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Serological and coprological comparison for rapid diagnosis of *Fasciola hepatica* infection in small ruminants from sub-tropical area of Pakistan

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ABSTRACT

The objective of present study was to determine the prevalence of *Fasciola hepatica* infection in small ruminants by using indirect ELISA and sedimentation techniques and made a comparison between both techniques for rapid diagnosis. A total of 1200 serum and fecal samples from 612 sheep and 588 goats were analyzed for IgG antibodies and fecal egg count. Other parameters such as breed, age and sex were also taken into consideration. The results showed that the infection was significantly ($p < 0.05$) higher in sheep as compared to goats. In sheep, a prevalence of 39.2% was found using the indirect ELISA and 28.43% for the fecal analysis, while in goat the prevalence was 4.08% and 5.01%, respectively. The results showed that there was a significant ($p < 0.05$) difference in prevalence between breeds of sheep and goats. The results also indicated that in goat there was no significant ($p > 0.05$) difference in prevalence between age and sex groups. After contrasting data from ELISA and fecal analysis, 5.5% of the sera analyzed had positive values of indirect-ELISA and negative by fecal analysis. In conclusion, the findings suggest that indirect ELISA may be an efficient technique for early diagnosis of infection compared to coprological examination. The combination of both techniques was very helpful for demonstrating the current status of *F. hepatica* infection, and can be recommended for epidemiological surveys and for anthelmintics treatment to minimize the major health hazard affecting the production potential of small ruminants.

1. Introduction

Parasitism is a major constraint to livestock production all over the world (Vercruysse and Claerebout, 2001), especially in developing countries like Pakistan where 65.2% of the population live in rural areas (Population Census Organization, 2007) and depend on livestock for their livelihoods. Among helminths, *Fasciola hepatica* is very prevalent due to a wide spectrum of definitive hosts (Rondelaud et al., 2001). This digenetic trematode parasite has a worldwide distribution, and is mostly encountered in temperate cooler areas of high altitude and also in tropical and subtropical regions (Andrews, 1999; Torgerson and Claxton, 1999; Okewole et al., 2000; Mas-Coma et al., 2005). The worldwide loses in animal productivity due to fasciolosis was estimated USD$3.2 billion per annum (Spithill et al., 1997).

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In Pakistan, fasciolosis has been reported in all parts of the country (Azam et al., 2002; Bhutto et al., 2002; Ahmed et al., 2005; Raza et al., 2007; Khan et al., 2009; Kakar et al., 2011). Some localities of Punjab, Sindh and Khyber Pakhtunkhwa are badly affected by fasciolosis. The precise extent of economic losses due to fasciolosis in livestock in Pakistan is not yet available. In Punjab, *F. hepatica* infection in sheep reported 14.67% around Lahore (Ijaz et al., 2009) and 21.41% in southern Punjab (Lashari and Tasawar, 2011), while in goats *F. hepatica* infection 28.75% around Multan (Tasawar et al., 2007) and 7.58% (Khan et al., 2010) in Toba Tek Singh.

The economic importance of fasciolosis to the livestock industry has various aspects limiting the productivity due to the morbidity, mortality, cost of treatment (Mas-Coma et al., 2009; Raza et al., 2010) condemned livers (Ahmadi and Meshkehkar, 2010), reduced milk and meat production and fertility disorders (Rokni et al., 2010; Hossain et al., 2011). One important aspect is to develop appropriate technique to diagnose infection at early stages. The usual method for diagnosis of fasciolosis is the fecal egg count during the patency period. Moreover, low infection can pass undetected, and parasitized animals may be the source of new infections (Urquhart et al., 1996; Reichel, 2002). In the present study the indirect ELISA was used for diagnosis of *F. hepatica* infection along with coprological examination. The advantage of serological diagnosis by indirect ELISA is to be able to indicate infection earlier than coprological analysis (Sanchez-Andrade et al., 2000; Reichel, 2002).

Preliminary studies carried out on the prevalence of fasciolosis in five provinces of Pakistan were all based on coprological analysis and no serological data has ever been reported. The agro-climatic conditions of sub-tropical area of Pakistan are highly conducive and are prone to water-borne trematode infection. This has necessitated developing rapid and cost effective diagnostic tool to reduce the impact of fasciolosis on animal health by selecting the appropriate anthelmintic treatment. The aim of the present study was to estimate the prevalence of fasciolosis in small ruminants and its relationship among breed, age and sex. For this purpose an indirect ELISA test and coprological analysis were compared.

2. Materials and methods

2.1. Experimental site and climate

The present study was conducted from June 2008 to August 2009 at different public and privately owned farms located in the Pothwar region of Pakistan. This area is situated in climatic region regarded as sub-tropical. The Pothwar Plateau is situated between latitude 30 and 34°N and longitude 70 and 74°E. The climate of the region is semi-arid, influenced mainly by summer monsoon (July–September) and partly from winter precipitation as well.

2.2. Flock management

The small ruminants grazing in Pothwar region of Pakistan follow extensive and semi-extensive farming production systems. The flocks of sheep and goat are generally kept separate but they closely follow each other sharing the grazing lands. The flocks of sheep and goats are taken out in the morning for grazing along the communal lands, roadsides and land along foot-hills and brought back to their kraal in the evening. The rotational grazing patterns are followed in order to avoid overgrazing and heavy contamination of pasture. The flocks also graze on small trees, shrubs and stubbles left after crop harvesting season. In the kraal, the flocks are usually supplemented with tree-leaves and shrubs, but during winter season due to lack of forage they are feed with concentrates comprises of oilseed cakes. Supplementation and prophylactic measures was low and irregular. Age, sex and breed of each examined sheep and goats obtained from owners and/or farm attendants were recorded during the study.

2.3. Animal data, study design and sampling

The homogeneity of the samples is attained by taking the samples from flocks under same management conditions, especially the grazing/feeding habit. The study population consisted of 612 sheep belonging to salt range ($n = 348$) and Afghani ($n = 264$) breeds and 588 goats belonging to Local Hairy ($n = 336$), Beetal ($n = 192$) breeds and a crossbreed of Beetal and Hairy ($n = 60$). A stratified random sampling method was used to select animals according to their breeds, sex and age groups. The sheep were stratified into two age groups: below 2 and above 2 and the goats were into three age groups (below 3, 3–6 and ≥ 6). Furthermore sheep were also grouped into two breeds, Afghani and Salt range and goats were into three breeds (Local Hairy, Beetal and Cross).

2.4. Serological protocol

2.4.1. Serum collection

Blood samples were obtained from the jugular vein of animals in non-EDTA coated vacutainers, centrifuged at 3000 rpm for 15 min and sera was separated and kept at −20 °C until used for antibodies detection.

2.4.2. ELISA

Serum IgG-antibodies were assayed for specific *F. hepatica* antigens by using a commercially available indirect ELISA Kit (DRG instruments GmbH' Germany). The ELISA was performed according to manufacture instructions on 96 well microtitration plates, whose odd columns were coated with specific *F. hepatica* antigens whereas even columns were used to control the specificity of the test. The sera were diluted at 1:100 in dilution buffer and were added (100 μl of each) in duplicate to the microplate wells, incubated for 1 h at 37 °C. The microplates were then rinsed with washing buffer. After washing, 100 μl of conjugate, a peroxidase-labeled anti-bovine IgG1 monoclonal antibody, was incorporated to microplate wells at the dilution of 1:50 and incubated for 1 h at 37 °C. The conjugate was then washed with washing buffer and 100 μl of chromogen-substrate mixture was added to microplate wells and was incubated for 10 min at room temperature. The reaction was stopped by adding 50 μl of stop solution per microwell. The optical density (OD) was read at 450 nm filter using a plate reader.

2.5. Parasitological techniques

Three grams of feces were taken from the rectum of each animal placed in plastic bags and examined by processed using a modified sedimentation techniques to determine the presence of fluke eggs (Urquhart et al., 1996). Trematode eggs were identified on the basis of morphology (Yamanguti, 1975; Soulsby, 1982).

2.6. Statistical analysis

The association between prevalence and examined animals' data (age, sex and breed) were compared using Pearson's chi-square (χ^2) test. Prevalence was calculated as percentage value. Statistical comparisons were carried out using SPSS 17.0 statistical software. The hypothesis tested is antibody-detection ELISA might be sensitive as compare to modified McMaster sedimentation techniques among sheep and goats, and some of the epidemiological risk factors (age, sex and breed) that might be contribute for fluke infections. A statistically significant association between variables was considered to exist if the calculated p-value is less than 0.05 with 95% confidence level.