



Detection of *Fasciola gigantica* antibodies using Pourquier ELISA kit

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Abstract

A cross sectional study using Pourquier Enzyme Linked Immunosorbent Assay (ELISA) screening kit for *Fasciola* antibodies was conducted in breeding herds in two Local Government Areas of Adamawa state. The objectives were to determine the presence of *Fasciola gigantica* antibodies as a way of demonstrating the use of the ELISA screening method in breeding cattle, compare the occurrence of the disease in the two areas and to also determine if the kit can serve as a feasible antemortem diagnostic and screening approach in breeding herd in a tropical developing country with possible under current infection. Two hundred and twenty five (225) serum samples from breeding herds were randomly collected and used. The samples were drawn from both matured and immature male and female cattle of white Fulani, Adamawa Gudali, Red Bororo and Sokoto Gudali breeds. An indirect ELISA analysis method was employed using the protocol designed by the manufacturer. The validity of the test for each plate was carried out. Results confirmed the presence of *Fasciola gigantica* antibodies: 162 positive cases out of the 225 cattle sampled, which gave an overall prevalence of 72%. Antibodies detected by the ELISA reader indicates that the cattle were affected by the disease at different infestation levels (0=28%, +=11.1%, ++=13.7%, +++=47.1%). Analysis with a t-test paired sample statistics showed that there was no significant difference in fasciolosis ($p>0.05$) between the 2 local government areas. The assay detected antibodies in sera from cattle with natural *Fasciola gigantica* infection. Therefore the kit was found very effective in detection of fasciola infection in live animals. We conclude that the kit will be a valuable tool for diagnosing *Fasciola gigantica* infection in cattle.

Keywords: Cattle, Fasciolosis, Gigantica antibodies, Pourquier ELISA, Prevalence, Sera sample.

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Introduction

Fasciolosis is a parasitic liver infection of humans, wild and domestic ruminants caused by trematodes of the genus *Fasciola*. *Fasciola gigantica* & *Fasciola hepatica* are responsible for the disease in tropical and temperate regions respectively (Tongson, 1978; Soulsby, 1986). They are normally transmitted by *Lymnae* snail (*Lymnae natalensis* & *Lymnae truncatula*) and do infect the animals at any stage of their life (Dorchies *et al.*, 2000; Lopez *et al.*, 2004). Fasciolosis constitutes an important public health problem in many parts of the world with an estimated 2.4 million people affected (WHO, 1995). The disease is transmitted to humans from contaminated plants and the parasites become

lodged in the biliary ducts of the liver after 3-4 months (Mohammad *et al.*, 2010.). The disease caused up to 12.5% infection among humans (Ekong *et al.*, 2012). Three (3) partially overlapping clinical syndromes of the disease have been identified as acute which occurs due to the prevalence of liver-flukes in the liver and characterized by lethargy and jaundice, depression or death in some cases. Chronic fasciolosis is essentially a wasting disease with the presence of flukes in the bile-duct and characterized by debility and anemia with sub-cutaneous edema in the lower portion of the abdomen, face and thorax while sub-acute fasciolosis is marked by anaemia due to young adult flukes emerging from the liver

into the bile duct (Losos, 1995).

Huge economic losses to cattle rearers in Nigeria through mortalities, abortion, decrease in weight gain, reduce milk, unthriftiness, condemnation of infected livers and cost of treatment, as a result of fasciolosis, have been reported (Fabiya *et al.*, 1980). World Association for the advancement of veterinary parasitology (2005) reported that up to 700 million cattle are exposed to liver-flukes annually causing an economic loss of 3.2 billion dollars.

Apart from these economic losses, there is also the problem of parasite diagnosis at the right time (Mira & Ralph, 1994). The commonest diagnostic method in Nigeria is the traditional/coprological/egg count method as well as the post mortem liver examination. The traditional egg count method is cumbersome and labour intensive with sensitivity as low as 30% in animals shedding small number of eggs (Happich & Boray, 1969; Burger, 1992; Reichel, 2002). In addition, the method is incapable of detecting early infection as eggs do not appear in faeces until between 77 – 84 days post infection (Jemli *et al.*, 1992; Reichel, 2002).

In contrast, the use of ELISA kit manufactured by Institut – Pourquier which employs the “f2” antigen is capable of detecting antibodies of fasciola hence it allows early detection of the disease as fluke specific antibodies can be detected in the serum of most animals by 14 days post infection (Hillyer, 1999; Castro *et al.*, 2000; Kaplan, 2001; Reichel, 2002). During the migration of liver flukes through the liver parenchyma (7-35 days after infection), the immature flukes released antigens (which stimulate the production of antibodies) which are detectable in the serum but once the flukes reached the bile ducts, antigenic release ends and antigens are no longer present in sera hence the demonstration of circulating antigens in sera definitely allows the early detection of live immature flukes (Sanchez-Andrade *et al.*, 2001). Also, the presence of liver flukes is not determined necessarily by a positive antibody serum result which normally indicates that an animal is, or has been infected by the parasite (Reichel, 2002). The use of the ELISA method is justified as it has been proven to be more sensitive compared to the traditional egg count method. This sensitivity was demonstrated in France when 1,308 heifers were subjected to faecal examination and specific ELISA's were performed on sera samples at the same time. Results showed that cattle shedding eggs were specific in only 20% of the herd while specific antibodies (from ELISA) gave 93% (Dorchies, 2006). Thus, Pourquier ELISA often detect infections due to

immature flukes when faecal egg output is nil. Muhammad *et al.* (2010) also observed that the coprological technique lacks sensitivity and the results usually mislead physicians. Salimi- Bejestani *et al.*, (2011) reported that the diagnostic sensitivity and specificity of the ELISA test is in the region of 98% in cattle and sheep; hence serological and immunological methods are at the fore front of investigating fasciolosis. This study was carried out to demonstrate the use of the ELISA screening method which has been reported to have high sensitivity and specificity (implies that when it is applied properly, treatment can be administered in good time before irreparable damage occurs to the liver).

Materials and methods

Study area

Yola south (which stretches over the upper Benue river) and Girei Local Government areas of Adamawa state are located in north eastern Nigeria at an altitude of 800m above sea level within the northern guinea savannah/sub-humid region between latitude 9°14'N of the equator and latitude 12°18'E of the Greenwich Meridian and with a mixed farming system. The annual rainfall is between 750mm – 1,100mm and rainy season is from May – October with temperatures ranging from 15.2°C – 40°C (Adebayo & Tukur, 1999).

Sample collection

Blood samples were collected in the field from 225 cattle (112 from Girei and 113 from Yola south) using the random sampling method (Plews, 1979). Samples were collected from both male and female White fulani, Red bororo, Adamawa gudali and Sokoto gudali breeds in September and October, 2013. Syringes were used to puncture the jugular vein after restraining the animals and the blood samples obtained were allowed to coagulate and in some cases centrifuged to obtain the sera which were dispensed into vacutainer bottles and stored at -20°C until the time of analysis.

Sample analysis

The serum samples were analyzed based on the method proposed by Soule *et al.* (1989), Levieux *et al.* (1992); as well as Crowther (1998) and adopted by Institut-Pourquier. Briefly, the even numbered microplates were already coated with “f2” antigen from the company. The controls were diluted to 1/20 by dispensing 190 µl of dilution buffer per well followed by 10 µl of undiluted negative control and

10 µl of undiluted positive control. The sera samples were also diluted to 1/20 by dispensing 190 µl of dilution buffer per well and 10 µl of each serum in one coated well and 10 µl of each serum in one uncoated well; using the multichannel micropipette in each case. Any antibodies in the sample specific to the “f2” antigen in the coated plate formed an “f2” antigen – antibody immune-complex and remained bound in the wells. This was followed by washing, addition of conjugates and other steps as specified in the protocol. The optical densities were read at 450nm (OD 450).

Statistical analysis

Data was analyzed using non-parametric inferential statistics particularly paired sample t-test to determine whether the disease differs with or without significant differences between the 2 local government areas. Descriptive statistical tools like tables, simple percentages and multiple bar charts were also used.

Results

Table 1 shows the validity of the test. The criteria for a valid test for each plate is that the mean

uncorrected OD450 value of the positive control should be >0.350 and the ratio between the mean uncorrected OD450 value of the positive control and corrected OD450 value of the negative control should be ≥ 3, 5.

Table 2: In plate 1, 20 samples are negative while 25 are positive at various antibody levels of infestation (+=5, +=7, +++=13) while plate 2 had 19 negatives and 26 positives (+=3, +=8, +++=15). Plate 3 recorded 13 negatives and 32 positives (+=4, +=0, +++=28). For plate 4, the negatives were just 6 while 39 were positive (+=8, +=5, +++=26). Plate 5 had 5 negatives (1 for Girei and 4 for Yola south) and 40 positives (21 for Girei and 19 for Yola south) also at various antibody levels of infestation (+=5, +=11, +++=24). Table 3 showed that the overall prevalence rate for the 2 local government areas is 72%. The multiple bar charts in figure 1 and 2 showed the levels of antibody infestations as well as the negative and positive values for each plate. Analysis with paired sample t-test showed that there is no significant difference (P > 0.05) for fasciolosis between the 2 local government areas.

Table 1: Validation values for the tests.

Plates	Validation values	
	Mean uncorrected OD 450 Value of +ve control	Mean OD 450 of positive control/ corrected OD 450 of –ve control
1	0.581	48.625
2	0.576	13.720
3	0.796	11.030
4	0.612	20.558
5	0.624	20.311

Table 2: Frequency of antibodies detected by Pourqueir ELISA kit.

L.G.A	Plate	Level of infestation				Total
		0	+	++	+++	
Girei	1	20	5	7	13	45
Yola south	2	19	3	8	15	45
Girei	3	13	4	0	28	45
Yola south	4	6	8	5	26	45
Girei (22), Yola south (23)	5	5	5	11	24	45
	Total	63	25	31	106	225
Degree of Infestation (%)		28	11.1	13.7	47.1	

Table 3: The overall prevalence of *Fasciola gigantica* antibodies detected (2010).

Month	Number examined	Number +ve	Prevalence rate
September	90	51	56.7%
October	135	111	82.2%
Total	225	162	72%

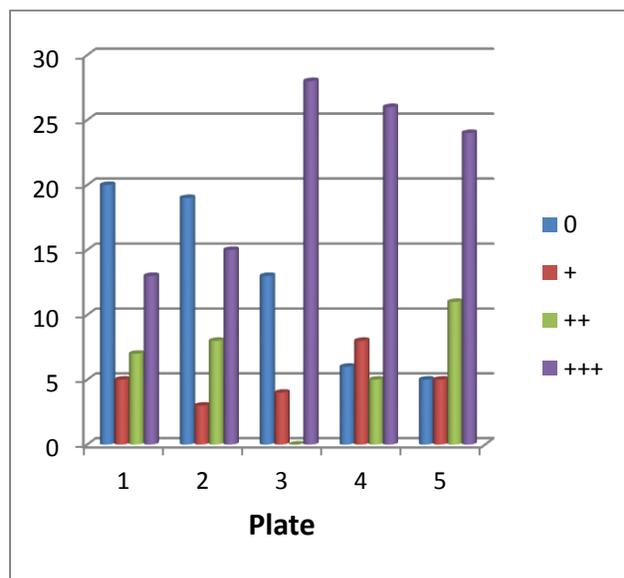


Figure 1: Chart showing level of antibodies detected by each plate.

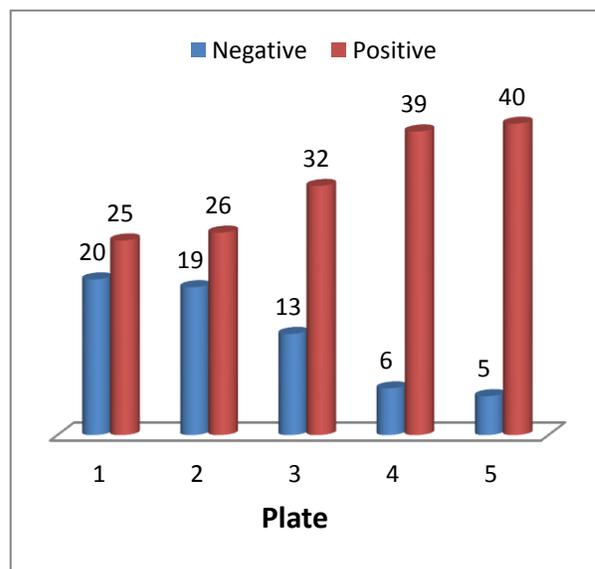


Figure 2: Chart showing level of positive & negative antibodies detected by each plate.

Discussion

The detection of *Fasciola gigantica* antibodies which occurred in the samples indicating different levels of infestation as indicated in table 2, figure 1 and 2 shows that the ELISA screening method is dependable. This is in line with the findings that serological/ELISA tests are the most dependable diagnostic methods (Hilliyer, 1999; Kaplan, 2001) and with high sensitivity and specificity, for example 95%, 98.2% & 98.5% respectively with f2-antigen (Reichel, 2002). Thus, as a screening tool, the ability to diagnose early leads to early treatment which is a big advantage because it minimizes tissue damage in the infected animals caused by immature flukes as they migrate through the liver (Sanchez-Andrade *et al.*, 2001).

The overall percentage prevalence of 72% as shown in table 3 also indicates that fasciolosis is still endemic within the study area despite various attempts at deworming cattle. This situation confirms with the report that despite repetitive treatment using efficient drugs, the prevalence of parasites have remained high (Maizels, 2004). Since

statistical analysis showed that there is no significant difference ($p > 0.05$) in the disease between the 2 local government areas; it implies that there is little or no variation in the factors responsible for fasciolosis in the 2 areas such as the ecological conditions of altitude, rainfall, temperature and livestock management system. This contrast with the result obtained in Ethiopia where differences in the prevalence of fasciolosis occurred from locality to locality due to variation caused mainly by altitude (Graber & Daynes, 1974).

It is therefore recommended that cattle should be treated based on their level of infection so as to ensure specificity thereby avoiding overdose or wastage of drugs.

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