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Epidemiological studies of *Fasciola gigantica* in cattle in Zaria, Nigeria using coprology and serology

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Fasciolosis is an important helminth disease of livestock and other ruminants. A cross sectional study to determine the prevalence of *Fasciola gigantica* in cattle was carried out in 9 randomly selected farms and 1 slaughter house between February and May, 2012. Faecal and blood samples were collected from 186 cattle in the farms and 200 cattle at slaughter. The faecal samples were analysed using the formol-ether sedimentation technique and the blood by Indirect ELISA kit (Bio-X-Diagnostic, ID VET Jemelle-Belgium) to detect *F. gigantica* eggs and antibodies to *F. gigantica* antigens, respectively. Of the 200 faecal samples collected at slaughter, 39 (19.5%) had *F. gigantica* eggs, as compared to 27 (14.5%) positives out of the 186 samples collected from the farms, giving an overall prevalence rate of 66 (17.1%). There was no significant difference (P>0.05) between prevalence of infection of cattle sampled in the farms and slaughter house. 23 (11.5%) of the sera prepared from the 200 blood samples obtained at slaughter had antibodies to *Fasciola hepatica* antigens, as against 5 (2.6%) for sera from 186 blood samples collected in the farms, giving an overall seroprevalence of 28 (7.3%). There was significant difference (P<0.05) between infection at slaughter and on farms. Out of the 200 cattle from slaughter, 20 (10.0%) had *F. gigantica* eggs and also were seropositive for *F. hepatica* antigens, and of the 186 cattle from farms, only 5 (2.7%) had *Fasciola* eggs and were also seropositive for *F. hepatica* antigens. Both at slaughter and on farms, infection was more prevalent in females than in males. The overall prevalence for females using coprology and ELISA were 19.3 (41/212) and 7.5% (16/212), respectively. The respective values for males were 13.7 (24/174) and 6.89% (12/174). However, the difference in the prevalence of females and males obtained was not statistically significant (P>0.05). No statistical difference was observed in breed prevalence. This study has established *F. gigantica* prevalence of 17.1 and 7.3% by coprological and serological examinations of faeces and blood of cattle in Zaria. It is recommended that cattle should be dewormed regularly and further serological screening be embarked in other local government areas of Kaduna State, so as to know the current status of *F. gigantica* infection in cattle.

Key words: Cattle, fasciolosis, prevalence, farms, slaughter house.

INTRODUCTION

Fasciolosis also known as fascioliasis, distomatosis and liver rot, is an important helminth disease caused by trematode species, *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica*. The disease belongs to the plant-borne trematode infection and the definitive host rangeisverybroadandincludesmanyherbivorousmammals

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and humans (Mas-Coma et al., 2005). *F. hepatica* has a worldwide distribution due to its capacity to infect many different species and the ability of the intermediate snail host to adapt to wide range of ecological niches (Garcia et al., 2007).

F. hepatica infects more than 300 million cattle and 250 million sheep worldwide and together with *F. gigantica*, causes significant economic losses to global agriculture; estimated at more than US$3 billion annually through lost productivity, such as a reduction of milk and meat yields (Mas-Coma, 1997).

Fasciolosis is a disease of public health and economic importance (Ashrafi et al., 2006). It causes serious disease of cattle, sheep, goats, buffalo and other ruminants. In cattle, the disease is debilitating, decreasing production of milk and result in losses due to condemned livers when the animals are slaughtered (Vassilev and Jooster, 1991). It can also lead to chronic low-grade anaemia and emaciated carcasses at slaughter.

In any country of the world, the goal of cattle production is to make money or provide some advantages such as a cheap source of labour and animal protein for the populace (Dixon et al., 2001). They also play an important role in the social and cultural life of most of the communities in the northern part of Nigeria (Tewe, 1997). However, the productivity of cattle has been limited by parasitic infections including fasciolosis (Keyyu et al., 2005) and this threatens the food security of the population and its social balance.

Apart from its veterinary importance throughout the world, fasciolosis is now recognized as an important emerging zoonotic disease of humans. Prior to 1992, the total number of reported human cases of fasciolosis was estimated to be less than 3000. More recent figures suggest that between 2.4 and 17 million people are currently infected, with a further 91.1 million living at risk of infection (Keiser and Utzinger, 2005). Drinking untreated water may be a source of infection due to the presence of free-floating metacercarial cysts. Vegetables washed in contaminated water may also become a source of infection (Taira et al., 1997; Mas-Coma et al., 2005). Although reported incidence and prevalence of the disease varies widely from country to country, prevalence rate in developed countries can reach up to 77%, but ranges from 30 to 90% in cattle in tropical countries (Spithill et al., 1999).

In Nigeria, the first incidence of fasciolosis was reported by Burke (1939) when about 3000 goats died of the disease in the then Borno province. In a South-Western State of Nigeria, a gross total liver loss of 8.292 kg was observed with about 75% loss of value in 29.952 kg of partially condemned livers in a single abattoir over a three-year period (World Bank, 2006). Estimating that each of the 36 states and the Federal Capital Territory will record similar losses in at least one abattoir per state, this will translate to huge loss of resources (US$ 5,762,010) for the country. These enormous losses are especially important for a low-income food-deficient country (LIFDC) like Nigeria (World Bank, 2006).

In Zaria, Kaduna State, cattle population was reported to be 1,144,000 (KDSG, 2008) and about 99% of these cattle were being managed/reared under semi-intensive, extensive or pastoral system. Most herdsmen graze their herds along the river banks during the prolonged dry season when the upland pasture is poor in quantity and quality. These animals are thus exposed to high risks of *Fasciola* infection (Olumide and Mpoko, 2001).

Most prevalence studies in Zaria and other parts of the country have been based mainly on abattoir records, with few on faecal examination (Ademola, 2003; Kamani et al., 2007). A serological method like enzyme-linked immunosorbent assay (ELISA) which detects all stages of the infection will be needed to have a more reliable figure on prevalence of the infection. This study on prevalence will therefore provide information on the seroprevalence and the current status of *F. gigantica* at slaughter and on farms in Zaria, and also to determine association between *F. gigantica* infection and age, sex and breed of cattle in Zaria.

MATERIALS AND METHODS

Study area

The study area is Zaria, a major city in Kaduna State in Northern Nigeria located within latitudes 11°7, 11°12 N and longitudes 07°41 E. It has an estimated population of 547,000 and a growth rate of 3.5% per annum. Zaria is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7°C and an annual rainfall of 1092.8 mm. It is approximated that about 40 to 75% of its working population derive their principal means of livelihood from agriculture (ABU, 2000). Agricultural activity in Zaria can be divided into two types: rain-fed (from May to October) and irrigation farming in the dry season (from November to April).

Sampling procedure

Samples were collected from cattle in 7 Fulani herds and 2 farms located in Basawa, Dogarawa, Dakachi, Hanwa, Jaja, Tukur-tukur and Zango, an institutional farm (belonging to Ahmadu Bello University and located in Samaru, a private farm (located in Zaria city) as well as in slaughtered cattle). Animals to be sampled were selected based on the simple random (without replacement) method (Fatimah, 2003). At least 20% of the animals in the herd were sampled. Slaughter cattle from Zaria abattoir (Zango) were also selected based on systematic random technique, the first forty cattle slaughtered were sampled (Fatimah, 2003).

Visits were made to the farms and nomadic herds between March and May, 2012, while visits to the abattoir took place once a week (7:00 to 10:00 am), the period when animals are slaughtered in the abattoir between February and April, 2012.

Sample collection and handling

From each animal sampled, blood and faecal samples were collected. The estimated age by dentition (Pace and Wakeman, 2003), sex and breed were recorded. On farms, each animal was properly restrained and 10 ml of whole blood was drawn from the jugular vein using a 10 ml syringe and 18G " 1.5" needle. Blood was

Table 1. Prevalence of *F. gigantica* at slaughter and on farms in Zaria by coprology.

Site	No. examined	No. Positive (%)
Abattoir	200	39 (19.5)
Farms	186	27 (14.5)
Total	386	66 (17.1)

χ^2= 1.689, P value=0.1938.

then transferred into clean, plain, labeled 10 ml bottles and placed in a receptacle allowing for clotting. Serum was then carefully extracted using sterile Pasteur pipettes and deposited in clean 5 ml serum vials which were properly labeled and stored at -25°C in a deep freezer, for subsequent serological analysis. About 4 g of faecal samples were collected from farm and abattoir prior to slaughter using a polythene bag worn over the fingers. All the samples were properly labeled and transported to the Parasitic Zoonosis Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for examination.

Enzyme-linked immunosorbent assay (ELISA)

The serum samples were analyzed using indirect ELISA Kit obtained from Bio-X-Diagnostics (Jemelle, Belgium) according to manufacturer's instructions. The format of the ELISA plates was such that alternate columns (1, 3, 5, 7, 9, and 11) were coated with *F. hepatica* antigen and the even columns (2, 4, 6, 8, and 12) contained only the monoclonal antibody. This is a genuine negative control to differentiate specific anti-*F. hepatica* antibodies from non-specific ones.

The test blood sera were diluted 1:100 in the dilution buffer and 100 µl was applied to a coated and uncoated well respecting the following pattern. Positive serum: wells Al and A_2, sample 1: wells B1 and B2, sample 2: wells C1 and C2 and the plate incubated at 21°C for 1 h.

After the incubation period, plates were washed and 100 µl of conjugate, a peroxidase-labeled anti-bovine lgG1 monoclonal antibody, was added to each well and incubated at room temperature for 1 h and was washed again. 100 µl of substrate chromogen tetramethylbenzidine (TMB) was added to each well and incubated for 10 min at 21°C protected from the light and uncovered. 50 µl of the stop solution (1 M phosphoric acid) was added and the plate read with an ELISA plate reader at 450 nm according to manufacturer's instruction. The test was considered valid if the ratio of the mean optical density (OD) values of the positive and negative controls (OD Pc and OD) was greater than 1.26, following the manufacturer's instruction.

Coprological analysis of sample

Detection of eggs was performed using the formol-ether sedimentation technique as described by Arora and Brij, (2010). Four grams of fecal sample was thoroughly mixed in 10 mm of water and strained through two layers of gauze in a funnel. The filtrate was centrifuged at 2,000 rpm for 2 min. The supernatant was discarded and the sediment resuspended in 10 mm of physiological saline. It was again centrifuged and the supernatant was discarded. The sediment was resuspended in 7 mm of formol saline, after which 3 ml of ether was added. The tube was closed with a stopper and shaken vigorously. The stopper was removed and the tube centrifuged at 2,000 rpm for 2 min. Four layers became visible: the top layer of ether, a second layer of plugs of debris, a third layer of formalin and a fourth layer of sediment. The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid was discarded leaving a small amount of formal saline for resuspending the sediment. A little was transferred to a clean glass slide at a time, covered with a cover slip and examined under the microscope at ×10 magnification to view the eggs. The procedure was repeated until the whole sediment was examined.

Statistical analysis of results

Data collected were reduced to contingency tables and Statistical Package for Social Science (SPSS), version 17.0 (SPSS Chicago Inc) was used to determine Chi-square or Fisher's exact test where appropriate. P values less than 0.05 (P<0.05) were considered to be statistically significant.

RESULTS

Prevalence of *F. gigantica* infection in Zaria by coprology

During the study period, a total of 386 faecal samples were collected, made up of samples collected from 200 individual cattle at slaughter and 186 individual cattle on farms. 39 (19.5%) of the cattle sampled at slaughter and 27 (14.5%) of those sampled in the farms had *F. gigantica* eggs in their faeces with overall prevalence rate of 17.1% (Table 1). Prevalence of *F. gigantica* was thus higher in cattle at slaughter than in cattle on farms, although this was not statistically significant (χ^2=1.689, df=1, P=0.1928).

Of 106 female cattle sampled in the abattoir, 25 (23.6%) had *F. gigantica* eggs, as compared to 14 of the 94 males (14.8%) that were sampled. Of the 106 female cattle sampled in the farms, 16 (15.1%) had *F. gigantica* eggs; while 10 of the 80 (12.5%) sampled males had *F. gigantica* eggs (Table 2). There was no association between sex and infection for cattle sampled at slaughter and on farms (χ^2=2.397, df=1; p=0.1215; χ^2=0.2552, df=1, P=0.6134).

Out of the 173 local breeds sampled in the farms, 27 (15.6%) had *F. gigantica* eggs; while no egg was detected in the samples collected from the 13 foreign breeds (Table 3). Of the 56 young cattle (2 years and below) that were sampled in the farms, 13 (23.2%) had *F. gigantica* eggs in their faeces. Of the 130 adult cattle (>2 years) sampled, 14 (10.8%) had *F. gigantica* eggs (Table 4). There was statistically significant association between the age of cattle and infection (χ^2=4.885; df=1; P=0.0271).

Seroprevalence of *F. gigantica* in Zaria

A total of 23 (11.5%) of the sera collected at slaughter and 5 (2.7%) of those collected from the farms were seropositive for *F. gigantica* infection; giving an overall

Table 2. Sex-specific prevalence of *F. gigantica* at slaughter and on farms in Zaria by coprology.

Sex	Abattoir		Farms	
	No. examined	No. positive (%)	No. examined	No. positive (%)
Male	94	14 (14.9)	80	10 (12.5)
Female	106	25 (23.6)	106	16 (15.1)
Total	200	39 (19.5)	186	26 (13.9)

χ^2= 2.397, P value=0.1215; χ^2= 0.2552, P value=0.16134.

Table 3. Breed-specific prevalence of *F. gigantica* on farms in Zaria by coprology.

Cattle breed	No. examined	No. infected (%)
Local breed	173	27 (15.6)
Foreign breed	13	0 (0)
Total	186	27 (14.5)

Table 4. Age-specific prevalence of *F. gigantica* on farms in Zaria by coprology.

Age	No. examined	No. positive (%)
Young cattle (2 years and below)	56	13 (23.2)
Adult cattle (above 2 years)	130	14 (10.8)
Total	186	27 (14.5)

χ^2= 4.885, P value=0.0271.

seroprevalence rate of 7.3%. Seroprevalence was higher in cattle at slaughter than in cattle on farms. There was statistically significant difference (χ^2=11.12; df=1, P=0.0009) (Table 5).

Of the 106 female cattle sampled in the abattoir, 13 (12.3%) were seropositive for infection; while 10 of the 94 sampled males (10.6%) were seropositive. Of the 106 female cattle sampled in the farms, 3 (2.8%) were seropositive for infection; while 2 of the 80 males (2.5%) were seropositive. There was no significance difference (χ^2=0.1294, df=1, P=0.7191, P=1.0000) (Table 6). Out of the 173 local breeds of cattle examined in the farms, 5 (2.9%) were seropositive, while none of the foreign breeds were infected, giving an overall seroprevalence rate of 2.7% (Table 7).

Of the 56 young cattle (2 years and below) that were sampled in the farms, 3 (5.4%) were seropositive for infection, while 2 (1.5%) of the 130 adult cattle (age above 2 years) were seropositive, giving a seroprevalence rate of 5 (2.7%). Infection rate did not differ significantly (P=0.1616, df=1) between young cattle and adult cattle (Table 8).

Overall prevalence of *F. gigantica* by coprology and serology in Zaria.

The overall prevalence obtained by coprology (17.1%) was higher than the 7.3% obtained using ELISA (Table 9). It was statistically significant (χ^2=11.12, df=1, P=0.000029).

DISCUSSION

Fasciolosis and other helminthes infections have been reported in Northern Nigeria to be wide spread (Elkanah et al., 2006). Studies on the prevalence of fasciolosis due to *F. gigantica* have been carried out in different parts of Nigeria (Ademola, 2003; Kamani et al., 2007). There is however, a general paucity of information regarding seroprevalence of *F. gigantica* at slaughter and on farms in and around Zaria, Nigeria. This study therefore provides current status on the prevalence of *F. gigantica* at slaughter and on farms in the area.

In this study, coprology gave a higher prevalence than ELISA which is a far more sensitive technique. The likely reason for this unusual result is that the antigen and monoclonal antibody used in the ELISA were those of *F. hepatica*. Although they can also detect antibodies to *F. gigantica*, the level of detection will be much lower, since the two species might have marked dissimilarities in their antigenic epitopes. This is in tandem with the findings of Meshgi et al. (2008) in Iran where they found differences in the excretory and somatic antigen of *F. hepatica* and *F. gigantica*.

The prevalence of *F. gigantica* as determined by coprology and serology was higher at slaughter than on

Table 5. Seroprevalence of *Fasciola gigantica* in cattle at slaughter and on farms in Zaria.

Location	No. examined	No. positive (%)
Abattoir	200	23 (11.5)
Farms	186	5 (2.7)
Total	386	28 (7.3)

χ^2= 11.12, P value=0.0009.

Table 6. Sex-specific seroprevalence of *F. gigantica* at slaughter and on farms in Zaria.

Sex	Abattoir		Farms	
	No. examined	No. positive	No. examined	No. positive
Male	94	10 (10.6)	80	2 (2.5)
Female	106	13 (12.3)	106	3 (2.8)
Total	200	23 (11.5)	186	5 (2.7)

χ^2=0.1294, P value=0.7191; P value=1.0000.

Table 7. Breed-specific seroprevalence of *Fasciola gigantica* on farms in Zaria.

Cattle breed	No. examined	No. positive (%)
Local breed	173	5 (2.9)
Foreign	13	0
Total	186	5 (2.9)

Table 8. Age–specific seroprevalence of *Fasciola gigantica* on farms in Zaria.

Age	No. examined	No. positive (%)
Young cattle (2 years and below)	56	3 (5.4)
Adult cattle (above 2 years)	130	2 (1.5)
Total	186	5 (2.7)

P value=0.7191.

Table 9. Comparison of infection detection by coprology and ELISA.

Technique	No. examined	No. positive (%)
Sedimentation	386	66
ELISA	386	28
Total	772	94

χ^2=17.47 P value=0.000029.

farms in Zaria. This could be attributed to the period during which sampling took place, which was the dry season. During this period of the year, the upland pasture is poor both in quantity and quality, as a result of which herdsmen graze their herds along the river banks, where the pasture may be contaminated with metacercariae (the infective stage of *F. gigantica*) which encyst from cercariae released from infected digenea snails which are abundant at the river banks during the dry season. Since most of the slaughtered animals were from the field, a higher prevalence rate may be expected as the on-farm cattle are less exposed to infection. In addition, the on-farm cattle are treated routinely with anthelmintics. Some of the farms sampled in this study for instance, the private and the institutional farms do not allow their cattle to drink water outside, thus reducing their chances of exposure to the infective parasitic stages.

In this study, female cattle had a higher prevalence rate compared to their male counterparts. This trend agrees with the results of studies in Egypt (Dhar et al., 1988; Fatima and Chislti 2008) and Nigeria (Ulayi et al., 2007). Studies as Schillhorn Van Veen (1997), Soulsby (1982)

and Ibrahim et al. (2001) have suggested that there is hormone-controlled relaxation of immunity in female animals during pregnancy and lactation, which increases their susceptibility to infection. Since coprology and the type of ELISA (antibody-detecting) employed in this study cannot differentiate between a new, recent and old infection, the female animals in this study might have contracted the infection during pregnancy and lactation, hence, the observed higher prevalence rate. This higher prevalence in females than males could also be attributed to the fact that more females were sampled than the males.

This study revealed that the local breed had high infection than the foreign breeds on farms. This result is in agreement with the findings of Ulayi et al. (2007). The higher prevalence obtained for the local breed could be due to the fact that cattle of this breed is the most predominant in the study area and very often, the extensive system of management under which they are reared, coupled with the dwindling grazing lands owing to increased food crops farming, compels them to graze in areas that could be heavily infested with the intermediate hosts of the liver fluke in the late dry season when there is acute shortage of feed.

In this study, the prevalence of *F. gigantica* was higher in the young cattle (<2 years old) than adult cattle (>2 years old). The reason for this could be the development of acquired immunity in the older animals which results in resistance, as opined by earlier investigators (Phiri et al., 2005).

The prevalence of 17.1% obtained in this study is much lower than the 71.1% reported by Fabiyi and Adeleye (1982) on the Jos Plateau; the 65.4% reported by Schillhorn Van Veen (1980) and 52.1% reported by Olusegun-Joseph et al. (2000) in Zaria. This could suggest that herdsmen and herd owners are now much aware and seek for anthelmintic intervention. On the other hand, the prevalence rate obtained in this study was higher than the 10.51 and 10.0% reported, respectively by Ekwunife and Eneanya (2006) and Ngwu et al. (2004) both in South Eastern Nigeria. This may be because animals in the South Eastern region of Nigeria are not subjected to dry season fadama grazing since the upland pasture is available all year round. Also, since most of the animals slaughtered in the region are from the northern part of Nigeria, it is not unlikely that only very healthy cattle are transported down South for sale and slaughtered.

Conclusion

This study has, through coprological and serological means, established the current prevalence (17.1 and 7.3%) of *F. gigantica* at slaughter and on farms in Zaria, Kaduna State. Coprology gave a higher prevalence than the more sensitive ELISA technique. Hence, proper meat inspection at abattoirs and public health enlightenment on the disease should be intensified.

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