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Copro-ELISA Prevalence of Fasciola hepatica in Cattle in Van, Turkey

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Aysegul Bostanci & Bekir Oğuz

ABSTRACT

Background: Fasciolosis is an important food borne zoonotic disease caused by *Fasciola* trematode parasites. There are two types of *Fasciola* spp. namely *F. hepatica* and *F. gigantica*, widely distributed across the globe, affecting both human and animal hosts. In endemic regions, it is possible to base the diagnosis of fasciolosis on clinical signs and the season, however, it could be more useful to support these data with fecal examination and various hematologic and serological tests. The aim of this study was to determine the prevalence of fasciolosis in cattle in Van province by copro-ELISA technique.
Materials, Methods & Results: Fecal samples from 140 cattle were technically collected and examined by sedimentation-zinc sulphate flotation technique. Modified McMaster sedimentation technique was applied to the egg positive samples to determine the EPG values. *Fasciola hepatica* coproantigens in samples were investigated by ELISA. The coprological and antigen ELISA prevalence of fasciolosis were determined as 5.07% and 30.7%, respectively, which shows the significant difference between these methods in examining the rate of infection. The highest prevalence of fasciolosis infection was observed in 1-2 age groups (41.9%), and this prevalence was followed by 3-5 (31.2%) and ≤6 age group (5%). The differences between age groups were found significant ($P < 0.05$). The prevalence in female and male cattle was found as 30.1% and 35.3% This difference was not found statistically significant ($P > 0.05$). The highest prevalence was observed in Brown Swiss with the ratio of 40% and this was followed by 31% in Crossbreed and 22.6% in Rubia Gallega. The differences among breeds were not statistically significant ($P > 0.05$).
Discussion: *Fasciola hepatica* is the most common species of liver flukes, and its pathogenicity leads to significant impact on the economy of the livestock industry. The economic losses consist of costs of anthelmintics, drenches, labor, liver condemnation at meat inspection; and losses in production due to mortality, reduction in meat, milk and reduction in growth rate, fertility and decreased feed intake, conversion and lower resistance to other disease.To diagnose fasciolosis, eggs can only be detected in feces after the tenth or twelfth week of infection once the parasites have matured. It is reported that routine microscopic methods used before this stage do not provide sufficient information about the current infection status. Therefore, serological tests have been introduced for the early diagnosis of the disease. Among these tests, the ELISA test based on detecting antigens has become the most commonly used test. It is known that the probability of ELISA to cross-react with parasites that carry similar immunogenic features and the similarities between antibodies generated in previous infections and new infections pose a challenge to making the definitive diagnosis. Therefore, it is reported that to predict the parasitic potential of the host and the success of treatment beforehand, the presence of *Fasciola* spp. antigens can be investigated in serum instead of antibodies. In conclusion, this study has established prevalence of fasciolosis in cattle raised in Van province using the copro-ELISA technique for the first time. It has been concluded that copro-ELISA could serve as a useful technique for herd diagnosis of fasciolosis in cattle in addition to fecal examinations particularly with respect to fasciolosis.

Keywords: cattle, Copro-ELISA, fasciolosis, Van, Turkey.

INTRODUCTION

Fasciolosis, a serious infectious parasitic disease infecting domestic ruminants and humans, tops all the zoonotic helminthes worldwide [12]. *Fasciola hepatica* and *Fasciola gigantica* are the two parasitic species most commonly implicate as the etiological agents of the fasciolosis. *F. hepatica* has a worldwide distribution while *F. gigantica* is found on most continents primarily in tropical region [10].

In endemic regions, it is possible to base the diagnosis of fasciolosis on clinical signs and the season, however, it could be more useful to support these data with fecal examination and various hematologic and serological tests [1]. Also, it cannot detect infection at the prepatent period, because eggs are found in faeces when the flukes are already matured (usually between 10 and 12 weeks of infection) [25,33]. Thus, alternative immuno-serological methods have been developed for early diagnosis of fasciolosis. The ELISA test is easy to perform for herd monitoring at early stage of fasciolosis. Especially, the copro-ELISA test, based on determination of ES antigens in feces, has high specificity as confirmed by necropsy and high sensitivity as confirmed by lacking cross-reaction with antigens from other helminthes [25].

In Turkey, epidemiology studies on fasciolosis are mostly based on fecal examination or inspection at slaughterhouse, and few researchers have used immuno-serological methods[34,37,39,40]. Thus, the aim of this study was to determine the prevalence of fasciolosis in cattle in Van province by copro-ELISA technique.

MATERIALS AND METHODS

Study area and animal sampling

The study was conducted in 5 districts of Van province. The city is located at 42º40'E and 44º30'E longitude and 37º43'N and 39º26'N latitude, it is at an altitude of approximately 1,725 m and has close borders to eastern Iran [16]. Fecal samples from 140 cattle along with questionnaire based data on age, sex and breed were collected in directly from the rectum. Animals were grazed and watered on communal areas during the day and housed around households in barns at night.

Coprological Examination

The presence of *F. hepatica* eggs in faecal samples was evaluated by a sedimentation-flotation technique that was performed on 10 g of faeces. Briefly, the amount of faeces was weighed, mixed in with 200 mL water in a measuring cup and filtered 3 times through a tea sieve. The filtrate was allowed to stand for 30 min after which the sediment was collected in a test tube and centrifuged at 700 × *g* for 3 min. After centrifugation, the supernatant was removed, the sediment was suspended in zinc chloride (density 1.56) and centrifuged at 180 × *g* for 3 min. Next, zinc chloride was added until a meniscus appeared on top of the test tube and the floating material was collected underneath a cover slip that was allowed to stand on the test tube for ≥ 2 min. Finally, the samples were microscopically investigated under a 100 × magnification [6]. The number of eggs per gram of fecal (EPG) was estimated according to a general method [7].

Copro-ELISA detection of Fasciolosis

A sandwich ELISA using Bio-X Bovine Fasciola hepatica Antigen ELISA Kit (Bio K 201)[1] was performed on all faecal samples. Each individual faecal sample was homogenized with 2 mL dilution buffer (Bio K 201)[1] by mixing with a pipette and vortexing. For cattle 2 g of each faecal sample was used. The homogenized samples were incubated overnight at 4°C. Supernatant was collected from all samples after centrifugation for 10 min at 1000 *g* and stored at -20°C. The format of the ELISA plates was such that alternate wells (A,C,E and G) were sensitised with a specific polyclonal antibody againts *Fasciola hepatica* or left (B,D,F and H) unsensitised. Supernatants were incubated on the microplate for 2 h at 21°C +/- 3°C. After this first incubation step, the plate was washed and incubated for 1 h with the first conjugate (a specific monoclonal antibody against an antigenic determinant of coupled to biotin), then the plate was incubated at 21°C +/- 3°C for 1 h. The plate was then washed, the second conjugate (a peroxidase-coupled avidine specific to biotin) was applied, and the plate was incubated at 21°C +/- 3°C for another hour. After this second incubation, the plate was washed again and the enzyme substrate (hydrogen peroxide)[1] and the chromogen (tetramethylbenzidine)[1] were added. The intensity of the resulting blue colour is proportionate to the sample's coproantigen titre. The optical densities in the microwells were evaluated spectrophotometrically (Bio-Tek Instruments, MicroQuant micropleyt reader)[2], using a 450 nm filter and the

absorbance of the unsensitised well was subtracted from the absorbance of the sensitised well. Then the corrected absorbance values for each sample were divided by the corresponding positive control signal optical density and the results were multiplied by 100 to express it as a percentage.

Statistical analysis

Pearson's chi-square (X^2) test was performed to compare prevalence among sex, age and breed categories. Statistical comparisons were carried out using SPSS 22.0 statistical software. Significance was defined as $P < 0.05$.

RESULTS

The overall prevalence of fasciolosis was 5.7% (8) and 30.7% (43) as assessed by the fecal egg examination and copro-ELISA method, respectively (Table 1). All positive results with fecal egg examination were also positive with copro-ELISA. The mean number of egg per gram of faeces (EPG) in infected cattle was 60.4 (min 16,6 max 100). The results of the association analysis of different factors with the prevalence of *F. hepatica* are presented in Table 2. Significant difference was found among cattle regarding age whereas no significant differences were found related to sex and breed.

Table 1. Diagnostic yield of fecal coproscopy and Copro-ELISA in detection of Fasciolosis in cattle.

Local	Examined cattle	Infected cattle		Copro ELISA (+) Coproscopy (+)		Copro ELISA (+) Coproscopy (-)		Copro ELISA (-) Coproscopy (-)	
	N	N	%	N	%	N	%	N	%
Van	140	43	30.7	8	5.7	35	25[*]	-	-

*Prevalence rate of prepatent *Fasciola hepatica* infections.

Table 2. Age, sex and breed related prevalence of *Fasciola hepatica* based on Copro-ELISA in cattle.

Parameter	Examined cattle N	Infected cattle N	rate	χ^2	P
Age (years)					
1-2	43	18	41.9[a]		
3-5	77	24	31.2[b]	8.732	0.013
≥6	20	1	5[b]		
Sex					
Male	17	6	35.3[a]	0.191	0.662
Female	123	37	30.1[a]		
Breed					
Brown Swiss	45	18	40[a]		
Simmental	53	12	22.6[a]	3.447	0.178
Crossbreed	42	13	31[a]		
Total	140	43	30.7		

[a,b]The different letters within the same column indicate significant difference among groups. χ^2: Pearson's chi-squared test.

DISCUSSION

It is known that parasitic diseases have an important place in cattle breeding in Turkey as they also have worldwide. Helminths account for a significant portion of parasitic diseases of cattle. The environmental and geographic conditions of Turkey are suitable for the development of most helminth species [35]. Particularly, the *Fasciola* spp. are distributed widely, and their intermediate hosts are snails of the *Lymnaea* genus. The causing agents of *Fasciola* also known as liver flukes are zoonotic trematodes that localize in the biliary ducts of various herbivores such as sheep, goats, and cattle. *F. hepatica* is the most common species of liver flukes, and its pathogenicity leads to significant impact on the economy of the livestock industry. The economic losses consist of costs of anthelmintics, drenches, labor, liver condemnation at meat inspection; and losses in production due to mortality, reduction in meat, milk and reduction in growth rate, fertility and decreased feed intake, conversion and lower resistance to other disease [15,32].

The prevalence of the disease in cattle in different countries is variable depending on some factors such as the region's soil structure, precipitation, and seasons [34]. It has been reported that this disease is prevalence by rates of 10.9% in cattle in North India [11], 25.5% in Pakistan [17], 75% in France [9], 4.9% in Greece [21], 11.1% in Italy [8], 75% in Brazil [3], 18% in Switzerland [13], 3.3% in Iraq [23], 45.6% in Cameroon [27], 27.7% in Nigeria [22], and 3.68% in Iran [19]. Although most of the studies conducted on bovine fasciolosis in Turkey used conventional methods [17,38], serological studies have also been performed to determine prevalence [34,39,40]. It is reported that the prevalence of fasciolosis calculated by different detection methods is between 0.5% and 69.2% in cattle in several regions of Turkey [4,39,40]. However, to the best of our knowledge, no investigation has been conducted to determine prevalence of bovine fasciolosis in Van province in Turkey. This study is the first to investigate fasciolosis with the copro-ELISA technique in cattle raised in Van province, Turkey. In the present study, the prevalence of fasciolosis in cattle was determined as 30.7% and 5.7% by the copro-ELISA and fecal egg examination, respectively. These results are conformity with some findings reported from different regions of Turkey [5,20], and it was concluded that the little differences present are because the studies were conducted in different cities.

The primary risk factor that affects the prevalence of fasciolosis is the animal's age. Although it is recorded that the infection risk varies depending on the age factor, cattle in advanced ages (> 2-3 years) are more prone to fasciolosis than those in younger ages (< 2 years) [24,26,31,39]. In the present study, the highest prevalence was observed in the age group of 1-2 years as 41.9%, and this was followed by 31.2% in the age group of 3-5 years and 5% in the age group of ≥ 6 years. The result of present study showed that age has significant effect ($P < 0.05$) on the prevalence of bovine fasciolosis, being higher in young animals than the adult. There was a decrease in infection rate as age increased. This may be due to the result of acquired immunity with age which is manifested by humoral immune response and tissue reaction in bovine liver due to previous challenge. There are some additional reports confirming that the increased resistance against fasciolosis (low prevalence) with age is most likely related to the effect of acquired immunity in adult cattle or liver fibrosis which prevention the passage of immature flukes by the calcification of the bile ducts [2,29,30].

It is reported that gender has no effect on fasciolosis in cattle or this parasite is generally more common in female than male [2,29,31,34]. The higher percentage of infection in the females can't be explained exactly but it might be assumed that hormonal influence as well as stress leading to immune-suppression may be associated with this phenomenon [31]. However, it has been reported that significantly higher prevalence rates in males [26]. In the present study, the fecal examination was positive in 6 of the 17 male cattle and 37 of the 123 female cattle tested by copro-ELISA. The result of present study revealed that the sex of animals did not have significant effect ($P > 0.05$) on the occurrence of bovine fasciolosis.

It was reported that the prevalence based on copro-ELISA technique as 3.48, 2.81 and 2.43% in Holstein, Simmental and Brown Swiss breed cattle, respectively [34]. They showed that there was no statistical difference between infection rates in relation to breed. In Kayseri, was reported that *Fasciola* spp. antibodies as 68.6% in Holstein, as 76.9% in Simmental, as 58.8% in Brown Swiss breed, and as 70.9% crossbred cattle breeds [39]. Researchers re-

corded that there was no statistical difference between the infection rates in relation to breed. Likewise, in this study *Fasciola* spp. antigens were identified at rates of 40% in Brown Swiss, 31% in Crossbreds, and 22.6% in Simmental. The difference between breeds did not show significant differences in prevalence of *F. hepatica*.

One study used that the ELISA test to detect *F. hepatica* antigens in the feces of cattle and sheep [25]. Their review of the test results yielded a 38.8% positivity even in feces of cattle in which *F. hepatica* eggs were not detected. The same researchers reported that the fasciolosis could be detected at its early phases by this method that is highly sensitive. Similarly, in another study found that the sensitivity of the copro-ELISA test used to detect fasciolosis in cattle was 94% [6]. Previous study investigated that the sensitivity and specificity of the copro-ELISA method in sheep, cattle, and horses infected with *F. hepatica* in Western Australia [28]. They determined that its specificity as 100%, and its sensitivity as 100% in sheep, 87% in cattle, and 28% in horses. Researchers report that the copro-ELISA method is quite sensitive in detecting the presence of fasciolosis in cattle and sheep, but in horses, they recommend that using the sedimentation method because the sensitivity of ELISA is lower than 30% for horses. It was examined that 198 cattle in the Derinkuyu district of Nevsehir with respect to fasciolosis using the copro-ELISA method [34]. They identified a 3.03% rate of positivity. In the current study, to detect fasciolosis in cattle, the copro-ELISA test which is defined as an alternative method of detecting excrete/secrete antigens of *F. hepatica* in feces on microplates covered with polyclonal antibodies was used. The infection rate that was identified as 5.7% by fecal examination was found to be 30.7% by coproantigen ELISA. This result shows that the copro-ELISA test is more specific than fecal examination.

To diagnose fasciolosis, eggs can only be detected in feces after the tenth or twelfth week of infection once the parasites have matured. It is reported that routine microscopic methods used before this stage do not provide sufficient information about the current infection status. Therefore, serological tests have been introduced for the early diagnosis of the disease [34]. Among these tests, the ELISA test based on detecting antigens has become the most commonly used test. It is known that the probability of ELISA to cross-react with parasites that carry similar immunogenic features and the similarities between antibodies generated in previous infections and new infections pose a challenge to making the definitive diagnosis. Therefore, it is reported that to predict the parasitic potential of the host and the success of treatment beforehand, the presence of *Fasciola* spp. antigens can be investigated in serum instead of antibodies [14].

CONCLUSIONS

In conclusion, this study has established prevalence of fasciolosis in cattle raised in Van province using the copro-ELISA technique for the first time. It is important to use the copro-ELISA test which has high sensitivity and specificity for prepatent infections that may be overlooked by fecal examination methods. It has been concluded that copro-ELISA could serve as a useful technique for herd diagnosis of fasciolosis in cattle in addition to fecal examinations particularly with respect to fasciolosis.

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