
PATTERNS OF TREMATODE INFECTION IN GALL BLADDER FROM CATTLE SLAUGHTERED AT ISHERI-OLOFIN, OGUN STATE, NIGERIA

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Abstract

A survey of gall-bladder of slaughtered cattle was carried out to determine variation pattern of trematode infection. A total of 1,240 gall-bladders of cattle were examined for trematode eggs and adult worms between August 2008 and March 2009. Fifty questionnaires were randomly administered to cattle handlers to investigate possible influence of knowledge, attitudes and practice in prevalence pattern of trematode parasites. Eggs and adults of two trematode species were identified. Overall prevalence of infection was 75.32%. The more prevalent *D. hospes* (60.24%), followed by *Fasciola gigantica* (45.16%). There was an inverse pattern in single infection, where *F. gigantica* prevalence declined, *D. hospes* prevalence inclined and a reverse pattern was observed between January and March. The prevalence of adult worms from the gall bladders were 2.26% and 6.69% for *F. gigantica* and *D. hospes* respectively. *Chi*-square analysis showed there was a significant difference in infection rate between the months of survey in single infection ($p < 0.05$). A positive correlation ($r = 0.61$) was present between the egg load and adult worm in the gall bladder. The variation in intensity of infection with *F. gigantica* and *D. hospes* in relation to months of survey was not significant ($p > 0.05$), but there was a similar trend in intensities of *F. gigantica* and *D. hospes*. Seventy-two percent of the cattle-handlers reported awareness of worms in liver of cattle and significant percentage (56%) grazed-cattle around streams particularly during the dry season. At the abattoir, 64% of the cattle-handlers claimed they seek prompt medical care immediately animals show signs of weakness or loss of appetite.

Keywords: trematode eggs, *F. gigantica*, *D. hospes*, prevalence.

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Introduction

Cattle play an important role in improving the national economy of Nigeria (Biu and Adindu, 2004; Biu and Babagana, 2004) and are reared for their meat, milk, hide and skin, manure and as draught animals for work on the farms. Furthermore, some of the products derived from cattle serve as raw materials in industries, for example, hide and skin for making belts, shoes, bags and other leather goods, blood and bones used for animal feeds; bones, blood and offal of cattle for fertilizers and the like. Cattle-raising also provides employment and income for the people, especially among the cattle-rearers in the northern part of Nigeria, and can as well be a source of revenue for government (Iweka, 2000). However, parasitic diseases which include fasciolosis,

dicrocoeliosis, cysticercosis, and hydatidosis which could be zoonotic constitute a major economic and public health problem by lowering the productivity of cattle, in addition to losses from condemnation of affected organs while humans can accidentally ingest infective metacercariae and become infected (Cook, 1989; Schentz, 1990; Biu and Adindu, 2004).

Fasciolosis is an important helminth disease caused by trematodes belonging to the genus *Fasciola* commonly known as liver fluke (*Fasciola hepatica* and *Fasciola gigantica*). Fasciolosis may be categorized as plant-borne trematode infection with zoonotic potential (Mas-Coma *et al*, 2005). The disease is cosmopolitan in distribution; in Europe, the Americas and Oceania. *Fasciola hepatica* is the major concern, but the



distribution of both species overlap in many areas of Africa and Asia (Esteban *et al* 2003; Mas-Coma *et al* 2005). The definite host range is very broad and includes many herbivorous mammals, and humans. Fasciolosis is a well known parasitic disease because of its veterinary importance and the great losses it causes in livestock production. However, fasciolosis was only considered a secondary disease by public health officials, with only approximately 2,000 human cases reported between 1970 and 1990 worldwide (Chen and Mott, 1990). As human cases were increasingly reported, the total estimated number of people infected was 2.4 million in 61 countries and the number at risk was more than 180 million throughout the world (FAO, 2008). The estimated number of people having fasciolosis is 360,000 in Bolivia; 20,000 in Ecuador; 830,000 in Egypt, 10,000 in Islamic Republic of Iran, 742,000 in Peru, and 37,000 in Yemen.

Researchers have attempted to provide data on economic and public health aspects of fasciolosis in Nigeria. Estimates of economic losses due to fasciolosis had been mainly derived from records of organs and carcasses' condemnation. Bui *et al* (2006) observed that in Maiduguri Abattoir 41.7% of condemned organs consisted mainly of livers, were due to fasciolosis. Major surveys of abattoirs from different parts of the world reported huge economic losses caused as a result of infection by fasciolosis with projection of financial losses based on average weight (Froyd, 1975; Subianto *et al* 1978; Ogunrinade, 1980; Schenz, 1990; Okoli *et al* 2000; Bui and Adindu, 2004 and Bui and Babangana, 2004). The worldwide economic losses in animal productivity due to fasciolosis were estimated as over US\$ 3.2 billion (Spithill *et al* 1997) and US\$ 2 billion (Spithill and Dalton, 1998). FAO (2008) put the incidence of *F. gigantica* in cattle in Nigeria at 60%, total losses for Nigeria was calculated to be 32.5 million pounds sterling, though this represents a substantially lower estimate according to FAO.

Most of the available records on helminth infections were based on the examination of faecal samples. This has numerous limitations and was reported by Misrel *et al* (1972) to be insufficient for indicating actual prevalence. Knowing that an under-estimation of intensity of infections and rate of helminth infections in livestock may result in failure of disease control due to the adoption of inappropriate control measures. The procedure of bile examination is known to give a higher percentage of positive finding (Bui *et al* 2006; Oyeduntan *et al* 2008).

This study was designed to determine the prevalence and intensity of helminth infection, the relationship between months of survey and infection rate, relationship between egg load and adult worm in bile, and to assess knowledge, attitude and practice with reference to *F. gigantica* in Nigeria.

Materials and methods

Sample collection and adult worm count

A total of 1,240 gall-bladder samples were collected from cattle slaughtered at Isheri Olofin Abattoir, Ifo North, Ogun State, between August 2008 through March 2009. Each of the gall-bladders were incised with dissecting scissors and the contents emptied into individual glass beakers. Forceps were used to pick and count adult worms observed from the naturally infected cattle gall-bladders. Helminths were washed extensively, free of debris, in physiological saline. Each worm was identified morphologically according to existing keys and descriptions (Yamaguti, 1958; WHO, 2008 and Wikipedia, 2008).

Examination of bile and egg count

Each bile sample collected was mixed thoroughly for random distribution of eggs and 10 ml samples were transferred into specimen bottles from which 0.1 ml was distributed on a chamber counter using micropipette. Slides were examined under the optical microscope. The ova of helminths were identified according to the FAO guide to veterinary diagnosis of parasites and Miller (1975) using morphological descriptions and counted.

Prevalence was determined using the number of infected bile samples which was expressed as percentage of the total number of sample examined. The prevalence by month and parasite types was determined. Intensity was obtained from the mean number of eggs per millimeter (ml) of bile sample. Mean egg per ml per month and parasite types were also estimated.

Questionnaire survey

Informed consent was obtained from individual. Standardized questionnaire and interview were used to obtain information from each of 50 randomly selected participants between the ages of 25 and 60 years. Responses for nature of occupation, that is, whether a cattle-dealer, butcher or meat-seller, years of experience on the job, management practices prior to slaughtering of cattle, mode of transportation of cattle to the market and abattoir, first-line-of-action when animals appear weak or sick, observation of worm infestation and local names were recorded in Excel Spreadsheet.

Data analysis

Differences among prevalences regarding months of survey were tested by *chi*-square, Analysis of variance (ANOVA) was carried out to determine level of significance in variation due to intensity of parasite species and month of survey. Correlation analysis was used to evaluate the relationship between the egg load and worm burden in gall-bladder. Questionnaire data were

analysed for percentage of occurrence of responses proportions.

Results

Study population and questionnaire analysis

Cattle were brought to the abattoir on the morning they would be slaughtered. One hundred percent (100%) of the cattle were transported from northern part of the country, having their origin from Chad or Niger. Prior to slaughtering, cattle were held in ranches for short duration varying from few days (3) to few weeks (2-4).

Cattle-handlers reported that while in waiting, preventive treatments were provided once or twice a month by veterinary practitioners. Also when required, 64% of cattle-handlers interviewed reported that animals received immediate treatment whenever there were signs of weakness or loss of appetite. Other respondents claimed to cull animals in order to avoid or reduce losses.

Questionnaire's investigation revealed that significantly high proportion, 72%, of the cattle-handlers were aware of trematode infection in cattle liver and gall-bladder (local names were reported for *F. gigantica*, that is "Chiwa Anta" and "Ajani eran" in Hausa and Yoruba languages respectively. Observations revealed a general awareness of animal feed being the suspected source of infection. Despite the high level of awareness, 56% reported they could not avoid grazing their cattle around streams especially during dry season, because water is usually very scarce.

Helminth species occurrence

Two species of helminths eggs were recorded and 934 cattle gall-bladders (75.32%) harboured at least one of the species. Of the helminth species found, *D. hospes*, eggs (60.24%) were the more prevalent, followed by eggs of *F. gigantica* (45.16%). Twenty eight (2.26%)

gall-bladders had adult *F. gigantica* and 83 (6.69%) had adult *D. hospes*, which is significantly higher than the occurrence of adult *Fasciola* ($p < 0.05$). There was no incidence of both *D. hospes* and *F. gigantica* adult worm occurring simultaneously in the gall bladder.

Prevalence pattern of trematode infection

The majority of cattle (44.19%) had one type of egg species, that is either *D. hospes* or *F. gigantica* eggs; while 306 (24.68%) and 386 (31.13%) had zero egg count and single infection respectively (Figure 1). Overall monthly prevalence of infection ranged from 63.13% in September 2008 to the highest 86.11% which was observed in January 2009. There were marked variations in the rates of infection in relation to months of survey.

In single infections, prevalence of *D. hospes* inclined between August 2008 and December 2008, peaked in January 2009 and declined until the end of survey in March 2009, while prevalence of *F. gigantica* declined from August 2008 to the lowest prevalence recorded during the survey period in December 2008 followed by a gradual increase till end of the study (Figure 2a). The *chi-square* tests for differences in prevalence by months of survey were significant ($\chi^2 = 51.44$ and 90.3 ; $p < 0.05$, for *F. gigantica* and *D. hospes* respectively).

The prevalence of *F. gigantica* and *D. hospes* in double infection (35% in November 2008 to 63.75% in August 2008 and 48.13% September 2008 to 77.32% in January 2009 respectively) showed a similar trend to single infections only (Figure 2b). The mean monthly rates were $46.54 \pm 6.16\%$ and $59.73 \pm 7.45\%$ for *F. gigantica* and *D. hospes* respectively. *Chi-square* showed there was a high significant difference in prevalence ($\chi^2 = 17.22$, $p < 0.05$).

Intensity pattern of trematode infection

Overall mean number of eggs per ml of bile harboured per host ($n = 1,240$) was 60.34 ± 2.35 eggs per ml of bile. The trends of mean monthly intensity of *F. gigantica* and *D. hospes* eggs are shown in Figure 3. Both species showed lowest intensity in eggs in gall-bladder in November 2008 for *F. gigantica* and March 2009 for *D. hospes* which corresponded to periods ending the rains and early rains respectively in a typical tropical climate, while the double peaks were observed first between August and September 2008 and the lower peak in January 2009. However, two-way ANOVA test showed that the variations in intensities due to sampling time were not significant ($f = 0.78$; $p > 0.05$). Generally, a lower range of intensity, 216-348 eggs/ml of bile, was observed in *D. hospes* infection compared to a range of 243-414 eggs/ml of bile for *F. gigantica* infection, variations in mean monthly egg load were not significantly different ($p > 0.05$).

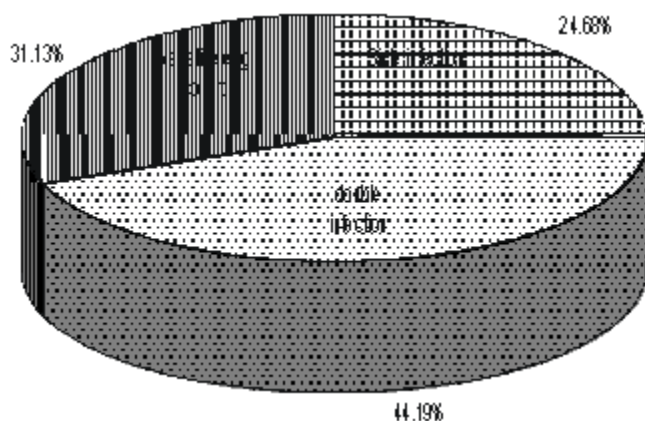


Figure 1: Percentage occurrence of trematodes egg types in cattle gall bladder ($n = 1240$).

Figure 2a: Single infections.

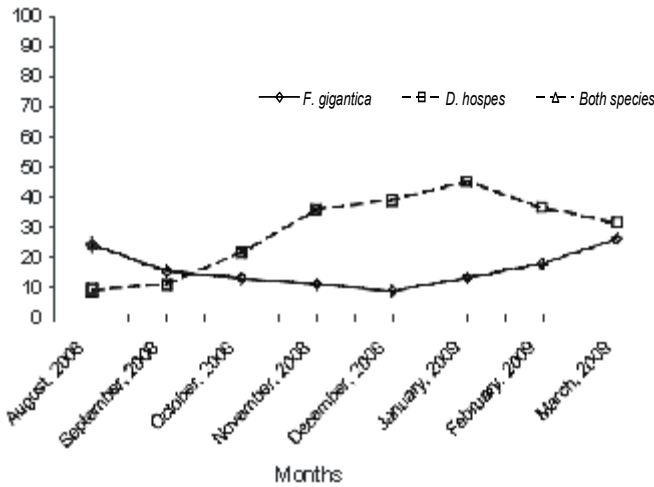
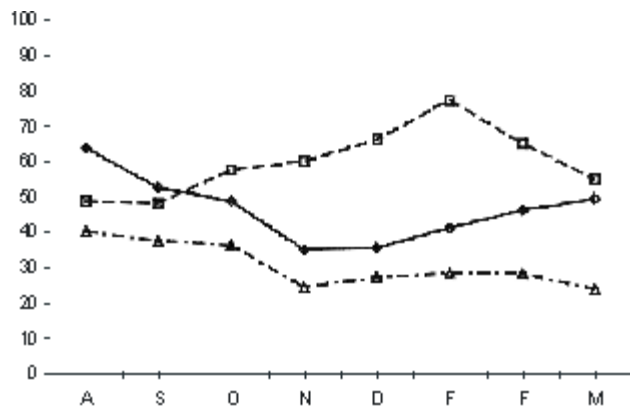


Figure 2b: Mixed infections.



Figures 2a and 2b: Monthly prevalence of trematode infection. (a) single infection (b) mixed infection.

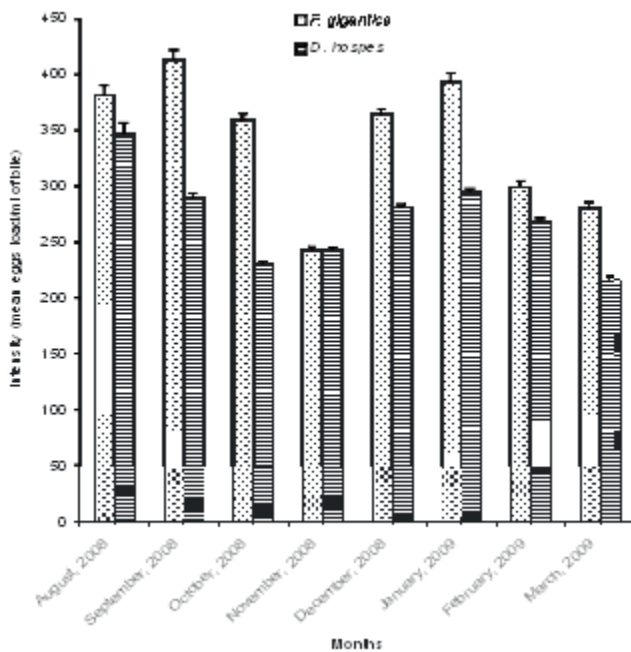


Figure 3: Mean monthly intensity of trematode eggs in gall bladders of cattle of *F. gigantica* and *D. hospes*.

Table 1: Monthly egg count per ml bile and worm load in gall bladder.

Month	Trematodes' species			
	<i>Fasciola gigantica</i>		<i>Dicrocoelium hospes</i>	
	Egg-load	Worm-load	Egg load	Worm load
August	0	0	1655	58
September	2250	18	1340	66
October	2005	39	950	47
November	485	11	1040	79
December	1080	19	1620	128
January	1910	33	1575	110
February	810	15	1375	61
May	670	10	1525	100

Total number of adult *F. gigantica* infected gall-bladders = 28.
 Total number of adult *D. hospes* infected gall-bladders = 83.

Egg intensities were significantly lower ($p < 0.05$) when adult worms were found in corresponding gall-bladders than the intensities observed when adult worms were not present in gall-bladders. In both *F. gigantica* and *D. hospes* infection, positive correlations were observed between the egg load and adult parasite in the gall-bladder ($r = 0.61$). Table 1 shows that egg intensities in relation to worm load in gall-bladder. A significant association between *F. gigantica* and *D. hospes* was observed in double and single infections with respect to expected values ($X^2 = 14.15, 3.84, p < 0.05$). The observed number of *F. gigantica* egg output in double infection exceeded the expected value and for *D. hospes* observed egg output exceeded the expected value in single infection.

Discussion

The overall prevalence of trematode parasites eggs in gall-bladder of cattle observed in this study (75.32%) was significantly high ($p < 0.05$). This study agrees with findings from major surveys of abattoirs from several parts of the world (Schenz, 1990; Okoli *et al* 2000; Bui and Babagana, 2004; Phiri *et al* 2006 and FAO, 2008). These findings further reported huge economic losses caused as a result of various trematodes' diseases: fasciolosis, dicrocoelosis, hydatidosis, cysticercosis and amphistomiasis.

The findings of the present study could imply that, in spite of major concerns about economic losses and large volume of documentation on animal health implications of helminthic infections, efforts to alleviate problems of animal health and productivity are yet to make any significant impact. Therefore, the need to investigate the pattern of prevalence of trematodes' infections in cattle

slaughtered at abattoir cannot be over-emphasized. On the other hand, the high overall prevalence was most probably not a true reflection of the status of trematodes' infection in cattle in Nigeria, as majority of slaughtered cattle were imported into the countries from countries like Chad and Niger, according to responses from cattle-handlers. This further explains the need to undertake surveys over a long duration to be able to predict proper intervals for deworming and discourage the practice of prescribing prophylactic treatment (levamisole) given orally twice or thrice in a month by veterinary drug vendors as observed at the studied abattoir — a practice which is a major public concern for drug resistance. Furthermore, acquiring infection by the cattle is not limited to the source, cattle are raised long enough at slaughtering location and this could influence prevalence patterns. The overall prevalence of *F. gigantica* in mixed infection at the studied-abattoir was 46.54% and is comparable to 41.76% reported by Biu *et al* (2006) at Maiduguri Abattoir. The prevalence obtained in this study was higher than the values reported by Biu *et al* (2006) and Wayi *et al* (2007). The differences in prevalence may be due to differences in resistance to infection because of the host breed and grazing habits (Tasawar *et al* 2007). The observed low prevalence of single infection with *F. gigantica*, 15.08%, conforms to the low prevalence of 15.52% reported by Idowu *et al* (2006) in Abeokuta, Ogun State and Phiri *et al* (2006) in Zambia. The low prevalence may be attributed to reported routine cattle management procedure adopted. The majority of respondents (64%) claimed to seek prompt medical care when necessary. Furthermore, veterinary consultants were reported to routinely prescribed prophylactic treatment (levamisole) given orally once or twice a month depending on how long cattle are kept before slaughtering.

The FAO (2008) reported a range of 17-56% prevalence of *D. hospes* in Nigeria, the present study showed a 30.16% prevalence. The overall single trematodes' prevalences reported in this study were 46.16% for *F. gigantica* and 60.24% for *D. hospes*. The higher *D. hospes*' prevalence is noteworthy considering reports that dicercarioeliasis is poorly known, often underestimated by researchers and practitioners in many countries availability of few effective drugs, difficulty of diagnosis and presence of poly-parasitic infections which mask the pathology of the disease (Schillhorn van Veen *et al* 1980; FAO, 2008) are also part of the problem. This trend may constitute a significant animal health issue of an emerging disease.

The prevalence patterns of infection with both single infections and mixed infections (Figure 2) with similar inversed increase and decreased trends could be explained by the fact that the period of sampling falls in the late rainy months and early dry months. This

corresponded to the reported most favourable period for the snail, while the wet months adversely influence the incidence of *D. hospes*, that of *Fasciola* is less affected (Oyeduntan *et al* 2008). In this study, the difference in prevalence by month was statistically significant ($p < 0.05$).

The infective intermediate hosts of *D. hospes* are reported to be infective from the first half of the dry season, when they occur in the same habit with the snail (Schillhorn van Veen *et al* 1980; Ukoli, 1982). The definitive host are thus expected to show high or increased incidence rate and intensity of infection as from the second month of acquiring infection, which corresponds with the late dry and early rainy season.

According to earlier reports, there was no build up of flukes' eggs in cattle (Kendall, 1965). This suggests that intensity of infection with respect to egg load is expected to decrease gradually to a low level until egg production stops. The monthly variations observed in the present study could be attributed to the expected decline in egg production and possible re-infection pattern. The observed lower intensities of *D. hospes* infection could be due to the fact that the rate of infectivity of the ants could be lower than the rate of occurrence of *F. gigantica* metacercariae on vegetation. Reduction in rainfall between December and March, which favoured presence of ants, could explain the second peak period of intensity of infection. Variation in intensities (eggs per ml of bile) in relation to sampling time and trematode species were however not significant.

In conclusion, the prevalence pattern and the co-occurrences of trematode parasites of cattle are undoubtedly influenced by climatic conditions (dry and wet months). Furthermore, the influence of management procedures adopted on different dairy and local farms cannot be excluded. There are also interactive influences between the two dominant trematodes' species, *F. gigantica* and *D. hospes* which are reflected by higher observed value of *F. gigantica* egg load than the expected value in double infections and the reverse in single infection as well as lower observed value of *D. hospes* egg load in double infection than the expected value and the reverse in single infection. The results of the present study have shown that more enlightenment is needed to enhance knowledge, attitudes and practice among cattle handlers for better management of trematode infections on cattle.

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