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PATHOMORPHOLOGICAL EXAMINATION OF THE LIVER IN CATTLE IN THE COURSE OF A NATURAL INFESTATION WITH *FASCIOLA HEPATICA* (L.)

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K e y w o r d s: *Fasciola hepatica,* cows, liver, pathomorphology, ACP, SDH.

A b s t r a c t

An evaluation of lesions in the livers of Black and White cattle, aged 4 to 7, naturally infested with *Fasciola hepatica* was performed. The studies included a comprehensive analysis of histological lesions and liver enzyme activities determined with histochemical methods depending on the degree of natural *Fasciola hepatica* infestation. The studies revealed increased number of reticular fibres, an increase of acid phosphatase (ACP) activity, a reduced activity of succinic acid dehydrogenase (SDH) and a decrease in the content of glycogen in the hepatocytes. The damage of the hepatocytes was potentiated together with increasing severity of fluke infestation, which caused mononuclear cell and eosinophilic granulocyte infiltrates, abscess formation and connective tissue proliferation. The presented lesions in liver were determined by duration of the pathogenic effects of flukes infestation, the number of fluke parasites and the age of the animals.

BADANIE PATOMORFOLOGICZNE WĄTRÓB BYDŁA W PRZEBIEGU NATURALNEJ INWAZJI *FASCIOLA HEPATICA* (L.)

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S ł o w a k l u c z o w e: *Fasciola hepatica,* krowy, wątroba, patomorfologia, ACP, SDH.

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Abstrakt

Przeprowadzono ocenę zmian patologicznych w wątrobach bydła rasy czarno-białej, w wieku od 4 do 7 lat naturalnie zarażonego *Fasciola hepatica*. Badania obejmowały kompleksową analizę zmian histologicznych i aktywności enzymów wątrobowych oznaczanych metodami histochemicznymi w zależności od stopnia naturalnego zarażenia *Fasciola hepatica*. Badania wykazały rozplem włókien retikulinowych, wzrost aktywności fosfatazy kwaśnej (ACP), zmniejszoną aktywność dehydrogenazy kwasu bursztynowego (SDH) i spadek zawartości glikogenu w komórkach wątrobowych. Wraz z wzrostem intensywności inwazji przywr uszkodzenia komórek wątrobowych nasilały się, co powodowało nacieki komórek jednojądrzastych, granulocytów kwasochłonnych, tworzenie się ropni i rozplem tkanki łącznej. Opisane zmiany w wątrobie były zależne od czasu chorobotwórczego oddziaływania przywr, liczby pasożytów i wieku zwierząt.

Introduction

Fasciolosis (liver fluke disease) in cattle is relatively common in Poland and still presents a significant economic issue, as it does in many other countries (CARRADA-BRAVO 2003, GAJEWSKA et al. 2005, KOZŁOWSKA-ŁÓJ and ŁÓJ-MACZULSKA 2013, MAGE et al. 2002, NOVOBILSKY et al. 2014). Animals infested with *Fasciola hepatica* are much less efficient in feed conversion, weight gains and milk production, and their resistance to infection is reduced. Apart from economic losses, these flukes cause disturbances in a number of organs and systems, e.g. the reproductive system (ROMANIUK 1977). Hepatic dysfunctions mainly involve the protein system and liver enzymes. During fluke infestation, the α- and β-globulin fractions in the blood significantly increase and their level is correlated with liver damage. In livers infested with flukes, a significant reduction in glycogen and reductive sugars is detected (NANSEN 1971, FURMAGA and GUNDŁACH 1972).

Migrating juvenile flukes form „migratory tunnels" which, over time, transform into connective tissue scars damaging the hepatic parenchyma over large areas. Bile ducts in the portal areas are significantly enlarged. Diffuse proliferation of the epithelial cells in the bile ducts, especially adjacent to blood vessels was reported (RAHKO 1971).

Flukes have a strong affinity to the hepatic tissue and bile ducts. Resultant lesions in the liver depend on the severity and stage of infestation (GAJEWSKA et al. 2005, KONRAD 1968). DOY et al. (1984) in a gross examination, found multiple red and brown or, locally, white foci under the liver capsule. The majority of these foci (approximately 80%) were situated in the left hepatic lobe, particularly under the capsule. Histology revealed inflammatory cell infiltrates consist of lymphocytes and neutrophils located under the capsule. Additionally indistinct lobular architecture of the parenchyma and haemorrhages were noted. DOY et al. (1984) on 14 days post infection, observed infiltrates of numerous eosinophilic granulocytes in the hepatic parenchyma.

After 21 days post infection, fibrous adhesions of the left hepatic lobe with the diaphragm, infiltrates of numerous eosinophilic granulocytes and regressive lesions in the hepatocytes were observed. Apart from the discussed lesions, DOW et al. (1968) also found hypertrophy and fibrosis of the intima in the hepatic blood vessels. Mechanical damage caused by migration of juvenile stages of liver flukes resulted in thrombosis and inflammatory processes in the blood vessels.

In the available literature, there is a lack of reports on a comprehensive analysis of histological lesions and liver enzyme activity determined with histochemical methods, depending on the degree of a natural infestation with *Fasciola hepatica* flukes.

The objective of the studies was to evaluate the pathomorphological lesions in the liver and to perform a histochemical assessment of the activity of selected enzymes in the hepatocytes in cows naturally infested with *Fasciola hepatica* flukes.

Materials and Methods

The studies were carried out with 22 Black and White cows, aged 4 to 7 years, slaughtered in an abattoir and divided into two groups: those with a liver fluke infestation and those without infestation ($n=9$). The group of animals with liver fluke infestation was further divided into three subgroups depending on the degree of severity and the type of lesions detected macroscopically. Subgroup I included 9 animals with slightly thickened bile ducts, whereas subgroup II consisted of 7 animals with significantly thickened bile ducts, calcification foci and parenchymatous degeneration of the liver; subgroup III included 6 animals with diffuse calcification of bile ducts, single abscesses and lipidosis of the liver.

Two samples of the left hepatic lobe of approximately 100 grams each were collected from the animals infested with *F. hepatica* ($n=22$) and without infestation ($n=9$). One sample for histopathology was fixed in 10% buffered formalin (pH = 7.4). The second was put in a vacuum flask with dry ice for testing succinic acid dehydrogenase (SDH) and acid phosphatase (ACP) activity and for detecting lipids.

The liver samples for histopathology were embedded in paraffin and 5μm-thick paraffin sections were prepared. These paraffin sections were stained with haematoxylin and eosin (HE) according to the PAS method by McManus and silvered according to the method by Gomori (BANCROFT and GAMBLE 2008).

The frozen liver sections were stained for lipids with Sudan IV according to Lillie-Ashhurn (BANCROFT and GAMBLE 2008). In addition, the cryostat liver

sections were tested for succinic acid dehydrogenase activity (BANCROFT and GAMBLE 2008) and acid phosphatase activity (BANCROFT and GAMBLE 2008).

The evaluation of reticular fibres in the liver was achieved by dividing them into three groups: 1 – normal reticular fibres; 2 – an increased number of branched reticular fibres; and 3 – an increased number of reticular fibres of different thickness and extensively branched.

The contents of polysaccharides in the hepatocytes was determined according to a established 3-degree scale: (+) – moderate content of polysaccharides (slightly more intensive pigmentation of the hepatocytes); (++) high content of polysaccharides (intensive pigmentation of the hepatocytes); (+++) – very high content of polysaccharides (very intensive pigmentation of the hepatocytes).

The content of lipids in the hepatocytes was determined microscopically by distinguishing two groups: 1 – a lack of lipid vacuoles in the hepatocytes; and 2 – the presence of lipid vacuoles in single hepatocytes.

The histochemical activity of acid phosphatase and activity of succinic acid dehydrogenase was determined according to a personal 3-degree scale: low (+); moderate (++), and high (+++).

Results

The livers of all 9 animals from the control group had a regular tawny colour and dense texture. The microscopic examination of the liver in these animals revealed hyperaemia in 3 animals and parenchymatous degeneration of the hepatocytes in 2 animals.

In the experimental group, in a majority of cases the number of liver flukes ranged from 6 to 10 (26.3%) and from 36 to 45 (22.3%). In 22 examined cows with evidence of fluke infestation, a microscopic examination showed small clusters of mononuclear cells in the lobules and mononuclear cell infiltrates with eosinophilic granulocytes situated perivascularly, with proliferation and oedema of hepatic stellate cells and hyperaemia of intralobular veins and central veins, subcapsular and interlobular vessels. Furthermore, there was proliferation of intralobular and interlobular connective tissue and connective tissue proliferation around the central veins and around migratory tunnels in the hepatic parenchyma. In addition, parenchymatous and vacuolar degeneration of single hepatocytes and necrosis of the hepatocytes were observed. The foci with adult flukes were located within the bile ducts and surrounded by connective tissue, and there was fatty degeneration of the hepatocytes. The hepatic stellate cell proliferation as well as dissociation of the hepatocytes were observed. The foci composed with neutrophils (abscessation foci) were also found in the hepatic parenchyma. Fatty degeneration of the hepatocytes in the

cows with liver fluke infestation was recorded in 27% of the specimens in subgroup I, in 18% of the specimens in subgroup II and in 22% of the specimens in subgroup III. Few foci of coagulative necrosis in the hepatic parenchyma were found in the cows from subgroup I, slightly more in subgroup III, and the most were found in subgroup II. Hepatic cell dissociation was only found in the liver from the cows in subgroup III, whereas hyperaemia was seen in all liver specimens from subgroups I, II and III in 87%, 82% and 100% of the sections, respectively. Mononuclear cell and eosinophilic granulocyte infiltrates were observed in 70% of the specimens in subgroup II, compared to 80% in subgroup I and 89% in subgroup III. Proliferation or atrophy of hepatic stellate cells was observed in approximately 27% of liver specimens in the subgroup I and in about 35% in subgroups II and III, whereas connective tissue proliferation was demonstrated in 50% of the liver samples in subgroup II.

An increase in the number of extensively-branched reticular fibres was detected in the cows from all experimental groups (Table 1). The results of polysaccharide content in the hepatocytes are shown in Table 2. In the control cows, a moderate amount of polysaccharides was demonstrated in 25% of the examined samples and a high content in 75% of the examined samples, while in subgroup I the content was 53% moderate, 33% high and 14% very high in the samples. These results were 65%, 25% and 10%, respectively, in subgroup II and 77 %, 18 % and 5 %, respectively, in subgroup III.

Table 1

The structure of reticular fibres in the cattle liver

The kind of changes	Affirmed changes in % of preparations			
	K	I	II	III
The normal reticular fibres	85	–	–	–
The increased number of branched reticular fibres	15	80	47	11
The increased number of reticular fibres of different thicknesses and extensively branched	–	20	53	89

Explanation: K – control group, I group – weak thickened bile ducts, II group – strongly thickened bile ducts, focal calcification, parenchymatous degeneration, III group – calcified bile ducts, single abscesses, fatty degeneration

Table 2

Contents of the polysaccharides in the liver hepatocytes (%)

Contents of the polysaccharides in the hepatocytes	Group of animals			
	K	I	II	III
Moderate	25	53	65	77
High	75	33	25	18
Very high	–	14	10	5

The results for lipid content in the hepatocytes demonstrate that no lipid vacuoles were observed in the hepatocytes in the control group samples, whereas single hepatocytes with lipid-containing vacuoles were found in the cows naturally infested with *F. hepatica*.

The results of histochemical assays are presented in Tables 3 and 4. The activity of acid phosphatase (ACP) in the cows from all experimental groups was significantly lower compared to the animals from the control group. The activity of succinic acid dehydrogenase (SDH) in subgroup I was average and low in the subgroup II and III in comparison to the control group, in which it was very high.

Table 3

Activity of acid phosphatase (ACP) in the cattle liver infested by *Fasciola hepatica* flukes

Acid phosphatase activity	Group of animals			
	K	I	II	III
Low	–	+	+	+
Moderate	+	+	+	+
High	–	–	+	+

Table 4

Activity of succinic acid dehydrogenase (SDH) in the cattle liver infested by *Fasciola hepatica* flukes

Succinic acid dehydrogenase activity	Group of animals			
	K	I	II	III
Low	–	–	+	+
Moderate	–	+	–	–
High	+	–	–	–

Discussion

The described lesions in the liver were found in the cows with liver fluke infestation, which was not reported in the control cows. The performed assays indicate that the least apparent lesions in the liver were found in the cows from subgroup I, slightly more severe in subgroup III and the most severe in the subgroup III. These lesions were determined by the pathogenic effects of flukes, age of the animals and the number of parasites. The results of personal studies are consistent with findings reported by other authors (DOW et al. 1968, GAJEWSKA et al. 2005, RAHKO 1971, RAHKO 1973, RAHKO 1973).

In the cows from the subgroups I and II, there was a increased number of reticular fibres with an increased number of branches fibrils surrounding hepatocytes. It was found that the reticular fibres of connective tissue in-

creased in number together with an increasing number of parasites damaging the hepatic parenchyma. These lesions were particularly evident in the subgroup III cows. A similar type and nature of the reticular fibres was reported by ROMANIUK et al. (1973). Similar results were reported by MARCOS et al. (2007), who found increasing amounts of connective tissue together with an escalating intensity of parasitic infestation, which was also found in our studies.

In the liver sections from the subgroups I and II, a moderate content of polysaccharides was detected in approximately 50% of the samples, whereas in about 70% of the samples in the subgroup III; the content was very high in 14%, 10% and 5%, respectively. The demonstrated lesions indicate that the level of polysaccharides in the cattle liver significantly decreases with increasing severity of liver fluke infestation (damage to the hepatocytes). Similar lesions in the course of *F. hepatica* infestation were reported by RAHKO (1971).

Single hepatocytes with lipid-containing vacuoles were observed. These cells were located around the bile ducts. Degenerated hepatocytes were more often found in subgroup III in comparison with the other groups of animals.

The activity of acid phosphatase (ACP) is linked to the function of lysosomes in the cells. Under pathological conditions, its activity increases, which indicates damage to the intracellular structures. In the analysed cows, ACP assay was enhanced in the central zones of hepatic lobules and near the parenchyma damaged by flukes and was intensified together with the degree of infestation. The activity of this enzyme was clearly increased in the cows from subgroups II and III. A similar acid phosphatase activity was demonstrated by THORPE (1967).

The succinic acid dehydrogenase (SDH) assay in the cattle infested with *F. hepatica* was the most evident in the central lobular zone. Reduced SDH activity was detected in the liver, adjacent to microabscesses. This assay was determined by the number of parasites, which is consistent with the findings reported by THORPE (1967).

The parasites exert a mechanical impact (by damaging with tiny spikes on the cuticle) on the host, as well as a chemical impact (mainly through their metabolic products such as toxins) and a direct impact (by introducing pathogenic microorganisms). The presence of *F. hepatica* in the host initiated protective mechanism interactions in a very complex way (OLDENBORG et al. 1976), resulting in biochemical changes (in the internal organs and body fluids), clinical symptoms and pathological changes (FURMAGA and GUNDŁACH 1972). A comprehensive analysis demonstrated that in the liver of cows in which the severity of *F. hepatica* infestation was highest, a low content of polysaccharides (reserve material and energy source), a high number of

reticular fibres (responsible for fibrosis of the organ), a high activity of acid phosphatase (ACP) and a low enzymatic activity of hepatocytes (manifested by a poor reaction of succinic acid dehydrogenase (SDH) reaction) were found.

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