocytes and metamylocytes were recorded in those specimens affected by a considerable invasion. Increased neutrophil percentages accompanying bothriocephalosis in *Ctenopharyngodon idella* were observed by Syrow (1968) and by Kozačenko and Klemow (1970), Slicher (1961), Einszporn-Orecka (1973), Hines and Spira (1973), Golovina (1976), and Golovina et al. (1977) observed increased neutrophil percentages proportional to intensities of invasion of various parasites.

The present results on morphological changes in neutrophils supply an evidence of increased production of these phagocyte cells (Plate 3b).

The affected fish show a well-marked increase in the eosinophil percentage. This is a reaction typical not only of fish; it occurs in worm invasion can serve as an auxillary index in the assessment of *Bothriocephalus acheilognathi* pathogeny. As can be seen from Fig. 13, the degree of eosinophilia is independent of invasion intensity and fish age. Eosinophilia was found also in other parasitic diseases: Golovina (1976) and Golovina et al. (1977) recorded the condition in carp affected by dactylogyrosis and ichthyophthyriosis.

In the present study, monocytosis was found to accompany the cestode invasion of a considerable extent. This finding is corroborated by morphological changes in the blood cells discussed (Plate 4a). Increased percentages of monocytes, the phagocyte cells, can be accounted for by the intensification of cellular defense mechanism. The phenomenon was observed also by Golovina in dactylogyrosis and ichthyophthyriosis.

The lymphocyte percentage was found to decrease markedly in the affected fish. It seems that the situation resulted from harmful effects of the invasion rather than from decreased lymphopoiesis, the suggestion being supported by the highly advanced degeneration of the blood cells (Plates 4c, 4d, 4e).

Decreased lymphocyte percentages were reported by Hines and Spira (1973) in carp ichthyophthyriosis, Einszporn-Orecka (1973) in ergasilosis, and Golovina (1976) in dactylogyrosis.

CONCLUSIONS

1. The fish affected by branchionecrosis show well-marked quantitative and morphological changes in blood cells; some changes occur in both forms of the disease (acute and chronic), while other alternations concern one form only. In both forms of branchionecrosis, decreased MCH and MCHC values are observed along with reduced percentages of eosinophils and degenerative changes in those blood cells; decreased percentages of basophils and monocytes, the latter showing degenerative changes; and increased percentage of lymphocytes and degenerative changes in them. Typical of the acute branchionecrosis is reduced erythrocyte count and erythrocyte lysis as well as decreased hematocrit and hemoglobin level. Additionally, increased MCV and leukocyte count are observed. Blastic forms of leukocytes (myeloblasts and promylocytes) appear in the peripheral blood. In the chronic branchionecrosis, changes in shape and size of erythrocytes (anisocytosis and poikilocytosis) are recorded along with decreased percentage of neutrophils.
2. Invasion of the cestode *Bothriocephalus acheilognathi* may result in marked changes of the carp hematologic parameters. The condition brings about decreased erythrocyte count and increased amounts of immature erythrocytes in the peripheral blood; decreased hematocrit and hemoglobin level; and decreased lymphocyte percentage, the latter cells showing highly advanced degenerative changes leading to disintegration of cells. Leukocyte counts increase as do percentages of myelocytes, metamyelocytes, eosinophils, and monocytes. The latter cells show marked degenerative changes.

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Translated: Dr Teresa Radziejewska

Antonina Sopińska

WPŁYW CZYNNIKÓW FIZJOLOGICZNYCH, STRESSOWYCH I CHOROBOWYCH NA PARAMETRY HEMATOLOGICZNE KARPIA ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OBRAZU BIAŁOKRWINKOWEGO. III. ZMIANY W KRWI WYSTĘPUJĄCE PRZY BRANCHIONEKROZIE I BOTRIOCEFALOZIE

STRESZCZENIE

Badania hematologiczne wykonano u ryb w wieku 12., 14., 24. i 26 miesięcy z objawami formy ostrej oraz przewlekłej martwicznego zapalenia skrzeli (branchionekrozy). Ryby kontrolne każdorazowo w tym samym wieku nie wykazywały żadnych zmian w skrzelach.

Postać ostra choroby charakteryzowała się niedokrwistością niedobarwliwą oraz cytolizą krwinek czerwonych.

U ryb chorych na formę przewlekłą obserwowano anizocytozę, poikilocytozę i zatarcie struktury jądrowej.

Przy obu formach wystąpiła wysoka leukocytoza oraz wzrost procesu limfocytów. Natomiast obserwowano niższy procent eozynofili, bazofili i monocytów jak również zmiany degeneracyjne w budowie wszystkich tych krwinek.

Do badań nad wpływem inwazji tasiemca *Bothriocephalus acheilognathi* użyto karpia w wieku 9., 12., 14., 16., 24., 26., 28., miesięcy.

Wynikiem działania tej tasiemczycy jest występująca u ryb niedokrwistość i pojawienie się we krwi obwodowej zwiększonej liczby form niedojrzałych krwinek.

Obserwowano w przebiegu tej choroby leukocytozę oraz wyraźny wzrost procentu eozynofili. Procent limfocytów u ryb zarażonych uległ znacznemu obniżeniu; w krwinkach tych stwierdzono daleko zaawansowane zmiany degeneracyjne.

Wyniki (opracowano statystycznie) wykazały, że badanie parametrów hematologicznych jest celowe i może służyć jako pomocnicza metoda określająca stan zdrowotny ryb.

Antonina Sopińska

ВЛИЯНИЕ ФИЗИОЛОГИЧЕСКИХ, СТРЕССОВЫХ И БОЛЕЗНЕННЫХ ФАКТОРОВ НА ГЕМАТОЛОГИЧЕСКИЕ ПАРАМЕТРЫ КАРПА, С ОСОБЫМ УЧЁТОМ КАРТИНЫ БЕЛЫХ КРОВЯНЫХ ТЕЛЕЦ. III. ИЗМЕНЕНИЯ В КРОВИ, НАСТУПАЮЩИЕ ПРИ БРАНХИОНЕКРОЗЕ И БОТРИОЦЕФАЛОЗЕ

Р е з ю м е

Гематологические исследования проводились у рыб 12, 14, 24 и 26 месяцев с признаками острой и хронической формы некрозного воспаления жабр (бранхионекрозы). Контрольные образцы рыб одного возраста не показали каких-либо изменений в жабрах.

Острая форма болезни характеризовалась гипохромной анемией, а также цитолизом красных кровяных телец.

У рыб, болезнь которых протекала в хронической форме, наблюдались аницитоз, поикилоцитоз и нечёткость ядерной структуры.

При обеих формах имел место высокий лейкоцитоз, а также рост процентного содержания лимфоцитов.

Зато, наблюдали меньшее процентное содержание эозинофилий, базофилий и моноцитов, а также дегенеративные изменения в строении всех этих кровяных телец. Для исследования влияния вторжения солитера Bothriocephalus acheilognathi использовали карпа в возрасте 9, 12, 14, 16, 24, 26, 28 месяцев. Результатом действия заражения солитером являлось появление у рыб анемии и увеличение количества несозрелых форм кровяных телец в периферийной крови.

В процессе болезии наблюдали лейкоцитоз и явный рост процентного содержания эозинофилий. Процентное содержание лимфоцитов у заражённых рыб значительно уменьшилось; в этих кровяных тельцах отмечены далеко зашедщие дегенеративные изменения.

Результаты –(статистически обработанные) показали, что исследование гематологических параметров целесообразно и может служить вспомогательным методом, определяющим состояние здоровья рыб.

Перевод: к.т.н. Мариуш Лучак

Author's address:
Dr Antonina Sopińska
Zakład Chorób Ryb
Akademia Rolnicza w Lublinie
ul. Akademicka 12
LUBLIN 20-033
Polska (Poland)

Received: 83.01.25