

Hematological and biochemical changes in European bison naturally infected with *Fasciola hepatica*

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Abstract: The aim of this study was to evaluate changes in selected hematological and biochemical parameters in European bison with confirmed chronic fasciolosis, living in Białowieża Forest.

Twenty one animals culled in winter season of 2013 were included in this study. In 14 of 21 cases (67%) the chronic fasciolosis was confirmed anatomopathologically. Blood samples for analysis were collected just after culling. The following parameters were determined using Horiba Scil Vet ABC plus automated hematology analyzer: number of white cells (WBC), number of red cells (RBC), haematocrit (Ht), hemoglobin concentration (Hb), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). In all cases blood smear was also performed. The following biochemical parameters were determined in blood serum using Mindray BS-130 biochemistry analyzer: urea, total cholesterol (Chol), total protein (TP), albumin (Alb), total calcium (Ca), magnesium (Mg), phosphorus (P) and enzyme activities: aspartate transaminase (AST), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK). Globulin concentration was calculated by the difference between the total serum protein and the albumin values.

No changes of hematological and biochemical parameters typical for chronic fasciolosis were found in infected animals. Hematological examination revealed low RBC as well as increased MCV and MCH. Absolute eosinophilia was found in two cases. Biochemical examination showed an increase activity of GGT, decreased levels of TP, Alb, Chol, P and AST activity. The concentration of serum magnesium extended beyond the reference values.

Key words: chronic fasciolosis, hematology, biochemistry, *Bison bonasus*

Introduction

Fasciolosis is the most common parasitological infection of European bison and domestic ruminants. Natural environment of Białowieża Forest creates favorable conditions for development of intermediate host of *Fasciola hepatica* i.e. freshwater snail of Lymnaeidae family – *Galba truncata*. Rapid growth of beaver population causes flooding of increasingly larger area of forest and its buffer zone. Because of technical and economical reasons anti-fluke prophylaxis is not performed in European bison.

Material and Methods

Twenty one European bison of different age and both sexes were included in this study. All animals were culled during winter-spring season of 2013. On the basis of results of anatomopathological examination all animals were divided into two groups. The group A consisted of 14 individuals with confirmed *Fasciola hepatica* infection while group B was comprised of animals without the presence of parasitic worms in the liver. Blood samples for analysis were collected just after culling. All blood analyses were performed with Horiba Scil Vet ABC plus automated hematology analyzer. The following parameters were determined: number of white cells (WBC), number of red cells (RBC), haematocrit (Ht), hemoglobin concentration (Hb), platelet count and red blood cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). In all cases blood smear was also performed. The following parameters were determined in blood serum using Mindray BS-130 biochemistry analyzer: urea, total cholesterol (Chol), total protein (TP), albumin (Alb), total calcium (Ca), magnesium (Mg), phosphorus (P) and enzyme activities: aspartate transaminase (AST), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK). Globulin concentration was calculated by the difference between the total serum protein and the albumin values.

All results are presented as range values and mean values \pm standard deviations (sd). Statistical analyses were carried out using one-way ANOVA ($P < 0,05$) using Statistica 10.0 digital software (Statsoft Inc., Tulsa, USA).

Results

Symptoms of prevalent bone fractures, *balanoposthitis*, extensive interstitial pneumonia with emphysematous foci and pneumothorax, chronic nephritis, lung and gastrointestinal nematode infections and liver damages with presence of adult flukes in bile ducts were observed during anatomopathological examination. Of the 21 examined European bison, 14 animals (67%) including 4 calves suffered from chronic fasciolosis.

Table 1 Hematological changes in the European bison with fasciolosis (group A) and without the presence of parasitic worms in the liver (group B).

Parameter	Group A (n = 14)			Group B (n = 7)			Difference between groups
	Mean value	sd	Range of values	Mean value	sd	Range of values	
WBC [$\times 10^9/l$]	5.36	3.43	1.5–13.1	6.50	2.87	4.2–11.1	P<0.05
RBC [$\times 10^{12}/l$]	7.16	1.97	3.26–9.40	8.71	2.50	4.38–10.47	P<0.05
Hemoglobin [g/dl]	13.54	3.47	7.2–18.4	14.40	4.20	8.1–18.2	ns
Haematocrit [%]	39.55	11.48	22.9–55.1	43.36	13.60	22.2–55.3	ns
Neutrophils [$\times 10^9/l$]	1.85	2.15	0.2–7.79	2.82	2.90	1.11–7.99	P<0.05
Eozynophils [$\times 10^9/l$]	0.68	0.54	0.2–1.83	0.39	0.20	0.16–0.63	P<0.05
Lymphocytes [$\times 10^9/l$]	2.92	2.38	1.10–8.91	3.68	1.54	2.39–3.73	P<0.05
Trombocytes [$\times 10^9/l$]	622.18	558.03	202–2032	210.20	107.47	83–355	P<0.05
MCV [fl]	55.27	5.66	43–63	49.8	5.63	43–56	P<0.05
MCH [pg]	19.22	2.56	43–63	16.74	2.26	13.9–18.6	P<0.05
MCHC [g/dl]	34.71	2.65	32.4–40.7	33.52	1.69	32.4–36.5	ns

Average WBC in European bison with chronic fasciolosis was significantly higher ($p < 0.05$) than in animals without invasion. No significant changes in hematological parameters except lowering of the RBC were found in most of animals from the group A (with chronic fasciolosis). Features of anemia including decrease in RBC ($4.21 \times 10^{12}/l$ and $3.26 \times 10^{12}/l$), Hb (7.2g/dl and 9.3 g/dl) and Ht (19.3% and 22.9%) counts below the reference values were found only in 2 adult individuals. These abnormalities were associated with increase in MCH (22.2pg and 22.1pg) and MCHC (37.5g/dl and 40.7g/dl). Increased absolute eosinophil count was found in two European bison from the group A, while in animals from the group B eosinophil number was within the reference ranges (Peinado *et al.* 1999) (Tabl. 1).

Regarding protein metabolism, the concentration of albumin, total serum protein and globulin were significantly ($p < 0.05$) higher in group A than in group B,

Table 2 Biochemical changes in the European bison with fasciolosis (Group A) and without the presence of parasitic worms in the liver (Group B).

Parameter	Group A (n = 14)			Group B (n = 7)			Difference between groups
	Mean value	SD	Range of values	Mean value	SD	Range of values	
Albumin [g/l]	42.10	5.39	34.1–51.5	37.95	10.54	23.4–49.2	P<0.05
Total protein [g/l]	73.68	12.06	55.4–95.3	64.73	16.28	41.7–83.0	P<0.05
Globulin [g/l]	31.58	8.71	17.3–47.6	26.78	10.89	15.6–33.8	P<0.05
Urea [mmol/l]	5.92	1.43	3.26–8.39	5.79	1.80	3.93–8.67	ns
AST [U/l]	84.92	4.55	73–103	103.00	25.50	76–138	P<0.05
GGT [U/l]	22.33	8.97	12–43	23.00	27.56	9–79	ns
CPK [U/l]	514.0	402.6	124–1280	771.4	662.4	59–1840	P<0.05
Cholesterol [mmol/l]	2.12	0.41	1.45–2.80	4.09	3.03	0.85–4.90	P<0.05
Calcium [mmol/l]	2.52	0.23	2,11–2,94	2.70	0.44	2.46–3.28	ns
Phosphorus [mmol/l]	3.33	0.80	2.11–4.30	4.30	1.06	3.07–5.46	P<0.05
Magnesium [mmol/l]	1.30	0.38	0.97–2.42	1.19	0.26	0.74–1.52	ns

but in both cases were within the reference ranges. Among parameters indicating liver injury increase of AST and GGT activity were observed in the examined animals. High GGT activity was found in all individuals, while increased AST activity above the reference values (Peinado *et al.* 1999) was noted only in 9 from the 14 European bison. However, compared to group B, AST activity was significantly lower in the group affected by the invasion. CPK activity in group A was significantly lower ($p < 0.05$) than in group B. Increased CPK activity was observed in 2 cases.

The serum levels of calcium, cholesterol and phosphorus were within the reference ranges. However, magnesium level was above the reference values in all European bison included in this study (Tabl. 2).

Discussion

Fasciola hepatica infection leads to liver damage, progressive cachexia and overall malnutrition. Diagnosis of fasciolosis is based on the clinical examination, coproscopy and hematological and biochemical blood tests. Serological tests are currently used for the diagnosis of fasciolosis. However, in free-living animals diagnostic possibilities are limited. Pharmacological immobilization is required to safe collection of blood samples. Interpretation of results of laboratory diagnostic tests is difficult because other numerous concomitant disorders should be taken into account. Lung and gastrointestinal nematode infections, chronic nephritis, necrotic inflammation of the prepuce (*balanoposthitis*), liver damages and numerous purulent inflammations of various tissues are common in European bison.

Anemia is common hematological feature of chronic fasciolosis, and is frequently observed in domestic ruminants. However, in the European bison with confirmed fasciolosis included in this study, changes in hematological parameters (RBC, Ht, Hb, MCV, MCH and MCHC) have not been demonstrated. Only in two cases anemia with mentioned above typical changes in hematological parameters have been shown. However, in both animals numerous concomitant diseases including purulent posthitis, pneumothorax and emphysema pulmonum, pyometra as well as purulent bronchopneumonia have been confirmed during anatomopathological examination. So the infection of *Fasciola hepatica* had not been the sole etiological factor of anemia. One case of anemia in the group of animals without features of fasciolosis confirms this hypothesis. In this animal, anemia was caused by chronic purulent inflammation of pharynx area which prevents food intake.

A typical phenomenon for parasitic diseases including fasciolosis is an increase of relative and absolute eosinophil counts. Persistent eosinophilia in course of parasitic diseases results from prolonged and recurring exposure of T lymphocytes to parasite antigens. However, in the European bison included in this study, eosinophilia was found only in two individuals. Lack of eosinophilia in animals with fasciolosis, as well as in cases with confirmed lung and gastrointestinal nematode infections, is difficult to explain. In domestic ruminants eosinophilia occurs only in the initial phase/early stage chronic fasciolosis, but completely regress in the final phase of infection (Gil *et al.* 2006; Raadsama *et al.* 2007).

During the life cycle of *Fasciola hepatica*, a damage of liver parenchyma by migrating larvae and intrahepatic cholestasis occur at the end/late-stage of infection. AST is a sensitive indicator of liver cell damage. However, slightly elevated AST was found only in 9 of the 14 European bison examined. Results of studies conducted on domestic ruminants with *Fasciola hepatica* infection have shown that increase of AST and GGT activities indicates hepatic injury either in acute or chronic fasciolosis (Mitchell 2002). However, E. bison affected by fasciolosis had lower values of AST. Our results are contrary to those observed in domestic animals (Marczuk *et al.*

2014). As a reason for the lower AST values considered is an extensive and chronic inflammatory process in the liver, leading to atrophy of hepatocytes (*cirrhosis*). In the course of fasciolosis, intrahepatic cholestasis occurs. Long-term presence of adult flukes in host's biliary system results in bile ducts hyperplasia and calcification. Hepatocyte-specific enzyme GGT is a sensitive indicator of liver cholestasis. In all animals with fasciolosis included in this study, GGT activity exceeded the reference value.

Increased activity of CPK was found in two cases. This enzyme is specific for muscle tissue and elevated CPK level in these animals appears to be due to lifetime injuries of skeletal muscles.

Chronic infection with *Fasciola hepatica* in animals leads to disturbances of mineral, protein and energetic metabolism. Changes in the protein metabolism resulting in decreased total serum protein and albumin concentrations, are observed most commonly. However, in the examined European bison this phenomenon was not recorded. Decreased albumin, total cholesterol and urea concentrations are indicators of liver damage caused by flukes. However, in animals included in this study these changes were not observed.

No changes in serum calcium and phosphorus concentrations were found in terms of mineral metabolism. Interestingly, increased serum magnesium concentration was found in all European bison examined in this study.

There are minor hematological and biochemical changes limited primarily to an increase in GGT and slightly to AST activity in European bison infected with *Fasciola hepatica* compared to livestock (cattle and sheep). Anemia as the typical hematological feature of chronic fasciolosis does not occur in European bison. However, in European bison similarly to domestic ruminants, eosinophilia occurs occasionally in fasciolosis (Marczuk *et al.* 2014).

It is believed that the organism of E. bison created adaptive mechanisms to chronic liver fasciolosis. Therefore, the results of standard laboratory tests for the diagnosis of this disease should be treated with caution.

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Hematologiczne i biochemiczne wskaźniki u żubrów zarażonych motylicą wątrobową *Fasciola hepatica*

Celem badań było określenie zmian w wybranych wskaźnikach hematologicznych i biochemicznych krwi u żubrów żyjących na terenie Puszczy Białowieskiej z potwierdzoną przewlekłą postacią motylicy wątrobowej.

Badaniami objęto 21 żubrów poddanych eliminacji, z których u 14 (67%) na podstawie badania anatomopatologicznego stwierdzono przewlekłą formę motylicy wątrobowej. Krew do badań pobierano jednorazowo bezpośrednio po odstrzale. Wykorzystując analizator hematologiczny Horiba Scil Vet ABC plus, oznaczono: liczbę leukocytów (WBC), liczbę erytrocytów (RBC), hematokryt (Ht), stężenie hemoglobiny (Hb), liczbę płytek krwi oraz wskaźników charakteryzujących krwinki czerwone: MCV, MCH, MCHC, a po wybarwieniu wykonano badanie rozmazu. Analizatorem biochemicznym Mindray BS-130 wykonano badanie stężenia w surowicy krwi następujących parametrów: mocznika (Urea), cholesterolu całkowitego (Chol), białka całkowitego (TP), albumin (Alb), wapnia całkowitego (Ca), magnezu (Mg), fosforu (P) oraz aktywności enzymów: AST, GGT, CPK. Z różnicy stężeń białka całkowitego i albumin wyliczono stężenie globulin.

Na podstawie wykonanych badań nie stwierdzono u żubrów z przewlekłą postacią motylicy wątrobowej typowych zmian wskaźników hematologicznych i biochemicznych, które można zaobserwować w podobnej sytuacji u domowych przeżuwaczy. Badaniem hematologicznym nie wykazano anemii, a tylko w 2 przypadkach występowała bezwzględna eozynofilia. W badaniu biochemicznym poza niewielkim wzrostem aktywności AST i GGT oraz wzrostem stężenia Mg nie wykazano istotnych zmian.
