

Validity of a commercial kit for detection of antibodies in bovine serum in an endemic area for fasciolosis

Validade de um kit comercial para detecção de anticorpos no soro bovino em área endêmica de fasciolose

Aline Nunes Simões^{1*}; Sayanne Luns Hatum de Almeida¹; Áquila Flávia da Rocha Braga¹;
Isabella Vilhena Freire Martins¹; Dirlei Molinari Donatele¹; Graziela Barioni¹

¹ Laboratório de Parasitologia Veterinária, Departamento de Medicina Veterinária, Centro de Ciências Agrárias e Engenharias, Universidade Federal do Espírito Santo – UFES, Alegre, ES, Brasil

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Abstract

Fasciolosis is caused by *Fasciola hepatica* that affects the bile ducts and liver parenchyma of ruminants, which can result in economic loss. This study aimed to carry out the validity of the commercial kit ELISA[®] indirect front of the simple fecal sedimentation test used as the standard. 143 samples were collected blood and feces of cattle from Jerome, south of the Espírito Santo. Serum samples were left at -80 °C and used to perform the ELISA kit IDEXX[®]. All animals to stool examinations were also positive to the ELISA (22) and negative samples to test stool (121), 52 animals reacted positively against the antibody research. The frequency of fasciolosis was 15.4% in the stool examinations and 51.8% by ELISA. The validity was calculated by sensitivity (100%), specificity (57%), positive predictive value (29%) and negative predictive value (100%), and the correlation between the tests calculated using the kappa index of 0.35. The better sensitivity of the ELISA commercial kit should not be a separately evaluated, since the cost benefit and the technical facility must be considered.

Keywords: Cattle, ELISA, fluke.

Resumo

Fasciolose é causada pela *Fasciola hepatica*, parasito que acomete os ductos biliares e o parênquima hepático dos ruminantes e pode resultar em perdas econômicas. Objetivou-se realizar a validade do kit comercial ELISA[®] indireto frente ao teste de sedimentação fecal simples utilizado como padrão. Foram coletadas 143 amostras de sangue e fezes de vacas provenientes do Sul do Espírito Santo. As amostras de sangue foram centrifugadas para separação do soro. As amostras de soro foram congeladas a -80°C e utilizadas para análise com o kit de ELISA IDEXX[®]. Todos os 22 animais positivos ao exame coproparasitológico também foram positivos ao ELISA e, das 121 amostras negativas ao exame de fezes, 52 reagiram positivamente frente à pesquisa de anticorpos. A frequência de fasciolose foi de 15,4% no exame coproparasitológico e 51,8% pelo ELISA. A validade foi calculada pela sensibilidade (100%), especificidade (57%), valor preditivo positivo (29%) e valor preditivo negativo (100%), sendo considerada medíocre a concordância entre os testes, calculada pelo índice kappa (0,35). A maior sensibilidade obtida para o kit comercial ELISA não deve ser avaliada isoladamente, uma vez que o custo benefício e a facilidade da técnica devem ser considerados.

Palavras-chave: Ruminantes, ELISA, trematoda.

The diagnosis of fasciolosis is made in order to detect infection by *Fasciola hepatica* and is done through examination of excrement from the definitive host. The objective of the present study was to evaluate the validity of a commercial kit for the ELISA test, for detecting antibodies against *F. hepatica*, in relation to the simple fecal sedimentation test to search for eggs.

Blood samples were collected from the caudal vein using Vacutainer tubes and feces were sampled directly from the rectum of 143 lactating cows in Jerônimo Monteiro (20°47'22.5" S; 41°23'20.2" W), a municipality in the south of the state of Espírito Santo, Brazil. The samples were forwarded to the Parasitology Laboratory of the Veterinary Hospital of the Agrarian Sciences Center, Federal University of Espírito Santo (HOVET, CCAE-UFES). The serum was used to perform the ELISA test, using the IDEXX[®] commercial kit for IgG antibody detection, in accordance with the manufacturer's instructions. The feces samples were stored in a refrigerator at 4 °C and were later processed using the fecal sedimentation technique described by Foreyt (2005).

*Corresponding author: Aline Nunes Simões. Laboratório de Parasitologia Veterinária, Departamento de Medicina Veterinária, Centro de Ciências Agrárias e Engenharias, Universidade Federal do Espírito Santo – UFES, Alto Universitário, s/n, Guararema, CEP 29500-000, Alegre, ES, Brasil. e-mail: anunesmv@hotmail.com

The relationship between animals that were positive through the coproparasitological test and those that were positive through the ELISA commercial test was analyzed by means of the chi-square test, taking the significance level to be 5%. Validity indicators (sensitivity, specificity and positive and negative predictive values) were calculated using the fecal sedimentation test as the standard.

A total of 143 samples were processed. All the 22 animals that were positive through the coproparasitological test were also positive through the ELISA test. Among the 121 samples that were negative through the feces test, 52 showed a positive reaction to the antibody test. The frequency of fasciolosis was 15.4% through the coproparasitological test and 51.7% through the ELISA test, and the association between them was confirmed by means of the chi-square test ($\chi^2 = 24.24$; $p < 0.05$) (Table 1).

The sensitivity of the ELISA IDEXX® commercial kit for investigating antibodies against *F. hepatica* in bovine serum was 100%, with specificity of 57%. The negative predictive value (NPV) of the kit was 100% and the positive predictive value (PPV) was 29%. The kappa coefficient presented mediocre concordance (0.35) between the tests studied. In the present study, the low positive predictive value (29%) may be related to the standard used, i.e. the simple fecal sedimentation test, due to the lack of monitoring during animal slaughtering, given that production in this region is concentrated within dairy farming. Pereira (2008) explained that predictive values were influenced by the sensitivity and specificity of different techniques. According to Martins et al. (2008), the sensitivity of the fecal sedimentation technique regarding eggs of *F. hepatica*, i.e. the method used in the present study as standard, was 59.8%.

The ELISA is considered a valuable tool for the diagnosis of fasciolosis in ruminants for many authors. Molloy et al. (2005) used the commercial ELISA for the detection of antibodies against *F. hepatica* in bovine serum and obtained the sensitivity and specificity of 98, 2% and 98.3%. The ELISA test detects infection earlier than the coproparasitological test, since the antibodies appear before in the circulation than the eggs in the faeces. However, even after successful treatment, antibodies may remain in circulation. This shows that antibody presence does not necessarily indicate active infection (FAIRWEATHER, 2011). Brockwell et al. (2013) artificially infected cattle and used the ELISA test for diagnosing the disease. Twenty-eight days after infection, the animals reacted positively to searches for antibodies in serum. However, even after treatment with triclabendazole, the immunoglobulin G levels persisted. According to Mezo et al. (2010), antibodies against *F. hepatica* can be detected between one and four weeks after infection.

The existence of animals that were negative through the coproparasitological test and positive through the ELISA test can be explained because when animals are in the acute phase of the disease or the prepatent period, there is no egg elimination because there is no egg production (ALMAZÁN et al., 2001). Leclipteux et al. (1998) explained that confirmation of the diagnosis of fasciolosis through feces examination could only occur approximately ten weeks after ingestion of metacercariae.

The present study was conducted in an area that is considered endemic for bovine fasciolosis (incluir referencia). Prevalences of 15.4% through the coproparasitological test and 51.7% through the ELISA test were observed. In a previous study, Martins et al. (2014) found prevalence of fasciolosis of 66.7% in the municipality of Jerônimo Monteiro through the fecal sedimentation test. This difference in prevalence can be explained through a real reduction in the prevalence of fasciolosis in this area due to raised awareness among producers. They may have started to implement control measures against the mollusks that transmit the disease and/or to treat local herds with fasciolicides.

As in the present study, Ibarra et al. (1998) compared the sensitivity and specificity of indirect ELISA in order to determine the serum prevalence of fasciolosis among cattle in Mexico and obtained 96.5% and 98.8%, respectively. They also made a parallel comparison with the fecal sedimentation test. The result showed that serological tests were more sensitive and detected a higher percentage of positive animals, similarly to the present study. However, in an investigation on antibodies through the indirect ELISA test, for detecting *F. hepatica*, Ferre et al. (1995) did not observe any cross-reaction in relation to *Dicrocoelium dendriticum*. There are no studies on occurrences of cross-reactions between *F. hepatica* and other parasites in serum samples.

About costs, a IDEXX® commercial kit can be used to sample 225 animals, which results in an approximate cost of R\$ 18 (Brazilian real) per animal, while the simple sedimentation test costs R\$ 15 per animal. Use of the indirect ELISA test depends on an imported kit and therefore depends on approval from the Ministry of Agriculture, Livestock and Food Supply. This makes it inaccessible and consequently unviable for routine use among veterinarians. In addition of these factors, the fecal test is considered to be easy to performance, in comparison with the processing stages of the indirect ELISA test.

Considering the economic impact of fasciolosis for bovine production in endemic regions, the veterinarians need to adopt early and effective diagnostic methods. Although more sensitive, serological tests have their drawbacks and need to be further studied to better serve the field veterinarians and producers, since the latter need to know the situation on their properties and thus promote the appropriate preventive measures.

Table 1. Relationship between the ELISA test (IDEXX) for detecting antibodies in serum and the fecal sedimentation test for identifying fasciolosis in dairy cattle from Jerônimo Monteiro, Espírito Santo.

ELISA test	Coproparasitological test		
	Positive	Negative	Total
Positive	22 (15.4%)	52 (36.3%)	74 (51.7%)
Negative	0 (0%)	69 (48.2%)	69 (48.2%)
Total	22 (15.4%)	121 (84.6%)	143 (100%)

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