

Detection of Fascioliasis in sheep and cattle by using of ELISA technique

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Abstract

The aim of present study detection of Fascioliasis in sheep and cattle immunologically in dewania city by using ELISA test. The results showed high percentage of seropositive sheep and cattle as 76.7% and 74.5% respectively .

The results of this study revealed an association between the percentage of infected animals and age , so the cattle with age more than and less than one year give 82.3% and 17.6% respectively while the sheep in the same above ages recorded 44.1% and 55.8% respectively .

Introduction

Bovine fascioliasis caused by the digenic trematoda *Fasciola hepatica* is a world-wide parasitic disease common in ruminants, the two-host life cycle parasite is classically found in farms where all conditions for the survival and the multiplication of the snail intermediate host (*Lymnea truncata*) are fulfilled. This snail is mainly found in damp meadows (watering-places , brooks , springs , ..) (1).*Fasciola* egg shedding occurs with feces . Hatching follows in water and gives rise to infectious metacercariae fixed on a plant holder, once the metacercaria are ingested by a ruminant, young flukes migrate through the liver to reach bile ducts. The prepatent period is 8 to 10

weeks . Adults appear in the bile ducts and start to lay eggs (2).Liver damage and acute disease especially in sheep are caused by migrating immature parasites , chronic disease occurs in cattle during the biliary phase ,the disease hampers zootechnical characteristics , decrease milk yield weight loss , intermittent diarrhea. anemia and infertility problems , acute distomatosis of the sheep is characterized by anemia and sometimes sudden mortality and chronic distomatosis by anemia , reduction of the dairy production . reduction of the average daily profit and oedemas (3).This study aimed to detect of the seropositive sheep and cattle of fascioliasis in Diwaniya .

Material and Methods

Ninety four blood samples were collected from sheep and cattle.

Collection of samples:The cypress *Fasciola hepatica* test uses 96 – well microtitration plates sensitized by a monoclonal antibody specific to one protein of *Fasciola hepatica*. This antibody is used to trap the protein as well as to purify it from lysate of the parasite.The test blood sera are diluted in the buffer for dilution . then incubate and washed. Then the conjugate- a peroxidase labeled anti-ruminant IgG1 monoclonal antibody-is added to the wells (4).The plate is then incubated a second time at room temperature (18-24°C) , washed again and the enzyme's substrate hydrogen peroxide and the chromogen tetramethylbenzidine (TMB) were added.If specific *Fasciola hepatica* immunoglobulins are present in

the test serum , the conjugate remains bound to the microwell that contains the antigen and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportional to the titer of specific antibody in the sample the signal read off the negative control microwell is subtracted from that of the positive microwell sensitized by the antigen. The interpretation of the results is done by comparing the signals of the samples serum with those of the positive controls.

Material :

Microplates_: Two 96 –well microtitration plates . The columns are sensitized by the *Fasciola hepatica* antigen that is captured by a monoclonal antibody .

Washing solution : one 100 ml bottle of 20x concentrated washing solution . the solution crystallizes spontaneously when cold. If only part of the solution is to be used, bring the bottle to room temperature until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or dematerialized water . store the diluted solution at 4°C .

Dilution buffer: One 50 ml bottle of 5x concentrated buffer for diluting the blood sera, milks and conjugate . the bottle's contents are to be diluted with distilled or demineralised water. this solution will keep at 4°C for at least 3 months

Conjugate : one bottle of anti-bovine immunoglobulin- peroxidase conjugate .

Positive reference : One bottle of Fasciola hepatica positive serum .

Chromogen solution: One 2 ml drop-dispenser bottle of the chromogen tetramethylbenzidine. Store at 4°C.

Substrate solution : One 30 ml bottle of the hydrogen peroxide substrate solution Store at 4°C.

Stopping solution : One 15 ml bottle of the 1 M phosphoric acid stop solution .

Methods :

1. We brought all the reagents at room temperature (18-24°C) at least half an hour before use.
2. Removed the microplate from its wrapper.
3. Placed 1ml aliquots of the dilution solution, prepared as instructed in the "Composition of the Kit " section, in 5 or 10 ml hemolysis tubes. Added 10 µl of the serum_sample to each of these tubes and shaken briefly on a mechanical agitator. Proceed in the same manner for the positive serum .
4. Added 100 µl aliquots of the 1:100 diluted sample 2 in wells c1 and c2 etc ..
5. Incubated the plate at room temperature (18-24°C) for one hour.
6. Rinsed the plate with the washing solution, prepared as instructed in the " Composition of the Kit " section , as follows : Empty the microplate

of its contents by flipping it over sharply above a sink . Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill the used wells with the washing solution using a squeeze bottle or by plunging the plate in a vessel of the right dimensions. Then empty the wells once more by turning the plate over above a sink .Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times go on to the next step.

7. We diluted the conjugate 1:50 in the buffer for dilution added 100 µl of the dilute conjugate solution to each well. incubate at (18-24°C) temperature for 1hour.
8. Washed the plate as described in step 8 above .
9. Prepared the indicator solution extemporaneously as follows : Added 12 drops of chromogen to 9.5 ml of the substrate solution . Mixed thoroughly , and then applied to the plate immediately in volumes of 100 µl per microwell . At the time of distribution of the chromogen-substrate mixture on the plates the solution must be completely colorless.
10. Incubated for 10 minutes at room temperature(18-24°C) this time is given as a guideline only , for in some circumstances it may be useful to lengthen or shorten the incubation time
11. Added 50 µl of stop solution to each microwell .
12. We readed the optical densities in the microwell using a plate reader and a 450 nm filter . results must be read fairly soon after the stopping solution has been added since the chromogen may crystallize in wells with strong signals and thereby distort the data (4).

Results

The results of the present study showed that seropositive cattle and sheep were (74.5%) and (76.7%) respectively while the negative results of cattle and sheep were (25.4%) and (23.2%) respectively table (1). The results showed also there were relationship between

seropositive animal and their age group so the rate of infection was increased in cattle with ages more than and less than one year which recorded (82.3%) and (17.6%) respectively table (2). also in sheep and in the same way revealed (44.1%) and (55.8%) respectively table (3).

Table (1) the percentage of seropositive animal of *F. hepatic* in sheep and cattle

sample	Serum N.	+	%	-	%
Cattle	51	38	%74.5	13	%25.5
sheep	43	33	%76.7	10	%23.3

Table (2) the relationship between seropositive cattle and age group .

Age of cattle	Sever infection +++	Moderate infection ++	Positive +	Suspected +/-	0	Total N.	%
cattle \leq 1 year	0	1	3	3	2	9	17.6%
cattle $>$ 1 year	8	7	11	5	11	42	82.3%
						51	

Table (3) the relationship between seropositive sheep and age group .

Age of sheep	Sever infection +++	Moderate infection ++	Positive +	Suspected +/-	0	Total N.	%
Sheep \leq 1 year	3	6	5	3	7	24	55.8%
Sheep $>$ 1 year	7	2	6	1	3	19	44.1%
						43	

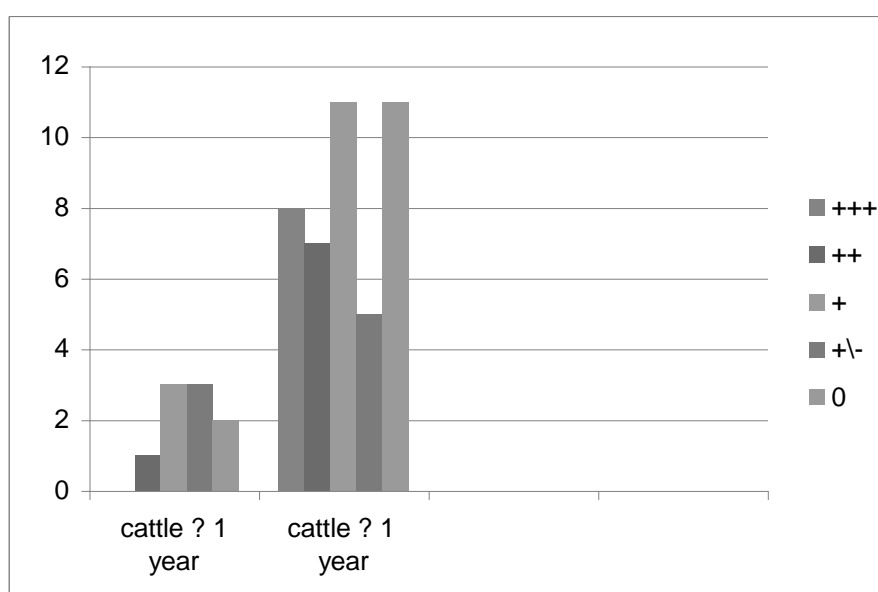


Fig (1) the relationship between seropositive cattle and age group.

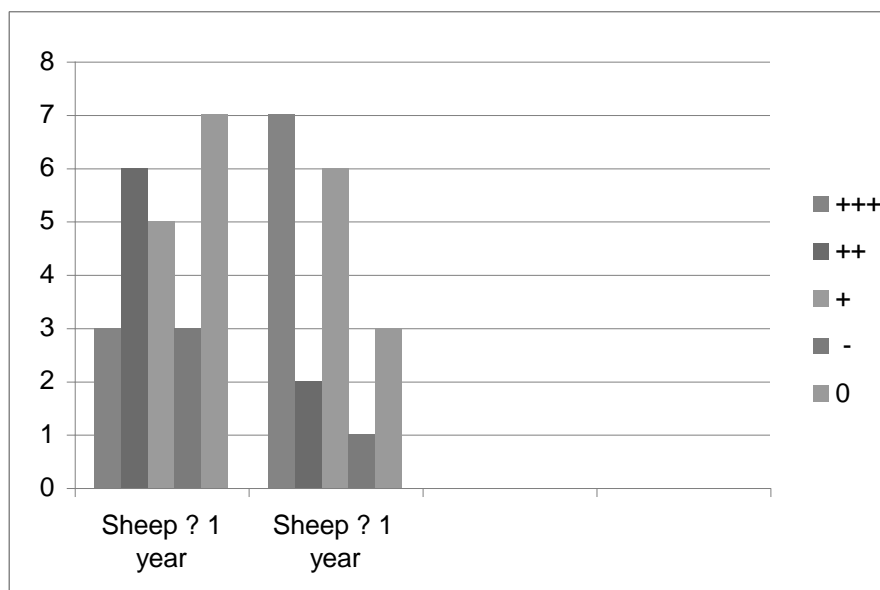


Fig (2) the relationship between seropositive sheep and age group.

Discussion

F. hepatica is a worldwide parasitic disease common in sheep and cattle. Diagnosis of *F. hepatica* in cattle can only be performed after 8 -10 weeks by coprological examination of fecal material however sometimes even repeated fecal examination cannot identify any *F. hepatica* infection due to lack of sensitivity of this method. The result of this study showed high prevalence of seropositive sheep and cattle 76.7% & 74.5% respectively which were in agreement slightly with (4) who found 90% seropositive result. This result is higher than our results may be due to variation in geographical area and climatic condition and presence of low lying area which is important to complete the life cycle. (4) mention that the sensitivity and specificity of ELISA in detection of fascioliasis in sheep and cattle were 90% and 80% respectively. It has been conducted that ELISA is an authentic test for diagnosis of infected animals with *F. hepatica*. The only confirmatory test for diagnosis of fascioliasis is finding the parasite egg in feces or duodenal lavage of infection patient, however other test like complement fixation test and skin test are also used for diagnosis of *F. hepatica* but they are characterized by lower sensitivity than above confirmatory test, for example only

after 7 – 11 weeks since the beginning of infection, it is possible to find parasite egg in feces, it has been shown that patients ingest animals' liver containing parasite egg and produce false positive result in stool microscopic examination (pseudo fascioliasis) (5). So one of definitive diagnosis is ELISA test. The employment of ELISA test for diagnosis of fascioliasis is possible after 2 - 4 weeks post infection and the sensitivity of ELISA is about 98% (6). (7) they reported that the positive result in ELISA and fecal examination were 86% and 51% respectively and that is in agreement with our study. Also the ability to diagnose and treat infection is a high advantage of the ELISA because it minimizes tissue damage in the infected animals caused by immature flukes as they migrate through the liver, early treatment prevents shedding of egg in feces, thus contributing to effective management by reducing the rate of infection (8). The result of this study related to age group and seropositivity relationship showed that cattle more than one year and less than one year age group 82.3% and 17.6% respectively while sheep more than one year and less than one year 44.1 % & 55.8% respectively. (9) recorded that detection of *F. hepatica* in human by ELISA were all positive cases

occurred in children between the age of 9 – 12 years old because children may become incontact with *F. hepatica* metacercaria by drinking contaminated water or eating vegetables growing near ditches because

they are responsible for taking herds of sheep to grazing field and that in agreement with our study as infection occur when there is suitable condition and suitable chance to complete the life cycle .

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تشخيص ديدان الكبد في الأغنام والأبقار بواسطة استعمال تقنية الأليزا

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الخلاصة

استهدفت الدراسة الحالية التحري عن الإصابة بديدان الكبد في الأغنام و الأبقار مناعيا في مدينة الديوانية باستخدام فحص المقايسة المناعي المرتبط بالأنزيم ELISA . أظهرت النتائج نسب عالية للإصابة في الأبقار والأغنام 76.7% و 74.5% على التوالي كما تبين من نتائج الدراسة أن هنالك علاقة بين عمر الحيوان ونسب الإصابة فقد كانت نسبة الأبقار الأكثر والأقل من سنة 82.3% و 17.6% على التوالي بينما كانت في الأغنام الأكثر والأقل من سنة 44.1 % و 55.8 % على التوالي .