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ORIGINAL ARTICLE

Hematological indicators of liver damage during the subclinical phase of fasciolosis in steers from Northeastern Argentina

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Abstract The aim of this study was to determine eventual changes in blood parameters that characterize liver damage during the subclinical phase (asymptomatic) of liver fluke disease in cattle from Northeastern Argentina. Blood samples of 56 apparently healthy half-blood Brahman × Angus steers from slaughterhouses of enzootic areas of fasciolosis were collected and later analyzed. After death, from the total of animals, it was found that 29 harbored slight amount of fasciolas in the hepatic parenchyma. With conventional laboratory techniques, erythrogram, leukogram, proteinogram, and enzymogram (alkaline phosphatase, gamma-glutamyltransferase (GGT), and aspartate aminotransferase (AST)) were determined, as well as concentrations of iron, glucose, bilirubin, and prothrombin time. Parasitized animals revealed higher values for leukocytes, eosinophils, gamma globulins, and GGT activity when compared to controls ($P < 0.05$). Such parameters are inflammatory indicators attributable to an incipient cholangitis that characterizes the subclinical phase of distomatosis. Changes that may indicate cholestasis or liver dysfunction were not verified.

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Introduction

Distomatosis or fasciolosis is a parasitic disease that affects the liver and may cause death of the host due to severe complications. The etiologic agent is the trematode *Fasciola hepatica* L. (liver fluke, Distoma, *duela del hígado*, and *saguaypé*) that parasitizes different mammalian species, including human beings (Boero 1967; Kassai 2002). World Health Organization considers that almost 2.5 million people are parasitized worldwide (Rim et al. 1994). Populations from South America are at high risk because of their eating habits and sometimes deficient hygiene conditions (Mas-Coma et al. 1999). The first case of human infestation was reported in Argentina in the northeastern region in the decade of 1950; later, many cases were registered in the rest of the country (Ale et al. 2000; Rubel et al. 2005). These early communications bear witness that biochemists as well as physicians were pioneers in establishing the changes in blood parameters caused by this parasitosis. In veterinary medicine, distomatosis principally affects ruminants, causing important losses in animal production systems (Boero 1967). In some areas of Argentina, the prevalence of this parasitosis is 82% in sheep, 86% in cattle, and 100% in goats (Rubel et al. 2005). For the northeast of the country (Corrientes Province), prevalence of fasciolosis in ruminants significantly increased during the last two decades; nowadays, the affected region covers a vast area over the Parana River shore (Moriena et al. 2002), from where many samples that were analyzed for this assay come from. For this region, animals with distomatosis show the early signs of the disease by the end of fall, when metacercariae invade the liver parenchyma (colics and fever). Hyperplasia of canalicular mucosa, as well as the presence of the parasite itself, leads to obstruction of bile ducts and jaundice. Blood-feeding habits and toxic substances from the parasites destroy circulating erythrocytes, whereas migrations of flukes through the parenchyma and canalicular hypertension cause inflammation and necrosis of hepatic tissue, with the consequent organ dysfunction. When glycogen is exhausted, adynamia becomes evident, causing metabolic changes in order to obtain energy from other sources such as lipids or proteins. Depletion of these sources will lead to cachexia that characterizes this disease. Hypoproteinemia will cause edema and ascites. Finally, inflammatory and necrotic areas of the liver suffer fibrosis and atrophy and may develop cirrhosis (Boero 1967). Such changes may alter blood parameters that typically indicate anemia (erythrocytes, hemoglobin, hematocrit, iron, and hematimetric indexes), cholestasis (total and direct bilirubin, alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) activities), inflammation (leukocitosis and aspartate aminotransferase (AST)), appearance of natural antiparasitary indicators (eosinophilia), increment of antibodies (gamma globulins), and development of liver insufficiency (prothrombin, glucose, total protein, and albumin; Coppo 2001; Kaneko et al. 2008).

In ruminants, it is also advisable to consider the activity of glutamate dehydrogenase (GLDH), which rise may indicate severe hepatocyte damage (Hutchinson et al. 2009; Kassai 2002; Urquart et al. 1999). In human beings, liver fluke infestation may increase the activities of alanine aminotransferase and sorbitol dehydrogenase (SDH; Kassai 2002). Arginase was found to be increased in cows (Haroun and Hussein 1975) but not in calves (Wyckoff and Bradley 1985). Values of amylase, creatinine, and serum electrolytes would not be affected (Maco et al. 2003). It has been proposed that for the diagnosis of distomatosis, serum enzymes may be considered as early indicators of the disease, rather than the finding of eggs of the parasite in feces (Wyckoff and Bradley 1985).

Fasciolosis typically develops from an acute phase, which begins 2 weeks after the infestation and is characterized by the increment of AST, SDH, and GLDH to a chronic phase several months after with anemia and hypoalbuminemia. However, in cattle, a very common state known as "subclinical phase" may exist, with mild cholangitis and no associated signs (Kassai 2002).

The aim of this study was to determine the eventual changes of blood parameters that characterize liver damage caused by *F. hepatica* during the subclinical phase of the disease in asymptomatic half-blood Brahman × Angus steers from northeastern Argentina, which crossbreeding is supposed to give animals an innate resistance to the parasite.

Materials and methods

Blood samples were collected from 60 half-blood Brahman × Angus steers. During the procedure, animals did not show signs compatible with liver fluke disease. Nutritional status of the animals was acceptable, and live weight ranged from 350 to 450 kg. Jugular venopunction was performed prior to death in slaughterhouses and meat processing plants. In a posteriori macroscopic examination of the liver, 30 negative and 30 positive samples to fasciola infestation were selected. In four cases, samples had to be discarded due to hemolysis.

Erythrogram and leukogram were determined in whole blood treated with EDTA. Hematocrit, hemoglobin concentration, erythrocytes, and leucocytes, as well as determination of hematimetric indexes (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration), were analyzed by hematology counter. Differential leukocyte count was obtained from blood smears stained with Giemsa and serum iron concentration by means of a photocolorimetric determination (ferrozine method, Wiener Lab). Activities of ALP (phenyl phosphate), GGT (nitroanilide), and AST (aspartate–oxoglutarate) were obtained spectrophotometrically from sera, as well as total protein concentration (Biuret), glucose (oxidase–peroxidase), and total and direct bilirubin (sulfanilic diazoreactive). Rates of albumins and alpha, beta, and gamma globulins were obtained by electrophoresis and densitometry; albumin/globulin ratio (AGR) was determined by calculation. Prothrombin time was determined according to Quick's method. Wiener Lab's reagents were used in all the determinations.

For the statistical analysis, a completely randomized design was used. Normal distribution was verified by the Shapiro–Wilk test. Once Gaussian symmetry was confirmed, parametric descriptive statistics of central tendency (arithmetic mean) and dispersion (standard deviation) were applied. Analysis of variance (ANOVA) of each dependent variable was performed by means of a one-way linear model upon verification of homogeneity of variance by Bartlett's test. Calculations were performed using the software *Statistix* 1996. For all the inferences, it was stipulated a type I error of 5%, rejecting the null hypothesis for values less than this significance level.

Results

From the total samples, 29 corresponded to steers that harbored juvenile and adult liver flukes in the hepatic parenchyma, whereas the 27 remaining animals were negative. None of the animals showed concomitant diseases that could change blood indicators of liver damage. Positive

Table 1 Hematic values of red and white cells from animals positive and negative to *Fasciola hepatica* infestation

Parameter	Ref. interv.	− (*n*=27)	+ (*n*=29)	Signif.
Hematocrit (%)	40–45	43.2±2.6	41.7±3.5	–
Hemoglobin (g/dl)	13–15	13.6±1.4	12.9±1.1	–
Erythrocytes (T/l)	7.8–8.8	7.97±0.6	7.88±0.7	–
MCV (fl)	45–55	54±4	52±6	–
MCH (pg)	15–20	17±3	16±2	–
MCHC (%)	26–33	32±2	31±2	–
Iron (ug/dl)	106–127	120±12	116±14	–
Leukocytes (G/l)	9–10	9.22±1.7	12.6±2.2	0.05
Neutrophyls (%)	26–30	30.9±2.8	28.4±2.5	–
Lymphocytes (%)	57–65	59.2±4.1	55.2±3.8	–
Monocytes (%)	4–6	4.8±0.3	4.0±0.5	–
Eosinophyls (%)	5–7	5.1±0.4	12.4±1.7	0.02
Basophiles (%)	0–1	–	–	–

− negative, + positive, *ref. interv.* reference interval (Coppo 2001), *signif.* signification, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *CHCM* mean corpuscular hemoglobin concentration, *T/l* tera per liter, *G/l* giga per liter

cases were considered as slight, according to the scarce amount of parasites found and mild macroscopic lesions.

On each dependent variable, distribution of data (n=56) was normal, permitting the use of parametric statistics. The Bartlett test revealed the homogeneity of variances, allowing the use of ANOVA.

Table 1 exhibits blood parameters of red and white cells. Analysis of erythrocytic values shows no significant differences between positives and negatives, yet it is evident that parasitized animals revealed lower values when compared to controls. On the contrary, total leukocytes and eosinophyls were significantly higher in animals that harbored parasites rather than controls, with no other significant differences in the rest of the differential count. Neutrophyls did not show a shift to the right.

Parameters related to biliary obstruction are shown in Table 2. Serum activity of ALP as well as direct, indirect, and total fractions of bilirubin did not reveal significant differences among groups, although for all the cases, values were higher in steers positive to *F. hepatica* infestation. However, GGT revealed values significantly higher in parasitized animals, exceeding the upper limit of the interval reference.

Table 3 includes the biochemical markers of inflammatory-necrotic lesions of the liver, emphasizing the significant increment of gamma globulins of the parasitized lot. On the contrary, alpha and beta globulins as well as serum activity of AST remained among their reference intervals, although beta globulins and AST were slightly higher in animals parasitized with liver flukes.

Indicators of liver dysfunction are listed in Table 4. Prothrombin time and serum concentrations of glucose and albumins did not register significant differences between lots. In contrast, for the parasitized group, total proteins were higher and AGR lower, beyond their upper and lower reference intervals, respectively.

Discussion

Red cell parameters were between the reference intervals for cattle and geographic area (Coppo 2001). Even though values for hematocrit, erythrocytes, hemoglobin, iron, and hematimetric indexes were lower for the parasitized animals, such a fall did not configure the anemic state reported for cattle with fasciolosis (Boero 1967; Kassai 2002).

In human beings, falls as low as 31% in hematocrit and 10 g/dl for hemoglobin have been reported (Maco et al. 2003). In cattle with fasciolosis versus healthy animals, significant falls for iron, hematocrit, hemoglobin, MCH, and CMCH with normal MCV were registered, thus characterizing hipocromic anemia. On the contrary, for other research falls in iron, hematocrit and erythrocytes were accompanied with increment in MCV; therefore, anemia was macrocytic (Haroun and Hussein 1975).

Data gathered for white cells in parasitized animals with *F. hepatica* are similar to those reported in other publication, in which affected cattle showed leukocytosis and

Table 2 Values of cholestasis serum indicators in animals positive and negative to *Fasciola hepatica* infestation

Parameter	Ref. interv.	− (*n*=27)	+ (*n*=29)	Signif.
ALP (UI/l)	205–248	216±11	224±17	–
GGT (UI/l)[a]	25–33	27.4±2.8	39.1±4.6	0.02
Indirect bilirubin (mg/l)	Up to 4	2.88±0.2	3.10±0.3	–
Direct bilirubin (mg/l)	Up to 1	0.76±0.03	0.97±0.04	–
Total bilirubin (mg/l)	Up to 5	3.64±0.4	4.07±0.3	–

– negative, + positive, *ref. interv.* reference interval (Coppo 2001), *signif.* signification, *ALP* alkaline phosphatase, *GGT* gamma-glutamyltransferase

[a] It may also increase in inflammatory process of the liver

Table 3 Serum indicators of liver inflammation and necrosis in animals positive and negative to *Fasciola hepatica* infestation

Parameter	Ref. interv.	− (*n*=27)	+ (*n*=29)	Signif.
AST (UI/l)	39–57	41.7±3.6	44.1±3.9	–
Alpha globulin (g/dl)	0.6–0.7	0.64±0.08	0.61±0.06	–
Beta globulin (g/dl)	0.8–0.9	0.85±0.13	0.83±0.11	–
Gamma globulin (g/dl)	2.3–2.6	2.41±0.36	3.60±0.41	0.03

– negative, + positive, *ref. interv.* reference interval (Coppo 2001), *signif.* signification, *AST* aspartate aminotransferase, *AGR* albumins/globulins ratio

eosinophilia (Boero 1967). Arithmetic media of eosinophyls in parasitized steers in our work (12.4%) was lower than that obtained by Boero (1967; 17%).

In human infestation, eosinophilia and leukocytosis reach values as high as 46% and 24,000 G/l, respectively (Rubel et al. 2005), although lower values have been reported, 4% and 11,300 G/l for each parameter (Maco et al. 2003). Increment of eosinophyls appears to be a constant in human distomatosis (Ale et al. 2000). In parasitized cattle, eosinophilia was associated with decrease in neutrophils, lymphocytes, and monocytes (Haroun and Hussein 1975).

Absence of jaundice in parasitized steers was in concordance with the invariability of bilirubinemia. Increment in serum bilirubin concentration is characteristic in cases of severe biliary obstruction, especially for the direct fraction (Boero 1967; Coppo 2001; Kaneko et al. 2008). In human distomatosis, total bilirubin remained normal in some cases (Rubel et al. 2005), whereas in others, it raised four times at the expense of the direct fraction (Maco et al. 2003). In cattle with signs of distomatosis, hyperbilirubinemia frequently occurs together with bilirubinuria (Boero 1967). Some researchers state that the increment of the biliar pigment is not a common finding in serum of parasitized ruminants (Kassai 2002).

Serum elevations of ALP and GGT activities basically indicate cholestasis state, although the latter of the enzymes can also increase due to canalicular or parenchymal inflammation (Coppo 2001). In humans affected by liver fluke, ALP increased two times in some cases (Maco et al. 2003) and up to six times in others (Rubel et al. 2005). In some studies, it was also increased in cattle (Lotfollahzadeh et al. 2008).

Elevations of GGT in distomatosis take place in humans as well as cattle, behaving for both cases as an indicator of the subacute phase of the disease, when trematodes obstruct bile ducts and cause inflammation (Hutchinson et al. 2009; Kassai 2002; Lotfollahzadeh et al. 2008; Wyckoff and Bradley 1985).

The activity of AST, an indicator of parenchymal damage in human distomatosis (Maco et al. 2003; Rubel et al. 2005), was also increased in parasitized cattle (Hutchinson et al. 2009; Lotfollahzadeh et al. 2008; Wyckoff and Bradley 1985), especially during the acute phase (Kassai 2002).

Rise of gamma globulins may indicate the immunologic response to the presence of *F. hepatica* by increment of circulating immunoglobulins (Coppo 2001). Such circumstance was formerly confirmed in cattle, with the consequent decrease of AGR (Haroun and Hussein 1975). Nevertheless, such finding was not recorded for humans, in which total protein concentration was normal (Maco et al. 2003). In parasitized cattle, total proteins might decrease due to their utilization for energetic purposes (Boero 1967). The significant increment of total proteins registered in the present study must be attributed to the previously mentioned rise of gamma globulins, which in turn was responsible for the AGR decrease (Kaneko et al. 2008).

As increment of prothrombin time or decrements in albumin and glucose levels were not found, existence of hepatic dysfunction is unlikely to occur in parasitized cattle (Coppo 2001). In ruminants that are severely parasitized by *F. hepatica*, decrement of glucose (Boero 1967) and albumins (Haroun and Hussein 1975) were found, mainly during the chronic phase of the parasitosis (Kassai 2002). In a communication of human distomatosis, glucose and albumins remained normal, while prothrombin time reached twice its reference value (Maco et al. 2003).

Table 4 Serum indicators of liver dysfunction in animals positive and negative to *Fasciola hepatica* infestation

Parameter	Ref. interv.	− (*n*=27)	+ (*n*=29)	Signif.
Prothrombin time (s)	10–25	16.4±1.8	19.2±1.5	–
Glucose (g/l)	0.58–0.71	0.61±0.07	0.68±0.08	–
Albumins (g/dl)	3.3–3.8	3.72±0.38	3.31±0.35	–
Total proteins (g/dl)	7.0–8.0	7.62±0.81	8.35±0.90	0.05
AGR (score)	0.7–0.9	0.95±0.09	0.66±0.07	0.05

– negative, + positive, *ref. interv.* reference interval (Coppo 2001), *signif.* signification, *AGR* albumins/globulins ratio

From the changes recorded in this work for the biochemical indicators of asymptomatic steers harboring liver flukes, it emerges that cholestasis and liver dysfunction syndromes were absent. However, leukocytosis together with hypergammaglobulinemia and increment of GGT activity only prove the existence of an inflammatory state that may match up with the "subclinical phase" (incipient colangitis) proposed by Kassai (2002) for animals recently parasitized and harboring scarce amount of parasites, which still appear healthy. The so-called greater resistance of half-blood Brahman × Angus steers to liver flukes (Sardar et al. 2006) may not protect them against the parasitosis.

In conclusion, our study reveals changes in blood indicators compatible with hepatic alterations due to parasitism by *F. hepatica* in steers during the subclinical phase, characterized by the presence of a mild inflammatory process interpreted as cholangitis, with no evident symptoms during the development of the disease.

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