



BIOCHEMICAL MARKERS OF LIVER DAMAGE IN CATTLE NATURALLY INFECTED WITH *FASCIOLA HEPATICA*

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ABSTRACT

Fascioliasis is a worldwide spread parasitic disease with a high impact on the economy and public health in many countries. Therefore, it is important to apply prevention, effective treatment, and education campaigns, to prevent the disease. This work aimed to determine the biochemical markers of liver damage caused by *Fasciola hepatica* during 21 days (D0 – D21) post albendazole (ABZ) therapy in the blood sera. This study included 24 cows naturally infected with *Fasciola hepatica*, randomly divided into a control group ($n = 12$) and a group treated *per os* with ABZ (7.5 mg per kg body weight) ($n = 12$). The activity of hepatic enzymes – aspartate aminotransferase (AST), alanine aminotransferase (ALT) and cholestatic enzymes – gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP), were measured in both groups. The activity of AST has significantly increased in the course of the experiment (D0 – D21) in both groups ($P < 0.001$). ALT activity during D0 – D21 was significantly decreased in both groups ($P < 0.001$). Activity of GGT was not

significantly changed in the control group in the course of the experiment ($P > 0.05$). Contrarily, the activity of GGT in the treated group was significantly decreased ($P < 0.01$) during D0 – D21. The activity of GGT in the blood sera of the treated group was significantly lower in comparison with the control group in D7 and D21 ($P < 0.01$). This decreased GGT activity in the blood sera of the treated group may be caused by the effect of albendazole resulting in fasciola elimination and bile flow improvement. The results of ALP determination show no significant changes in ALP activity between groups. This can lead to the conclusion that the most useful marker of cholestasis caused by *F. hepatica* is the GGT activity and may help to evaluate the ABZ therapy effect in cattle.

Key words: alanine aminotransferase; albendazole; alkaline phosphatase; aspartate aminotransferase; fascioliasis; γ -glutamyl transferase

INTRODUCTION

Fascioliasis is a common zoonotic parasitic disease that affects ruminants worldwide and is known to cause significant economic losses in the livestock industry. The most important species of liver flukes are *F. hepatica* and *F. gigantica*. *Fasciola hepatica* is the most widespread species, may be found in more than 70 countries around the world [10]. The only fasciola free area is Antarctica [1]. Lymnae snails as intermediate hosts are required for complete life cycle of *F. hepatica* [2, 29]. Therefore *F. hepatica* is limited to areas where high soil moisture, high rainfall and poorly drained soils leading to standing waters are present [6, 10, 11]. There is also need for temperature range of 10–25 °C for development of both *F. hepatica* and the snail *L. trunculata* [11]. The disease has a high negative impact on economic conditions in the livestock industry annually [2, 4, 5, 10, 28]. There is a decreased milk production in dairy cattle, decreased fertility, nutrients conversion and requirement for anthelmintic drugs administration [2, 28]. Distribution of fascioliasis around the world is a dynamic process and may change with years, therefore monitoring of its prevalence is important to determine the risk [2]. Increasing risk of fascioliasis is caused by multiple factors – climate changes, anthelmintic resistance and absence of clinical signs in cattle [16, 22]. Humans living in the endemic areas of fascioliasis are at high risk of the infection. Fascioliasis is defined as plant/food-borne disease [18]. Additionally, individuals living in endemic regions for this disease are at considerable risk of contracting it. Studies showed that roughly 50 million people are currently affected, with over 180 million more at risk of infection annually [4, 13, 18]. The incidence of human natural fascioliasis is mostly correlated with regions of occurrence of animal fascioliasis [2, 23]. As such, it is crucial to take preventative measures in both dairy and meat livestock production to minimize the spread of this disease.

In cattle the infection can be more severe to develop evident clinical signs. The liver and biliary system are mainly affected in infected cattle. The liver parenchyma may show pathological changes such as fibrosis, haemorrhage, or necrosis, and bile ducts may become obstructed [21]. Therefore, it is important to monitor a herd for the presence of the infection. There are many diagnostic methods available to identify the presence of fascioliasis. Several diagnostic methods are available to identify the presence of fascioliasis, such as detecting fasciola eggs or

antigens in the faeces, antibodies in blood serum, or using bulk milk tests for antibodies in milk. Biochemical analysis may be very helpful and enhance laboratory diagnostics of fascioliasis [31]. In addition to diagnostic methods it is important to use effective treatment of the infection. The treatment of fascioliasis based on the use of anthelmintics. The efficacy of the drug against different stages of liver flukes should be considered [25]. However, there has been an increasing risk of anthelmintic resistance in fascioliasis treatment in the last few decades. In the studies by Novobilský and Höglund [17], Overend and Bowen [20], and Sanabria et al. [24] the authors discussed the presence of resistance of *F. hepatica* resistance to various anthelmintics (triclabendazole, closantel, albendazole).

The aim of this study was to evaluate the biochemical markers of liver damage hepatic and cholestatic enzymes – caused by fascioliasis during 21 days post the albendazole (ABZ) therapy in the control and the treated group.

MATERIALS AND METHODS

Animals

Our experimental study included 24 Charollais cattle aged 3–7 years, weighing from 400 to 900 kilograms, non-lactating. The cattle were located in the endemic zone of fascioliasis in the North-East part of Slovakia with semi-extensive farming system. All 24 animals were positive for *Fasciola hepatica* eggs. The last anthelmintic drug was administered 12 months before this study. All 24 animals were divided randomly to two equal groups. In the untreated group (control group), no anthelmintic drug was administered for fasciola infection treatment study. Animals in the treated group (ABZ) were administered albendazole orally at a dose of 7.5 mg per kg body weight (Albendavet 10 %®, DIVASA-FARMAVIC S.A., Spain).

Sampling

In both groups blood samples were obtained at the beginning of the experiment (D0) and animals in the treated group were administered albendazole afterwards. Later, on the 7th (D7) and 21st (D21) day of the experimental study, additional blood samples were collected from individuals in both groups (Figure 1). Serum was obtained from these blood samples for biochemical analysis.

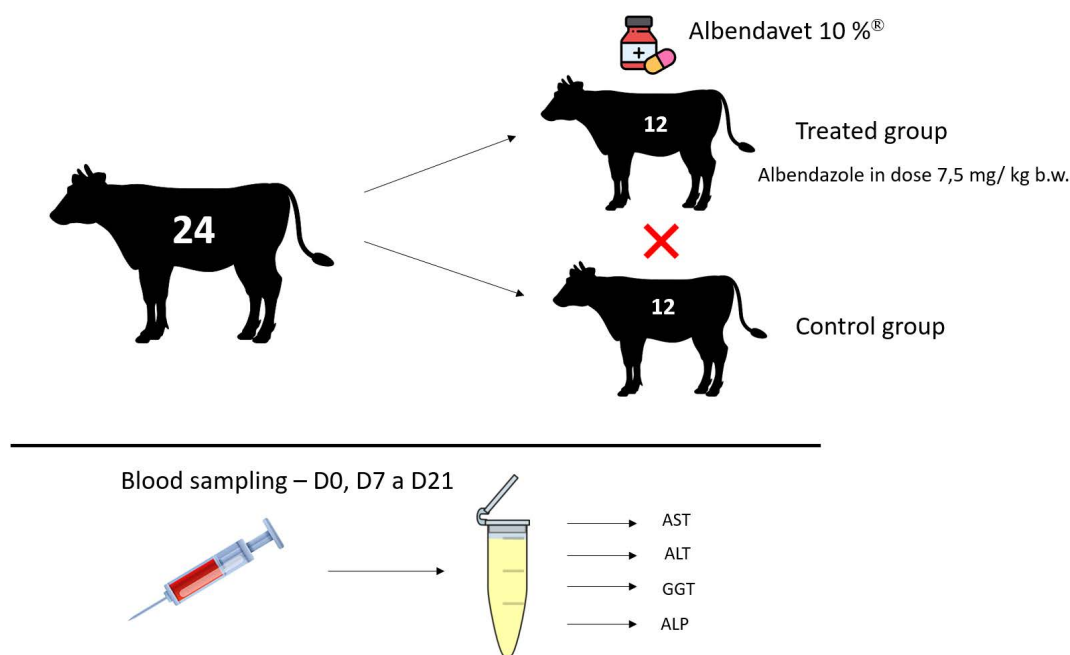


Fig. 1. Scheme of the experiment

Biochemical analysis

To monitor the development of liver injury and the effects of anthelmintic treatment, levels of four biochemical parameters were determined in the blood serum. The following hepatic and cholestatic enzymes were detected: aspartate aminotransferase (AST); alanine aminotransferase (ALT); gamma-glutamyl transferase (GGT); and alkaline phosphatase (ALP). Commercial kits from Erba Lachema (Brno, Czech Republic) and a spectrophotometer ONDA V-10 PLUS from Labbox (Spain) were used to determine all parameters.

Calibration curves were used to determine the activities of hepatic enzymes (AST and ALT), while kinetic spectrophotometric methods were used to determine the activities of cholestatic enzymes (GGT and ALP). The enzymatic activity was calculated using the following formula:

$$\text{GGT/ALP}(\mu\text{kat/l}) = \frac{\Delta_{\text{sam}}}{\Delta_{\text{cal}}} \cdot C_{\text{cal}}$$

Δ_{sam} = absorbance change of sample

Δ_{cal} = absorbance change of calibrator

C_{cal} = calibrator concentration

Statistics

Statistical analysis of the obtained data was performed using Student t-test in the Microsoft Excel 2021 program (Table 1).

Ethical consideration

The study was approved by the Ethics Committee of the Institute of Parasitology of the Slovak Academy of Sciences in Košice, Certificate of approval No. 03/2021.

RESULTS

The activity of AST was significantly increased in the course of the experiment (D0 – D21) in both control and treated groups ($P < 0.001$). ALT activity during D0 – D21 was significantly decreased in both groups ($P < 0.001$). The activity of GGT was not significantly changed in the control group in the course of the experiment ($P > 0.05$). Contrarily, the activity of GGT in the treated group was significantly decreased ($P < 0.01$) during D0 – D21. The activity of GGT in the blood sera of the treated group was significantly lower in comparison with the control group in D7 and D21 ($P < 0.01$). All measured serum enzymatic activity levels and statistical analysis are shown in Figure 2.

Table 1. Statistical analysis

	Mean 1	Mean 2	t-value	P	Valid N1	Valid N2	SD 1	SD 2
Control (1) - treated (2) group								
ALT (D0)	0.2002	0.1020	5.0441	0.000172	10	10	0.054	0.030
ALT (D7)	0.1697	0.1374	1.4314	0.170600	10	9	0.050	0.049
ALT (D21)	0.1033	0.0534	4.3267	0.000407	10	10	0.026	0.026
AST (D0)	0.4744	0.5168	-1.8755	0.077650	10	10	0.055	0.045
AST (D7)	0.7057	0.7952	-3.2708	0.007396	10	9	0.035	0.075
AST (D21)	0.7747	0.8619	-6.3661	0.000006	10	10	0.034	0.027
GGT (D0)	0.4560	0.3980	1.1263	0.279100	10	10	0.143	0.078
GGT (D7)	0.3814	0.2825	3.5475	0.002496	10	9	0.066	0.055
GGT (D21)	0.3842	0.2833	3.1476	0.008501	10	10	0.094	0.038
ALP (D0)	0.8436	0.8331	0.0380	0.970100	10	10	0.581	0.657
ALP (D7)	0.8621	0.8026	0.2364	0.816000	10	9	0.592	0.504
ALP (D21)	1.1205	1.1995	-0.2051	0.839900	10	10	0.787	0.932
Control group								
AST D0 - D21	0.4744	0.7747	-14.6932	3.00E-10	10	10	0.0552	0.0336
ALT D0 - D21	0.2002	0.1033	5.1400	0.000189	10	10	0.0536	0.0260
GGT D0 - D21	0.4560	0.3842	1.3281	0.203300	10	10	0.1430	0.0939
ALP D0 - D21	0.8436	1.1205	-0.8948	0.383700	10	10	0.5807	0.7873
Treated group								
AST D0 - D21	0.5168	0.8619	-20.5512	2.85E-12	10	10	0.0455	0.7873
ALT D0 - D21	0.1020	0.0534	3.8842	0.001135	10	10	0.0302	0.0256
GGT D0 - D21	0.3980	0.2833	4.1726	0.001084	10	10	0.0781	0.0381
ALP D0 - D21	0.8331	1.1995	-1.0167	0.324300	10	10	0.6568	0.9315

DISCUSSION

Internal parasites as *Fasciola hepatica* are likely to influence general health status in infected animals. Fascioliasis affects mainly the liver and biliary system function. The determination of biochemical parameters can help identify pathological changes in the hepatobiliary system of affected animals [2]. In the case of severe infection, loss of hepatic a biliary system function may occur. Those pathological changes can be detected by analyses of some biochemical markers when elevation of their activities are expected in blood sera. Increased activity of enzymes indicates damage to those tissues in which the enzymes are found. Cell death or tissue damage and therefore subsequently increased permeability of cell membranes and the transfer of enzymes into the intercellular space and then blood circulation occur. As enzymatic markers of hepatic and biliary function are AST,

ALT, GGT and ALP determined commonly [7]. Since AST and ALT are not enzymes specific for the liver parenchyma, they can be examined together with GGT and ALP to monitor liver damage [27]. In the early stage of *F. hepatica* infection, changes occur mainly in the activity of AST and ALT as enzymes related to hepatic function when juvenile forms of flukes migrate in the liver parenchyma [19]. According to O t t e r [19], activity of AST is high in the liver parenchyma and is an indicator of acute and chronic liver injury. Increased serum activity of AST occurs 3–4 weeks post infection. However, the increase in AST serum activity may be also associated with pathological changes in kidneys, pancreas, skeletal muscles or erythrocytes. Therefore, the increase in AST serum activity is not specific to liver injury. Serum activity of ALT is normally measured to monitor hepatic function in animals. However according to H o f f m a n and S o l t e r [9] serum ALT activity is decreased in cattle, horses, sheep

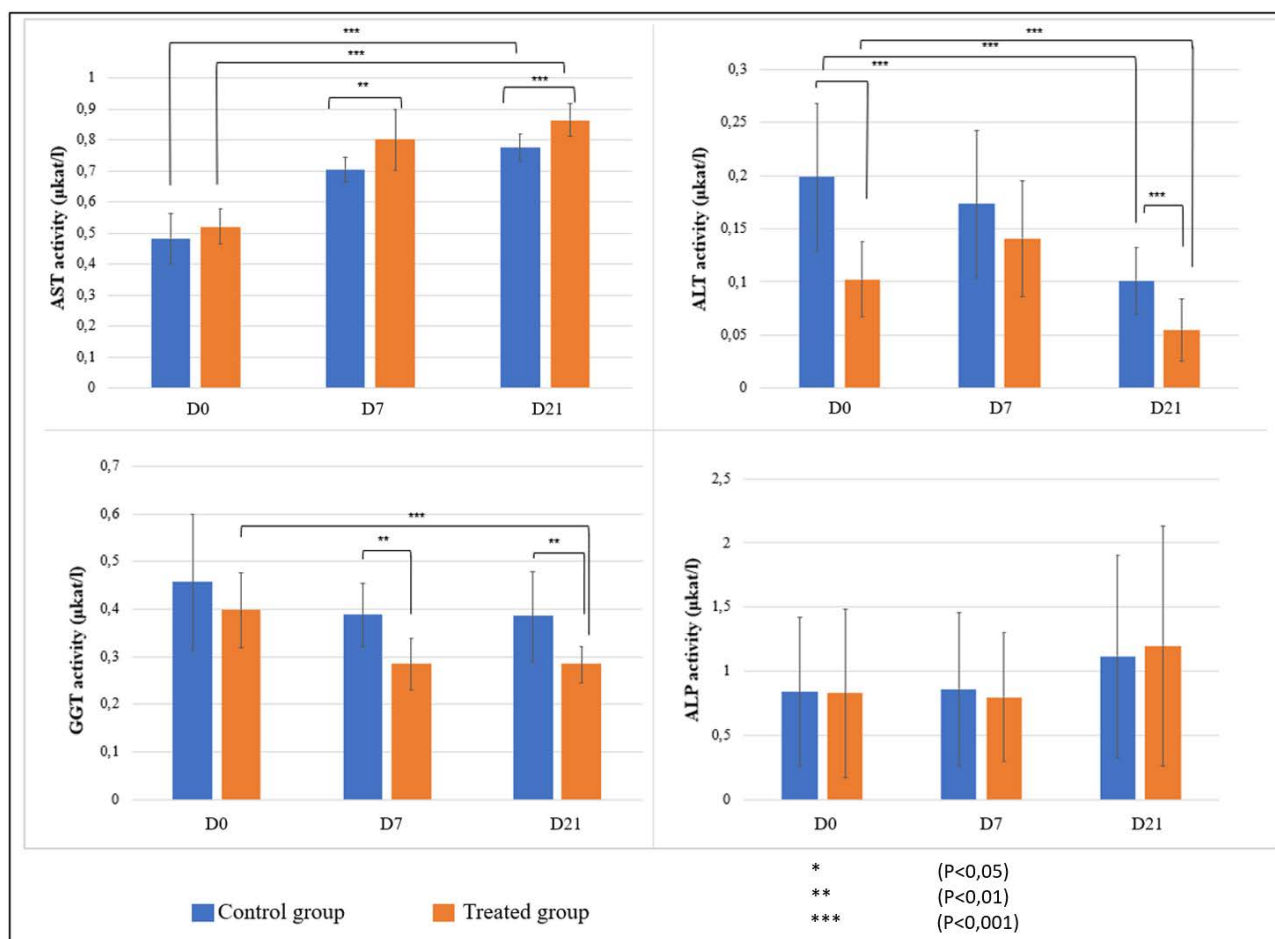


Fig. 2. Serum enzymatic activity levels during 21 days (D0 – D21) post albendazole therapy and statistical analysis
 AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma glutamyl transferase; ALP – alkaline phosphatase

and pigs in comparison with other animal species. Therefore, it is not routinely monitored in these animal species. In our study we noticed significantly decreased activity of ALT and increased activity of AST in the course of the experiment in both groups. Our results of AST activity resemble those of Simesen and Nansen [26]. On the contrary, Kowalczyk et al. [12] and Matanović et al. [14] reported increased serum AST activity in infected group comparing with the control group. Hodžić et al. [8] did not note any difference in AST activity between infected and control groups. These findings of researchers support the claim made by Hoffman and Solter [9] that the AST activity has a limited diagnostic sensitivity for fascioliasis (46 % sensitivity).

High activity of GGT is present in the biliary system and kidneys. In kidney injury, GGT is eliminated mainly by urine [3, 30]. Therefore, increased blood GGT activity is related primarily to liver and pancreas pathologies. In our study, we noticed decreased serum GGT activity

in the treated group compared to the control group in D7 and D21. In the course of our study, the activity of GGT was significantly decreased only in the group treated with albendazole. The decreased level of serum GGT activity may be associated with the flukicidal effect of albendazole administration in this group of cattle. Albendazole affects the adult forms of the liver fluke present in the biliary ducts. After albendazole administration, elimination of flukes from biliary ducts occurs, the bile flow is improved and serum GGT activity is decreased. Similar findings were observed in the study by Bulgín et al. [3], Hodžić et al. [8], Kowalczyk et al. [12] and Matanović et al. [14]. These authors reported increased GGT serum activity in infected group in comparison with the control group. However, Kowalczyk et al. [12] noted that GGT serum activity may be affected also by other factors like age of cattle or raising cattle on pasture. ALP is present in several body tissues such as the liver, bones, kidneys, intestinal mucosa and placenta. Infestation with

a small number of flukes may not be sufficient to cause an increase in ALP serum activity. Additionally, bile stasis usually causes increase in ALP activity but cattle and sheep are an exception [3]. According to Hoffman and Solter [9], in comparison with ALP activity the serum GGT activity is a more sensitive marker of extrahepatic cholestasis, cholangiohepatitis and liver cirrhosis. In our study, we did not note any statistically significant changes in ALP activity during the experiment. Bulgin et al. [3] pointed out that compared to the GGT activity more severe infection is needed to produce change in ALP serum activity.

Increased serum activity of hepatic enzymes, ALT and AST, is associated mainly with the acute stage of liver injury. However, other organ tissues responsible for increased serum activity should be considered. On the contrary, increased GGT and ALP activity in the blood serum is associated with the chronic stage of the infection when adult forms of *F. hepatica* are located in the biliary system.

CONCLUSIONS

The monitoring of hepatic and biliary system injury in cattle with *F. hepatica* infestation revealed that determining the GGT activity is the most reliable parameter. GGT activity can serve as a highly effective diagnostic tool for identifying pathological changes in these systems. Regular blood biochemistry monitoring can aid in the diagnosis and monitoring of fascioliasis in cattle. It is recommended to monitor also other blood biochemical parameters, such as globulins, total proteins, urea, and glutamate dehydrogenase (GLDH). For the most accurate diagnosis, a combination of coprological examination and blood biochemical analysis should be used.

Conflict of interest

The authors declare that they have no conflict of interest.

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REFERENCES

1. Atalabi, E. T., Laval, T. O., 2022: Fascioliasis: A foodborne disease of veterinary and zoonotic importance. Bacha, U., (Ed.): *Rural Health*, IntechOpen, 2022, 2–16. DOI: 10.5772/intechopen.91361.
2. Brahmabhatt, N. N., Kumar, B., Thakre, B. J., Bilwal, A. K., 2021: Haemato-biochemical characterization of fasciolosis in Gir cattle and Jaffrabadi buffaloes. *J. Parasit. Dis.*, 45, 683–688. DOI: 10.1007/s12639-020-01342-1.
3. Bulgin, M. S., Anderson, B. C., Hall, R. F., Lang, B. Z., 1984: Serum gamma glutamyl transpeptidase activity in cattle with induced fascioliasis. *Res. Vet. Sci.*, 37, 167–171. DOI: 10.1016/S0034-5288(18)31899-X.
4. Cwiklinski, K., O'Neill, S. M., Donnelly, S., Dalton, J. P., 2016: A prospective view of animal and human fasciolosis. *Parasit. Immunol.*, 38, 558–568. DOI: 10.1111/pim.12343.
5. Forbes, A., 2017: Liver fluke infections in cattle and sheep. *Livestock*, 22, 250–256. DOI: 10.12968/live.2017.22.5.250.
6. Fox, N. J., White, C. L., McClean, C. J., Marion, G., Evans, A., Hutchings, M. R., 2011: Predicting impacts of climate change on *Fasciola hepatica* risk. *PLOS ONE*, 6, 1, e16126. DOI: 10.1371/journal.pone.0016126.
7. Hauptman, K., Tichý, F., Knotek, Z., 2001: Clinical diagnostics of hepatopathies in small mammals: Evaluation of importance of individual methods. *Acta Vet. Brno*, 70, 297–311. DOI: 10.2754/avb200170030297.
8. Hodžić, A., Zuko, A., Avdić, R., Alić, A., Omeragić, J., Jažić, A., 2013: Influence of *Fasciola hepatica* on serum biochemical parameters and vascular and biliary system of sheep liver. *Iran. J. Parasitol.*, 8, 92–98.
9. Hoffmann, W. E., Solter, P. F., 2008: Diagnostic enzymology of domestic animals. *Clinical Biochemistry of Domestic Animals*. Elsevier, 351–378. DOI: 10.1016/B978-0-12-370491-7.00012-X.
10. Howell, A. K., Williams, D. J. L., 2020: The epidemiology and control of liver flukes in cattle and sheep. *Vet. Clin. North Am. Food Anim. Pract.*, 36, 109–123. DOI: 10.1016/j.cvfa.2019.12.002.
11. Köstenberger, K., Tichy, A., Bauer, K., Pless, P., Wittek, T., 2017: Associations between fasciolosis and milk production,

- and the impact of anthelmintic treatment in dairy herds. *Parasitol. Res.*, 116, 1981–1987. DOI: 10.1007/s00436-017-5481-3.
12. Kowalczyk, S. J., Czopowicz, M., Weber, C. N., Müller, E., Kaba, J., 2018: Accuracy of a diagnostic model based on serum biochemical parameters in detecting cows at an increased risk of chronic fascioliasis. *Vet. Parasitol.*, 254, 15–20. DOI: 10.1016/j.vetpar.2018.02.038.
 13. Mas-Coma, S., Valero, M. A., Bargues, M. D., 2019: Fascioliasis. *Adv. Exp. Med. Biol.*, 71–103. DOI: 10.1007/978-3-030-18616-6_4.
 14. Matanović, K., Severin, K., Martinković, F., Šimpraga, M., Janicki, Z., Barišić, J., 2007: Hematological and biochemical changes in organically farmed sheep naturally infected with *Fasciola hepatica*. *Parasitol. Res.*, 101, 1657–1661. DOI: 10.1007/s00436-007-0709-2.
 15. McManus, D. P., 2020: Recent progress in the development of liver fluke and blood fluke vaccines. *Vaccines*, 8. DOI: 10.3390/vaccines8030553.
 16. Mezo, M., González-Warleta, M., Carro, C., Ubeira, F. M., 2004: An ultrasensitive capture Elisa for detection of *Fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (MM3). *J. Parasitol.*, 90, 845–852. DOI: 10.1645/GE-192R.
 17. Novobilsky, A., Koudela, B., 2005: Treatment and control of *Fascioloides magna* infection in cervids – review (In Czech). *Veterinářství*, 55, 98–102.
 18. Nyindo, M., Lukumbagire, A. H., 2015: Fascioliasis: An ongoing zoonotic trematode infection. *BioMed Res. Int.*, 2015, 1–8. DOI: 10.1155/2015/786195.
 19. Otter, A., 2013: Diagnostic blood biochemistry and haematology in cattle. *In Practice*, 35, 7–16. DOI: 10.1136/inp.e8719.
 20. Overend, D. J., Bowen, F. L., 1995: Resistance of *Fasciola hepatica* to triclabendazole. *Aust. Vet. J.*, 72, 275–276. DOI: 10.1111/j.1751-0813.1995.tb03546.x.
 21. Pérez-Caballero, R. et al., 2018: Pathological, immunological and parasitological study of sheep vaccinated with the recombinant protein 14-3-3z and experimentally infected with *Fasciola hepatica*. *Vet. Immunol. Immunopathol.*, 202, 115–121. DOI: 10.1016/j.vetimm.2018.07.006.
 22. Reigate, C., Williams, H. W., Denwood, M. J., Morphew, R. M., Thomas, E. R., Brophy, P. M., 2021: Evaluation of two *Fasciola hepatica* faecal egg counting protocols in sheep and cattle. *Vet. Parasitol.*, 294, 109435. DOI: 10.1016/j.vetpar.2021.109435.
 23. Robinson, M. W., Dalton, J. P., 2009: Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiasis. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 364, 2763–2776. DOI: 10.1098/rstb.2009.0089.
 24. Sanabria, R., Ceballos, L., Moreno, L., Romero, J., Lanusse, C., Alvarez, L., 2013: Identification of a field isolate of *Fasciola hepatica* resistant to albendazole and susceptible to triclabendazole. *Vet. Parasitol.*, 193, 105–110. DOI: 10.1016/j.vetpar.2012.11.033.
 25. Shokier, K. M., Aboelhadid, S. M., Waleed, M. A., 2013: Efficacy of five anthelmintics against a natural *Fasciola* species infection in cattle. *Beni-Suef Univ. J. of Basic and Appl. Sci.*, 2, 41–45. DOI: 10.1016/j.bjbas.2013.09.006.
 26. Simesen, M. G., Nansen, P., 1974: Serum- γ -glutamyltranspeptidase (γ -GT) and aspartate-aminotransferase (AspAT) activities in adult cattle with chronic *Fasciola hepatica* infection. *Acta Vet. Scand.*, 15, 239–43. DOI: 10.1186/BF03547484.
 27. Taibi, A., Aissi, M., Harhoura, K., Zenia, S., Zait, H., Hamrioui, B., 2019: Evaluation of *Fasciola hepatica* infections in cattle in Northeastern Algeria and the effects on both enzyme and hepatic damage, confirmed by scanning electron microscopy. *Acta Parasitol.*, 64, 112–128. DOI: 10.2478/s11686-018-00013-9.
 28. Toet, H., Piedrafita, D. M., Spithill, T. W., 2014: Liver fluke vaccines in ruminants: strategies, progress and future opportunities. *Int. J. Parasitol.*, 44, 915–927. DOI: 10.1016/j.ijpara.2014.07.011.
 29. Walker, S. M., Makundi, A. E., Namuba, F. V., Kassuku, A. A., Keyyu, J., Hoey, E. M., et al., 2008: The distribution of *Fasciola hepatica* and *Fasciola gigantica* within southern Tanzania – constraints associated with the intermediate host. *Parasitology*, 135, 495–503. DOI: 10.1017/S0031182007004076.
 30. Yang, Q., Mao, W. H., Ferre, I., Bayón, J. E., Mao, X. Z., González-Gallego, J., 1998: Plasma aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and gamma-glutamyl transpeptidase (GGT) activities in water buffaloes with experimental subclinical fasciolosis. *Vet. Parasitol.*, 78, 129–36. DOI: 10.1016/S0304-4017(98)00134-4.
 31. Zeng, M., Wang, X., Qiu, Y., Sun, X., Qiu, H., Ma, X., et al., 2023: Metabolomic and systematic biochemical analysis of sheep infected with *Fasciola hepatica*. *Vet. Parasitol.*, 313. DOI: 10.1016/j.vetpar.2022.109852.

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