Consequences of Concurrent Infections with *Ascaridia Galli* and *Eimeria* in Broiler Chickens

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ABSTRACT---- ***Ascaridia galli and Eimeria species constitute the aetiology of ascaridosis and coccidiosis respectively in chickens, and the two diseases are the most important internal parasitic infections that militate against the development of the poultry industry. Natural infections of both parasites are known to occur in chickens but it is not yet fully known how conjoint infections with both parasites can influence the pathology they inflict cause to the chickens. A. galli has been reported to enhance the pathogenicity/pathology of Pasteurella multocida and Escherichia coli when the organisms occurred concurrently with the nematode. This study was therefore conducted to investigate the influence of A. galli to the response of two-week old broiler chickens to single and concurrent infections with the parasites either given at the same time or at different times with the hope that the results would greatly inform decisions to be taken in mitigating the infections.***

Forty two broiler chickens were used for the study. The birds were allocated into six groups of 7 birds each and placed in separate pens. Five of the groups were either infected with A. galli only, caecal Eimeria isolate only, A. galli and caecal Eimeria isolate at the same time, A. galli and later caecal Eimeria isolate or caecal Eimeria isolate and later A. galli while the sixth group remained as uninfected controls. The dose levels used were 1000 embryonated A. galli eggs and 12,000 sporulated Eimeria oocysts per chicken by oral administration. Packed cell volume (PCV), body weight (BW), feed consumption (FC), faecal egg counts (EPG), faecal oocyst counts (OPG), caecal gross lesion score (GLS) for Eimeria infected birds, clinical signs and worm burdens (WB). The infections had a negative impact on the birds evident by low PCV, low BW gain, reduced feed consumption, listlessness, bloody diarrhoea and mortalities when compared with the uninfected controls that did not exhibit such effects.

A drop in the PCV from day 8 to day 12 of the birds infected with A. galli and later Eimeria group occurred following the administration of the protozoan infection. The A. galli and later Eimeria group also had the least weight gain from day 12 to day 42. Similarly their feed intake was also least from day 10 to day 26 in the A. galli and later Eimeria group in comparison with all the other infected and the uninfected control birds. The greatest mortalities (5 birds) also occurred in the A. galli and later Eimeria group.

It was concluded that the infections lead to poor performance of the chickens, however, the effect of the infection was severest in the chickens that were infected with A. galli and later with Eimeria. It was suggested that A. galli caused immune down regulation of the chickens allowing the Eimeria to exert maximally its pathogenic effects on the birds. It was also suggested that the initial A. galli infection possibly attracted influx of large numbers of macrophages into the lamina propria where they in turn greatly enhanced the transportation of sporozoites from the lamina propria into the glands of Lieberkuhn. It is therefore strongly recommended that the infections should be diagnosed regularly and controlled promptly when present particularly if they are concurrent as the effects can be disastrous in situations where A. galli infections precede Eimeria infections as in the semi intensive and free range systems of poultry production.

1. INTRODUCTION

The poultry industry plays a crucial role not only in the provision of protein of animal source to man but also as a contributor to the development of the economies of many nations through provision of revenue and employment[1, 2]. Poultry production is both widespread and the most developed and gainful animal production enterprise today[3, 4]. Its importance in improving the nutritional status and income of many small scale chicken producers and those with limited or even no land has long been acknowledged by various scholars and rural development agencies [2, 3, 5]. In Nigeria, the poultry industry contributes up to 15% to the gross domestic product (GDP) and accounts for 36% of total protein intake of the country [6].

In spite of the pivotal role of poultry in the economic development of many countries, diseases of poultry have been a major threat to profitable poultry production [7, 8, 9]. Parasitic diseases are among the most dreadful diseases that influence poultry production and amongst which ascaridosis and coccidiosis are the most important**.**

Coccidiosis is an intestinal parasitic disease caused by intracellular protozoan parasites of the genus *Eimeria*[10]. Seven major species of *Eimeria* are widely recognized as the causative agents of coccidiosis in chicken, namely *E. acervulina, E. brunetti, E. maxima, E. mitis, E. praecox, E. necatrix* and *E. tenella* [11], although *E. hagani* and *E. mivati* are also recognized [12]. The two most pathogenic species are *E. tenella* and *E. necatix* [13, 14].

Birds of all ages are susceptible to coccidiosis but most birds get infected in the early few weeks of life [15]. Most *Eimeria* species affect birds between 3 and 8 weeks of age and can cause high mortality [16]. Coccidiosis is endemic in greater parts of both tropical and subtropical areas where ecological and management conditions are conducive for the transmission of *Eimeria* throughout the year [17]. The disease has been reported in most parts of Nigeria [18, 19, 20, 21]. The prevalence rate of coccidiosis is higher during the rainy season as the most important biotic requirements of warmth and moisture are readily attained during the rains thereby enhancing the development and availability of the infective oocysts to the birds [9, 22].

On the other hand ascaridosis is an intestinal parasitic disease with aetiology as *A. galli* of the phylum Nematoda [23]. Birds of all ages can be infected by the nematode however the greatest degree of damage is common in young birds less than 12 weeks old [24]. Heavy infections have lead to reduced weight gain or even weight loss and reduced egg production in affected birds [24]. Heavy infections often result in obstruction of the intestine [24], however, the bulk of the pathology of *A. galli* is due to its developmental stages in the lining of the gut in form of haemorrhagic enteritis.

Although most epidemiological studies have focused on single infections, poultry are actually exposed to a multitude of pathogens in nature. Unfortunately, concurrent infections are known to influence the response of the host to the co-infecting parasites. This has been demonstrated in layer chickens where birds infected with both *A. galli* and *Pasteurella multocida* were shown to suffer greater impact of the infections than those with single infections of *A. galli* or *P. multocida* alone[25]. In like manner, chickens concurrently infected with both *A. galli* and *Escherichia coli* showed more severe clinical signs and pathology than the singly infected ones [26]. Furthermore, it was also reported that the timing of the co-infecting parasites had an impact on the severity of the infection [26]. For example, in both cases cited above, the pattern of pathogenesis became more severe where *A. galli* preceded the co-infecting parasites, a situation that led to the conclusion that *A. galli* had an immune suppressing effect [26]. However, unlike Dahl and Permin [25] and Permin and Christensen [26],. Norton *et al.* [27] had reported that in concurrent *A. dissimilis* and *E. meleagritis* infections in turkeys the parasites interacted in such a manner to suppress each other leading to lower oocyst production and fewer third-stage larvae than in turkeys that were infected with either *E. meleagritis* or *A. dissimilis* respectively alone. The importance of concurrent infections of pathogens is not only limited to poultry as it has also been demonstrated in humans [28, 29], wildlife [30,31] and extensively farmed livestock species [32].

Unlike the above studies, there are no properly documented controlled experimental studies on *Eimeria* and *A. galli* concurrent infections known to the authors. The few cases of concurrent *Eimeria* and *A. galli* infections documented are largely prevalence studies without any relation as to how one affects the other. However, given the high prevalence, pathogenicity and endemicity of both parasites in the study area, it was deemed necessary to bridge the dearth of information that currently exists by finding out how both parasites affect the host response to the infections. The current study was therefore designed to investigate the response of broiler chickens to single and concurrent experimental infections of *Eimeria* and *A. galli* either given simultaneously or *A. galli* superimposed on *Eimeria* and vice versa. If it is shown that concurrent *Eimeria* and *A. galli* infections are more pathogenic than single infection with the parasites, and that it is particularly more dangerous when *A. galli* infection precedes the *Eimeria* infection, then emphasis would be made in the early prophylactic control of ascaridosis beginning from early life of the birds to minimize the pathogenicity of the highly pathogenic ubiquitous *Eimeria* infections which are able to cause clinical coccidiosis even from about two weeks of age.

2. MATERIALS AND METHODS

Experimental birds

Broiler chicks of Anak 2000 breed which are commonly reared in Nigeria because of their big size, body conformation and white color were obtained from a reputable source at day old, brooded and managed as described by Ngongeh *et al.* [33]. Briefly, birds were managed on deep litter which was maintained dry by replacing old litter with dry wood shavings every two days. Birds were duly vaccinated against viral diseases (Newcastle disease and infectious bursal diasease). Prophylactic treatment against *Eimeria* infections was done with toltrazuril (Baycox 2.5% w/v Oral Solution) which was withdrawn before experimental infections.

All relevant laws and codes of practice governing the experimental studies with life animals were complied as stipulated by Ward and Elsea [34], and the experimental protocol was approved by Michael Okpara University of Agriculture, Umudike Animal Ethics Committee.

Eimeria isolate

The *Eimeria* used for the study was isolated from the caeca of 3 to 4 weeks old broiler chickens clinically sick of coccidiosis as described by Ngogeh *et al.* [33] but with slight modifications. The caeca were collected during post mortem and incised and the contents which were largely blood and caecal cores were washed into sieves placed in small bowls and caecal cores crushed with a spatula. Caeca with much faecal material were avoided in order to obtain a clear *Eimeria* oocyst suspension for easy quantification and dose determination. The filtrate was poured in 400ml beakers and washed thrice with water in order to remove the red colour of blood by allowing the suspension to sediment for 10 minutes before decanting each time. Following washing, the sediment was examined to confirm the presence of the oocysts. The sediment was resuspended in 100ml of water and 20ml of the *Eimeria* suspension was dispensed into each Petri dish. 1ml of the sporulating agent, 2.5% potassium dichromate ($KCrO_4$) was then added into each Petri dish and incubated at room temperature (27-28^{0}c) on bench tops in the Parasitology Laboratory in MOUAU for five days.

Following sporulation, the suspensions were recollected in a 400ml beaker and more water was poured into the suspension, allowed to sediment for 20 minutes and then decanted in order to wash out the $KCrO_4$. The suspension was allowed to sediment for 20 minutes before decanting each time and this was repeated twice. The caecal *Eimeria* isolate was then put into a 50ml Teflon tube and preserved in the refrigerator (4^{0}C) until it was used for the experimental infections two weeks later.

A. galli embryonated eggs

Embryonated *A. galli* eggs used for the study were obtained from *A. galli* egg cultures. *A. galli* adults were obtained from the intestines of spent layers slaughtered at Ekpa market in Nsukka local government of Enugu state. Briefly, following slaughter, intestines were collected and the small intestine were separated and incised to harvest the adult *A. galli*. The worms were then rinsed with distilled water and the females were placed in a sieve in a Petri dish and macerated with a few drops of PBS/distilled water. More distilled water was added to the crushed worms in order to wash the eggs into the Petri dish while the residue was discarded. The egg suspension/filtrate was then transferred to a 200ml beaker and washed **5** times with distilled water and allowed to sediment. The supernatant was siphoned and sediment was re-suspended in normal saline and 20ml of it were placed in each Petri dish and cultured on desktops at 27-28ºC in the laboratory for 21 days. Aliquots/drops were collected at 5 days interval and checked for the extent of development. Embryonated eggs were washed with fresh normal saline and preserved in 50ml Teflon tubes in the refrigerator (4^{0}C) until they were used within four weeks.

Experimental design and infection

Forty two broiler chickens were used for the study as shown on the experimental design summarized on Table 1. Briefly, each experimental group was comprised of 7 chickens. Some groups were infected with both parasites on day (D) 0 or at one week interval while some were infected with *A. galli* or *Eimeria* alone and one group remained as uninfected controls. Four chickens from the *A. galli* infected groups were necropsied for total worm counts on D60 of the study.

Table 1: Groupings and infection protocol

Group	Number of birds	Day of infection with *Eimeria*	Day of infection with *Ascaridia*	Day of autopsy
1. Infected with 12,000 sporulated caecal *Eimeria* oocysts only	7	0	-	60
2. Infected with 1,000 embryonated eggs of *A. galli* only	7	-	0	60
3. Infected with *Eimeria* and *A. galli* at the same time	7	0	0	60
4. Infected with *A. galli* and later *Eimeria*	7	8	0	60
5. Infected with *Eimeria* and later *A.galli*	7	0	8	60
6. Uninfected controls	7	-	-	60

The course of infection was monitored using the parameters feed intake, body weight, PCV, clinical signs, mortalities, faecal worm egg counts, faecal oocyst counts, gross lesion scores, microscopic lesions and worm counts.

Feed intake

Feed was weighed with a weighing balance (Camry Emperors, China) each time before introducing it into the feed troughs and the remnant weighed at the end of the day, and the difference was determined and recorded as the feed consumed each day for each group.

Body weight

The birds were each weighed using a weighing balance (Camry Emperors, China) on day zero of the experiment and subsequently every four days till the end of the study.

Packed cell volume

The packed cell volume (PCV) was taken on day zero and later every four days till the end of the study. Blood was collected via the wing vein directly into heparinized capillary tubes (Camlab Ltd, Cambridge) for determination of PCV.

Clinical signs

The birds were monitored closely for possible observable signs (such as droopy wings, bloody faeces, morbidity and mortality) throughout the course of the experiment starting on the second day post infection.

Mortalities

The number of Birds that died before the end of the experiment were also recorded.

Faecal worm egg counts

Faecal worm egg counts, expressed as eggs per gramme (EPG) of faeces, were conducted on freshly collected faecal samples from individual chickens using the floatation technique and where suitable by the modified McMaster technique [35, 36].

Faecal oocyst counts

Faecal oocyst counts were conducted daily on freshly collected faecal samples from each chicken beginning from day 3 of the infection until patency and thrice a week following patency using both the centrifugal floatation in saturated salt solution and where suitable by the modified McMaster technique [35, 36].

Gross lesions

Post mortem examination was carried out on birds (*Eimeria*-infected birds) that died before the end of the experiment and the gross lesions scores (GLS) of the caeca were recorded. Gross lesions such as caecal haemorrhages, necrotic foci and bloody caecal contents and/or caecal cores were checked. Coccidial lesion scoring was conducted for the caeca as a means of assessing the severity of the infection and gross lesions were graded from 0 to 4 based on lesion score key [37]. A lesion score of zero represents absence of lesions while a lesion score of four indicates very severe caecal mucosa lesion.

Worm counts

At the end of the experiment, 4 birds from each of the experimental groups (infected with *A. galli*) including the uninfected control group were humanely sacrificed by cervical dislocation and the small intestines were harvested for post mortem worm counts. Following a longitudinal incision the contents of the small intestine were washed with water into bowls and allowed to settle for ten minutes before decanting. 10% formol saline was then added to the worm suspension and preserved for worm counts using a stereo microscope at 40X magnification. All the worms in the worm suspension from each chicken were counted by pouring aliquots of 20ml each time in a ruled Petri dish and scanning and counting any worm sighted until the entire suspension was exhausted.

Microscopic lesions

Caecal tissues were collected for histopathology at postmortem. Histopathological studies of the caeca were made from chickens randomly chosen from each of the groups with *Eimeria* infections at the end of the experiment. Tissues were fixed in 10% formol saline solution. Following the fixation process, samples were dehydrated in alcohol, cleared in xylene, and then embedded in paraffin wax. The tissues were sectioned at 5 micrometer and stained by haematoxylin and eosin (H & E). Stained tissues were mounted and examined under a light microscope.

Worm burden

Chickens were necropsied humanely on day 60 of the study and the small intestines were recovered for worm counts according to Ngongeh *et al* [38].

Data analysis

Summary data are presented as mean ± standard error of the mean (S.E.M.). Data were analysed univariate aunivariate analysis.

Summary data are presented as mean ± standard error of the mean (SEM) and Probabilities (P) of 0.05 or less were considered significant. Data were analyzed using both the student t-test and analysis of variance (ANOVA)

3. RESULTS

Clinical signs

Chickens in the *Eimeria* infected groups exhibited dullness, off-feed or reduced feeding, bloody diarrhoea, droppiness, listlessness, ruffled feathers and mortalities from day 5 of infection. Morbidity was high in the concurrent infected groups while some mortalities occurred in the *Eimeria* only, *Ascaridia later Eimeria*, and *Eimeria* and and *Eimeria* later *Ascaridia* infected groups.

Faecal oocyst counts

Eimeria infections became patent on day 4 evident by presence of oocysts in the faeces of birds infected with the *Eimeria* isolate while there were no oocysts in the faeces of uninfected control birds as expected. The faecal oocyst counts (OPG) rose sharply in all infected groups following patency with peaks on day 8 for *Eimeria* later *Ascaridia,* day 12 for *Eimeria* and *Ascaridia and Eimeria* only, and day 16 for *Ascaridia* later *Eimeria* groups. Following the various peaks, OPG dropped sharply on day 16 for *Eimeria* only, *Eimeria* later *Ascaridia* and *Eimeria* and *Ascaridia* infected groups before gradually fluctuating and generally reducing to almost zero by day 44. OPG of *Ascaridia* later *Eimeria* dropped at day 20 and mildly fluctuated up to day 44 (Fig 1). The variation in OPG over time was significant ($P < 0.05$). There were variations in mean OPG of the different groups but the difference was not statistically significant ($P > 0.05$).

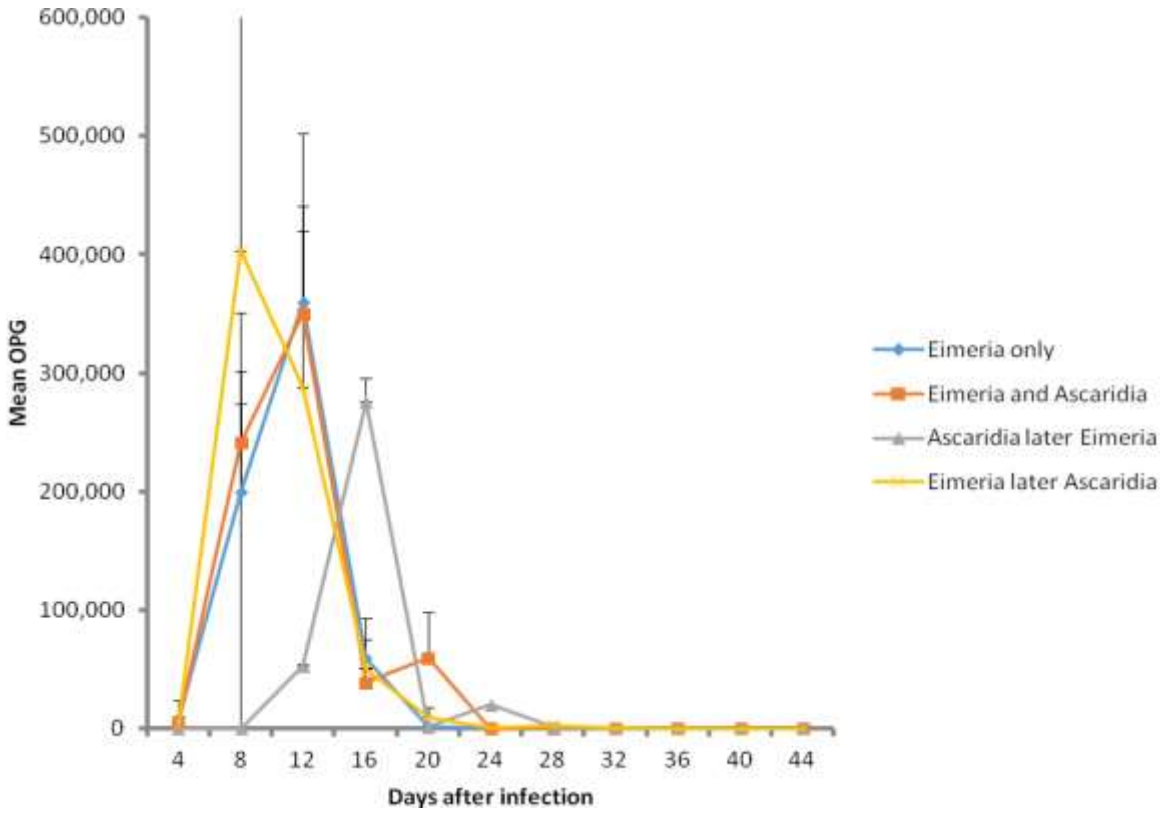

Figure 1: Mean oocyst counts of broiler chickens infected with either sporulated *Eimeria* oocysts alone or *Eimeria* oocysts in combination with *A. galli* at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeri*a oocysts.

***A. galli* faecal egg count**

A. galli infections were patent on day 40 of infection with the nematode. The EPG fluctuated significantly with time ($P < 0.05$) with peaks on days 43, 52 and 58 (Fig 2). The *Ascaridia* and *Eimeria* infected chickens had the highest

EPG on day 43 with mean EPG of 29, followed by *Ascaridia* only group with mean EPG of 18 (Fig 2). The *Eimeria* and later *Ascaridia* group became patent later on day 48 and had very low EPG with a small peak on day 52 with mean EPG of 2 (Fig 2).

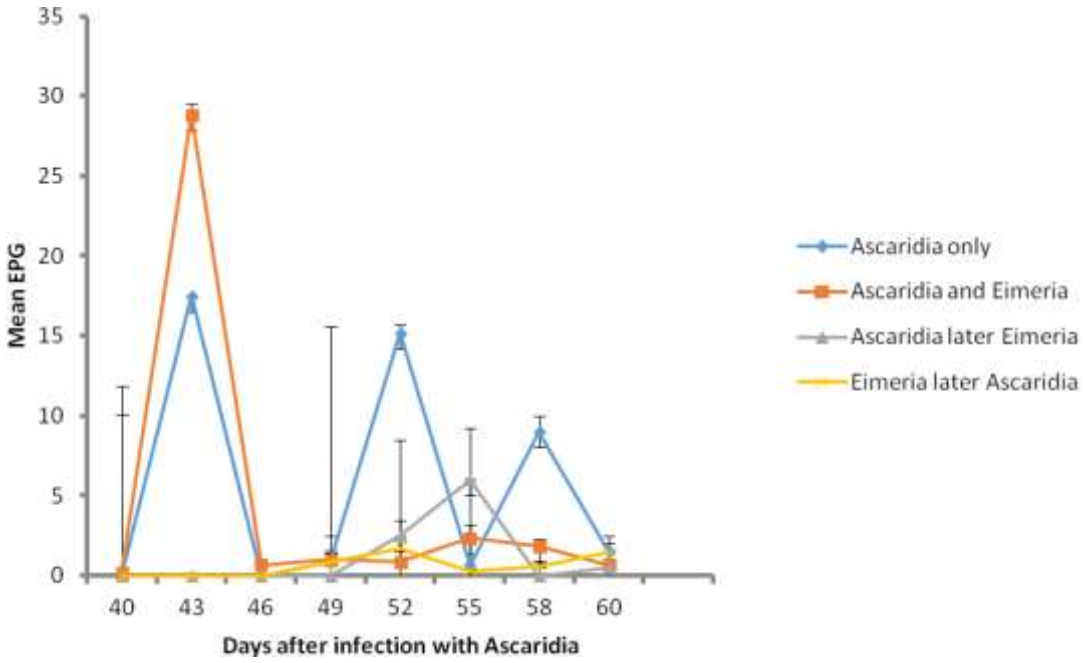

Figure 2: Mean faecal egg counts of broiler chickens infected with either *A. galli* alone or *A. galli* in combination with *Eimeria* oocysts at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeria* oocysts.

Packed cell volume

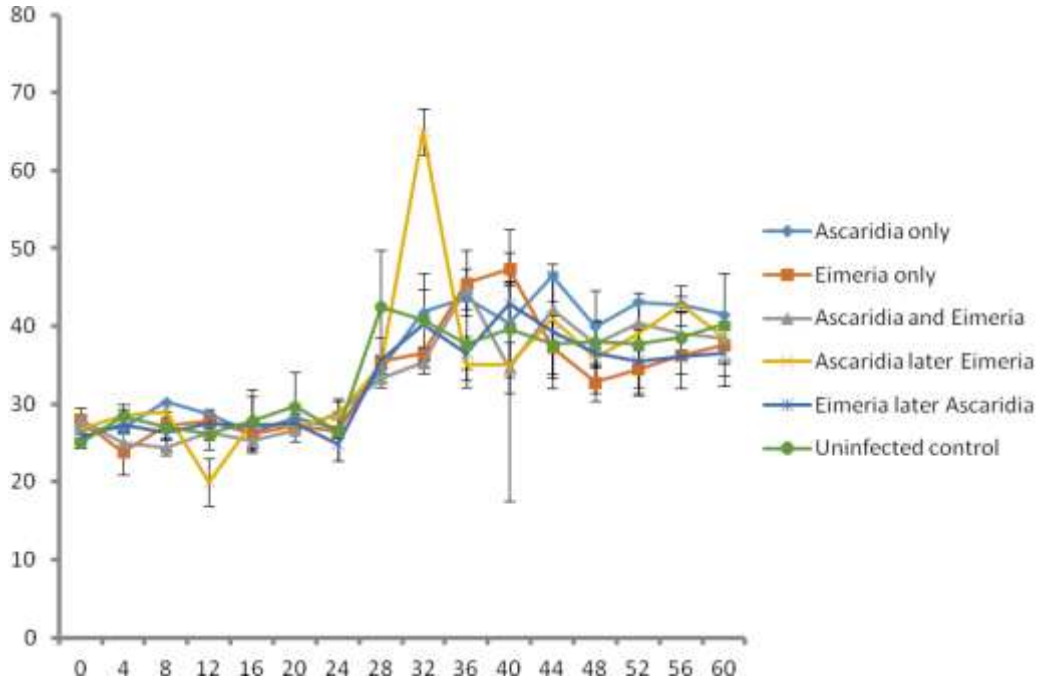

Figure 3: Mean PCV of broiler chickens infected with either *A. galli* or *Eimeria* oocysts alone or *A. galli* in combination with *Eimeria* oocysts at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeria* oocysts.

Figure 3 illustrates the significant fluctuation of PCV with time ($P < 0.05$). PCV somewhat assumed two plateaus, the lower plateau from day 0 to day 24, and the higher from day 28 to day 60 occurring in the earlier and later parts of the life of the birds respectively (Fig. 3). Chickens in all the *Eimeria* infected groups experienced a drop in PCV either on day 4, 8, or 12, that is, in the early phase of the infection (Fig. 3) compared to the uninfected control, with the drop in *Ascaridia* later *Eimeria* group occurring later on day 12.

Body weight

The body weights of the chickens generally increased in all groups as the study progressed although the weight gain was clearly lower in the *Ascaridia* later *Eimeria* group from day 20 to day 40 (Fig. 4). The rate of weight gain of the uninfected control was clearly higher than of the infected groups from day 28 to day 60 when the study ended although the difference was not **statistically significant on day 60 ($P > 0.05$)**.

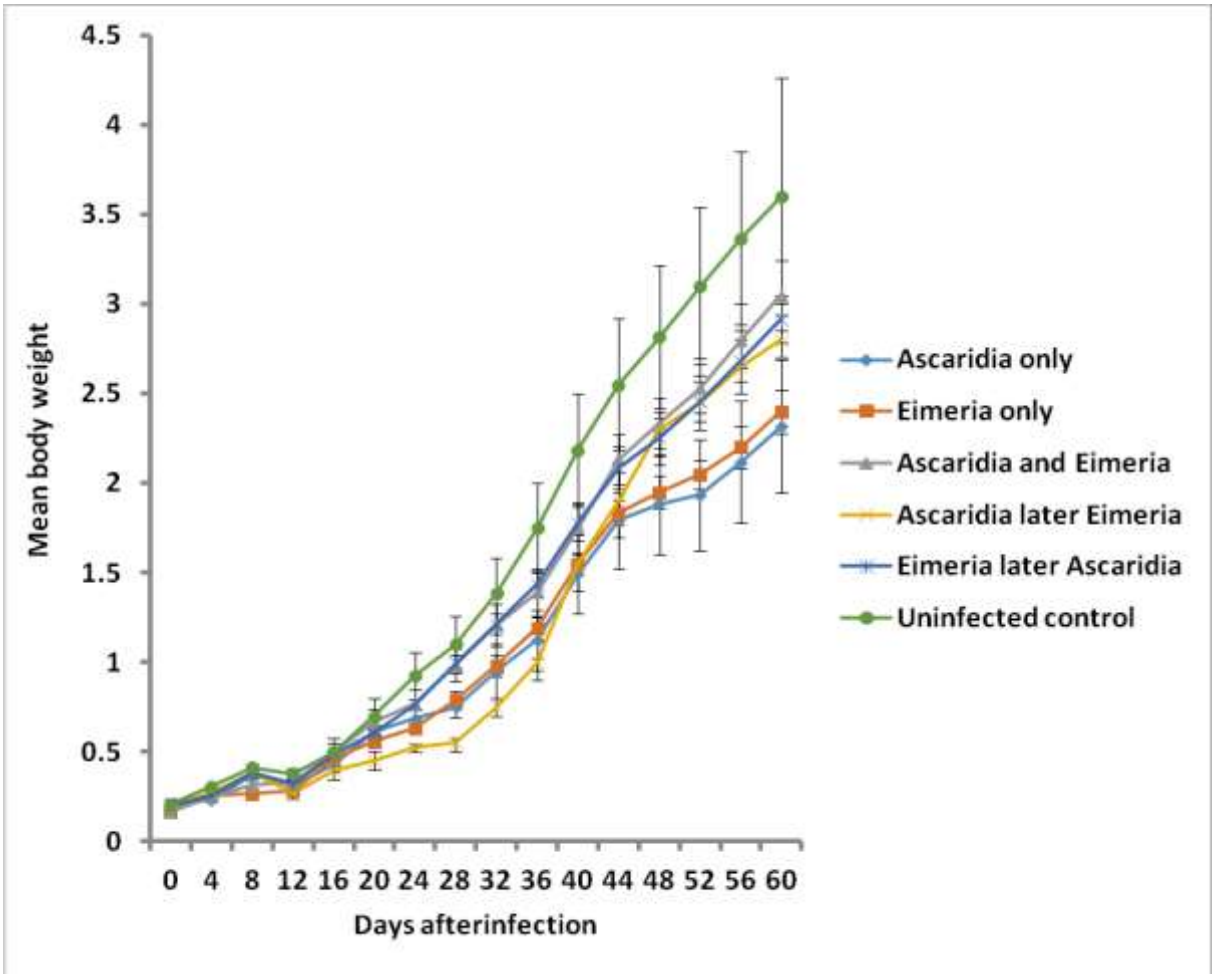

Figure 4: Mean body weights of broiler chickens infected with either *A. galli* or *Eimeria* oocysts alone or *A. galli* in combination with *Eimeria* oocysts at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeri*a oocysts.

Feed consumption

There was an increase in feed consumption in all the groups with time although there were some fluctuations as the study progressed (Fig. 5). However, the uninfected control chickens consumed more feed than their infected counterparts followed by *Ascaridia* later *Eimeria* group in the later part of the study (days 42 and 58). Feed consumption at the end of the study was highest for the uninfected control birds with a mean feed consumption of 0.42**g**. The feed consumption for *Ascaridia* later *Eimeria* dropped sharply from day 12 to day 18 (Fig 5). The feed consumption of *Eimeria* later *Ascaridia* dropped briefly on day 16 before recovering on day 18 (Fig 5).

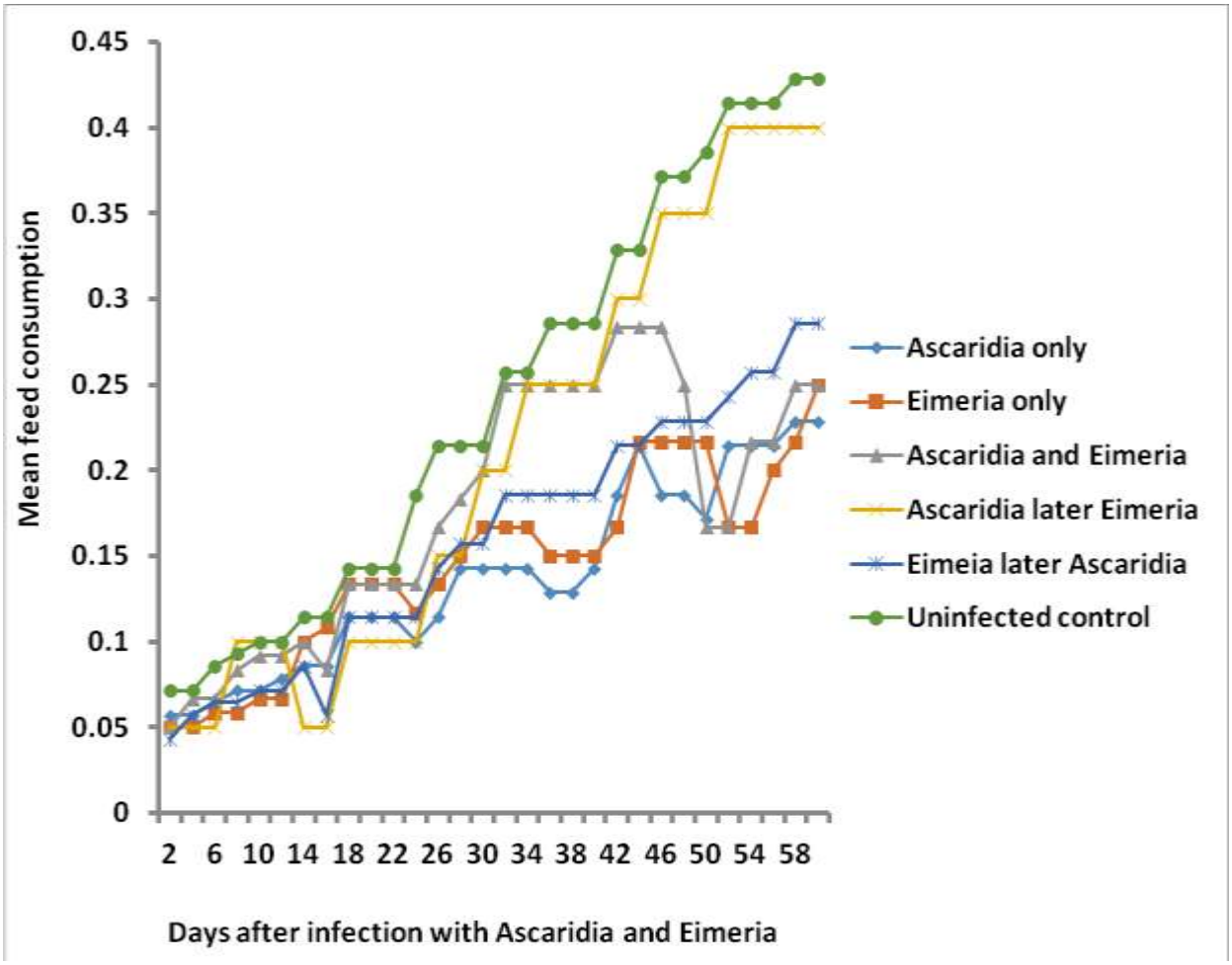

Figure 5: Mean feed consumption of broiler chickens infected with either *A. galli* or *Eimeria* oocysts alone or *A. galli* in combination with *Eimeria* oocysts at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeri*a oocysts.

Mortalities

Some birds died in the early phase of the study, with the *Ascaridia* later *Eimeria* group suffering the greatest mortality rate with as many as 5 of the 7 birds in the group dying (71.4%), while *Eimeria* only and *Ascaridia* and *Eimeria* groups each lost one bird (14.3%) while no mortality occurred in the *Ascaridia* only and the uninfected control groups (Fig. 6).

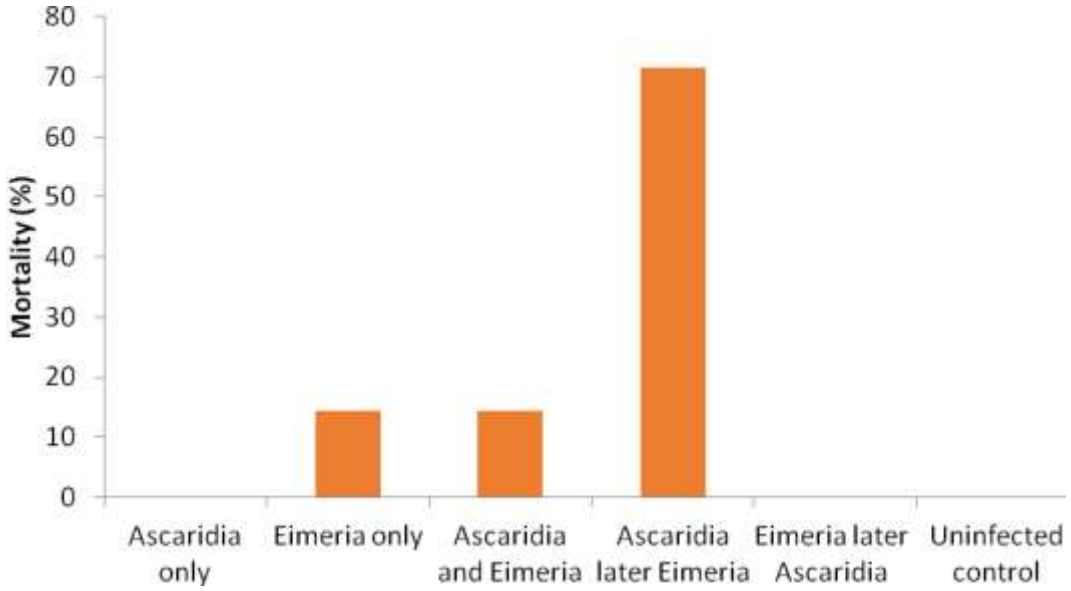

Figure 6: Mortality rate of broiler chickens infected with either *A. galli* or *Eimeria* oocysts alone or *A. galli* in combination with *Eimeria* oocysts at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeri*a oocysts.

Worm burden

The worm burdens varied between groups with the *Eimeria* later *Ascaridia* group having the highest worm burden while the *Ascaridia* only and the *Ascaridia* later *Eimeria* group had intermediate worm burdens, and the *Eimeria* and *Ascaridia* group had the least worm burden (Fig. 7).

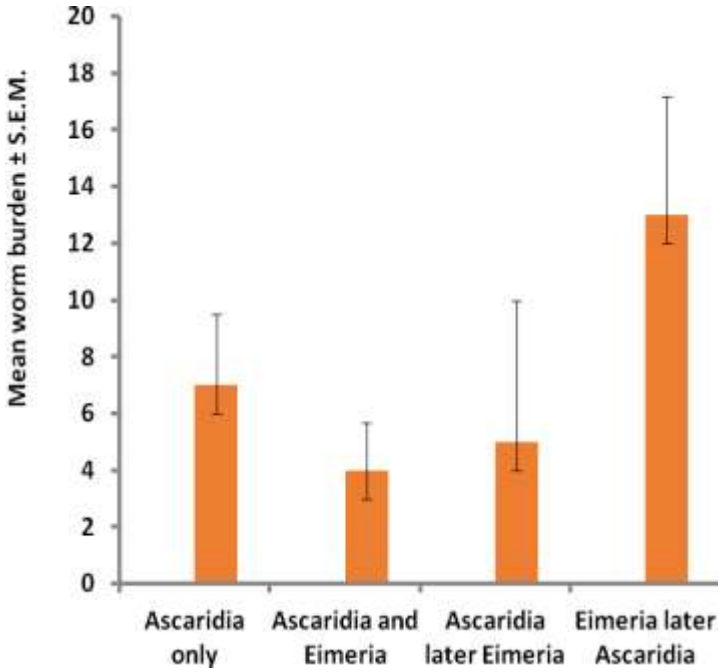

Figure 7: Mean worm burden of broiler chickens infected with either *A. galli* alone or *A. galli* in combination with *Eimeria* oocysts at the same time or *A. galli* given one week before *Eimeria* oocysts or one week after *Eimeri*a oocysts.

***Ascaridia* infected groups**

Gross lesion examination and lesion scores

The gross pathology in the caeca following necropsy of the birds that died before the end of the study was summarized by the following features. There were haemorrhages and caecal cores in the caeca of birds infected with *Eimeria* oocysts either alone or in combination with *A. galli* (Fig. 8, 9, 10, and 11). The GLS were similar in individual birds that died before the end of the study, the caecum of each bird having a GLS of 4.

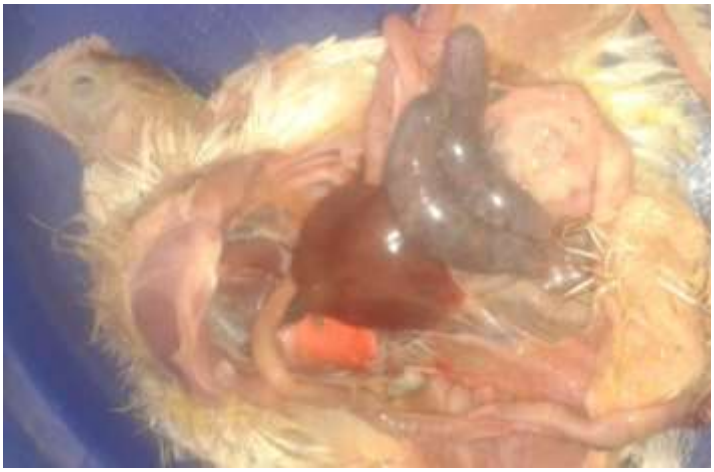

Fig 8: Caeca of a broiler chicken infected with *Ascaridia later Eimeria* distended with blood (as obvious from the serosal surface).

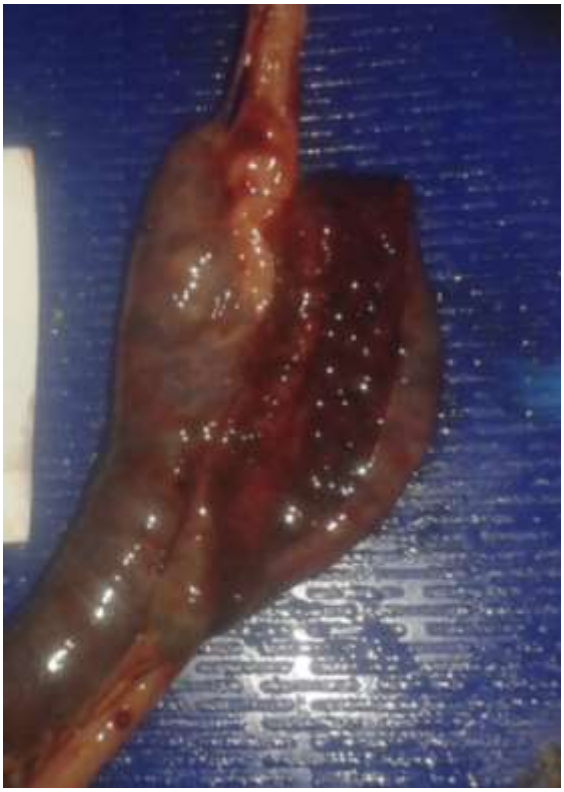

Fig 9: Caeca of a broiler chicken infected with *Ascaridia* later *Eimeria* with haemorrhages and caecal cores.

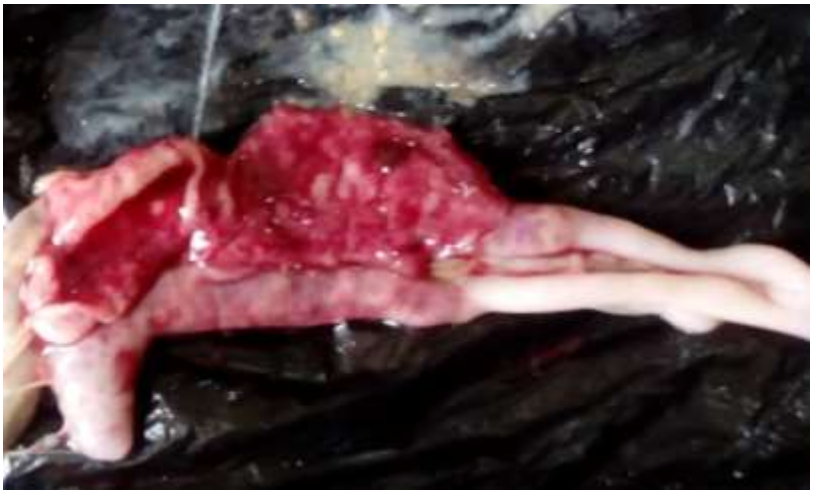

Fig 10: Caeca of a broiler chicken infected with sporulated *Eimeria* oocysts only with bloody caecal content.

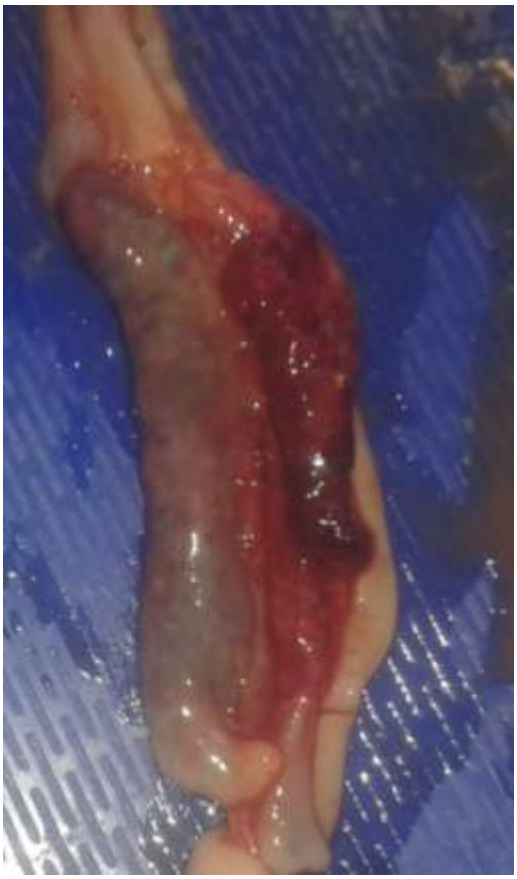

Fig 11: Caeca of a broiler chicken infected with *Ascaridia* and *Eimeria* with bloody caecal content.

Histopathology

The caecal mucosa of all the *Eimeria*-infected chickens that died before the end of the study showed various degrees of pathology evident by oedema, mononuclear cell infiltration (inflammation) and erosions/ulcerations. For example in chickens infected with *Ascaridia and Eimeria* on the same day, there was mild oedema, cellular infiltration and mucosal erosion (Fig. 12). The caeca of chickens infected with *Eimeria* only showed both cellular infiltration and erosions (Fig. 13). Chickens infected with *Ascaridia* and later *Eimeria* displayed severe cellular infiltration and erosions of the caecal mucosa (Fig 14a and b). The caeca of broiler chickens infected with *Eimeria* and later *Ascaridia* exhibited ulcerations of the mucosa and there were massive mononuclear cell infiltration (Fig 15a and b).

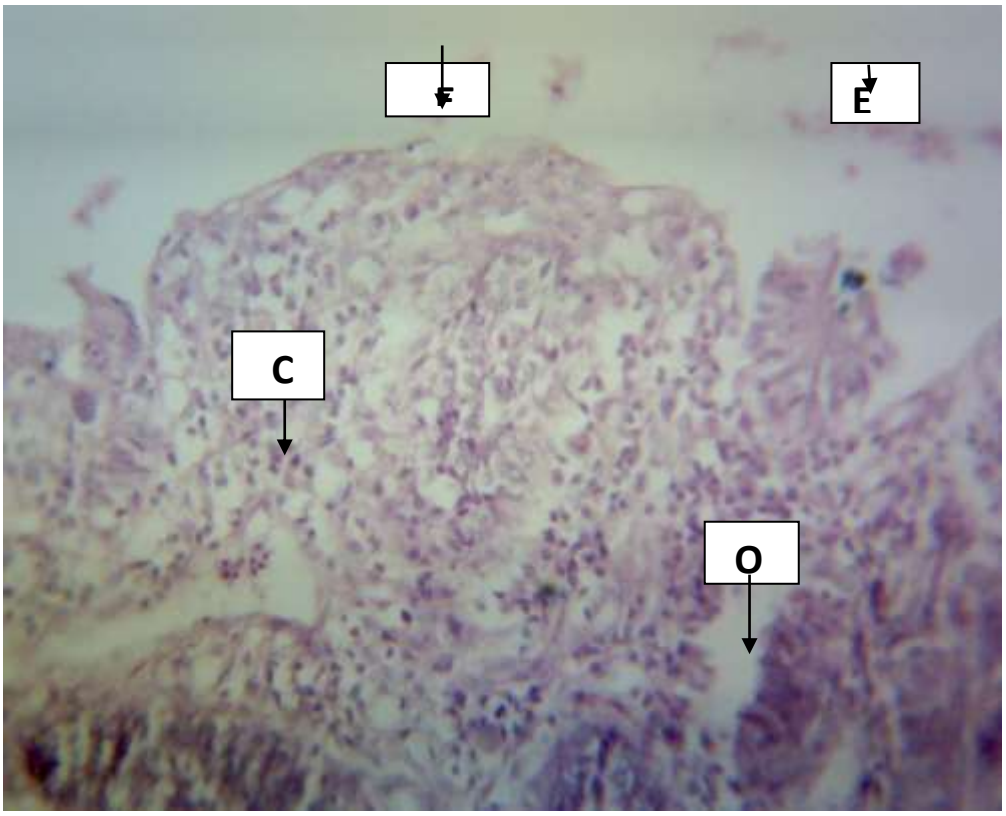

Fig 12. The mucosa of a boiler chicken infected with *Ascaridia* and *Eimeria* showing mild oedema (O), mononuclear cell infiltration (C) and erosions (E) (H & E X400).

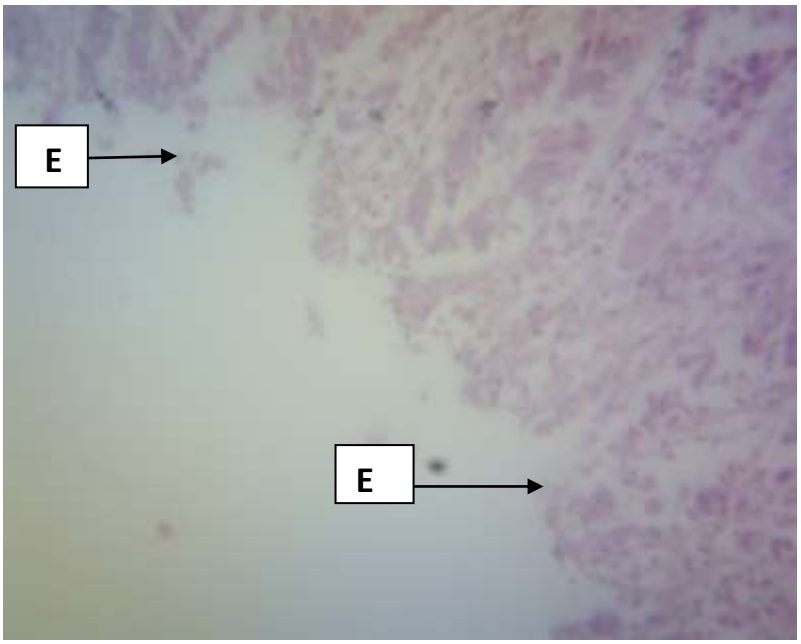

Fig 13. Caecum of a broiler chicken infected with *Eimeria* only showing erosions of the caecal mucosa (E) (H & E X 400).

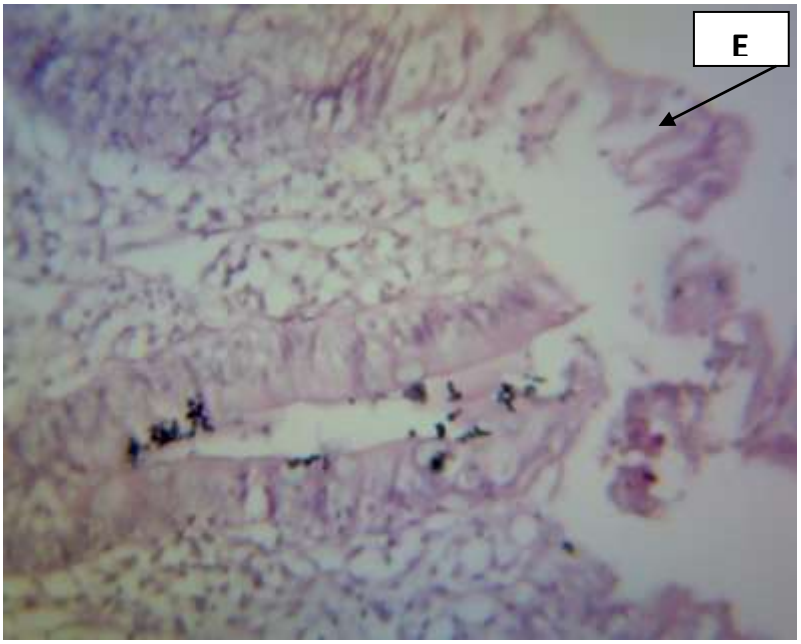

Fig 14a. Caecum of a broiler chicken infected with *Ascaridia* and later *Eimeria* showing massive erosions (E) (H & E X 400).

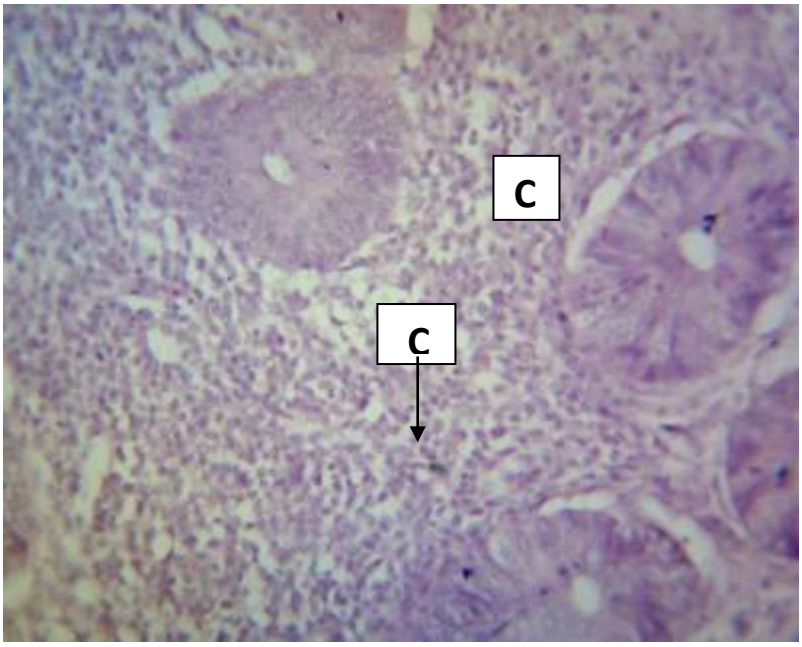

Fig 14b. Caecum of a broiler chicken infected with *Ascaridia* and later *Eimeria* showing massive mononuclear cell infiltration (C) (H & E X400).

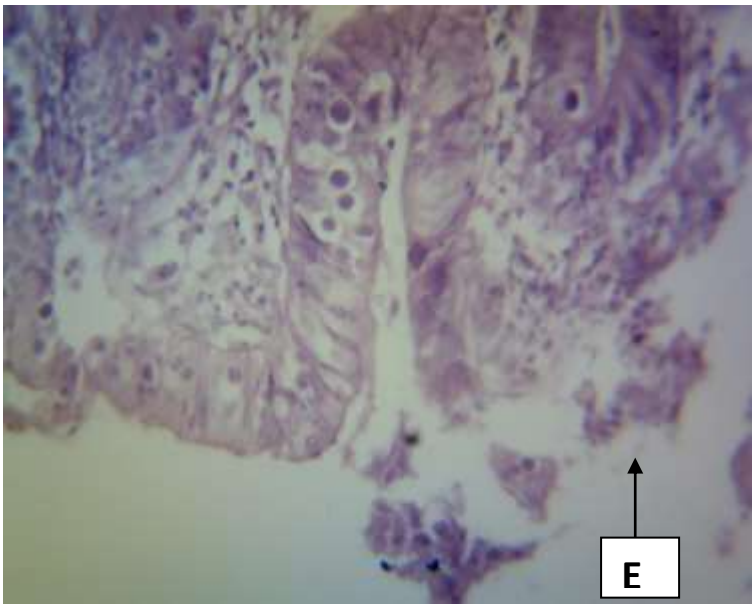

Fig 15a. Caecum of a broiler chicken infected with *Eimeria* and later *Ascaridia* showing erosions of the caecal mucosa (E) (H & E X 400).

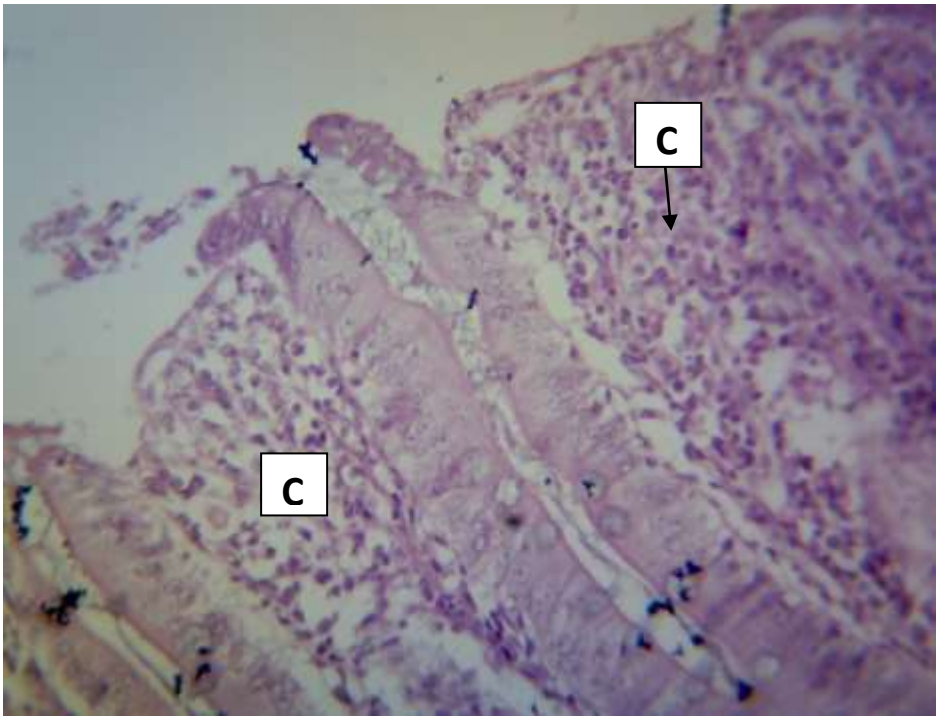

Fig 15b. Caecum of a broiler chicken infected with *Eimeria* and later *Ascaridia* showing massive mononuclear cell infiltration (C) (H & E X 400).

4. DISCUSSION AND CONCLUSION

A distinct effect of concurrent infections with *Eimeria* and *A. galli* was manifested in the chickens by variation in faecal oocyst counts (OPG), FEC (EPG), weight gain, feed consumption, PCV, clinical signs, mortalities, gross lesion scores and microscopic lesions and post mortem worm counts at autopsy. The voidance of *Eimeria* oocysts and *A. galli* eggs in the faeces of infected birds and the clinical signs (dullness, off-feed or reduced feeding, bloody diarrhoea, droppiness, listlessness, ruffled feathers and mortalities) manifested by the infected birds in comparison to uninfected control birds that did not show such signs were indications that the infections established and caused some pathology in

the birds. Most of the clinical signs adopted here have been reported as being typical clinical signs often exhibited in clinical coccidiosis [39]. The absence of pronounced clinical manifestations in *Ascaridia* only infected group of birds apart from being a pointer that concurrent infections did influence the course of response, their low worm burden further suggests low worm establishment since the dose level of infection was high (1000 embryonated eggs). The current observation supports the findings of Permin and Hansen [40] who had theorized that birds with low level of *A. galli* infections may not show overt clinical symptoms.

The reduced feed intake or being off-feed altogether is likely responsible for the reduced weight gain or even weight loss in some of the infected groups compared to the higher weight gain of the uninfected control birds. Ascaridosis and coccidiosis have been associated with reduced appetite[41].

Nevertheless, all the chickens gained weight at the end of the study although the gain was higher in the uninfected control than in the infected birds.

The gross lesions tended to be more severe in birds in which the *Eimeria* infection was involved whether singly or in combination with *A. galli* than when *Ascaridia* alone was present. This is because chickens with *Eimeria* infection either singly or in combination with *A. galli* exhibited more severe intestinal haemorrhages, caecalcores and bloody faeces while orange or sulphur-yellow coloured intestinal contents was more common in birds infected with *A. galli* alone. It was observed however, that the haemorrhages and caecal cores in *Eimeria* infected chickens were more severe in the group infected with *A. galli* followed by *Eimeria* than all in other infected groups suggesting that greater pathology was inflicted by that combination.

The bloody diarrhoea was most likely as a result of the haemorrhages and erosions in caecal mucosa due mainly to the infection *Eimeria* although mild haemorrhagic enteritis in the small intestine could also occur due to the *A. galli* larvae emerging from the mucosa. *E. tenella* and *E. necatrix* have been established as the major causes of bloody diarrhoea in poultry coccidiosis [13, 14]. The haemorrhages due to the parasites would have constituted the major cause of the reduced PCV of some infected birds.

That the highest rate of mortality occurred in the *Ascaridia* later *Eimeria* group is a further pointer that the pathogenic effect of the infections in the chickens were more severe when the nematode preceded the protozoan as illustrated by the profound haemorrhages observed during post mortem and the overt clinical signs such as high morbidity and bloody diarrhoea. The *Ascaridia* later *Eimeria* group thus proved to be a treatment that produced the most severe negative impact on the birds. The mortalities occurred after the *Eimeria* infection was superimposed on the *A. galli* infected birds. *A. galli* likely suppressed the immunity of the birds leading to an increase in the establishment and subsequent increase in the pathogenic effect of the *Eimeria*. Roespstorff *et al.* [42] had earlier reported possible immunedownregulation of *A. galli* in birds leading to increase in the pathogenicity of *P. multocida* infection that was given after the nematode infection. However, it is also possible that the earlier *A. galli* infection might have triggered the influx of an enormous number of macrophages into the lamina propria where they in turn greatly enhance transportation of sporozoites from the lamina propria into the glands of Lieberkuhn. It has been reported that once in the lamina propria the sporozoites are further transported by macrophages to the gland of Lieberkuhn where further development occurs[43]. A much earlier study had also showed that concurrent infection of chickens with *P. multocida* and *A. galli* in which the nematode preceded the bacteria lead to more severe pathology than when both parasites and the bacteria were either given at the same time or the *P. multocida* preceded the *A. galli* and this was attributed to immunosupression of the birds by the nematode[25] In like manner, in concurrent infections of *E. coli* and *A. galli*, the *A. galli* was suggested to have immunosuppressive effect on the chickens allowing the *E. coli* to greatly establish causing enormous pathology [25]. Noteworthy too is the fact that Dahl and Permin [25] has further emphasized the role of *A. galli* in enhancing the establishment of other parasites or bacteria co-occurring with it provided that *A. galli* preceded such organisms and that in the occasion where the other organisms preceded the nematode infection the clinical picture would just be as if it were a single organism involved. It was also observed that the EPG, OPG and worm counts were not so high suggesting that both *A. galli* and *Eimeria* somewhat actually inhibit each other to an extent. This is in line with the report of Norton *et al.* who stated that in concurrent *A. dissimilis* and *E. meleagritis* infections in turkeys the parasites interacted in a manner to suppress each other leading to lower oocyst production and fewer third-stage larvae than in turkeys that were infected with either *E. meleagritis* or *A. dissimilis* respectively alone.

However, unlike Dahl and Permin [25] and Permin and Christensen [26], [27] had reported that in concurrent *A. dissimilis* and *E. meleagritis* infections in turkeys the parasites interacted in such a manner to suppress each other leading to lower oocyst production and fewer third-stage larvae than in turkeys that were infected with either *E. meleagritis* or *A. dissimilis* respectively alone.

Histopathology largely revealed the pathogenicity of the *Eimeria* infection. The infiltration of the intestinal mucosa by inflammatory (mononuclear) cells was likely in response to the invading *Eimeria*, an attempt to resist the

invading parasites. The mucosal erosions observed concord with the haemorrhages and caecal cores that were common findings at post mortem and the clinical signs of bloody diarrhoea observed.

In conclusion, the infections were generally pathogenic to the chickens and this was more pronounced in the chickens that harboured concurrent infections with both parasites. Strikingly, the effects of the infection became more severe when the *A. galli* (nematode) infection preceded the *Eimeria* (protozoan) infection compared to *Eimeria* followed by *A. galli* or when both parasites were given at the same time for reasons which remains to be fully investigated**.** It is therefore strongly recommended that the infections should be diagnosed regularly and controlled promptly when present particularly if they are concurrent as the effects can be disastrous in situations where *A. galli* infections precede *Eimeria* infections as in the semi intensive and free range systems of poultry production as the nematode is not only pathogenic but incapacitates the birds to resist the ubiquitous *Eimeria.*

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