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Research Article
Occurrence of Necrotic Enteritis (NE) Among Local Chickens Slaughtered at Muda Lawal Market in Bauchi Metropolis, Bauchi State, Nigeria

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Abstract
Background and Objective: Most poultry farmers and veterinarians focus on coccidiosis, which is a common disease in poultry of all ages. Necrotic enteritis (NE) and coccidiosis have almost the same clinical presentation and therefore, birds may be treated for coccidiosis when they are actually infected with NE. This study investigated the occurrence of NE in local chickens slaughtered at Muda lawal market in Bauchi metropolis. **Materials and Methods:** A total of one thousand (1000) whole intestines were randomly collected from different slaughter stands in the slaughterhouse at the Bauchi poultry market. Two separate samples of the intestinal content were obtained from each whole intestine and examined for the presence of *Clostridium perfringens* and *Eimeria* which are the aetiologic agents for NE and coccidiosis, respectively. **Results:** An overall occurrence of 55.7% was obtained for NE. The study revealed a significant association between the occurrence of the disease and the season, with the early rainy season having the highest occurrence (64.6). However, there were no significant differences in occurrence among different genotypes and sexes of local chickens. More than half of the birds (58.5%) had the unapparent form of the infection, while 30.5 and 11.0% had the moderate and severe forms, respectively. The results also showed that severity of NE was significantly ($p<0.05$) influenced by season, with the late rainy season recording the highest percentage of the most severe form (14.6). The McNemar test for significance of changes showed a significant ($p<0.001$) deviation from the speculation that coccidiosis is the major predisposing factor to NE. **Conclusion:** The results indicate that NE occurs in local chickens.

Key words: Necrotic enteritis, coccidiosis, *Clostridium perfringens*, *Eimeria*, intestine, muda lawal, poultry disease

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Following the European Union (EU) ban on in-feed growth promoters, necrotic enteritis (NE) has re-emerged significantly, causing reduced growth performance and increased feed costs[1]. The subclinical form of NE is more disastrous since it can be more pervasive within the flock but mostly goes unnoticed and therefore undetected because of the absence of evident clinical signs and/or symptoms[1]. This usually results in condemnation of carcasses at the time of slaughter. Skinner *et al*.[1] reported that it is becoming more apparent that the economic impact of subclinical NE has not been formally investigated. The direct results of the damage to intestinal mucosa caused by the subclinical form of the disease are decreased digestion and absorption, reduced weight gain and an increased feed conversion ratio[2].

Clostridium perfringens, a commensal in the gastrointestinal tract (GIT) of poultry, is the primary causative organism of NE. It is a gram-positive, anaerobic, spore forming bacterium that has been isolated from feed, litter, dust and faeces[3]. Despite the identification of numerous factors that promote the development of subclinical NE, the exact field conditions that precipitate outbreaks of NE remain a major problem. In addition, with a degree of co-infection with *Eimeria* species, the predisposing factors are mainly dietary in nature. In the modern poultry industry, NE remains a major problem, although in the past, it has been controlled by in-feed microbials and ionophore anti-coccidials[4]. Poultry farms cannot afford to ignore the economic losses caused by this disease. Despite its sporadic nature in developing countries, it is still causing large-scale outbreaks in chicken production units. However, the total impact of fully developed NE on chicken production is difficult to determine accurately due to the nature of subclinical NE. Currently, the only way to assess the degree of host response is by scoring gross pathological lesions within the intestine[4]. The financial cost of NE to the world's poultry industry has been estimated to be £1.6 billion per year[5]. The objective of this study was to determine the occurrence of NE in local chickens in the Bauchi metropolis.

MATERIALS AND METHODS

Study area: This study was carried out at a chicken slaughterhouse in Bauchi metropolis, the capital of Bauchi state. According to Bauchi state ministry of information[6] records, the state lies between latitude 9.3° and 12.3° north of the equator and longitude 8.5° and 11° east of the Greenwich meridian. The state is bordered by 7 states, Jigawa and Kano to the north; Plateau and Taraba to the south; Adamawa, Gombe and Yobe to the east; and Kaduna to the west. It covers a total land area of 49, 259.01 square kilometres, approximately 5.3% of Nigeria's total landmass and has a total population of 4,676,456 people based on the 2006 census.

Study animals: The animals used in this study were local chickens. The birds were mainly the indigenous breed *Gallus domesticus*. Although they are not well classified into distinct genotypes, the local chickens could be classified into the following groups based on phenotype (physical appearance): normal, naked neck, frizzle and dwarf[7]. Birds were kept following traditional husbandry practices, with chickens scavenging freely for feed during the daytime and shelter provided at night.

Sample collection: A simple random sampling technique was used for collecting samples from various slaughter stands. Thirteen stands were randomly assigned numbers and with the aid of the table of random numbers, samples were then collected. An average of 28 samples of the whole intestines of local chickens was collected weekly. Three seasons were covered in the course of this research: late dry season, February-April; early rainy season, May-July and late rainy season, August-October. There are no records that show the total number of local chickens being slaughtered at the Mudal Lawal market. As a result, having a defined sample size for this research was difficult. A total of 1,000 whole intestines from slaughtered local chickens were randomly collected from different slaughter stands in the slaughterhouse of the Bauchi poultry market. These were then put in separate polythene bags, appropriately labelled and transported to the laboratory in a flask. Each of the whole intestines was cut open and two samples of the intestinal content were collected, cultured and examined for the presence of *Clostridium* and *Eimeria* species at the National Veterinary Research Institute (NVRI) outstation laboratory in Bauchi. Of the two samples collected from the intestine, one was used for microscopic examination for the presence of coccidial oocysts using the simple floatation technique[8]. The second sample was then used to culture for *Clostridium perfringens*.

Laboratory analysis for *Clostridium perfringens*: To isolate sporulating strains, luminal materials from the intestines were enriched for 24 h in cooked meat broth under anaerobic conditions at 37°C and subjected to heat and alcohol shock. A loopful of culture was plated onto a blood agar base with 10% sheep blood and 70 μg mL^{-1} neomycin sulphates. The plates were incubated at 37°C for 24 h under anaerobic

conditions[9]. Typical colonies showing a double zone of beta haemolysis were picked and subcultured. The subcultured colonies were identified by colony morphology and Gram staining. The severity of infection was determined using a technique described by Byrne *et al.*[10].

Laboratory analysis for *Eimeria*: Each intestinal tract was examined microscopically for lesions due mainly to coccidiosis and the entire contents of the intestines were then transferred to a sterile plastic tube and examined for the presence of coccidial oocysts using the simple floatation technique[8].

Statistical analysis: The data were entered into Microsoft Excel and analysed using the Minitab Statistical Package version 16. Descriptive statistics were used to determine the prevalence of the parasite and the Chi-square test (χ^2) was used to calculate the differences in prevalence between season, strain and sex of local chickens. It was applied to 2×2 contingency tables with a dichotomous trait[11]. The McNemar test for significance of changes showed a significant ($p<0.001$) deviation.

RESULTS AND DISCUSSION

Occurrence of NE in the study area: The overall occurrence of necrotic enteritis (NE) and coccidiosis in local chickens slaughtered in the study area was 55.7 and 31.2%, respectively (Table 1). The high occurrence of NE in this study area maybe largely due to poor management practices in which local chickens scavenge for feed freely in their environment, thus increasing the probability of exposure of local chickens to the aetiologic agent of NE. The percentage occurrence (55.7) of NE observed in this study is very similar to the occurrence (57.9%) of NE reported by Osman *et al.*[12] for Egypt. Hermans and Morgan[13] conducted a survey in Canada and found that 32.8% of respondents indicated they had a case of NE, showing the high occurrence of this disease in poultry farms even in agriculturally advanced countries.

Effect of season on occurrence of NE: From the results shown in Table 2 on the occurrence of NE in local chickens based on the season, a significant ($p<0.001$) effect of season on the disease occurrence can be deduced. The early rainy season had the highest (64.6%) occurrence compared to the late rainy (62.3%) and late dry (48.0%) seasons. The high occurrence during the rainy season maybe due to the weather conditions of warmth and moisture which are favourable for the growth of *Clostridium perfringens* that causes NE. These results are different from a previous study conducted in Canada[14], where NE was more frequently observed during the dry periods of the year. NE often occurred more than once per year on a farm as observed by Mohajeri *et al.*[15], which also agrees with the findings of this research that NE occurs in both the dry and rainy seasons of the year.

Effect of genotype on occurrence of NE: The effect of genotype on the occurrence of NE in local chickens was not significant, as presented in Table 3. These results are in contrast to those reported by Mohajeri *et al.*[15], a study carried out in Iran, which found that the occurrence of NE varies significantly with different broiler genotypes. These contrasting findings may be due to lack of classification of the local chickens into distinctive genotypes in this study. A retrospective study revealed large differences between genotypes, where some were more resistant to NE compared to others in relation to mortality and hen day egg production in a NE-infected laying hen flock[16].

Effect of sex on the occurrence of NE: Table 4 shows that the effect of sex on the occurrence of necrotic enteritis was not large. The effect of sex on the disease occurrence was not

Table 1: Occurrence of necrotic enteritis and coccidiosis in local chickens slaughtered in Bauchi metropolis

	No. of samples		Percentage	
Diseases	Necrotic enteritis	Coccidiosis	Necrotic enteritis	Coccidiosis
Infected	557	312	55.7	31.2
Not infected	443	688	44.3	68.8
Total	1000	1000	100.0	100.0

Table 2: Occurrence of necrotic enteritis in local chickens based on season

Season	No. infected (% of total)	No. not infected (% of total)	Total	χ^2	Level of significance
Late dry	240 (48.0)	260 (52.0)	500	24.290	***
Early Rainy	155 (64.6)	85 (35.4)	240		
Late Rainy	162 (62.3)	98 (37.7)	260		
Total	557 (55.7)	443 (44.3)	1000		

χ^2: Pearson chi-square, ***Significant at $p<0.001$

Table 3: Occurrence of necrotic enteritis based on the genotype of local chickens

Genotype	No. infected (% of total)	No. not infected (% of total)	Total	χ^2	Level of significance
Normal	444 (55.4)	358 (44.6)	802		
Naked Neck	66 (57.9)	48 (42.1)	114		
Frizzle	45 (59.2)	31 (40.8)	76	3.7	ns
Dwarf	2 (25.0)	6 (75.0)	8		
Total	557 (55.7)	443 (44.3)	1000		

χ^2: Pearson chi-square, ns: Not significant

Table 4: Occurrence of necrotic enteritis based on the sex of local chickens

Sex	No. infected (% of total)	No. not infected (% of total)	Total	χ^2	Level of significance
Male	254 (57.2)	190 (42.8)	444		
Female	303 (54.5)	253 (45.5)	556	0.7	ns
Total	557 (55.7)	443 (44.3)	1000		

χ^2: Pearson chi-square, ns: Not significant

Table 5: Severity of necrotic enteritis in local chickens based on season, genotype and sex

		Severity					
Variables		Low	Moderate	High	Total	χ^2	Level of significance
Season	Late dry	145 (60.4%)	68 (28.3%)	27 (11.3%)	240		
	Early rainy	98 (63.2%)	45 (29.0%)	12 (7.7%)	155	31.3	*
	Late rainy	83 (50.6%)	55 (34.8%)	24 (14.6%)	162		
Genotype	Normal	258 (58.1%)	136 (30.6%)	50 (11.3%)	444		
	Naked neck	40 (60.6%)	18 (27.3%)	8 (12.1%)	66		
	Frizzle	27 (60.0%)	16 (35.6%)	2 (4.4%)	45	7.9	ns
	Dwarf	1 (50.0%)	0 (0.0%)	1 (50.0%)	2		
Sex	Male	155 (61.0%)	68 (26.8%)	31 (12.2%)	254		
	Female	171 (56.4%)	102 (33.7%)	30 (9.9%)	303	4.1	ns

χ^2: Pearson chi-square, *Significant at $p<0.05$, ns: Not significant

Table 6: Occurrence of necrotic enteritis in association with coccidiosis in local chickens

	Necrotic enteritis			
Coccidiosis	Not infected	Infected	χ^2	Level of significance
Not Infected	311[a]	377[b]	117.9	***
Infected	132[c]	180[d]		

χ^2: McNemar Test for significance of changes, ***Significant at $p<0.001$, where c and b cells are the changers

statistically significant and this finding could be due to equal exposure of both the male and female local chickens to infection. This is in contrast to a study carried out in Iran by Mohajeri *et al.*[15] that reported a significant difference in the prevalence of NE between males and females (52.9% vs. 47.2%).

Effects of Season, genotype and sex on severity of NE infection: Season had a significant ($p<0.05$) effect on disease severity (Table 5). Table 5 shows that more than half (58.5%) of the chickens had the unapparent form of the infection, while 30.2 and 11.3% had the moderate and severe forms of the infection, respectively. The results of this research concurred with the findings of Skinner *et al.*[1], who reported that the majority of NE outbreaks are subclinical or unapparent and occur unnoticed. This may possibly be due to the gradual accumulation of toxins produced by *Clostridium perfringens*. It is the most devastating of all the forms and has not been formally investigated[1]. The subclinical form of the disease causes damage to the intestinal mucosa leading to decreased digestion and absorption, reduced weight gain and increased feed conversion ratio[2].

Association between occurrence of NE and coccidiosis: The McNemar test for significance of changes showed a significant deviation ($p<0.001$) from the speculation that birds having NE were likely to have coccidiosis, thus indicating the lack of an association between the occurrence of necrotic enteritis and coccidiosis, as seen in Table 6. The study revealed that no relationship existed between necrotic enteritis and coccidiosis, in contrast to the speculation that the latter was a predisposing factor to the former[16]. This may be because the causative agents of NE and coccidiosis are completely different (bacteria for NE and protozoa for coccidiosis). However, in agreement with this research, several reports have indicated that coccidiosis does not always result in NE[13]. Williams[17] generally concluded that *Eimeria* may only provide

a gateway for the entry of *Clostridium perfringens* into the intestinal wall but do not have any direct effect on the growth of *Clostridium perfringens* and thus NE.

Some challenges encountered in the course of this study included alack of quick diagnostic kits, which may lead to changes in samples during long distance transport to the laboratory, lack of modern diagnostic equipment and no research grants from the government to encourage research. More research can be designed to study the complete course of NE in local chickens by inducing the disease in the laboratory. Studies can also be carried out on the predisposing factors of NE in local chickens.

CONCLUSION

An occurrence of necrotic enteritis of 55.7% was established in this research. There were seasonal variations in the occurrence, such that the highest occurrence was found in the early rainy season (64.6%). More than half of the birds had the unapparent form of necrotic enteritis, while the remaining birds had either the moderate or severe forms. Finally, there are no associations between necrotic enteritis and coccidiosis.

SIGNIFICANCE STATEMENT

This study examines the occurrence of necrotic enteritis in local chickens that presents clinical signs similar to coccidiosis. This study can be beneficial for local chicken farmers who usually do not think of diseases other than coccidiosis. This study will help researchers to develop a database for the occurrence of NE in order to achieve more effective control measures for this costly disease.

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