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Seroprevalence of *Fasciola gigantica* infection in sheep in Khouzestan province, Southwest of Iran

Galehdar, H[1]., Razi Jalali, M.H[2*]., Ghorbanpour, M[2]., Pourmahdi Broujeni, M[3]

1. *Graduate, Faculty of Veterinary Medicine of Shahid Chamran University of Ahvaz, Iran*
2. *Department of Pathobiology, Faculty of Veterinary, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
3. *Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*

***Corresponding author**: mh.jalali@scu.ac.ir*

Abstract

Tropical fasciolosis is regarded as one of the most important helminthic infections of ruminants in Asia and Africa. Throughout most of its geographical range, *Fasciola gigantica* is of great importance as a parasite in sheep. The aim of this study was to detect seroprevalence of fascioliasis in sheep in Khouzestan province. In this study, due to the importance of livestock diseases in Khozestan province, total of 519 sheep from different areas in Khouzestan province were randomly bleeded, and their sera were preserved at 20°C. All sera were evaluated for anti- *Fasciola gigantica* excretory-secretory antigen by an in-house ELISA test. The results obtained from sheep show that the prevalence rate is 15.2 % (prevalence in female and male sheep was 14.3% and 25%, respectively). The prevalence rate in the age group of 4-5 years was higher than other groups, and it was lower in the age group of 2-3 years. than other groups, in Baghmalek the prevalence rate was higher area than other areas of the province. According to the results and the importance of this disease and its high prevalence, it is necessary to use appropriate strategies for controlling, preventing and treating it in the sheep in Khouzestan.

Keywords*:* Fasciola gigantica, Sheep, Khouzestan

Introduction

Causing significant losses in livestock population, Fasciolosis is one of the most important helmintosis affecting the liver of ruminants (Darabus, *et al.*, 2006). In domestic ruminants, adverse effects of acute or chronic fasciolosis include decreased meat and milk production as well as decreased fertility (Kithuka,*et al.*, 2002; Swai and Ulicky, 2009; Theodoropoulos *et* al., 2003; Urquhart *et al.*, 2001).The early diagnosis of fasciolosis provides an opportunity to prevent great economic losses. (Charlier *et al.*, 2008; Espino *et al.*, 1990).

Fasciolosis diagnosis, due to low sensitivity of coprological diagnostic method, has been a challenge for a long period. To prevent the hepatic damage, several immunological methods have been developed for the detection of early and specific antibodies to *Fasciola*, especially in cattle and sheep (Price et al., 1993; Ruiz *et al.*, 2003). The diagnosis has been improved by the development of ELISA, using crude

extracts (Knobloch *et al.,* 1985), excretory-secretory products (Espino *et al.,* 1990), purified or recombinant antigens such as cathepsin l-1 (O'Neill *et al.,* 1998), and by the detection of circulating antigens and coproantigens by sandwich ELISA (Carnevale *et al.,* 2001). According to the previous study, the ELISA method with ES antigens has a high accuracy for diagnosis of sheep fasciolosis (Razi Jlali *et al.,* 2012. Since the *Fasciola gigantica* is dominant species in Khouzestan state, the potential for cross-reactivity in this study has decreased.

The objective of this study was to examine the prevalence of this disease in Khouzestan province by ELISA, using excretory-secretory antigens of *Fasciola gigantica.*

Materials and Methods

Collecting sera

Totally, 519 blood samples were collected simple randomly from sheep jugular vein from 6 different areas of Khouzestan province. Blood samples were centrifuged at 3000×g for 10 minutes and sera were stored at -20°C. Serum samples were tested with an in-house indirect ELISA method. Two blood samples from experimentally infected sheep (another study) were prepared and four sheep blood samples that had been confirmed after slaughter were used as positive control. Also, ten blood samples were collected from the newborn 2-3 month old lambs with no history of fasciolosis, used as negative controls. To increase assurance, positive and negative controls were tested by counterimmunoelectrophoresis.

Excretory-Secretory antigen preparation

*Fasciola gigantic*a excretory-secretory antigen was prepared according to Simsek *et al* (2006). Briefly, adult *Fasciola gigantica* worms were collected from the large bile ducts of sheep, and washed several times in PBS (pH=7.4) and 5 worms were incubated in 10 ml of PBS at 37°C for 6 hours. After incubation, all worms were removed and the collected medium was clarified by centrifuging at 10000 rpm for 30 min at 4°C. The supernatant was then filtered through a 0.2 µm filter. The filtered supernatant was dialyzed against distilled water for 24 h, aliquot and stored at -20°C as antigen.

Measurement of protein in prepared antigens

Protein concentration of antigen was measured using Lowry et al.'s method (1951).

ELISA

Optimal serum, antigen, and sheep anti IgG peroxidase conjugate (sigma, A3415, USA) concentrations were determined after preliminary checkerboard titration (Catty Raykundalia, 1989). ELISA plates were coated with 100µl (50µg/ml) of diluted antigens per well. The plates were sensitized overnight at 4°C. The wells were washed 3 times with PBS. Two hundred µl of 7% skimmed milk was added to each well as

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blocking and incubated for 90 minutes at room temperature. Three more washes were undertaken as before and one hundred µl of 1/25 dilution of serum was added to each well and incubated at room temperature for 1 hour.

After washing, 100 µl anti-sheep IgG peroxidase conjugate diluted at 1/6000 in PBS were added to each well. The plates were incubated at room temperature for 1 h and washed as previously described. A 100µl of substrate solution containing TMB/H_2O_2 was added to each well and the plate was incubated for 15 minutes at room temperature. The reaction was stopped with 1M sulfuric acid. The absorbance was measured at 450 nm in an ELISA reader (Dynatech, Netherlands). The samples with absorbance of at least two times more than the mean negative samples OD were considered as positive.

Data were evaluated with chi-square test to analyze the relationships between sex, age and location.

Results

Using chi-square test, the data were evaluated to analyze the relationships between sex, age and region. The overall prevalence of fasciolosis in the study was 15.2%. The specific prevalence of fasciolosis was found to be 14.3% in female sheep and 25% in male sheep. Statistical analysis of the data showed that there was a significant difference ($p<0.05$) between prevalence of fasciolosis among sheep of different ages (Table 1). A higher *Fasciola* spp. infection prevalence ($p<0.05$) was found among sheep of 4-5 years of age (21.8%) and lower *Fasciola* spp. Infection prevalence was found among 2-3-year-old sheep (11.8%).

Table1. Seroprevalence of Fasciola gigantica based on age groups in Khouzestan province.

prevalence / age	Positive		Negative		Total	
	Respective	Abundance	Respective	Abundance	Respective	Abundance
1<[ab]	13.3	6	86.7	39	8.67	45
1-2[ab]	16.1	9	83.9	47	10.78	56
2-3[b]	11.8	10	88.2	75	16.38	85
3-4[ab]	13.1	17	86.9	113	25.04	130
4-5[a]	21.8	26	78.2	93	22.91	119
>5[ab]	13.1	11	86.9	73	16.18	84
Total	15.2	79	84.7	440	100	519

The different lowercase letters represent a significant difference

The prevalence of *Fasciola* spp. in male sheep (25%) was higher than that among females (14.3%) but this difference was not statistically significant (Table 2).

Table 2. Seroprevalence of Fasciola gigantica based on sex in Khouzestan province

prevalence / sex	Positive		Negative		Total	
	Respective	abundance	respective	abundance	Respective	abundance
Male[a]	25	11	75	33	8.5	44
Female[a]	14.3	68	85.7	407	91.5	275
Total	15.22	79	84.7	440	100	519

The different lowercase letters represent a significant difference

The highest prevalence of fasciolosis (26.8%) was recorded in sheep originated in Baghmalek whereas the lowest prevalence (8.3%) was identified to be in sheep originated in Dezfoul.

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but this difference was not statistically significant (Table 3).

Table 3: Seroprevalence of Fascioa gigantica based on region in Khouzestan province

Prevalence location	Positive		Negative		Total	
	Respective	Abundance	Respective	Abundance	Respective	Abundance
Hendijan[a]	10.1	10	89.9	89	19.07	99
Baghmalek[a]	26.8	11	73.2	30	7.89	41
Dezfoul[a]	8.3	7	91.7	77	16.18	84
Sousangerd[a]	16.3	15	83.7	77	17.72	92
Ahvaz[a]	18.2	32	81.8	144	33.52	176

The different lowercase letters represent a significant difference

Discussion

Comprehensive knowledge of parasite ecology is crucial to sustainable control because the parasites interact differently with hosts in specific climatic and production environment (Almeria Uriarte, 1999; Papadopoulos, 2003). Our data indicated that the exposure of domestic sheep to *Fasciola* spp infections in Khouzestan province was common, with an overall prevalence of 15.2%. Fasciolosis has historically been a disease of ruminant worldwide and has caused serious economic losses in the animal husbandry industry (Kithuka,, 2002).

Climate conditions, particularly the rainfall, have frequently been associated with differences in the prevalence of *Fasciola* spp. In tropical areas such as Khouzestan province, *Fasciola gigantica* is fasciolosis agent. These conditions are suitable for intermediate hosts *lymnea* spp to reproduce and to survive which is in agreement with the present study. The variation in prevalence between different locations is also likely due to the difference in landscape, such as swampy areas and agricultural irrigation practices. During the rainy season, the amount of rainfall flooding Baghmalek creates a favorable condition, which favors the development of the intermediate host (snail) and the transmission of the diseases.

Solomon (2005) has suggested that fascioliasis equally affects both sexes. In his study, a higher prevalence of parasitic infection was not associated with sex ($p > 0.05$). Although the difference was not statistically significant, males actually had higher infection prevalence than females. This might be due to the fact that all the animals were grazing similar pasturelands (Solomon, 2005).

Young animals had a lower prevaleance of *Fasciola* spp. infections in this study. This finding is consistent with other reports, and it is not surprising because naïve kids have maternal immunity. Higher infection rates were found in adults in other age groups ($p< 0.05$). Based on this finding, it can be suggested that the higher exposure risk of adult may be due to physiological differences, such as stress, pregnancy, lambing, inadequate nutrition, and infectious diseases. Similar results were reported by Ayalew (1994).

We consider that the high specificity and sensitivity of the ELISA described above, as well as its practicability in seroepidemiology studies to detect infection

by *Fasciola gogantica* at the herd level, indicate that the indirect ELISA using *Fasciola gigantica* ES antigens could be adopted as the technique routinely used in veterinary diagnostic laboratories (Manga-Gonzalez *et al.*, 1991).

Different prevalence of *Fasciola* spp. in these reports could be explained by the different parasitological techniques used in these studies, the difference in the origin of the samples, or the geographical differences.

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