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website: http://jsci.utq.edu.iq Email: utjsci@utq.edu.iq

Evaluation Of *Fasciola gigantica* Excretory-Secretory Antigen on Immunodiagnosis For Cattle Fascioliasis in AL-Chebayish Marshes By Using ELISA

Sarmad A. M. AL-Asadi

Basrah University −Education College −Biology Department
Basrah - Iraq

Abstract

Enzyme-Linked Immunosorbent Assay (ELISA) was evaluated for ability to detect IgG anti- *Fasciola gigantica* antibodies in cattle by using *F. gigantica* excretory-secretory antigen (FgES).The presnt study revealed that the sensitivity and specificity were 95% and 100% respectively, when no cross-reactions with sera of cattle infected with other parasites was reported .At the mean time fifty seven random serum samples of cattle were examined by ELISA and revealed that the infection prevalence was 64.9% .

Introduction

Fascioliasis is an important zoonotic disease caused by liver flukes of the genus *Fasciola* of which *F.hepatica* and *F. gigantica* are the most common representatives . The disease is recognized as a serious public health problem by World Health Organization [1].

Diagnosis of fascioliasis in ruminants (sheep , cattle and goats) depends on detection of eggs in the feces of infected animals[2].However, early diagnosis is not possible because eggs are not found in the feces until 10-14 weeks after infection[3],when flukes reach maturity, and when hepatic injury has been produced[4].An alternative approach to overcome the difficulties of diagnosis is the detection of serum antibodies formed against a specific antigen of *Fasciola* spp. Several methods have been developed for detection of *F. hepatica* infection ,but only few are applied to *F. gigantica* infection . The best methods of serodiagnosis is by ELISA using antigens derived from adult fluke extracts or excretory-secretory products (ESP)[5,6,7]. Anti-*Fasciola* antibody can be detected as early as 2-4 weeks after infection[8,9,10,11,12].

The aim of the present study is to evaluate immunodiagnosis value of ELISA in AL-Chebayish cattle fascioliasis using FgES antigen .

Materials and methods

Preparation of the *F. gigantica* excretory-secretory antigen

Flukes were obtained from naturally infected of cattle livers and treated following the technique described by[13].Briefly , batches of about 20 adult worms were put into 100ml of phosphate buffered saline (PBS) (0.15M pH=7.2) and then incubated in fresh PBS for 1h at 37 C˚ .After incubation , the worms were removed and suspension containing the ES antigen of *F. gigantica* was centrifuged at 3600 rpm for 1h at 4 C˚ using cooling centrifuged type Chilspine 2 .

The supernatant was collected and stored at -20 C˚ until required . Protein concentration (31.515µg/ml) was measured by[14] method .

Blood samples collection

Blood samples were obtained by bleeding the animals from jugular vein ,at the period from February to June 2008 , then were allowed to clot for 1h at room temperature, centrifuged (30 min , 1500 rpm) and sera were obtained , placed into eppendorf tubes and stored at -20 C˚ until used .

Sera were divided into four groups : The first negative sera (n=13) were obtained from uninfected cattle of AL-Chebayish (fascioliasis from , the second positive slaughter in Thi-Qar) sera (n=20) were obtained from cattle of diagnosed with *F. gigantica* infection identified by fecal examined and observed AL-(of fluke in infected livers from Chebayish slaughter in Thi-Qar) , the third randomly sera (n=57) were obtained from cattle herd in AL-Chebayish marshes and the fourth cattle sera of infected with other parasites (n=10) : *Echinococcus granulosus* (3) , *Paramphistomium* sp. (2) , *Toxocara vitulorum* (3) and *Ornithobilharzia terkestanicum* (2) were obtained from (AL-Chebayish slaughter in Thi-Qar).

Enzyme – Linked Immunosorbent Assay (ELISA) method

The method used for ELISA was similar to that described by[15] with some modification . Briefly , ELISA plates were coated with 100µl of the optimum

concentration 20µg/ml of the FgES antigen solution per well and incubated overnight at 4 C˚ . The wells were washed three times with PBS containing 0.05% tween 20 and incubated with 100µl of 5% bovine sera albumen (BSA) in PBS for 1h at 37 C˚ . Another three washes were undertaken as before and 100µl of a 1:100 dilution of serum was added to each well before incubation for 1h at 37 C˚ . After washing again , 100µl of a 1:4000 dilution of rabbit anti-bovine IgG-horseradish peroxidase conjugate (Millegen) was added to each well and the plate was incubated for 1h at 37 C˚ . After washing again ,100µl of substrate (orthophenylene-diamine-OPD) was added to each well , incubation for 15min at 37 C˚ , then stopped with 25µl of 12.5% H_2SO_4 . The optical density (OD) was measured at 450nm in an ELISA reader (Bioteck).

The cut-off value adjusted to be equal to the mean OD value of negative (control) plus 2SD and any reading above it was considered positive .

Results

The results of the ELISA to detection anti-*F gigantica* antibodies are shown in Table (1).

A total of 100 serum samples from cattle were examined by ELISA method .This method revealed that , 19 (95%) out of 20 were positive , 13 (100 %) were negative (control) , 37 (64.9 %) out of 57 randomly sera were positive with fascioliasis and no cross-reaction was observed with sera (10) from cattle of infection with other parasites .

ELISA method showed a sensitivity of 95% and a specificity of 100% .

$$\text{Sensitivity (\%)} = \frac{a}{a+b} \times 100$$

$$\text{Specificity (\%)} = \frac{d}{c+d} \times 100$$

a = True positives
b = False negatives
c = True positive
d = True negative

Table (1) Evaluation of ELISA method in the diagnosis of cattle fascioliasis by using FgES antigen

Cattle sera	No.	ELISA method	
		Positive	Negative
Positive	20	19 (a)	1(b)
Negative	13	0	13
Randomly	57	37	20
Other parasites	10	0 (c)	10 (d)
Total	100	56	44

Discussion

In the present study , ELISA method had been 95% , 100% sensitivity and specificity respectively ,and this agreement with [6] when he found that the sensitivity and specificity of this method was 100% respectively , when used FhES antigen for the diagnosis of human fascioliasis . [16]found that sensitivity and specificity were 96.5% and 98.8% respectively, but [17]found that ELISAsensitivity was 82% in cattle ,[18]and [19] found that sensitivity were 86.1% and 83.33% in human respectively, and this difference may be due to they used FhES antigen to detection IgG anti-*F.*

hepatica antibodies in sera , While in the present study FgES antigen have been used . [20] Clean that variation in ELISA reactivity may be due to presence of common bands among the prepared antigen . These bands may be responsible for the cross-reactivity between *Fasciol*species [19].

With regard to the specificity of the ELISA method , our FgES antigen proved to be specific , since no cross-reactions were observed when sera from other parasitic infections in cattle were tested .

The current study uses ELISA to define infection prevalence in cattle in AL-Chebayish marshes of Thi-Qar , fifty seven serum samples were randomly collected and examined by ELISA . This method revealed that 37 out of 57 were positive with fascioliasis , which a infection prevalence 64.9% , this may be due to the animals mainly feeding on grazing and aquatic plants .[21]has been shown that feeding of herbage and semi-aquatic plants to guinea pigs resulted in *F. hepatica* worm burden recoveries of over 50 flukes per 100 sample of feed . this is an extremely high infections source and load .

Serological diagnosis is much more advantageous than directed diagnostic methods such as fecal egg examination , in that the infections can be diagnosed in their early stage . ELISA method capability to detect IgG anti-*F.gigantica* antibodies from the second week post-infection. [8] Found 2 fold over normal increases in antibody levels to FhES in experimentally infected cattle by 2 weeks of infection , with absorbance values peaking at 8-10 weeks of infection . In other studies , all ELISA methods were highly sensitive and specific when compared to diagnosing *F. hepatica* by coprological mean[16 , 18]. In conclusion , this study confirmed on FgES antigen used in immunodiagnosis by ELISA . This may aid in the study of epidemiological of fascioliasis in other regions of Iraq . Besides , the study proved the value of ELISA in diagnosis of cattle fascioliasis .

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تقيم المستضد الابرازي- الافرازي لطفيلي Fasciola gigantica في التشخيص المناعي لإصابة الماشية بمرض تعفن الكبد في اهوار الجبايش باستخدام ELISA

سرمد عواد موزان الاسدي

قسم علوم الحياة – كلية التربية – جامعة البصرة - العراق

الخلاصة

اظهر فحص الامتصاصية المناعية المرتبط بالانزيم (ELISA) قابليته للكشف عن الاجسام المضادة المتكونة ضد طفيلي *Fasciola gigantica* في الماشية باستعمال المستضد الابرازي الافرازي لطفيلي *F. gigantica* (FgES) . كما أظهرت الدراسة الحالية ان حساسية وخصوصية فحص الامتصاصية المناعية المرتبطة بالانزيم بلغ ٩٥% و ١٠٠% على التوالي ، ولم يظهر تفاعل تصالبي مع امصال ماشية مصابة بطفيليات اخرى . وفي الوقت نفسه فحصت ٥٧ عينة مصل عشوائية من ماشية باستعمال فحص الامتصاصية المناعية المرتبطة بالانزيم واظهر ان نسبة الاصابة بلغت ٦٤,٩%.