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Diagnosis of *Fasciola* spp. infection in cattle in El-Dakhla Oasis, Egypt by Intradermal test and Enzyme-Linked Immunosorbent Assay

Ahmed, I Hassan[1], Mohamed, Y Ramdan[2], Omar, S.F. A[1], Aliaa Balegh[1] and Lubna, M Elakabawy[2].

[1]*Agricultural Research Center, Animal Health Research Institute, Egypt.*

[2]*Parasitology Department, Fac. Vet. Med. Benha University, Egypt.*

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ABSTRACT

The present study aimed to evaluate both intradermal test (IDT) and ELISA test in the diagnosis of *Fasciola* spp. at El-Dakhla Oasis, El-wadi El-Gadid, Egypt. The intradermal test was carried out in 100 cattle that proved to be negative to *Fasciola* spp. infection by fecal examination. ELISA test was conducted on 96 sera samples from injected cattle. The prevalence of infection was 32% and 56.25% for the intradermal test and ELISA test, respectively. All positive results with the Intradermal test were also positive with ELISA. The results of this study revealed that the Intradermal test and ELISA could become a useful tool for the diagnosis of fascioliasis in cattle.

1. INTRODUCTION

Fascioliasis is a parasitic disease caused by *Fasciola hepatica* or *Fasciola gigantica*. For several years, it has posed a major problem in ruminant livestock production, but recently, largely due to climatic change, there has been awareness of the incidence and spread of the disease (Halferty et al., 2008). Moreover, fascioliasis is thought to be an emerging serious human health problem in some countries (Caravedo and Cabada, 2020).

Early and accurate diagnosis of *Fasciola* infection plays an essential role in the control of the disease. Utilization of skin tests for diagnosis of parasitic infections was described by Suree, (1995) who used worm crude extracts as antigen. Type I immediate hypersensitivity was used for the diagnosis of bovine fasciolosis. Skin tests are characterized by being used in the field, of simple execution, and the obtaining of rapid results (Salam et al., 2009).

Serological tests can examine a large number of sera and also detect the presence of infection as early as 2 weeks post-infection and earlier than fecal egg examination. Enzyme-Linked Immunosorbent Assay (ELISA) can detect serum antibodies to specific antigens of *Fasciola spp.* (Fagbemi and Guobadia, 1995). Concerning the detection of specific anti-Fasciola antibodies in serum, the sensitivity and the specificity of serological tests are affected by the antigens used; including *Fasciola* crude antigen, egg antigen, and ES antigen (Attallah et al., 2013). The second prospect depends on the detection of circulating parasitic antigens by ELISA; being positive after 4–6 weeks post-infection.

The current study aimed to make an early diagnosis of fascioliasis in apparently healthy cattle using the intradermal test and ELISA at El-Dakhla Oasis, El-wadi El-gadid, Egypt.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 100 cattle (60 female and 40 male) were subjected to post-mortem inspection in Mout main slaughterhouse, El- Dakhla province, El-Wadi El-gadid Governorate, Egypt, for detection of adult *Fasciola* spp.

The liver of 100 cattle was inspected for the presence or absence of liver flukes (Chesbrough, 2003). The adult flukes were collected from the infected liver and preserved in (0.9% Na Cl) in a plastic container clearly labeled with (owner name, locality, age, sex, and date).

The collected adult liver flukes were washed extensively in PBS (37 °c) and stored at (-20 °c) till used.

A total of 96 sera samples were collected from cattle which were found negative on fecal examination. Sera were kept at –20 °C until used.

2.2. Preparation of crude antigen

Preparation of crude antigen according to Balegh (2018). Preserved flukes were ground using a tissue homogenizer (Tempest Vertis hear) followed by sonication 60-80 pulse amplitude for 5min. (Sonics materials inc. Vibra cell™). Centrifugation in cooling centrifuge 12.000 rpm for 30min.

* Corresponding author: drahmed2034@yahoo.com

The supernatant fluids were collected, divided into small aliquots, and stored as crude antigens at -20 °c till use. Protein content was measured with the Folin phenol reagent (Lowry et al., 1951)

2.3. Intradermal test

A total of 100 cattle proved to be negative to *Fasciola* spp. infection by fecal examination and showed absence of any clinical signs to fascioliasis were subjected to the intradermal test according to (Oliveira and Antonio, 2010)

An area of 30 cm × 15cm at the skin of the neck was disinfected with ethyl alcohol 70% then subjected to intradermal injection of 0.1ml of phosphate buffer saline as negative control and 0.1ml of crude antigen in two sides in the skin of the neck.

The result of the IDT was determined through visualization of the diameter of the resulting wheels and by detection of presence or absence of skin reactions (erythematous swellings or ulcers) at 0.5 h, 1 h, 24 h, and 48hr.

2.4. Sero diagnostic studies through indirect immunosorbent assay (ELISA)

ELISA has been developed to detect antibodies against *Fasciola* spp. crude antigen according to Krishna and Souza (2015). The ability of crude *Fasciola* spp. antigen to detect low levels of anti-Fasciola spp. antibodies in serial dilutions of infested animals' sera were assessed through checkerboard titration in an indirect ELISA test. Checkerboard titration was carried out to determine the optimum dilution of the antigens and tested sera. Serial dilutions of the tested antigen were used in coating ELISA plate at 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90 and 1:100 versus different dilutions of tested sera (positive, negative sera) diluted, as 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, 1:5120, 1:10240 and 1:20480.

Serum dilution associated with the lowest protein concentration of antigen/well provided perfect differentiation between positive and negative (under-recognized dilution of conjugate and substrate) is considered as the optimum status which will be used in the ELISA test.

2.4.1. Procedure of Indirect ELISA

Sera of 96 apparently healthy cattle were tested by ELISA against the crude antigen of *Fasciola* spp. for detection of antibodies levels against *Fasciola* spp. in living cattle

According to the manufacturer's instructions.

- Sera were positive when the OD was as or more than the cut-off value (The cut-off = double fold of the mean negative control sera OD).
- The antibody percent was calculated for each serum sample by comparison with positive and negative control serum samples as follow:

$$\text{Antibodies (\%)} = \frac{OD\ (serum\ sample) - OD\ (negative\ control)}{OD\ (positive\ control) - OD\ (negative\ control)} \times 100$$

3. RESULTS

3.1. Intradermal test

Out of 100 cattle, 32 were positive for *Fasciola* infection by Intradermal test (Table 1). After injection, both the negative control and treated parts with crude *Fasciola* spp. Antigen showed minor swelling. This swelling disappears about 10 minutes after injection.

After 30-45 min. of injection, an area of redness appeared around the application site of both PBS and crude antigen in both infected and non-infected cattle

Also, visible swelling appeared at the application site of crude antigen in the infected cattle.

After one h of injection, there was an increase in the thickness of the skin that reaches its maximum dimension about 1.5 to 2 h after injection, **Fig. (1)**. 2-5 h, reduction in thickening of the skin that disappears about 4 to 5 hours of application. 24-48 h, no reaction or significant alteration of the skin was observed.

Table (1): The prevalence of *Fasciola* infection in cattle by intradermal test.

TEST	Number of examined cattle	Positive results	Negative results	Prevalence (%)
I/D	100	32	68	32

I/D: intradermal test

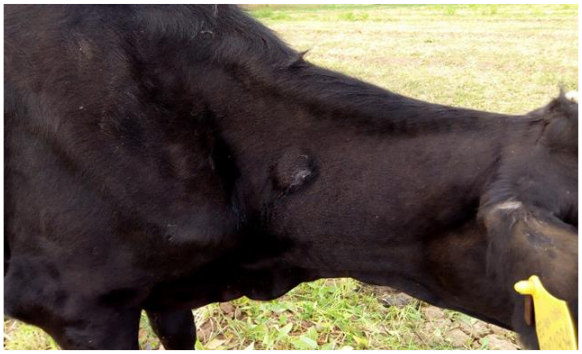

Fig. (1) Intradermal test in cattle infected with Fasciola spp. Infection. Note the skin thickening.

3.2. ELISA

Out of 96 *Fasciola* negative cattle up on fecal examination, 54 were positive to *Fasciola* spp. infection and 42 were negatives (Table 2).

Table (2): The prevalence of *Fasciola* species infection in cattle by ELISA test.

TEST	Number of examined cattle	Positive results	Negative results	Prevalence (%)
ELISA	96	54	42	56.25

4. DISCUSSION

In the present study, the prevalence of fascioliasis in cattle by the intradermal test was (32 %). This indicated that the intradermal test was the sensitive method for the diagnosis of fascioliasis. Similar result was recorded by (Mas-coma et al., 1995; Esteban et al., 1998). While the present prevalence was lower than that reported by Oliveira and Antonio. (2010) who recorded that 78.9% of sheep experimentally infected with 200 metacercariae of *F. hepatica* were positive. On the other side, it was higher than that recorded by Suree, (1995) who found that the prevalence rate of fascioliasis was 6.3%. ELISA results showed that the prevalence of fascioliasis in cattle was (56.3%) which have partial agreement with the findings reported by Nyera et al., (2002) who found a prevalence of 56.7%, Abou-Elhakam et al., (2013) who recorded that the prevalence of fascioliasis was 56% by Sandwich ELISA and the sensitivity was 97.3%, and the specificity was 95% in Giza, Egypt. Kuraa and Malek, (2014) stated that 60.9% out of 100 cattle and buffaloes investigated for fascioliasis were positive by ELISA in Assiut Governorate. Mufti et al., (2015) showed that the prevalence of infection based on

ELISA results was 60 and 50%, in both buffaloes and cattle, respectively.

On the other side, the present prevalence was lower than that reported, Loung et al., (1997) confirmed 17 (94 %) of 18 cattle were positive using ELISA and Akca et al., (2014) detect a prevalence of 66.6 % by an in-house ELISA. While the present prevalence was higher than reported by Suree, (1995) who recorded the prevalence of fascioliasis was 48.14% by ELISA, and Krishna and Souza, (2015) found that 34.0 % of cattle and 42.5% of buffalo's sera were positive by ELISA. Kelly et al., (2019) investigated that 12.7% of cattle sera were positive for *Fasciola* infection.

The higher prevalence of *Fasciola spp.* infection in this study especially by ELISA test was due to the area of study (Dakhla Oasis) which is highly endemic with *Fasciola* spp, this is because the irrigation system and water sources (wells) were endemic with the intermediate host *(Lymnea* snails) of *Fasciola spp.*

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