Treatment strategies for sheep with acute yellow athrophy of the liver caused by the fasciolosis


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The article presents results of studies of acute yellow athrophy of the liver development in sheep with fasciolosis. The general condition of sick sheep was suppressed; they had polypnoe, tachycardia and hypotension of the rumen, the reticulum, and the omasum. Total protein content (p<0.001), due to albumins (p<0.001) and α-globulins (p<0.001) was decreased in the blood serum of sheep with fasciolosis, which indicates protein synthesis dysfunction in the liver. At the same time, iblood serum levels of γ-globulins (p<0.01), total bilirubin (p<0.001), urea (p<0.01) and creatinine content (p<0.001) were decreased. Increase of cytolytic (AST, ALT) and cholestatic (GGT, ALP) enzymes activity in the blood serum of sick sheep was also established. Histological examination of liver biotapes from live animals indicated fatty infiltration of the parenchyma and protein dystrophy. These changes in blood parameters and histological studies indicate disruption of basic functions and structure of the liver. After administration of anthelmintic Rolenol to sheep with fasciolosis a decrease in albumins and α-globulins content was recorded. Accordingly, the content of γ-globulins in their blood increased. At the same time, the concentration of total and conjugated bilirubin increased, indicating diminished of hepatocyte function to conjugate and secrete pigment. Deworming led to an active increase of aminotransferases (AST, p<0.001, ALT; p<0.001) activity, as well as cholestatic (GGT and ALP) enzymes. Microscopic examination of sheep liver biotapes after Rollenol administration indicated an increase of structural changes in hepatocytes. Thus, in sheep with fasciolosis and after administration of anthelmintic Rolenol, basic functions and structure of the liver were disrupted, indicating the development of acute yellow athrophy of the liver. The treatment of sheep with acute yellow athrophy of the liver was conducted according to the following scheme: daily intravenous administration of a 10% glucose solution at a dose of 0.1 g/kg; subcutaneous administration of insulin at a dose of 0.25 U/kg of body weight; twice a day peroral administration of hepatoprotector Hepabene three capsules and brewer’s yeast with selenium four pills per animal; intramuscular injection of Trivit at a dose of 1 ml once a week was administered. After six days of treatment, improvements of general condition and recovery of basic functions and structure of the liver were established. In the blood serum of sheep increased albumins content decreased concentrations of total and conjugated bilirubin, as well as enzyme activity – AST, ALT, GGT and ALP were detected. The content of urea and creatinine in the blood serum did not differ from clinically healthy sheep, which may indicate a normalization of liver and kidneys function. Histological examination of liver biotapes indicated the restoration of their structure. However, a six-day treatment of sheep with acute yellow athrophy of the liver did not lead to normalization of some parameters of functional status and liver structure. Therefore, a rehabilitation period of 20 days was appointed, during which conditions of keeping were improved, and dietary feed was added into ration. At the end of rehabilitation the general condition of sheep was satisfactory, basic functions of the liver (protein synthesis, pigment, carbohydrate) were stabilized, the activity of indicator enzymes – AST, ALT, GGT and ALP was normalized, indicating the restoration of the hepatobiliary system structure.

Key words: Sheep; Fasciolosis; Rolenol; Acute yellow athrophy of the liver; Treatment

Introduction

Fasciolosis is one of the most common and dangerous helminthosis in animals. Mostly sheep, goats, cattle, rarely pigs, deer, rabbits and occasionally horses are affected (Mitchell, 2002; Mas-Coma et al., 2005; Knubben-Schweizer et al., 2015; Amer et al., 2016; Vázquez et al., 2016). Fasciolosis of ruminants is often diagnosed in Ukraine. The available data indicate that, first of all, ruminant fasciolosis occurs most frequently in the climatic zones of the Carpathians, Western Poliesie, and Forest steppe (Dakhno, 2001; Dovhi, 2005). The epizootic situation of ruminant fasciolosis is quite difficult in Western Ukraine. It was established that invasiveness of animals in this region ranges from 6.2 to 18.2% (Sobolta, 2009). Symptoms of fasciolosis are expressed to varying degrees, they depend on the intensity of the invasion, species, age and general condition of animals at the stage of disease. Parasitizing in the body of the host, fasciobes secrete vital and toxic products that damage the biological membranes of cells, inhibit cellular mechanisms of energy supply, activity of membrane-dependent enzymes and disrupt the processes of substances transport across membranes. There are changes in the liver of animals with fasciolosis that are characterized by traumatic hepatitis, hemorrhages, dystrophic changes in hepatocytes, cirrhosis and calcification of organ areas, blockage of parasites and mechanical damage of bile ducts (Dakhno, 2001; Sheliakyn et al., 2016). In turn, the prevention and treatment of fasciolosis using anthelmintic drugs causes decrease in body resistance and can be a contributing factor to increased liver damage (Matanoč et al., 2007; Levchenko et al., 2012). Therefore, after deworming of ruminants with fasciolosis, the primary task is to establish the functional
state and structure of the liver and, if necessary, to treat the animals. For the treatment of animals with liver pathology, a set of measures should be used that would restore the various functions and structure of the organ (Vilzio & Lewtschenko, 1992; Simonov & Vilzio, 2015; Mann et al. 2018).

The purpose of the work was to study the functional state and structures of the liver in sheep with fasciolosis, after administration of an anthelmintic drug – Rolenol, establish the effectiveness of treatment and to rehabilitate animals.

Materials and Methods

The material for the study were 24 sheep with fasciolosis, local breeds aged 2-6 years, and live weights 39-60 kg from farms of Western Ukraine. Fasciolosis was diagnosed by coproscopic examination and microscopy in McMaster egg counting chamber. 14 days after use of anthelmintic Rolenol, repeated coprological studies were performed. Antiparasitic drug Rolenol manufactured by Invesa (Spain), which contains 5% of clozanetel, is used for the treatment and prevention of ruminant fasciolosis (Swan, 1999). Animals received Rolenol at a dose of 2.7–3.4 ml once by subcutaneous injection (2.5 mg of active substance clozanetel per 1 kg of animal body weight). After deworming, sheep were treated to restore the function state and structure of the liver. Animals were treated according to the following scheme: daily intravenous administration of 10% glucose solution at a dose of 0.1 g per kg of body weight and subcutaneous administration of insulin at a dose of 0.25 U/kg of body weight; once a week, 2 ml of Trivit were injected intramuscularly (in 1 ml contains vitamin A – 30,000 IU, vitamin D3 – 40,000 IU, vitamin E – 20 mg); Hepabene hepatoprotector three capsules and beer yeast with selenium four tablets per animal were orally administered twice daily (1 tablet contains selenium – 0.0025 mg; vitamins: B<sub>1</sub> – 0.67 mg, B<sub>2</sub> – 0.63 mg, B<sub>5</sub> (niacin) – 5.88 mg, B<sub>6</sub> – 0.88 mg, B<sub>12</sub> – 0.0004 mg, E – 5 mg, vitamin C – 19.8 mg, folic acid – 0.13 mg, biotin – 0.05 mg, pantothenic acid – 3.8 mg). The treatment of sick sheep lasted for six days. Based on the analysis of blood biochemical parameters after treatment, a rehabilitation period of 20 days was carried out.

During the rehabilitation, the ration and conditions of sheep keeping were improved. The ration of sheep consisted of grass hay – 1.5 kg, fodder beet – 0.7 kg, wheat bran – 0.3 kg, oats – 0.2 kg, potatoes – 0.8 kg.

Throughout the experiment, sheep were clinically examined daily (Levchenko et al., 2017). In clinical study of animals, attention was paid to color of visible mucous membranes, sclera and skin, borders and pain of the liver. Blood for laboratory analysis was collected 24 hours before the experiment, and then 24 hours after Rolenol administration and on the 2nd, 4th, and 6th days after the start of the treatment and at the end of the rehabilitation period. Liver biopsy was performed before administration of Rolenol, 48 hours after its administration, at the end of treatment and rehabilitation.

The functional state and structure of the liver were evaluated by determining the blood serum total protein content (biuret method), protein fractions (polyacrylamide gel electrophoresis), total and conjugated bilirubin (Jendrassik and Grof method in modification of Levchenko and Vilzio, 1987), glucose (enzymatic glucose-oxidase method), urea (reaction with diacetylmonoxime), creatinine (Jaffe’s color reaction), enzyme activity: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – method of Raytman and Frenkel, γ-glutamyltranspeptidase (GGT) – reaction with L-γ-glutamyl-4-nitroaniline, alkaline phosphatase (ALP) – reaction with fenilphosphate and by results of histological examination of liver biopates (Vilzio et al., 2012). A biopsy of the liver was performed in the 11th intercostal space, previously determining the field of liver blunting using percussion. For biopsy of the liver we used needles of American production (Tru-cut-Biopsienadel, Travenol Laboratories Ins. firm), 1.6 mm diameter, 200 mm length, with 20 mm specimen notch. The obtained biopate was fixed in 10% formalin solution, made preparations that were stained with hematoxylin and eosin, and microscopically examined the liver structure.

Clinical and experimental animal studies were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) and the decisions of the First National Congress on Bioethics (Kyiv, 2001). The data were processed by Statistica v. 6.0 (Stat Soft, Tulsa, USA). Differences between the mean values were analyzed by ANOVA.

Results

In the study of sheep feces, a fasciolosis invasion was observed, which was characterized by an increase in extensity up to 6.0 (Stat Soft, Tulsa, USA). Differences between the mean values were analyzed by ANOVA.
In the study of serum urea in sheep with fasciolosis, an increase in its content by 38.4% (5.4 ± 0.38 mmol/l), compared to clinically healthy animals (3.9 ± 0.20 mmol/l); p<0.01. Obviously, due to fasciolosis, the kidneys are also affected and urinary excretion is delayed. The confirmation of this assumption was an increase of creatinine concentration in the blood. Thus, in the blood serum of sick sheep established its increase by 26.4% (126.8 ± 2.68 μmol/l; p<0.001), compared to healthy (100.2 ± 4.19 μmol/L). After deworming, the content of urea and creatinine in the blood serum decreased slightly, but was higher than in clinically healthy.

In the study of glucose in the blood serum of sheep with fasciolosis, it was established that its concentration was increased to 3.6 ± 0.11 (p<0.001), compared with 2.9 ± 0.09 mmol/L in clinically healthy. After the administration of Rolenol, the serum glucose content decreased to 2.8 ± 0.08 mmol/L.

In sheep with fasciolosis, the serum total bilirubin content (Figure 3) increased (p<0.05), and after deworming its level increased by 67% (p<0.01) and was 3.3 times higher (p<0.001) comparing to clinically healthy animals. The administration of Rolenol to sick animals caused an increase in conjugated bilirubin in the serum of sheep. Thus, its content is almost three times higher than in sheep with fasciolosis (p<0.001) and 5.7 times – clinically healthy (p<0.001).

At the same time, the activity of indicator enzymes – GGT and ALP (Figures 4 and 5) indicated the lesion of the hepatobiliary system. In serum of sheep with fasciolosis, the activity of GGT increased up to 40% (p<0.001), and after the introduction of anthelmintic, more than 2 times (p<0.001), compared to clinically healthy. The activity of ALP in the serum of sick sheep exceeded the level of clinically healthy by 4 times (p<0.001) and continued to increase after administration of anthelmintic (p<0.001).
The lesion of hepatocytes of sheep with acute yellow atrophy of the liver was indicated by high activity of AST (p<0.01) and ALT (p<0.001) in serum. In particular, it is necessary to note the active increase of enzyme activity after treatment of sheep with Rolenol (Figures 5 and 6).

Histological examination of liver biopsy specimens established development of protein dystrophy and fatty infiltration of hepatocytes (Figure 7). Thus, in sheep with fasciolosis, and after their deworming is violate basic functions and structure of the liver are compromised which can be interpreted as the development of acute yellow atrophy of the liver.

The next stage was the treatment of sheep with acute yellow atrophy of the liver. It was established that after six days of therapeutic measures in sheep body temperature (38.9 ± 0.23°C), pulse rate (73.2 ± 1.56, p<0.001), respiration (26.4 ± 1.24 breathing movements per minute; p<0.001) and rumen reduction (3.5 ± 0.20, p<0.001) were normalized. On the 4th day of treatment of sick sheep is significant (p<0.001) increase in total protein serum content was established (Figure 1). At the end of treatment, its content did not differ from clinically healthy. Increase in serum total protein content during treatment in blood was due to albumins (p<0.001, Figure 2) and α-globulins (11.6 ± 0.45%, p<0.001), indicating recovery of protein synthesis liver function. At the same time, the serum γ-globulin content decreased from 49.4 ± 0.94% to 28.2 ± 1.52 at the end of treatment (p<0.001). Accordingly, the albumin-globulin ratio increased (p<0.01-0.001) from the second day (0.53 ± 0.03) and continued to increase on the sixth day of treatment (0.91 ± 0.040).
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Consequently, within six days of treatment of sheep with secondary hepatopathy, caused by fasciolosis and anthelmintic Rolenol, restoration of the liver's protein synthetic function, normalization of the ratio between protein fractions and possible absence of pathological proteins were established. During THE treatment, urea concentration was within physiological values (3.0-6.0 mmol/l). At the same time, creatinine content in the blood serum at the fourth (107.3 ± 2.67 μmol/l) and sixth (103.0 ± 3.30 μmol/l) days of treatment (104.4 ± 3.08 μmol/l) was decreased (p<0.05–0.001) compared to sheep with fasciolosis (126.8 ± 2.68 μmol/l) and after deworming (120.2 ± 4.11 μmol/l), which indicates the restoration of filtration function of the kidneys glomeruli. It should be noted that glucose concentration in the blood serum of sheep after treatment is normalized (3.2 ± 0.07 mmol/l).

Figure 5. ALP activity in the blood serum of sheep (n=24).

During the treatment of sheep the concentration of total and conjugated blood serum bilirubin decreased (p<0.01-0.001), especially on the sixth day (Figure 3), indicating the restoration of the functional state of hepatocytes and elimination of cholestasis. This was also evidenced by a gradual decrease in serum GGT activity during the treatment of sheep with acute yellow athrophy of the liver (p<0.05). Similar changes were established in the study of serum ALP activity (Figure 5). Its activity in the blood decreased during treatment (p<0.01-0.001) on the fourth and sixth days. However, the activity of ALP in the blood serum of sheep remained quite high compared to healthy animals (61.2 ± 6.76 U/l), indicating that there was insufficient restoration of extrahepatic bile ducts. AST activity in the blood serum during the treatment of sheep decreased slightly (Figure 6) and only on the sixth day the indicator was significantly lower (p<0.05). It should be noted that the activity of AST in the blood serum of sheep after treatment was even higher compared to clinically healthy (p<0.01). The activity of ALT in the serum of sheep normalized during the treatment and did not differ compared to clinically healthy. The six-day treatment did not contribute to the complete normalization of blood parameters, and we conducted a 20-day rehabilitation of sick sheep. After the rehabilitation period, clinical examination of sheep indicated a satisfactory general condition. All sheep had stable protein synthesis function of the liver (Figures 1 and 2). The rates of total and conjugated bilirubin were lower compared to animals after deworming (p<0.001) and sheep with fasciolosis (p<0.05), and did not differ from the clinically healthy (Figure 3) indicating restoration of the functional state of hepatocytes and elimination of
cholestasis. This was also indicated by the results of GGT and ALP activity in the blood serum at the end of rehabilitation, which were (p<0.001) lower in sheep with fasciolosis and dewormed (Figures 4 and 5). The activity of cytolytic enzymes (AST, ALT) in the blood serum after rehabilitation was decreased (p<0.001), compared to sick sheep and even treated, and was within physiological values (Figure 6). When performing liver puncture in sheep after treatment and rehabilitation, it was established that macroscopically liver bioplates were solid, elastic in consistency, red-brown in color. At their histological examination restoration of the particles structure, beams and hepatocytes was established (Figure 7).

Figure 7. Liver structure of sick sheep before (a) and after rehabilitation (b). Hematoxylin-eosin. x 400.

Discussion
According to the literary data, the development of fasciolosis invasion among small ruminants is affected by the relative air humidity, total rainfall, air temperature. It is established that these factors are important in the development of larval stages of trematode, their infection with molluscs, the course of all stages of helminth reproduction, as well as the output of adolescarias. The spread of fasciolosis invasion among small ruminants is also influenced by such factors as the deworming of animals. The use of anthelmintic drugs eliminates the pathogen in the animal body and prevents the release of Fasciola hepatica eggs into the environment. This leads to break of epizootic chain in fasciolosis process and reduces the spread of invasion in animals. Studies of sheep with fasciolosis when using anthelmintic Rolenol, indicate that animals are not only negatively affected by helminths but also by antiparasitic drugs (Mooney et al., 2009). Therefore, in diagnosis and establishment of deworming effectiveness selective laboratory blood tests should be performed in the herd. (Vlizlo et al., 2012; Munita et al., 2019). Informative laboratory methods for the diagnosis of liver damage are study of basic functions and structure of the organ (Matanović et al., 2007; Vlizlo et al., 2014; Levchenko et al., 2017). In animals with fasciolosis, and during anthelmintic drugs intake, the acute yellow athrophy of the liver is developed (Kulyaba et al., 2019). In sick sheep there is protein synthesis dysfunction of the liver was observed, characterized by a decrease in serum total protein content due to albumins. Hypoproteinemia increased 24 hours after administration of an anthelmintic drug. It was caused by violation of albumins synthesis in hepatocytes as well as α-globulins, since all plasma albumins and up to 90% of α-globulins are synthesized by the liver (Levchenko & Vlizlo, 2019). At the same time, γ-globulins content in blood serum of sick sheep was increased, which was, obviously, due to accumulation of toxic products in the body, that are formed during the life of helminths, irritation of immunoreactive tissue of phagocytic mononuclear cells system by endotoxins which are formed in the body with liver pathology, and cause the active formation of coarse-dispersed globulin fractions and pathological proteins that are part of these fractions (Vlizlo, 1998; Chernushkin et al., 2010). That is why sick animals have decreased albumin-globulin ratio.

The development of acute yellow athrophy of the liver in sick sheep caused an increase in the blood serum concentration of not conjugated and conjugated laboratory blood tests should be performed in the herd. (Vlizlo et al., 2012; Munita et al., 2019). Informative laboratory methods for the diagnosis of liver damage are study of basic functions and structure of the organ (Matanović et al., 2007; Vlizlo et al., 2014; Levchenko et al., 2017). In animals with fasciolosis, and during anthelmintic drugs intake, the acute yellow athrophy of the liver is developed (Kulyaba et al., 2019). In sick sheep there is protein synthesis dysfunction of the liver was observed, characterized by a decrease in serum total protein content due to albumins. Hypoproteinemia increased 24 hours after administration of an anthelmintic drug. It was caused by violation of albumins synthesis in hepatocytes as well as α-globulins, since all plasma albumins and up to 90% of α-globulins are synthesized by the liver (Levchenko & Vlizlo, 2019). At the same time, γ-globulins content in blood serum of sick sheep was increased, which was, obviously, due to accumulation of toxic products in the body, that are formed during the life of helminths, irritation of immunoreactive tissue of phagocytic mononuclear cells system by endotoxins which are formed in the body with liver pathology, and cause the active formation of coarse-dispersed globulin fractions and pathological proteins that are part of these fractions (Vlizlo, 1998; Chernushkin et al., 2010). That is why sick animals have decreased albumin-globulin ratio.

The development of acute yellow athrophy of the liver in sick sheep caused an increase in the blood serum concentration of not conjugated and conjugated bilirubin. This may indicate a violation of the functional ability of hepatocytes to absorb, conjugate and secrete bilirubin into bile (Moritz et al., 2014; Simonov & Vlizlo, 2015; Levchenko et al., 2019). The lesions of the hepatobiliary system were also indicated by the high activity in the blood serum of sick sheep indicator enzymes – GGT and ALP (Vlizlo et al., 2012). This can be a sign of lesion and blockage of bile duct by fascioles. Particularly increased activity of ALP, as the fascioles affect, first of all, extrahepatic bile ducts (Macs et al., 1988). Increased activity of cytolytic (AST, ALT) enzymes was also established in the blood serum of sick sheep, indicating damage of liver cells (Vlizlo et al., 2014). Histological examination of liver bioplates confirmed organ cells damage and development fatty infiltration of hepatocytes and lesions of bile ducts in sheep with fasciolosis.

Not only the liver, but also the kidneys were affected by fasciolosis, since the content of urea and creatinine in the blood serum have been increased. This should be taken into account in treatment of animals. Thus, on the basis of the data obtained, we can conclude that in the case of fasciolosis invasion negative changes in the body of sheep occur. That is why the treatment of sheep should be carried out comprehensively, using drugs that would be involved in the restoration of protein synthesis function and functional state of the liver. Conducted therapeutic measures for liver diseases should be efficient and cost-effective (Vlizlo & Lewtschenko, 1992; Vlizlo, 1997; Simonov & Vlizlo, 2016).

For the treatment of sheep with acute yellow athrophy of the liver, we used intravenous injections of 10% glucose with insulin, intramuscular – trivit, oral – brewer’s yeast with selenium and hepatoprotective drug hepabene. The treatment of sick sheep showed a positive effect after 6 days. Thus, in sick sheep increased the content of total protein due to albumins and α-globulins. The absence of toxic substances that irritate immune cells of the liver indicated a decrease in the number of γ-globulins. Accordingly, the albumin-globulin ratio increased and dysproteinemia decreased. Particularly dynamic was the normalization of liver
pigment function. The concentration of total and conjugated bilirubin in the blood of animals decreased already after the second day of treatment, and at the end of therapeutic measures the pigment content did not differ on average from the clinically healthy. The high specificity of changes in bilirubin in diagnosis of liver lesions and control over treatment has been established in other studies (Vilzio, 1998; Simonov & Vilzlo, 2013; Simonov & Vilzlo, 2015). In post-rehabilitation period, the serum parameters of all the studied sheep responded to clinically healthy animals, indicating a recovery of hepatocyte functional status and elimination of cholestasis (Vilzio 1998; Levcchenko & Vilzlo et al., 2019).

This was also confirmed by a study of enzyme activity in the serum of sheep both cytolytic (AST, ALT) and cholestatic (GGT, ALP), the level of which significantly decreased during the six days of treatment. However, in the blood of some sheep, there were still low parameters of liver protein synthesis function, increased total and conjugated bilirubin, and high enzyme activity (AST, ALT, GGT, ALP). Conducting a 20-day rehabilitation period by improving the feeding and keeping conditions of sheep led to complete restoration of pigment, protein synthesis, urea liver functions, normalization of creatinine and glucose. At the end of rehabilitation, AST, ALT, GGT and ALP activity in the blood serum of sheep fluctuated within physiological limits. These changes in blood parameters indicate the restoration of liver cells structure, intra- and extrahepatic bile ducts and the elimination of cholestasis, which was also confirmed by histological examination of liver biopsates.

Conclusion

In sheep with fasciolosis and after their deworming, violated basic functions (protein synthesis, pigment) and structure of the liver (increase of AST and ALT enzymes activity, histologically – fatty infiltration and protein dystrophy) and the hepatobiliary system are detected (content of conjugated bilirubin and activity of GGT, ALP increase), indicating the development of acute yellow athrophy of the liver. During the treatment of sheep with acute yellow athrophy of the liver, caused by fasciolosis, and after administration of anthelmintic Rolenol, a positive result was obtained after 6 days of injection of glucose with insulin, administration of vitamins A, D, E, brewer’s yeast with selenium and hepatoprotector hepabene. It is efficient and cost-effective to carry out a 20-day rehabilitation period after the treatment with improved feeding and keeping conditions of sheep, during which the normalization of protein synthesis, pigment and urea formation functions of the liver as well is reduced activity of cytolytic (AST, ALT) and cholestatic (GGT, ALP) enzymes occurred, which indicates the restoration of liver structure.

References


**Citation:**