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A comparison of enzyme-linked immunosorbent assay and faecal egg examination for the diagnosis of bovine fascioliasis

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ABSTRACT: Enzyme-Linked Immunosorbent Assay (ELISA) for the diagnosis of fascioliasis due to F. gigantica was found to be more sensitive for the diagnosis of fascioliasis when compared with Faecal Egg Examination (FEE). An average monthly prevalence of 79.3% was observed for ELISA while FEE gave a prevalence of 28%. This indicates that parasitological methods of diagnosis underestimates parasite prevalence. There is therefore need to use conventional method of diagnosis in association with serological diagnosis. Using ELISA for Fascioliasis diagnosis did not show definite seasonal pattern of prevalence of *Fasciola* antibody. Anti-Fsciola antibody was present all year round. However, using FEE showed a seasonal pattern with the dry season showing a lower prevalence. This study demonstrate the advantages of immunological diagnosis over routine diagnosis ofparasitic infections.

Key Words: Fascioliasis; *Fasciola gigantica*; ELISA; Diagnostic methods.

Introduction

Fasciolisis is a disease caused by the digenetic trematode of the genus *Faciola*. It is a disease of great economic importance in cattle, sheep and goats husbandry worldwide, and it is recently gaining prominence as a major disease of man. Fascioliasis is widespread in Africa, prevalence rates is higher in countries south of the Sahara (Schillihorn van veen *et al.*, 1980). Ecological diversity and migration of livestock herds result in differences in prevalence rates within each country and between countries in the region.

Several prevalence rates have been recorded for fascioliasis in Nigeria. Pevalence rates as high as 65.4% in cattle has been reported in Zaria (Schillihorn van veen *et al*., 1980) 25-30% in cattle in South Western Nigeria (Ogunrinade, Okon and Fasanmi, 1981), 26% was recorded by Ikeme and Obioha 1973 in Eastern Nigeria while Nwosu and Srirastava (1993) reported 42.7% in cattle slaughtered at Maiduguri, Northeastern Nigeria.

Laboratory diagnosis of fascioliasis is mainly done by demonstration of *Fasciola* ggs in faeces of infected animals. Folaranmi (1975) considered coporological examination as the easiest method of diagnosis while Hillyer, Bermudez, and Arellano (1984) stated that coporological examination lacked sensitivity and was unreliable,because in acute fascioliasis eggs do not appear in faeces. In chronic

infections, repeated faecal examination as often needed to demonstrate fluke eggs in faeces. Serological diagnosis of fscioliasis was therefore found to be a better alternative for diagnosis. In an ttempt to improve the diagnosis of fascioliasis in the laboratory and in field condition, there is ned to compare coporological and immunological methods. This work compares Faecal Egg Examination (FEE) and Enzyme-linked immunoorbent Assay for the diagnosis of fascioliasis in slaughtered cattle in Ibadan municipal battoir, Bodija, Ibadan, Nigeria.

Materials and Methods

Sources of Cattle

The cattle examined for trematodiases were hose brought fo slaughter by local butchers in the Ibadan Municipal abattoir, Bodija, Ibadan, Nigeria between January and December, 1993. These were mostly adult white Fulani Bororo and mixed breed cattle transported to Ibadan in trailers.

Collection of Faecal Samples

Faecal samples were collected from the rectum of total of 700 adult cattle of these different local breeds. Sampling was done randomly weekly.

Coporological Examination for Fluke Eggs

Faeces from slaughtered animals were examind only for the presence or absence of *F. gigantica eggs, S. bovis eggs, P. microbothrium eggs, D. hospes eggs* and *Strongyle eggs* for the purpose of this study.

Faeces from cattle were examined for trematode eggs adopting the sedimentation technique of Theinpont, Rochette and Vanparijs (1979). 1g of faeces was thoroughly emullisified in 10ml of tap water and the suspension was filtered through a sieve into test tube. After allowing sedimentation of the eggs, for about 10 minutes, the supermatant was discarded and the tube refilled with water, The process was repeated three times in order to wash off most of the faecal debris. The resulting sediment was resuspended in 5ml of water and a drop o 2 (aaproximate 0.5ml) of the agitated test tube content was put on a clean glass slide and examined under the microscope for fluke eggs.

Preparation of antigens

Somatic/Adult whole worm antigen (F S)

Somati antigen of *F. gigantica* was prepared as described by Ogunrinade, (1983). 20 freshly collected adult flukes were rinsed thoroughly several times first with distilled water and later with PBS (pH 7.4). The flukes were then homogenized in 10ml of PBS (pH 7.4) with a glass homogenizer. Protease inhibitor was added to the homogenate, and left at 4^{o}C over night, then centrifuged for 10 minutes. The supernatant constituted the somatic antigen. The antigen was aliquoted into eppendorf tubes and stored at -20^{o}C until used. The protein content of the antigen was determined using th method of Bradford, (1976).

Determination of Protein Concentration of Somatic antigen

Protein concentration of the antigen was determined by the method of Bradford (1976) using Bovine Serum albumin (BSA) as standard.

Collection of blood and Sera

Approximtely 10ml of blood was obtained from the jugular vein of the slaughtered animal or by cardiac puncture from 144 of the 700 cattle from which faecal samples were collected. Care was taken to make sure the samples ere well labelled so that sera and faecal samples could be tied during analysis.

On getting to the laboratory the blood samples were left on the bench for about 2 hours and then transferred to the fridge and left over night. Sera samples were collected the next day and cleared by centrifugation at 5,000 rpm for 10 minutes.

Another 61 samples were collected. 10 had monospecific infection of *F. gigantica*, 8 samples with mixed infection of *F. gigantica and P. microbothrium*, 2 samples from animals with P. microbothrium infection alone, 1 sample from *D. hospes infected animal*. The remaining 40 samples were from apparently normal cattle.

Enzyme-linked immunosorbent assay (ELISA)

A chequer board titration is recommended for any ELISA system in order to determine an optimum antigen concentration for absorption into ELISA plate. Antigen, serum and conjugate standards for the test were first determined by titrating the dilutions of each of these ELISA while keeping the other variables at known dilutions. The optimal reading between the values obtained for the know positive and negative sera in the titrations were taken as the optimal dilutions for the test. Thus antigen was used at 10μg/ml, bovine sera at 1:20, conjugate dilution 1:2,000. All test plates consisted of positive and negative controls of antigen, antibody and conjugate.

Ninety-six (96) round well bottomed microtitre plates (Dynatech) were coated with 100μg/well of 10μg/ml antigen in sodium carbonate buffer (1:1,000 for FS, FM and FT, 1:50 for FEA). The coated plates were incubated on the bench for 2 hours and then was washed off with 0.05% Tween PBS pH 7.4 (washing buffer). The non-reactive sites were blocked with 5% non-fat milk. 0.1% BSA was incorporated in the antibody and conjugate steps. After washing (3 times each time) 100μg/well of bovine sera (1:20) was added o each well and left to incubate for 1 hour at 37^{o}C. The wells were washed thoroughly 3 times with ELISA washer. 10μg/well of conjugate (peroxidase labelled rabbit anti-bovine 1g G conjugate 1:2,000) diluted in 0.1% BSA Tween PBS was added to each well and incubated at 37^{o}C for I hour. Thereafter the content of each well was aspirated off by the washing machine and washed three times in washing buffer. 10μl of freshly prepared substrate (OPD, 40mg, 10μl of 30% hydrogen peroxide, 0.47g citric acid and 100ml distilled water) or (10mg of P-nitrophenyl phosphate in 10mls of 10% DEA) was added to each well. Plates were incubated at room temperature in subdued light for 15-30 minutes. The reaction was stopped with 1M, H_2SO_4. The optical density of each reaction mixture in the well was read at 492nM on an ELISA reader.

Results

Prevalence of Trematode Eggs in Faeces Using FEE

Results show that concurrent infection of cattle with *F. gigantica, P. microbothrium, S. bovis, D. hopes* is frequent and Strongyle eggs were also common during the survey period. Of the 700 cattle examined, 196 (28.2%) were infected with *F. gigantica*, 178 (25.0%) with *P. microbothrium*, 172 (24.6%) with *D. hospes*, 72 (10.3%) with *S, bovis* and 139 (19.49%) with *Strongyles sp.* (Table 1). Table 2 shows that the faecal egg output for the trematodes showed a seasonal pattern with the dry season recording lower incidence while the rainy season showed a higher incidence with July and August recording the highest incidence (50 – 60%) while November through January recorded the lowest incidence (8.3 – 12.5%) for *F. gigantica. P. microbothrium* and *D. hospes* had their highest incidence in April (83% and 50% respectively). At the peak of the rains (June and July) the incidence of *P. microbothrium* and *D. hospes* dropped , picking up again in August and September and fell again during November through January. It appears that the incidence of *P. microbothrium, D. hospes* and *S. bovis* follow a similar patter to that of *F. gigantica*. The incidence of Strongyle was high from April through October (17%) with only the dry season experiencing low incidence (0.8%). A few animals had single infection of either *F. gigantica, P. microbothrium, D. hospes* and *S. bovis*.

The frequency of occurrence of the trematodes in association with Strongyle species was highest for S. bovis. 99.4% of animals examined had mixed infection with either *P. microbothrium, D. hospes, S. bovis* or a combination of these three trematodes occurring with F. gigantica. Mixed infection of *F. gigantica*

with *P. microbothrium* was 14.3% while mixed infection of *F. gigantica* with *S. bovis* was 12.9%. 11.4% was recorded for D. hospes and F. gigantica. The overall prevalence determined by faecal examination between January and December 1993 were 28% (196) for F. gigantica, 25.4% (178) for P. microbothrium, 24.6% (172) for D. hospes, 10.3 (72) for S. bovis and 19.4% for Strongyle species.

Sensitivity and specificity of antibody ELISA using somatic antigen of F. gigantica

ELISA using somatic antigen from adult flukes was used to detect the presence of anti-*F. gigantica* antibody in serum samples from 144 of the 700 slaughtered cattle examined. Positivity of serum samples in ELISA was correlated with prevalence of *F. gigantica* eggs.

The results are presented in Figs. 1 and 2 as well as Table 2. All animals positive for fluke worm eggs in FEE were alsopositive for ELISA. However, some animals which were negative for fluke worm eggs were positive fro ELISA. Percentage positivity in ELISA for the presence of *F. gigantica* antibodies was high through the survey period with no definite seasonal pattern. Prevalence was between 70 and 100% with the highest percentages observed from November through February. The months of May to August which had the highest incidence of fluke worm egg also had consistently high seroprevalence. These results suggest that animals had chronic infections throughout the year and that most animals in the field are exposed to repeated infections. Furthermore, *Fasciola* antibodies are almost always present in circulation even when egg output is low or absent. The implication of cross-reactive nematode antigens is also observed as some animals which were positive for either *S. bovis* or *P. microbothrium* also showed high optical density values in some cases which was indicative of cross-reactivity of these antigens with *F. gigantica*. The implication of the presence of *Strongyle* sp. On cross-reactivity was however not investigated.

Table 2 shows the comparison between results obtained for the months of August, September and November for FEE and ELISA. Some of the positive samples which were missed by FEE were detected by ELISA. Some of the samples which were negative for fluke eggs may have been animals with acute fascioliasis or prepatent infection with eggs in faeces. Only one false negative was obtained in ELISA (sample 3, Aug.). Some false positives were also observed (samples 2, 6 (Aug.), sample 2 (Sept.) and sample 1 (Nov.)). These could be as a result of common antigen shared between *S. bovis, D. hospes* and *F. gigantica*.

Table 1: Incidence of Fasciola gigantica and intercurrent trematodes in slaughtered cattle at Ibadan Municipal abattoir, January – December, 1993.

Months	No. of cattle	*F. gigantica*	*P. microbothrium*	*D. hospes*	*S. bovis*	Strongyle
Jan	96	12 (12.5)	16 (16.7)	16 (16.7)	–	–
Feb	100	36 (36.0)	32 (32.0)	12 (12.0)	–	–
Apr	48	8 (16.7)	40 (83.3)	24 (50.0)	–	20 (41.7)
May	88	40 (45.5)	32 (36.4)	32 (36.4)	–	20 (22.7)
Jun	72	24 (33.3)	12 (16.7)	4 (5.6)	–	12 (16.7)
Jul	40	20 (50.0)	8 (20.0)	4 (10.0)	20 (50.0)	28 (70.0)
Aug	40	24 (60.0)	16 (40.0)	16 (40.0)	16 (40.0)	24 (60.0)
Sep	60	20 (33.3)	20 (33.3)	24 (40.0)	12 (20.0)	12 (20.0)
Oct	60	4 (6.7)	12 (20.0)	24 (40.0)	16 (26.7)	12 (20.0)
Nov	48	4 (8.3)	–	8 (16.7)	4 (8.3)	4 (8.3)
Dec	48	4 (8.3)	–	8 (16.7)	4 (8.3)	4 (8.3)
Total	700	196 (28.2)	178 (25.0)	172 (24.6)	72 (10.3)	136 (19.4)

Figures represent the number of positives with percentages in parentheses.

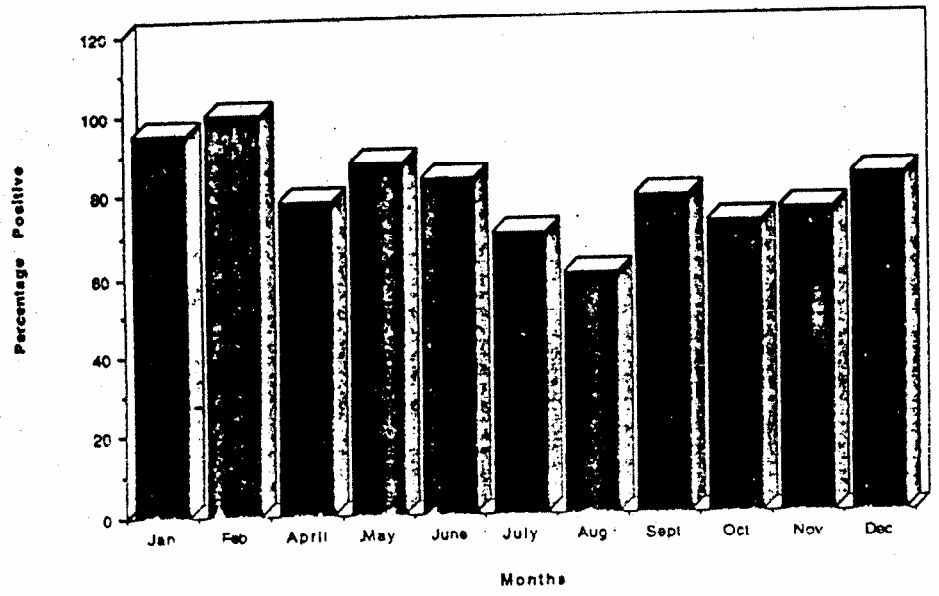

Fig. 1: Percentage positive of cattle sera for the seroprevalence of bovine fascioliasis in slaughtered cattle in Ibadan for 1993, using ELISA.

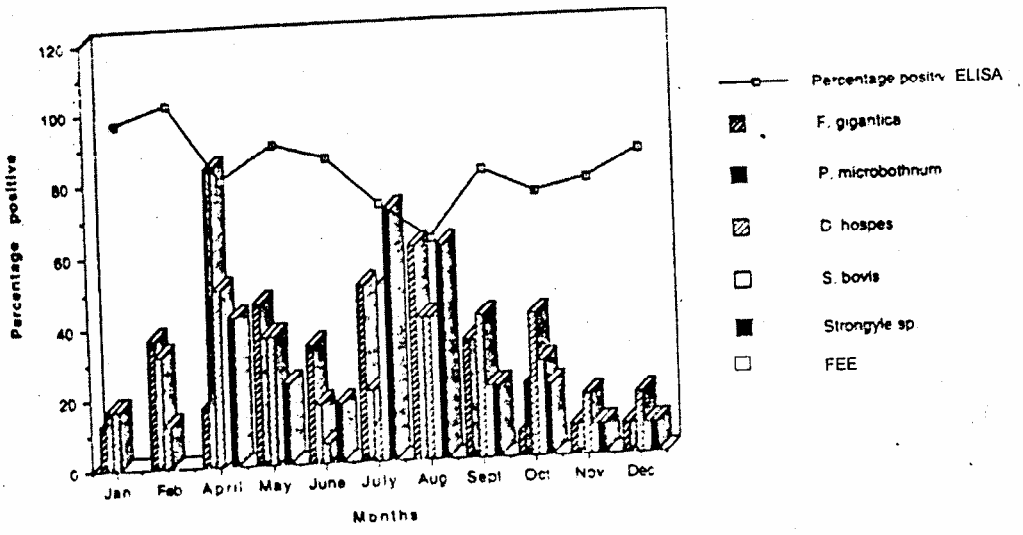

Fig. 2: Comparison of positive animals in ELISA and faecal egg examination for liver fluke infection in slaughtered cattle between January and December, 1993 at Ibadan, Nigeria.

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Discussion

This study compared the sensitivity of ELISA using somatic antigen with coporological examination or faecal egg examination for the diagnosis of fascioliasis.

The results of the ELISA test showed that antibody against *F. gigantica* could be demonstrated in the sera of naturally infected cattle all the year round. A monthly prevalence of between 60 and 100 per cent was observed with a monthly mean prevalence of 79.3% for the survey period. This prevalence rate observed in ELISA as against the 28% observed for faecal egg examination (FEE) highlights the advantage of serodiagnosis over coprological examination for fluke ova. This indicates that current parasitological methods of diagnosis consistently underestimate parasite prevalence (Parkhouse *et al*, 1987). There is, therefore, a need to use conventional methods of diagnosis in association with serodiagnosis. Using ELISA, there was, however, no definite seasonal pattern for the prevalence of *Fasciola* antibody. Anti-*F. gigantica* antibody was present all year round. This suggests that most animals had chronic infection throughout the year and long after the inducing worm population had been lost. Furthermore, present or past infection with cross-reacting helminthes or tematodes may be responsible for some of the positive results (false positives) in ELISA. Fagbemi and Obarisiagbon (1991) reported the cross-reactivity between antigens of *F. gigantica, D. hospes* and *S. bovis*. Samples 2 and 6 (August) and 1 (November) were positive fro *S. bovis* and *D. hospes* eggs respectively, but they were serologically positive in ELISA. This supports the findings of these workers. Some of the false positive results in ELISA were therefore due to exposure of animals to either *S. bovis, P. microbothrium, D. hospes* or acute fascioliasis. There was only one false negative in ELISA (Sample 3 of August). False negative results are difficult to explain.

Almost all the cattle (99.4%) examined in this study had multiple trematodiasis which is a common feature on the field. Some cross-reactions of *Fasciola gigantica* antigens with concurrent trematodes were observed. Therefore, there is need to purify this antigen so as to further enhance the specific diagnosis of bovine fascioliasis in a situation where there is need to distinguish between Fasciola gigantica and other trematodes.

Table 2: Representative data comparing FEE and ELISA for the serodiagnosis of fascioliasis.

Number	Aug FEE	ELISA	Sep FEE	ELISA	Nov FEE	ELISA
1.	F.g, Strongyle	+	–	+		+
2.	S.b, Strongyle	+	S.b	+		+
3.	F.g, P.m, Strongyle	–	P.m, D.h, Strongyle	–		+
4.	D.h, Strongyle	–	Strongyle	+		–
5.	D.h, Strongyle	–	F.g, D.h, P.m	+		–
6.	S.b	+	F.g, D.h	–		+
7.	F.g, Strongyle	+	D.h	–		+
8.	S.b, F.g, P.m, Strongyle	+	P.m, S.b, D.h	+	F.g, S.b	+
9.	F.g, S.b	+	–	+		+
10.		–	–	+		–
11.			–	–	D.h, Strongyle	+
12.			–	+		+
13.			F.g, S.b, D.h, Strongyle	+		
14.			F.g, P.m	+		

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