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Prevalence and Intensity of Fascioliasis in Cattle Slaughtered at Central Abattoir Gombe, Gombe State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Fascioliasis is one of the most prevalent and economically significant parasitic illnesses of domestic animals, particularly cattle, sheep, goats, and man. It is particularly widespread in countries with heavy cattle production especially places with low interest in vector-related diseases. Therefore, the study was designed to investigate the prevalence of this parasitic disease among the cattle slaughtered in central abattoir Gombe, Gombe state of Nigeria.

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Materials and Methods: A total of three hundred and eighty-four (384) faecal and gall bladder samples were collected at the Gombe municipal abattoir covering two seasons, from June to October 2022 during the rainy season and November, to May, 2023 during the dry season and examined using sedimentation techniques to detect *Fasciola* spp eggs, The number of eggs were counted and expressed per grams of faeces.
Results: It was observed that 72.1% were positive for fascioliasis out of the 384 slaughtered cattle examined. The prevalence of the infection based on the sex of the cattle was found to be lowest in males 34.3% and highest in Female 65.7%, although not statistically significant ($p>0.05$). The percentage of fascioliasis depending on the breed of the cattle also indicates high prevalence in white Fulani 69.3%, followed by Red Bororo16.7% and least in Sokoto Gudali 14.0% with an insignificant difference ($p>0.05$). Prevalence of fascioliasis based on the estimated age of animals was not statistically significant however; animals aged 2-4 years had the highest percentage 57.1%, followed by≥5 years29.2% and least in ≤1 year 13.7%. The prevalence was higher in dry season 72.9% than rainy season 27.1%, the analysis implied that there was a strong association between infection and season. Three categories of fascioliasis intensity were based on egg per gram of faeces (epg), 70.03% of the cattle sampled were lightly infected, with moderate infection in 20.57% and 9.38% with heavy infection.
Conclusion: The study concludes that fascioliasis among cattle in Gombe state was high causing great economic loss to the people rearing the animals. farmers/rearers should be encouraged on the importance of regular de-worming and watering of their animals using clean water for their economic benefit and health conditions of their consumer. Reduction of worm burden through chemotherapy and eradication should be encouraged.

Keywords: Fascioliasis; prevalence; intensity; cattle; Gombe; Nigeria.

1. INTRODUCTION

"Cattle (*Bos tauras*) are a multipurpose animal of semi-arid and, arid areas kept for a variety of purposes such as milk, meat, wool, transport and Agricultural purposes. Apart from camel no other domestic animal can provide as many variable services to humans as cattle" [1]. "The most important disease of cattle is fascioliasis which is a vector borne helminthic disease caused by *Fasciola hepatica* and *F. gigantica* with wide distribution throughout tropical and sub-tropical regions of the world" [2]. "Fasciolosis is an economically important parasitic snail-borne disease of ruminant and animals including cattle, goat and sheep that has public heath significant due to risk of infection transmission to humans. The diseases cause growth retardation, decreased milk, meat and wool production as well as liver damage in infected animals. Furthermore, humans may become infected by eating raw meat dishes made from fresh livers infected with immature flukes, since early migrating flukes present in the consumed and infected liver can retain the ability to restart intra-organic migration" [3]. "The disease is one of the important neglected tropical diseases that have gained significant global attention in recent years as a result of its associated morbidity and socio-economic impact" [4].

"The growing popularity of the disease is driven by the high prevalence among rural herding communities in resource-poor country and their constant close association with livestock. Although the disease (fascioliasis)fascioliasis begins as a subclinical disease similar to some bacterial infections, causing poor milk yield (milk volume), and quality, if unattended it may lead to more devastating outcomes such as severe weight loss, diarrhea, swelling under the jaw and sudden death if allowed to progress" [5]. "It has been reported to directly affect the liver through the migratory action of flukes in ductular tracts, blood-sucking and liver damage leading to metabolic disease. The snails belonging to the general lymnaea, Amphipela, Simimnaea, Golba, Fassoria, Stagniciola and Pseudosuccinea serve as imtermediate hosts, with Specific species restricted to different geographical regions of the world" [6,7]. "In Nigeria the most important snail is *Lymnaea natalensis*. These intermediate hosts are found in shallow water and are capable of migrating for long distance over mud and wet pasture, thereby increasing the chances of exposure of susceptible animals" [8,9]. "*Fasciola hepatica* infects more than 300 million cattle and 250 million small ruminants worldwide and together with *F. gigantica*, causes significant economic losses to global agriculture; through lost productivity such as a reduction in milk and meat yields, mortalities" [10,11]. A previous

report by [12] estimated a conservative amount of over US$ 3.2 billion per annum, as losses due to fasciolosis in production animals worldwide, which was slightly higher than the US$ 3 billion estimated by [13] in Nigeria. According to [2], "fasciolosis was limited in the past to specific and typical geographical areas, but is now widespread throughout the world". "Human cases occurred occasionally but are now increasingly reported from developing countries with a higher chance of exposure to the infective fluke stage during food preparation, predominantly in Africa, China, Korea, South America, North and South Asia" [14].

"Sellers and buyers of meat in Gombe are ignorant of the danger of consuming infected livers, due to lack of public health education. The animals are reared under the free-range extensive management system. The animals range freely grazing grasses in the fields. Their feeding on grasses is supplemented with fodder particularly during the dry season when there is a scarcity of grasses. The animals usually graze on grasses and leaves near water holes, rivers, lakes and temporary flooded areas within the grazing area or range. The animals may acquire infections from such areas that are infested with metacercaria. Information on whether or not the animals are treated or dewormed against fascioliasis is unavailable. Thus, untreated or un-dewormed animals will continue to shed *Fasciola* eggs in their faeces and could serve as a source of infection to healthy animals" [15].

"Among all the livestock, ruminants, comprising sheep, goats and cattle, constitute the largest group reared by farm families in the country's agricultural system. Nigeria has an estimated population of 34.5 million goats, 22.1 million sheep and 13.9 million cattle. However, about 90 per cent of the country's cattle population and 70 per cent of the sheep and goat populations are concentrated in the Northern part of the country" [16]. Most studies aimed at determining of the prevalence of *Fasciola* spp. conducted in Nigeria: [15,17] in northern Uganda, and [18] in South "Africa, have been based on examination of the liver at post-mortem. Hence, comparatively, fewer reports exist on the detection of *Fasciola* by coprology, the traditional detection method in the laboratory, than by post-mortem examination in the abattoir. The exact prevalence of fasciolosis is most likely underestimated due to the lack of comprehensive epidemiological surveys performed in potentially endemic areas. Furthermore, in some areas where reports exist, there is a time lag of up to a decade or more, thereby making such reports obsolete and probably not in tune with current realities, hence the need for this study in this area" [16].

The present study investigated the occurrence of cattle fascioliasis using parasitological (microscopic) identification techniques. The findings have been properly recorded and will be forwarded to the appropriate channel for planned control strategies. The study was limited to cattle brought to the abattoir however were often supplemented from cattle markets within the State. This study aims of this study is to determine the prevalence and intensity of *Fasciola* specie, based on sex, age, breed, and season, among cattle slaughtered at Gombe Central Abattoir, by detecting *Fasciola* eggs in bile and stool samples.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at the central abattoir of Gombe metropolis located in the North East region of the Country. The abattoir is the major abattoir in Gombe metropolis slaughtering an average of 100% cattle a day. Gombe State is located between latitudes 10016, and 60.00"N and longitude 110 9E. "The climate of Gombe is characterized by a cool dry (Harmattan) season with minimum temperature of about 23.480c from December to February, a hot dry season with annual maximum temperature of about 35.390c from March- May and warm wet season from June September, a less marked season after rains during the months of October to November, Characterized by decreased rainfall and gradual lowering of temperature" [15]. Gombe State has two main vegetation zones. The Guinea is Savannah zone in the southern part of the State and the Sudan Savannah zone in the northern part (Fig. 1).

2.2 Ethical Approval

The study protocol was conducted with the ethical approval of the Animal Ethical Committee (ACE) of the National Veterinary Research Institute (NVRI), Vom, (ACE/20/83/20), and with the full approval of authorities in the abattoir.

Image 1: Map of Nigeria showing the location of the study area

2.3 Study Design and Selection of Cattle

The survey of *Fasciola spp* in cattle slaughtered in the abattoir was carried out between February 2023- January2024. A total of 384 cattle were selected and tagged from the lots prepared for daily slaughter. The samples cut across both sexes and different breeds of cattle that include while Fulani, Sokoto Gudali and red Bororo.

2.4 Study Population

The Study was conducted on naturally suspected, infected and healthy cattle meant for slaughter in the main abattoir of the State capital. Most of the cattle Slaughtered in this abattoir were from Ngalda, Leggal, Gombe, Dukku and Bayo, however some were often supplemented from the rural cattle market within the state. The ages of the sample animals were estimated as described by [19]. Cattle ≤1 year were classified as young; 2-4 years adult while ≥ 5 years age were regarded as older. Breeds identification was done according to the identification keys as provided by [20] for traditional livestock breeds of West Africa. Similarly, the sexes were identified on the appearance of the external genitalia, as described by [21].

2.5 Sample Size Determination

The sample size was determined according to the number of animals presented for slaughter during the study period. The number of samples collected was determined using the formula of [22] with previous prevalence of 50% [15] was used in calculating the sample size.

N= Z 2 P (1-P)/d2

Where:

N=Sample size
Z=2 statistics for a level of confidence,
P= expected prevalence or proportion (50%)
D=Precision (5%, d=0.05)
Z Statistics (2): At a confidence level of 95% is 1.96

Therefore,

N=3.842x0.5x0.5x0÷0.0025
= 0.9604÷0.0025
= 384.16

Hence = 384 to the nearest whole number

2.6 Sample Collection and Transportation

Before sampling the abattoir was visited to make preliminary arrangements for collection of samples.

A total of three hundred and eighty-four (384) of fecal and gall bladder sample were collected at Gombe municipal abattoir covering two seasons from June to October 2022 during the rainy season and November, to May, 2023 during the dry season. “Convenient sampling technique was used for the sampling and about 2 g of faecal sample was collected directly from the rectum of each animal using hand (manually) with disposable hand gloves into a sample bottle for analysis. For bile collection, the whole gall bladder was removed from animal species through gentle excision from the liver using scalpel blade. Care was taken to prevent spilling of the bile from the gall bladder to the thoracic cavity of the slaughtered animal. The bile was

then emptied into a suitable container and 10% formalin solution was added for preservation. The stool and bile samples were taken to Gombe State University parasitology laboratory in a cool box for analysis and identification" [13].

2.7 Sample Processing

2.7.1 Phenotypic detection of *Fasciola* spp. in faeces

The processing was as earlier described by [8]. Four grams (4g) of faeces will be placed into labelled test tubes containing 6 mls of distilled water, and then strained to give a suspension, which was also strained through a tea strainer into a clean labelled Petri dish. The resultant filtrate was poured into a test tube and one millilitre (1ml) of 10% formalin was added, after which the suspension was allowed to stand for 5 minutes, followed by addition of diethyl ether (1ml). The test tube containing the suspension was corked and shaken to mix, then centrifuged at 2000 g for 8 minutes, after which the supernatant was decanted, leaving a drop of it with the sediment. Part of the sediment was placed on a glass slide and covered with cover slip, drops of methylene blue was added and then viewed at ×10 magnification of a stereomicroscope for *Fasciola* eggs, until the entire sediment examined and all eggs counted [23]. Prevalence was determined by expressing the number of positive samples as a % of total sample collected. The number of eggs counted was expressed per grams of faeces [24].

2.7.2 Detection of *Fasciola* spp in bile

Approximately 4 millilitres of the collected bile was poured into a labelled test tube and the procedure used for faecal sample analysis was repeated, followed by addition of 1ml of 10% formalin. After 5 minutes, diethyl-ether (1 ml) was added. The test tube containing the solution was corked, shaken to mix, and then centrifuged at 2,000 g for 10 minutes. The supernatant was decanted leaving few of it with the sediment. Parts of the sediment was placed on a clean glass slide and drops of methylene blue added before being viewed under a microscope using ×10 magnifications for *Fasciola* eggs, until the entire sediment examined [23]. Prevalence was determined by expressing the number of positive samples as a % of total samples collected. The number of eggs counted was expressed per millilitres used [24].

2.8 Data Analysis

The data were analyzed and presented using descriptive statistics such as means and tables. Chi-square was used to establish association between fluke infection and ANOVA to determine the significant of difference in mean distribution of the flukes between the variables**.**

3. RESULTS

3.1 Prevalence of Fascioliasis Based on Location of Cattle Studied

Among the five different locations examined in this study, the highest prevalence of *Fasciola spp* was recorded in Gombe (53.8%) followed by Dukku (15.6), Ngalda (12.6%), Leggal (10.1%) and the lowest prevalence was recorded in Bayo which had a prevalence of (7.9%). as shown in Table 1.

3.2 Prevalence of Fasciola Infections in Relation to Sex of Cattle Examined

Two hundred and sixty-four female (264) and one hundred and twenty male (120) cattle were sampled and examined for the presence of *Fasciola* spp. Ninety-five 95(34.3%) male cattle examined were infected while one hundred and eighty-two (65.7%) of the female cattle examined were found to be infected with Fascioliasis as shown in Table 2. The prevalence of infection among male and female cattle was found not be statistically significant (p>0.05).

Table 1. Distribution of Fascioliasis based on location of cattle in this study

Location	No. Examined	No. positive	Prevalence (%)
Gombe	184	149	53.8
Dukku	67	43	15.6
Leggal	41	28	10.1
Bayo	40	22	7.9
Ngalda	52	35	12.6
Total	**384**	**277**	**100**

p>0.05

Table 2. Prevalence of fascioliasis based on sex of cattle examined during the study

Sex	No. Examined (n=384)	No. Positive	Prevalence (%)
Male	120	95	34.3
Female	264	182	65.7
Total	**384**	**277**	**100**

p>0.05

3.3 Prevalence of Fascioliasis Based on Age of Cattle Examined

Age prevalence among cattle examined in this study showed that, the prevalence of *Fasciola spp* was higher in adult cattle age 2-4 years (57.1%) and the lowest prevalence was recorded in young cattle age less than 1 year ≤1 (13.7%). While older cattle age ≥5 had a prevalence of (29.2%) as shown in Table 3. The prevalence of infection based on the ages of cattle examined was found not to be statistically significant (p>0.05).

3.4 Prevalence of Fascioliasis Based on Breed of Cattle in the Study

Among the three different cattle breeds examined in this study, the prevalence of *Fasciola spp* was highest in White Fulani (69.3%) followed by Red Bororo (16.7%) and the lowest prevalence was recorded in Sokoto Gudali breed which had a prevalence of 14.0%. as shown in Table 4.

3.5 Prevalence of Fascioliasis Based on the Body Condition of Cattle in the Study

Prevalence among body condition of te cattle examined in this study showed that, the prevalence of Fasciola spp was higher in cattle with poor body condition (44.1%) and the lowest prevalence was recorded in good body condition (16.6%). while cattle with moderate body condition had a prevalence of (39.3%) as shown in Table 5. The prevalence of infection based on the ages of cattle examined was found to be statistically significant (p<0.05).

Table 3. Distribution of Fascioliasis based on age of cattle in this study

Age (years)	No. Examined (n=384)	No. Positive	Prevalence (%)
≤1	56	38	13.7
2-4	196	158	57.1
≥5	132	81	29.2
Total	**384**	**277**	**100**

p>0.05

Table 4. Distribution of Fascioliasis based on breed of cattle

Breed	No. Examined (n=384)	No. Positive	Prevalence (%)
White Fulani	272	192	69.3
Red Bororo	65	46	16.7
Sokoto Gudali	47	39	14.0
Total	**384**	**277**	**100**

p>0.05

Table 5. Distribution of fascioliasis based on body condition of cattle studied

Body condition	No. examined	No. infected	Prevalence (%)
Poor	130	122	44.1
Fair	143	109	39.3
Good	111	46	16.6
Total	**384**	**277**	**100**

(p<0.05)

3.6 Frequency of *Fasciola* Infection According to the Months of Sample Collection in the Study Area

Distribution of infection with fascioliasis based on the months in which samples were collected shows that prevalence increases from the months of June onwards and it is highest in August after which prevalence rates falls and decreases towards the Months of dry season from November to May (Fig. 1).

3.7 Intensity of Infection with *Fasciola* spp. among Infected Cattle from Gombe State

Three categories of fasciolosis infection intensity were based on epg. 194 (70.03%) of the 277 cattle sampled were lightly infected, with moderate infection in 20.57% (57/277) and 9.38% (26/277) with heavy infection (Table 6).

4. DISCUSSION

Fascioliasis is an infection of cattle and humans due to the consumption of meat by human beings. The total prevalence of Fasciola infections obtained in this research work on cattle slaughtered at Gombe market abattoir from five different locations was 277(72.1%). The high prevalence obtained in this work can be attributed to the high number of disease reservoirs (cattle, goat and sheep) and climatic conditions of Gombe which favours the survival of the intermediate hosts, the snail. This intermediate host prefers swampy areas with slowly moving water and small streams which also allow sufficient moisture for the survival of the infective metacercaria. This may not be unconnected with the fact that, north-eastern Nigeria is a zone of rampant uncontrolled grazing, with no clean water sources for the animals except open ditches and ponds which are factors aiding transmission.

"High prevalence of infection may also be due to poor herd sanitary conditions, host susceptibility to infection as a result of poor feed quality, ill-treated tributaries, careless attitude of both herdsmen and health supervisors, and lack of proper control programs, as well as poor veterinary services in the study area against the disease. Both cattle and people may suffer substantial economic consequences as a result of this high incidence. Such economic loss could include; costs of anthelminthics, drenches, labour, liver condemnation during meat inspection, and production losses owing to mortality, including reduced meat, milk, and wool output, as well as reduced growth rate and fertility. The disease could be public health significance, causing human fascioliasis" [17].

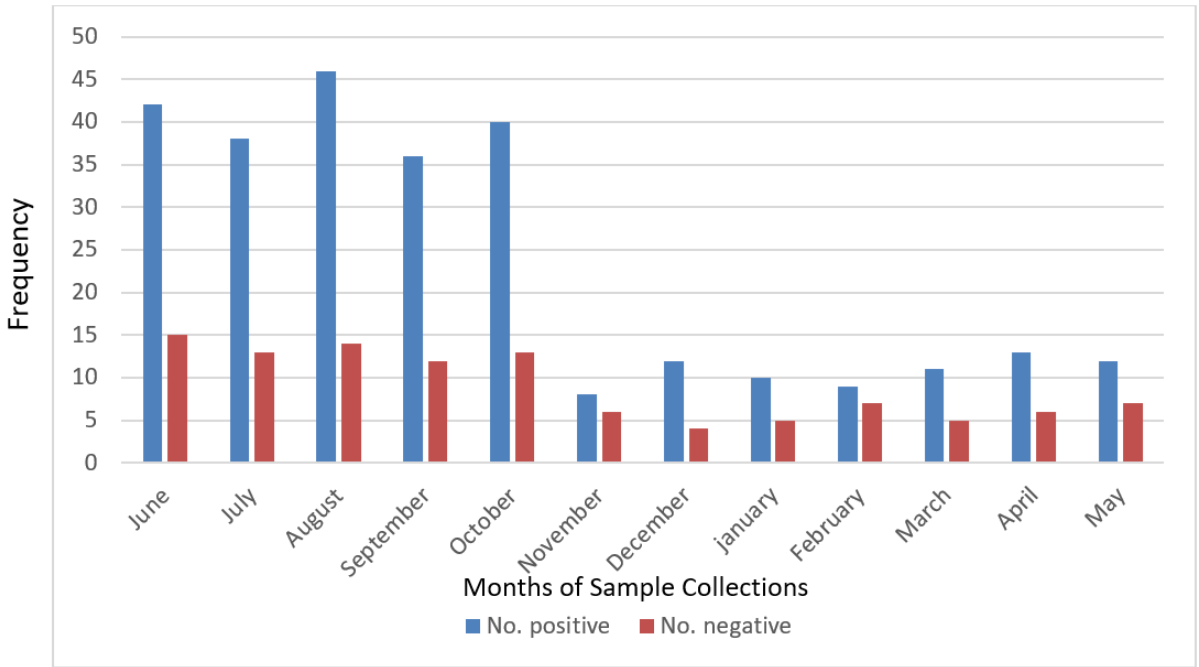

Fig. 1. Distribution of *Fasciola* infection according to the months of sample collection in the study area

Table 6. Intensity of infection with *Fasciola spp*. among infected cattle from Gombe state

Factor	Category	Frequency	Intensity of infection, frequency (%)		
			Light	Moderate	Heavy
Gender	Male	95	69(72.63)	20 (21.05)	6(6.31)
	Female	182	125(68.68)	37 (20.32)	20(10.98)
Age	1-2 years	47	28 (59.57)	12 (25.53)	7 (14.89)
	3-5 years	162	119 (73.45)	30 (18.51)	13 (8.02)
	6 and above	68	47(69.11)	15 (22.05)	6 (8.82)
Breed	White Fulani	192	141 (73.43)	32 (16.66)	19(9.89)
	Red Bororo	46	30 (65.21)	13(28.26)	3(6.52)
	Sokoto gudali	39	23(58.97)	12 (30.76)	4 (10.25)
Season	Rainy season	202	142 (70.29)	39(19.30)	21 (10.39)
	Dry season	75	52 (69.33)	18 (24.0)	5 (6.66)

This prevalence was generally high and approximately agrees with findings from similar studies by [25] who recorded a prevalence of 70% among cattle in the study performed at Yenagoa, Bayelsa state of Nigeria and 80% prevalence rate reported from cows slaughtered at Maiduguri, north-eastern Nigeria. Also study by [26] at Kampala City Abattoir, Central Uganda reported a prevalence of 84%. Despite several studies reporting high rates of prevalence, low prevalence was reported in several other studies as well. For example, low prevalence of 29.8% was reported by [27] in Bauchi, 18.0% was reported by [28] in Cameroon.

A prevalence of 34.3% and 65.7% was recorded for male and female cattle respectively. The distribution of the illness by sex indicated that female cattle were considerably more infected with Fascioliasis than male cattle in this investigation. This might be because more female animals were slaughtered at the slaughterhouse or because more females were sampled than males. Another reason for the discrepancy might be because females stay in the herd longer (for reproduction, breeding, and milk production), resulting in a larger illness load. The result is consistent with findings from research by [29] in kwara and [30] in Taraba of Nigeria. However, this finding conflicts with the findings of [15, 31] in Nigeria, who found greater prevalence in males than females.

“Adult cattle had a larger number of instances than younger animals, according to the age distribution of the illness. However, chi-square statistical analysis for significance (P>0.05) revealed that infection rate and age have no relationship. The increased incidence in adults compared to younger cattle may be due to the young animals' reduced exposure to the parasite and the type of feed they consume. Adults graze on grasses, leaves, and other flora, primarily in regulated pastures, especially along river banks, while young animals are given milk and chaff near the base of Fulani houses. During the dry season, they are also pushed from place to place in quest of lush pastures and water. In permanent water bodies, there is frequently a significant concentration of snail intermediate hosts, which can pollute the water and neighboring flora with encysted metacercariae” [31]. Also, Most of the cattle slaughtered at the abattoir are adults, while the young ones are being retained for fattening to adult size before slaughtering. This finding is consistent with [32] in Maiduguri, [33] in Adamawa and [34] in Bauchi. However, this conclusion conflicts with [35] findings in South Africa, where they found higher infection in young animals than adult animals.

In this investigation, it was found that the White Fulani cattle breed had the highest prevalence of fasciolosis (P<0.05) compared to the other breeds studied, followed by the Red Bororo and the Sokoto Gudali cattle breeds. This suggests that some breeds are more prone to infection than others. “This might be due to the large quantity of these breeds murdered in abattoirs. It is also possible that variations in genes, the environment, and human intervention are possible factors for these results. However, the management techniques used with specific cow breeds may have had an impact on the risk of exposure and subsequent transmission. This result is consistent with research from different authors in Nigeria” [15,33,36]. In contrast to the findings of this study, [37] in Birnin kebbi Nigeria reported that “prevalence was observed to be higher in Sokoto Gudali than in any other breed, while there was a statistically significant association observed between prevalence and breeds of cattle slaughtered”.

The result of the present study indicates that body condition of animals has a significant association with the occurrence of *Fasciola* infection. The prevalence was significantly higher ($P<0.05$) in poor body conditioned animals than in fair and good body condition groups. A similar observation was made by [38,27] in Taraba and Bauchi but is in contrast with the result of [39]. "Poor body conditioned animals resulting from nutritional or other health challenges are often associated with low resistance to parasitic infections; such animals when infected with liver flukes often end up with an exacerbated condition. This is because helminths infected animals give priority to the reversal of the pathophysiological consequences of parasitism over other body functions and growths. Nutrients are thus not effectively utilized for body maintenance and growth. This results in cachexia, particularly at the chronic stage of bovine fasciolosis" [27].

This study found that bovine fasciolosis is more common during the wet season. The association between bovine fasciolosis and the season was statistically significant. The reason for the high prevalence in the rainy season could be due to the existence of a positive relationship between the prevalence of fascioliasis and environmental characteristics such as rainfall, humidity and temperature. The rainfall determines the prevalence and intensity of fluke's infection more than any other factor. Studies by [40] in Zamfara State, [34] in northern Bauchi State, and [41] in Niger State all show similar patterns, lending credence to these findings. However, [42] reported that prevalence was higher in dry season (15.3%) than the rainy season (12.0%) although not statistically significant ($p>0.05$). Similarly, [43] found a higher frequency in Makurdi during the dry season of the year than in the rainy season.

The intensity of infection in this study was determined based on the total number of epg in feces, and the data revealed a low infection rate in the animals studied. The capacity of *Fasciola* spp. to survive and infect a host depends on the interaction between host and parasite factors, which are related to immunological systems [44]. The ability of the parasite to establish infection in a mammalian host depends on its ability to manipulate the host's physiological milieu by producing and releasing a complex of regulatory proteins, glycans, and microRNAs. The parasite's tegument also serves a vital function in defending it from assaults by the host immune system. The result is similar to the finding of Kurnianto et al. [43] in Indonesia who found Sixty-one (92.42%) of the 66 cattle sampled were lightly infected, with moderate infection in 6.06% (4/66) and 1.52% (1/66) with heavy infection.

5. CONCLUSION

The study concludes that fascioliasis among cattle is prevalent in Gombe state causing high economic loss to the people rearing the animals. This is due to the system of rearing in the study area extensive management which influenced the risk of fasciolosis grazing animals in swampy areas, where there is availability of snail intermediate hosts, watering animals using open water bodies as well as lack of routine deworming. Farmers should be encouraged on the importance of regular de-worming and watering of their animals using clean water for their economic benefit and health conditions of their consumer. Reduction of worm burden through chemotherapy and eradication should be encouraged.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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