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Short Communication

Seroprevalence of Fasciolosis in Equines of the Black Sea Region, Turkey

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ABSTRACT

This study determined the seroprevalence of fasciolosis in equines (n = 140) by enzyme-linked immunosorbent assay and Western blot. Blood samples were collected from different locations of the Black Sea Region of Turkey. The overall seroprevalence of fasciolosis in equines was 19.3%: 18% of horses (15), 22.6% of donkeys (7), and 19.2% of mules (5). The seroprevalence rates across provinces were as follows: 25% (5/20) in Samsun, 21.9% (7/32) in Tokat, 31.4% (11/35) in Amasya, 7.4% (2/27) in Ordu, and 7.7% (2/26) in Sinop. Three protein bands with molecular weights of 13, 30-40, and 40-65 kDa were determined in antigen clusters in sera from *Fasciola* spp-infected equine animals.

1. Introduction

Fasciola hepatica is occasionally found in the liver of equines and is detected during postmortem examination [1]. A few studies have been conducted on the prevalence of *F hepatica* in equines, or on its association with liver pathology and clinical signs [2-4]. Although the coprological examination of donkeys revealed the prevalence of fluke infection to be 44.4%, the postmortem finding showed it to be 41.9% [4]. One of the main problems in the study of the prevalence of fasciolosis in equines is the difficulty of diagnosis. Because most flukes do not reach the mature stage in equines, fecal examination for excreted eggs is not reliable [2]. *F hepatica* (range: 0.2%-41%) in pony foals was detected on postmortem examination [5].

A potential alternative is the detection of serum antibodies against *F hepatica* [2]. Using recombinant antigen, detection of equine fasciolosis before maturity of the fluke by using enzyme-linked immunosorbent assay (ELISA) contributes significantly to the knowledge of serological diagnosis of *F hepatica* [6]. Toparlak et al. [7] reported that of 495 cattle livers from an abattoir in Van in Turkey, 53.7% were infected with *F hepatica*, and 1.8% with *F gigantica*. In another study based on fecal examination of samples taken from Samsun, Turkey, *Fasciola* spp were detected in 15.43% of cattle [8]. Coprological testing of samples from the same area showed 24% of water buffaloes were infected with *Fasciola* spp [9]. In other studies [10,11], *F hepatica* was detected in 0.48% and 25.3% of livers from cattle in the Thrace and Samsun regions, respectively. Most studies on *Fasciola* spp in Turkey have been carried out by coprological and slaughterhouse analysis [12-15]. In a study by Burgu et al. [16], *F hepatica* was detected in 20% of donkeys slaughtered for zoo carnivores in Turkey.

According to one study [17], there were approximately 189,000 horses, 296,000 donkeys, and 68,000 mules in Turkey.

No serological investigations have been conducted on equine fasciolosis in the Black Sea region and other areas of Turkey. The aim of the present study was to determine the seroprevalence of *F hepatica* in equines in the Black Sea region of Turkey by using indirect ELISA and the Western blot test.

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2. Materials and Methods

2.1. Area of Study

The present study was conducted in 13 randomly selected villages of Amasya, Ordu, Samsun, Sinop, and Tokat provinces in the Black Sea region of north-central Turkey. The study area was located between 40-42°N and 35-38°E, and it ranged from 6 m to 1268 m in altitude (Fig. 1). Most of the substrate in the studied area is siliceous, whereas the calcareous terrain is located in peripheral zones. *Lymnaea* spp, the intermediate hosts of *Fasciola* spp, are found in hard waters having a high pH and containing nitrates and silica [18].

The Black Sea region has a distinct climate type that features mild temperatures; the average annual rainfall is 670-920 mm in the Kizilirmak and Yesilirmak deltas. The study area has various habitats such as rivers, lakes, swamps, meadow pastures, forests, sand, and agricultural lands that harbor high biological diversity. The majority of vegetation from the coast to adjacent inland areas is coastal dune vegetation, marshes, halophytes, flooded forests, forest vegetation, or tall grassland. Most of the equines are grazed on the same natural pastures as are other farm animals such as sheep, cattle, and water buffaloes.

2.2. Animals

The sample size was derived from a predicted 25% fasciolosis prevalence, with 5% deviation and 90% confidence interval. A total of 140 equines, including 83 horses, 31 donkeys, and 26 mules, were examined. Age, breed, and sex of all animals were recorded; animals aged 3 years or older were considered old (Table 1).

2.3. Fecal Sampling

The sample (n = 140) obtained from the rectum of each animal was placed in a plastic fecal pot, stored in a refrigerator, and examined within two days by the standard flotation and sedimentation method [19]. Fecal egg counts were carried out by using a modified McMaster technique

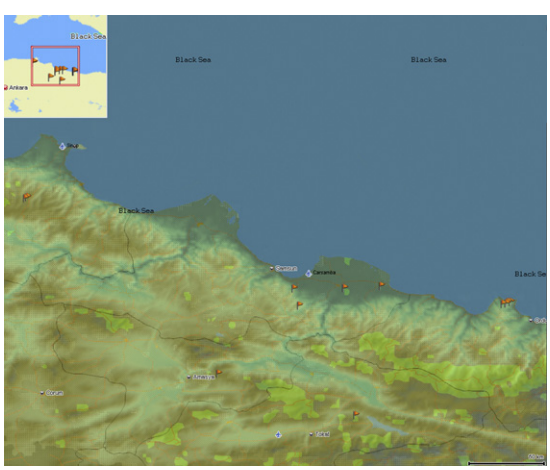

Fig. 1. Topographic map of the study area in the Black Sea region.

Table 1
The number of equines examined by ELISA for fasciolosis

Study Group	Horse				Donkey		Mule		Total
Breed	Arabian		Crossbred		Anatolian				
Age	♀	♂	♀	♂	♀	♂	♀	♂	
0-3	3	3	9	13	2	4	2	3	39
≥3	3	3	20	29	6	19	12	9	101
Total	6	6	29	42	8	23	14	12	140

[19], which showed a sensitivity of 50 eggs per gram (EPG) of feces. *Fasciola* spp eggs were identified on the basis of egg morphology and measurements, according to Georgi and Georgi [20] and Thienpont et al. [19].

2.4. Blood Sampling

Blood samples were collected in vacutainer tubes without anticoagulant, centrifuged, and then stored at −20°C until use.

2.5. Preparation of F hepatica Excretory-Secretory (E/S) and Somatic (S) Antigens

F hepatica E/S antigen was prepared by a modification of the technique described by Oldham and Williams [21]. Adult *F hepatica* specimens were collected from the bile ducts of cattle livers from local slaughterhouses, washed six times in physiological saline solution, and the flukes were incubated for 24 hours in phosphate-buffered saline (PBS; pH 7.2) with 100 IU penicillin and 100 μg streptomycin per milliliter (1 worm per 3 mL) at 37°C. After incubation, the supernatant was collected and centrifuged at 14,500 rpm for 30 minutes at 4°C. Somatic antigen was prepared by homogenizing adult flukes in PBS (pH 7.2) using a homogenizer, and the supernatant was filtered through a 0.45 μm membrane. Protein determination was carried out as described by Hartree [22], using bovine serum albumin as the standard.

2.6. Anti F hepatica Polyclonal Serum

To determine immunogenicity of the E/S antigens, a 2.0-kg New Zealand rabbit was immunized with the antigen of *F hepatica*, according to the method of Almazan et al. [23]. First, 1 mL (0.9 mg/mL) of the E/S antigen of *F hepatica* was emulsified with an equal quantity of Freund's complete adjuvant and then injected subcutaneously. Four additional immunizations were given at 15-day intervals, using 1 mL (0.9 mg/mL) of E/S antigen mixed with an equal quantity of Freund's incomplete adjuvant. After the eighth week, the rabbit had an antibody titer >100,000, as determined with the Dot blot technique.

2.7. Indirect ELISA

ELISA testing was performed on microtiter plates, as per the methodology of Zimmerman et al. [24], with some modifications. Briefly, polystyrene microtiter plates (Maxisorp; Nunc) were coated with 100 μL of the E/S antigen solution per well (10 μg/mL) and incubated for 1 hour at 37°C, and then overnight at 4°C. The plates were washed