

# Prevalence and risk factors associated with *Fasciola hepatica* in cattle from Kayseri province, Turkey

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## SUMMARY

A cross-sectional study was conducted to determine the prevalence of *Fasciola hepatica* and to investigate the related risk factors in cattle from Kayseri, Turkey. Faecal and blood samples were collected from 282 cattle from May 2004 to April 2005 and were examined by modified McMaster sedimentation and ELISA techniques to detect *Fasciola* sp. eggs and anti-*F. hepatica* antibodies, respectively. Of the total of 282 cattle, 184 were seropositive for *F. hepatica* with a prevalence of 65.2%. In addition 24.5% of seropositive cattle had fluke eggs in the faecal examination. The mean number of EPG in infected cattle was 42.8±4.4. The highest prevalence was observed in ≥6 age group (87.2%) followed by 3-5 (79.5%) and ≤2 age groups (51.6%). The differences between ≤2 and other age groups were found significant ( $p<0.001$ ), whereas no statistically significant difference ( $p>0.05$ ) was observed between 3-5 and ≥6 age groups. The infection was more prevalent in females (70.7%) than males (47.8%) and in cattle from the traditional farms (76.5%) than the small-scale dairy farms (37.2%). No statistically significant difference ( $p>0.05$ ) was observed related to breed. Our results highlight the importance of initiating a control program for fasciolosis based on regular treatment and prophylaxis in Kayseri Province.

**Keywords:** *Fasciola hepatica*, prevalence, cattle, Turkey.

## RÉSUMÉ

**Prévalence et facteurs de risque associés à *Fasciola hepatica* du bétail de la ville de Kayseri, en Turquie**

Une étude croisée a été menée pour déterminer la prévalence du *Fasciola hepatica* et pour rechercher les facteurs de risque du bétail à Kayseri, en Turquie. Les échantillons de sang et d'excrément ont été prélevés à partir de 282 animaux de Mai 2004 à Avril 2005 et ont été examinés par la méthode de sédimentation de McMaster modifiée et les techniques d'ELISA pour détecter respectivement des œufs de *Fasciola* sp. et des anticorps anti-*F. hepatica*. 184 animaux sur 282 étaient séropositifs pour *F. hepatica* (prévalence de 65,2%). En outre, 24,5% des animaux séropositifs avaient des œufs de trématodes révélés par l'examen fécal. Le nombre moyen d'EPG chez le bétail infecté était de 42,8±4,4. La plus grande prévalence a été observée chez le groupe d'âge ≥6 (87,2%), elle est suivie par le groupe d'âge 3-5 (79,5%) et le groupe d'âge ≤2 (51,6%). Les différences entre le groupe d'âge ≤2 et les autres groupes d'âge sont significatives ( $p<0,001$ ), tandis qu'aucune différence statistiquement significative ( $p>0,05$ ) n'a été observée entre les groupes 3-5 et ≥6. L'infection était plus importante chez les femelles (70,7%) que les mâles (47,8%) et chez le bétail des fermes traditionnelles que des laiteries (37,2%). Aucune différence significative n'a été observée en fonction de la race ( $p>0,05$ ). Nos résultats soulignent l'importance d'entamer un programme de contrôle de la fasciolose dans la ville de Kayseri, basé sur le traitement régulier et la prophylaxie.

**Mots-clés :** *Fasciola hepatica*, prévalence, bétail, Turquie.

## Introduction

Fasciolosis, caused by *Fasciola hepatica* is a helminth infection of ruminants with a cosmopolitan distribution, and is a cause of important economic losses [6]. Besides its great veterinary importance throughout the world, fasciolosis has recently been shown to be a re-emerging and widespread zoonosis affecting a number of human populations [9].

The prevalence of fasciolosis in many parts of Turkey has been determined mainly by abattoir surveys and by faecal examination. Although both *F. hepatica* and *F. gigantica* are present in Turkey, *F. hepatica* is more prevalent species in cattle (Table I). Parasitological diagnosis of fasciolosis is, however, possible only after 12 weeks and 13-14 weeks post-infection for *F. hepatica* and *F. gigantica*, respectively by demonstrating fluke eggs in faeces. By this time, major damages to the liver may have already occurred. To overcome this, efforts have been directed towards the immunological

detection of fasciolosis. A number of sensitive and specific serological tests for diagnosing infection have been described in recent years [11,19,24] culminating in the commercial availability of ELISA kits.

This study was performed to investigate the status of *F. hepatica* by antibody-detection ELISA and modified McMaster sedimentation techniques among cattle in Kayseri province and to assess the risk factors associated with the presence of infestation.

## Material and Methods

### STUDY AREA AND ANIMAL SAMPLING

The study was conducted in 12 districts of Kayseri province (Figure 1). Kayseri, is located in the central part of Turkey and surrounded by Erciyes, Hasan and Ali Mountains (37° 45' to 38° 18'N and 34° 56' to 36° 59'E). The city is located

at an elevation of 1050 meters above sea level and the climate is similar to that of the whole Central Anatolian Region. Summers are hot and dry while winters are cold and rainy.

A total of 282 cattle, 196 of which were from 14 traditional farms and 86 of which were from 8 small-scale dairy farms were randomly selected from May 2004 to April 2005. All of the traditional farms investigated were stationary herds where farmers also practiced agricultural activities. Traditional cattle were denied of any form of modern animal husbandry such as supplementation and effective disease control. Animals were grazed and watered on communal areas during the day and housed around households in barns at night. All of the small-scale dairy farms mainly involved zero-grazing with occasional semi-zero and tethering systems. In the semi-zero system the animals were housed during the rainy season and taken out for grazing during the dry season. The commonest feeding practice was the "cut and carry" system whereby grasses were obtained by cutting from various pastures and carried for feeding the housed animals. Supplementation and prophylactic measures was low and irregular. Age, sex and breed of each examined cattle obtained from owners and/or farm attendants were recorded during the study.

## BLOOD AND FAECAL SAMPLES

A 10 ml of whole blood was drawn from the jugular vein of each cattle to serum tubes and was allowed to clot. Serum was harvested following centrifugation of clotted blood and was stored at -20 °C until analysis. Faecal samples were collected per rectum using gloved fingers. The collected samples were labeled, placed in cool boxes and transported to the laboratory for examination. All samples collected were examined within 36 h.

## ELISA

The ELISA was obtained from Bio-X Diagnostics (BIO K 064, Jemelle, Belgium) and used according to manufacturer's instructions. The format of the ELISA plates was such that alternate wells (1,3,5,7,9,11) were coated with *F. hepatica* purified excretory and secretory (ES) antigens or left (2,4,6,8,10,12) uncoated. This is a genuine negative control to differentiate specific anti *F. hepatica* antibodies from non specific ones. The test blood sera were diluted 1:100 in the dilution buffer and each serum sample was applied to a coated and an uncoated well. After the incubation period plates were washed, then the conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, was added to the wells. The plates were then incubated a second time at room temperature, washed again and the enzyme's substrate (hydrogen peroxide) and the chromogen tetramethylbenzidine (TMB) were added. The intensity of the resulting blue color is proportionate to the titre of specific antibody in the sample. The optical densities in the microwells were evaluated spectrophotometrically (Bio-Tek Instruments, MicroQuant microplate reader), using a 450 nm filter and the absorbance of the uncoated well was subtracted from the absorbance of the coated well. Then the corrected absorbance values for each serum were divided by the corresponding positive

control serum optical density and the results were multiplied by 100 to express it as a percentage. The detected antibodies were categorized as negative and five levels of positive (from low to high) corresponding to 0-9.33%, 9.34-33.05%, 33.06-56.76%, 56.77-80.48%, 80.49-104.19% and  $\geq 104.20\%$  antibody levels, respectively according to the quality control data sheet provided with the ELISA kit.

## COPROLOGICAL EXAMINATION

Conceicao method [5] was used for the detection and quantification of *Fasciola* sp. eggs from collected faecal samples. Briefly, when compared with simple sedimentation method (McMaster), the sedimentation period was reduced to 10 min, the first step tap water was replaced by 5% water detergent solution (Sodium dodecyl sulfate) and 30 g instead of 10 g of faeces was examined.

## STATISTICAL ANALYSIS

Pearson's chi-square ( $X^2$ ) test was performed to compare prevalence among sex, age, management and breed categories. Statistical comparisons were carried out using SPSS 13.0 statistical software.

# Results

## PREVALENCE OF INFECTION

Of the 282 cattle tested, 184 were positive for *F. hepatica* with coprological and/or antibody detection ELISA tests resulting in a prevalence of 65.2%. The individual results of the antibody-detection ELISA and the modified McMaster sedimentation tests are shown in Table II. Overall, 45 (24.5%) of the 184 seropositive cattle had fluke eggs in coprological examination. In addition no other trematoda eggs such as *Paramphistomum* sp. or *Dicrocoelium dendriticum* were found in examined cattle. Fasciolosis was heterogeneous though the study area. The highest *F. hepatica* prevalence was detected in Sariz district (96.7%) followed by Bunyan (82.1%), Incesu (80.0%), Felahiye (77.8%), Pinarbasi (75.0%), Akkislá (71.2%), Sarioglan (66.7%), Yesilhisar (65.5%), Ozvatan (54.5%), Tomarza (53.8%), Erkilet (39.5%) and Develi (25.0%) districts. In relation to antibody titers, most of the infected cattle were determined to have lowest antibody level (Figure 2). In addition, only the cattle having  $\geq 56.77\%$  antibody level had fluke eggs in the faecal examination.

The mean number of egg per gram of faeces (EPG) in infected cattle was  $42.8 \pm 4.4$  (min=10, max=110).

## RISK FACTORS ASSOCIATED WITH FASCIOLOSIS

The results of the association analysis of different factors with the prevalence of *F. hepatica* are presented in Table III. Significant differences were found among cattle regarding sex, age and management system whereas no significant differences were found related to breed.

Cities-Years	Detection method	No of examined cattle	Prevalence (% positive)	References
Van-1989	PE	495	53.7 <sup>a</sup> 1.8 <sup>b</sup>	28
Adiyaman and Trakya-1993	PE	520	1.2 <sup>a</sup> 0.8 <sup>b</sup>	7
Samsun-1994	CE	470	15.4	4
Trakya-1999	PE	415	0.5 <sup>a</sup>	10
Eastern Turkey-2003	AD	378	51.8	27
Afyon-2003	PE	1001	13.6	17

PE: Postmortem examination; CE: Coprological examination ; AD: Antibody detection  
<sup>a</sup>: *F. hepatica* ; <sup>b</sup>: *F. gigantica*

TABLE 1: Fasciolosis prevalence in cattle previously reported from Turkey

	Not of examined cattle	Infected cattle		Antibody (+) Egg (+)		Antibody (-) Egg (+)		Antibody (+) Egg (-)	
		no	%	no	%	no	%	no	%
Kayseri	282	184	65.2	45	24.5*	-	-	139	75.5

\* Prevalence rate of patent *Fasciola* sp. infections.

TABLE 2: Overall *F. hepatica* prevalence in Kayseri.

	No of examined cattle	No of infected cattle	rate (%)	$\chi^2$	P
Sex					
Female	215	152	70.7 <sup>a</sup>	11.851	0.001
Male	67	32	47.8 <sup>b</sup>		
Age ( years)				29.906	<0.001
≤ 2	157	81	51.6 <sup>a</sup>		
3-5	78	62	79.5 <sup>b</sup>		
≥ 6	47	41	87.2 <sup>b</sup>		
Breed				5.006	0.287
Anatolian black	12	8	66.7 <sup>a</sup>		
Rubia Gallega	66	45	68.2 <sup>a</sup>		
Friesian	61	42	68.9 <sup>a</sup>		
Brown Swiss	70	38	54.3 <sup>a</sup>		
Crossbreed	73	51	69.9 <sup>a</sup>		
Management				40.382	<0.001
Traditional farms	196	150	76.5 <sup>a</sup>		
Small-scale farms	86	32	37.2 <sup>b</sup>		
Total	282	184	65.2		

<sup>a,b</sup> The different letters within the same column indicate significant difference among groups.  
 $\chi^2$  Pearson's chi-square test.

TABLE 3: The seroprevalence of *F. hepatica* correlated with sex, age, breed and management system

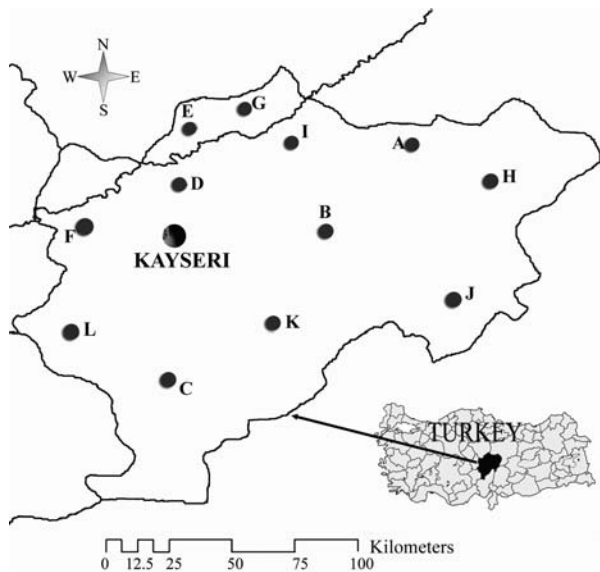


FIGURE 1: Map of Kayseri showing the geographical distribution of research districts (A, Akkışla; B, Bunyan; C, Develi; D, Erkiilet; E, Felahiye; F, Incesu; G, Ozvatan; H, Pınarbasi; I, Sarioglan; J, Sariz; K, Tomarza; L, Yesilhisar).

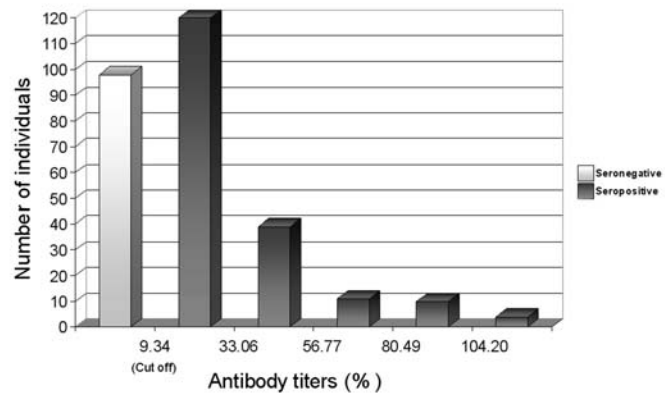


FIGURE 2: Distribution of seronegative and seropositive cattle according to antibody levels for *F. hepatica* infection.

## Discussion

The prevalence of fasciolosis in cattle in different regions is variable depending on some factors such as environmental and climatic conditions, snail population, choice of diagnostic method and situation of infection (patent or prepatent). Different prevalence rates (0.5-55.5%) reported in previous studies in Turkey [4,10,17,27] should be related to these factors. The prevalence in such animals in the present study is higher than previously thought in some provinces of Turkey. This difference is due to fact that earlier estimates were based largely on abattoir surveys which have a relatively low sensitivity. Rapsch *et al.* [22] indicated that approximately one-third of infected livers were not being detected in the abattoirs. The indirect ELISA is clearly more sensitive than the faecal egg count technique, partly because antibodies are present approximately 8 weeks before the infection matures and eggs are shed in the faeces [3,23], and partly because many animals with mature infections do not shed detectable numbers of eggs [12]. One disadvantage of the indirect ELISA is that positive results only imply exposure to the trematode at some time, but not necessarily current infection [8]. In addition antibody levels in most animals persist above the positive threshold of the indirect ELISA for about 12 weeks after treatment [3]. In the present study most of the infected cattle had lowest antibody levels. This may be due to early stage of the infection and/or the disadvantages of the indirect ELISA. SALIMI-BEJESTANI *et al.* [24] developed an ELISA based on E/S antigens of *F. hepatica* for the detection of anti *F. hepatica* antibody in serum of cattle and they found the diagnostic sensitivity and specificity of the test as 98% and 96% respectively at a cut-off value of 15% positivity. They also compared this test to the commercially available Bio-X bovine *F. hepatica* ELISA kit which was used in our study and indicated that the agreement between the two tests was almost perfect.

The present study has shown a significant influence of the type of management on the prevalence of fluke infections in cattle. The higher prevalence of *F. hepatica* in traditional farms as compared with small-scale dairy farms is in agreement with others [15,16]. Higher prevalence rate (76.5%) in traditional farms can be attributed to high contaminated pastures, the high biological potential of the intermediate snail host and insufficient treatment and control measures. The “cut and carry” system of obtaining food from pastures from valleys grazed by traditional cattle during the dry season and irregular usage of anthelmintics might have been responsible for the overall prevalence of *F. hepatica* (37.2%) in small-scale dairy farms.

When evaluating the prevalence of *F. hepatica* by age, data from all research areas indicated that animals older than 2 years of age had a higher prevalence than their younger counterparts and the results were statistically significant. Similar findings were reported previously by several researchers [1,15,18,25,26]. The higher incidence in older cattle might be due to grazing more frequently from pasture, enhancing the possibility of infection with *F. hepatica* metacercariae. However HILLYER *et al.* [13] commented on the resistance of adult cattle to reinfection, as they found a drop in faecal egg count after 20 weeks of primary infection.

Evaluation of fasciolosis prevalence in cattle by sex has yielded contradictory results. No significant differences between sexes were reported in some researches [1,18,20]. On the other hand some other researchers [2,21] have reported significantly higher prevalence rates in females. In our study, the prevalence of *F. hepatica* was found significantly higher in females than males. Higher prevalence rates in females can be attributed to the fact that most or all the female cattle attributed to the practice of retaining for breeding and milk productions as indicated by Phiri *et al.* [21].

In the present study the relation between breed and prevalence of *F. hepatica* is in agreement with the findings of Sanchez-Andrade *et al.* [25]. The infection risks for cattle in all breeds were equal in the study area. On the other hand KATO *et al.* [14] pointed a significantly higher prevalence in Japanese native cattle than in Friesian or Jersey probably due to the difference in cattle feed as the Japanese native cattle were fed on rice straw. They indicated that rice straw feeding is suggested to be related with the high rates of cattle fasciolosis in Japanese native cattle.

In conclusion, this study indicated that *F. hepatica* is endemic and widespread in Kayseri province. Owing to the public health and economic importance of fasciolosis, there is a need for more practical approaches to its control, to alleviate the burden of this disease on its cattle host and reduce economic losses. These initial findings also serve as baseline data for further studies related to seasonal transmission patterns and management of fasciolosis in the region.

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