

Fasciolosis in Cows in Espírito Santo, Brazil, and its Association with Risk Factors, Mastitis and Liver Enzymes

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Abstract

The objective of the present study was to determine the frequency of fasciolosis in cows in southern Espírito Santo, Brazil and its association with risk factors, mastitis and liver enzymes. Samples of faeces and blood of 928 dairy cattle from 72 properties of the municipalities of Caparaó region were collected. A questionnaire on risk factors was applied to property managers. Samples were processed by fecal technique of spontaneous fecal sedimentation for *Fasciola hepatica* eggs and biochemical analyzes for the detection of liver enzymes. Mastitis tests and clinical evaluations were performed to verify the presence of mastitis. Data were stored in spreadsheets and analyzed by descriptive statistics and chi-square test, with a significance level of 5%. From the total of 928 samples collected, 51 presented positive for eggs of *F. hepatica*, resulting in a frequency of 5.49%. The only variable related to the questionnaire to present a significant association ($p = 0.00104$) was the permanence of the animals with flooded areas. Regarding the association with other diseases, no relation ($p = 0.1667$) was found between the presence of *F. hepatica* and positive animals for mastitis. When hepatic enzyme alterations were studied, a significant association ($p = 0.0125$) was observed between animals infected with *F. hepatica* and animals with increased concentration of GGT hepatic enzyme in the serum. It was concluded that the fasciolosis is present in the studied region, and that the contact of the animals with flooded areas was the most relevant variable as a factor associated with fasciolosis. In addition, it was also possible to verify hepatic enzyme alterations in the animals infected with *F. hepatica*.

Keywords: Cattle; *Fasciola hepatica*; Frequency

Introduction

According to IBGE [1], the Brazilian cattle herd is estimated at 213 million head, of which almost 2 million head are in the state of Espírito Santo, of which approximately 250 thousand are intended for dairy farming.

Fasciolosis is a disease caused by the *Fasciola hepatica* and *Fasciola gigantica* flukes and the young forms parasite the liver parenchyma and the adult form the bile duct of several mammalian species, including bovine and human, being considered a zoonosis. *Fasciola hepatica* is the only species found in Brazil and is of great importance in the economy, since it causes several economic losses to producers [2-4].

In Brazil, fasciolosis has been described in the South, Southeast and Midwest regions, and the commercialization of cattle between owners of different regions is one of the main factors responsible for the increase of parasite distribution throughout the Brazilian territory in recent years [5,6].

In the state of Espírito Santo, Brazil, Bernardo, *et al.* [7] reported the presence of fasciolosis as an economic problem for producers in the southern region of the state due to the condemnation of the liver of infected cattle. And Martins, *et al.* [8] and Martins, *et al.* [9] described the existence of possible risk areas for fasciolosis in the southern region of the state, where the major foci of infection are concentrated and estimated the prevalence in dairy herds in the same region at 19%.

As for the association with other diseases, mastitis, as well as fasciolosis, is one of the diseases that most affects cattle in southern Espírito Santo, and its important to evaluate a possible association between animals infected with *F. hepatica* and positive animals for mastitis, but no reports were found in the literature with this association. However, Hodzic, *et al.* [3] stated that *Fasciola hepatica* causes cell wall damage and liver tissue necrosis and that these effects influence serum biochemical parameters, as damaged cell enzymes are released into the blood and their serum concentration increases.

Aim of the Study

The aim of the present study was to report the frequency of fasciolosis in cows in southern Espírito Santo, analyzing associations with risk factors for acquisition of parasitic infection and with mastitis and liver enzyme changes.

Materials and Methods

Seventy-two dairy cattle properties were randomly selected from the twelve municipalities in the Caparaó, Espírito Santo region. The animal use protocol for this study was approved by the Animal Use Ethics Committee (003/2014) and cattle were included in the study after the signed consent of the owner.

A total of 928 female dairy cattle of reproductive age and lactation period were studied. A structured questionnaire was applied to all those responsible for the properties, addressing questions about the origin of the water from the animals, flooded areas, animals dewormed, anthelmintic used and others related to the risk of fasciolosis.

Clinical evaluations of the udders of all lactating cows were performed and the black-bottomed mug test was performed for the detection of clinical mastitis, and then the presence of subclinical mastitis was analyzed by the CMT (California mastitis test). The data obtained were properly stored in spreadsheets for further evaluation of the association of the disease with fasciolosis.

Blood and stool samples from all lactating animals were included in the study. Blood was collected by puncture of the coccygeal vein or artery in a vacuum collection system with non-anticoagulant tubes, which were transported in isothermal boxes to the Veterinary Hospital of Federal University of Espírito Santo. The samples were centrifuged and the serum was stored at -20°C until liver enzyme perform. The analysis was performed in an automatic biochemical device following the recommendations of the commercial kits. Fecal samples were collected and sent in isothermal boxes to the Parasitology Lab, where the stool samples were processed according to the spontaneous fecal sedimentation technique for *Fasciola hepatica* egg research (Foreyt, 2005) validated by Martins, *et al.* [10].

The frequency was calculated by the relationship between the number of existing cases of fasciolosis and the number of animals studied. Data were stored in a database using the Open Epi program (https://www.openepi.com/Menu/OE_Menu.htm), and descriptive statistics were used and the associations of fasciolosis with risk factors and diseases (mastitis and liver enzyme alterations) were performed by chi square test (X^2), with significance level of 5% and odds ratio.

Results and Discussion

Considering the 12 municipalities studied in Espírito Santo state, from the total of 928 samples of fecal samples collected, 51 were positive for the fecal sedimentation test for *Fasciola hepatica* eggs, resulting in a frequency of 5.49%. These data are lower than those previously found by other authors in the southern region of Espírito Santo. Alves, *et al.* [11] and Martins, *et al.* [9] studying cattle in municipalities in the southern region of the state using the fecal sedimentation test for *F. hepatica* eggs, found rates of 21.3% and 19%, respectively. This difference can be explained by the decrease in rainfall in the region, since according to Incaper [12] data the rainfall

decreased from 1450 mm in 2013 to 800 mm in 2015, leading to the conclusion that there was a lack for the presence of *Lymnaea* mollusks, essential for the completion of the *F. hepatica* life cycle. This factor also decreases the dispersal of these mollusks making it difficult for animals to become infected.

Some municipalities of the study did not present positive animals, which according to Martins., *et al.* [9] can be explained by the location of these municipalities in Serra do Caparaó, a region with few floodplain areas, making it unfavorable for the presence of flooded areas. In addition, Martins., *et al.* [8] state that these municipalities are considered low risk areas for fasciolosis, mainly due to the high altitude in which they are located.

Of the 72 properties studied, 22 (30.5%) presented animals infected with *Fasciola hepatica*, and according to interview data, the great majority of the positive properties for fasciolosis are located in areas considered by Martins., *et al.* [8] as areas of risk or high risk for parasite development, due to the favorable environment and climate.

Table 1 shows the responses of producers to risk factors and changes found in animals with fasciolosis.

Risk factors	Percentage
From properties (n = 72)	
Water origin: watercourse	15,68%
Water origin: stream	11,76%
Water origin: dam	1,96%
Properties where animals had access to the river	58,82%
Property where animals had access to wetlands	50,98%
Properties in which animals received anthelmintic	21,56%
Properties in which animals received fasciolicide	7,84%
From animals with fasciolosis confirmed (n = 51)	
Presence of fasciolosis and increased AST enzyme concentration	7,84%
Presence of fasciolosis and increased GGT enzyme concentration	23,52%
Presence of fasciolosis and mastitis	39,21%

Table 1: Risk factors associated or not with fasciolosis found in the studied properties in Espírito Santo state, Brazil.

The chi-square test revealed a significant association ($p = 0.00104$) between the presence of fasciolosis and the permanence of animals in flooded places and animals that have contact with flooded areas. 2.5 times more likely to have fasciolosis (Odds Ratio = 2.5). This association was also cited by Martins., *et al.* [9] and Alves., *et al.* [11] studying fasciolosis in the municipalities of the southern region of the state of Espírito Santo. Takeuchi-Storm., *et al.* [13] also cited that heifers grazing on wet pastures was identified as risk factors associated with fasciolosis in Denmark. As other variables, water origin ($p = 0.1734$) and animals' contact with the river ($p = 0.3601$), did not find significant association with the presence of fasciolosis.

In the present study, of the 928 animals studied, 434 (46.76%) had positive effects for mastitis. However, the chi-square test showed no significant association ($p = 0.1667$) between a *F. hepatica* infection and presence of mastitis. No reports were found in the literature of this association in cattle, but in sheep Mavrogianni., *et al.* [14] concluded *F. hepatica* infection as a predisposing factor for post-animal mastitis, mainly in the early phase of the lactation period.

When studied as changes in liver enzymes, Hodzic., *et al.* [3] stated that *F. hepatica* parasitism causes cell wall damage and liver tissue necrosis, or influences non-serum biochemical parameters, or was observed in this study with an enzymatic dosage (gamma glutamyl

transferase - GGT). and aspartate aminotransferase (AST) from animals. Of the 51 animals positive for *F. hepatica*, four (7.84%) showed increased AST concentration and 43 (84.31%) showed increased serum GGT concentration. Similar result was reported by Lotfollahzadeh, *et al.* [15] analyzing the blood of *F. hepatica* infected cattle in northern Iran, which increased serum GGT and AST concentrations. Hodzic, *et al.* [3] suggest that the small number of animals with alteration in AST enzyme concentration is due to lack of hepatocellular damage, which causes a probable chronic fasciolosis (biliary phase), where non-parenchymal regenerative changes and normalization of liver function occur. The higher number of animals with increased GGT concentration may be explained by the fact that this enzyme is a more sensitive indicator of liver cell damage in chronic subclinical fasciolosis when compared to AST [16]. However, according to Dorchie [17] when the parasite load is very low, the dosage of this enzyme is not useful.

The chi-square test showed a significant association ($p = 0.0125$) between animals infected with *F. hepatica* and animals with increased serum GGT in the liver and (Odds Ratio = 2.4). Increasing serum AST concentration was not significantly associated ($p = 0.2062$) with the presence of *F. hepatica* [18,19].

Conclusion

It was concluded that fasciolosis is present in the studied region, and that the contact of animals with flooded sites was the most relevant variable as a factor associated with fasciolosis. In addition, it was also possible to verify alterations of liver enzymes in animals infected with *F. hepatica*. Regarding mastitis, no significant association was found.

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