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A comparative study on the concurrent infections with *Ascaridia galli* and *Eimeria* in broiler chickens

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Abstract

This study was conducted to investigate the response of two week old broiler chickens to single and concurrent infections with Ascaridia galli and Eimeria spp and the influence of timing of the infections with the hope that the results would greatly inform the improvement of control measures of the infections for better productivity of the birds. Six groups of 7 broiler chickens each were used. Five of the groups were either infected with A. galli only, caecal eimeria only, A. galli and caecal eimeria (A+E) at the same time, A. galli and later caecal eimeria (AE) or caecal eimeria isolate and later A. galli (EA). The sixth group remained as the uninfected control birds. The dose levels were 1000 embryonated A. galli eggs and 12,000 sporulated Eimeria oocysts per chicken. Packed cell volume (PCV), body weight (BWt), faecal egg counts (EPG), faecal oocyst counts (OPG), caecal gross lesion score (GLS), and worm burden (WB). The infections had a negative impact on the birds evidenced by low PCV, low weight gain, reduced feed intake, dullness, bloody diarrhea and mortalities when compared with their uninfected controls. A drop in the PCV from day 8 to day 12 of the birds infected with AE. The AE group also had the least weight gain and least feed intake in comparison with all the other infected and the uninfected control birds. The greatest mortalities occurred in the AE group. It was concluded that the poor performance of the chickens was due to the infections, however, the severity of the infections was greatest in the AE group. It was suggested that A. galli caused immune downregulation of the chicken allowing the Eimeria to exert maximally its pathogenic effects on the birds. It is therefore strongly recommended that the infections should be diagnosed and controlled promptly particularly if they are concurrent as the effects can be disastrous especially when A. galli infection precedes Eimeria infection.

Keywords: Protozoan, Nematode, Parasite, Haematological indices, Poultry.
Running title: Effect of single and conjunct *ascaridia* and *eimeria* infection on poutry

Une etude comparative sur les infections concurrentes a ascaridia galli et eimeria chez les poulets de grille

Résumé

Cette étude a été menée pour étudier la réponse de poulets de chair âgés de deux semaines à des infections uniques et concomitantes à Ascaridia galli et Eimeria spp et l'influence du

moment des infections dans l'espoir que les résultats contribueraient grandement à l'amélioration des mesures de contrôle des infections. pour une meilleure productivité des oiseaux. Six groupes de 7 poulets de chair chacun ont été utilisés. Cinq des groupes étaient soit infectés par A. galli uniquement, par eimeria caecal uniquement, par A. galli et par eimeria caecal (A+E) en même temps, par A. galli et plus tard par eimeria caecal (AE) ou par un isolat d'eimeria caecal et plus tard par A. .galli (EA). Le sixième groupe est resté constitué d'oiseaux témoins non infectés. Les niveaux de dose étaient de 1 000 œufs embryonnés d'A. galli et de 12 000 oocystes d'Eimeria sporulés par poulet. Volume de cellules concentrées (VCC), poids corporel (PC), nombre d'œufs fécaux (NŒF), nombre d'oocystes fécaux (NOF), score de lésion brute cœcale (LBC) et charge de vers (CV). Les infections ont eu un impact négatif sur les oiseaux, comme en témoignent un faible VCC, un faible gain de poids, une consommation alimentaire réduite, une matité, une diarrhée sanglante et une mortalité par rapport à leurs témoins non infectés. Une baisse du VCC du jour 8 au jour 12 des oiseaux infectés par AE. Le groupe AE présentait également le gain de poids le plus faible et la consommation alimentaire la plus faible par rapport à tous les autres oiseaux témoins infectés et non infectés. Les mortalités les plus importantes sont survenues dans le groupe AE. Il a été conclu que les mauvaises performances des poulets étaient dues aux infections, cependant, la gravité des infections était la plus élevée dans le groupe AE. Il a été suggéré qu'A. galli provoquait une régulation immunitaire négative du poulet, permettant à l'Eimeria d'exercer au maximum ses effets pathogènes sur les oiseaux. Il est donc fortement recommandé que les infections soient diagnostiquées et contrôlées rapidement, en particulier si elles sont concomitantes, car les effets peuvent être désastreux, en particulier lorsque l'infection par A. galli précède l'infection par Eimeria.

Mots-clés: Protozoaire, Nématode, Parasite, Indices hématologiques, Volaille.

Introduction

The poultry industry occupies an important position in the provision of animal protein to man and generally play a vital role in the national economy as a revenue provider and provides employment (Nnadi and George, 2010). Poultry is one of the most intensively reared of the domesticated species and is the most developed and profitable animal production enterprises (Al-Jamaien *et al*., 2013; Opara *et al*., 2014). Its importance in improving the nutritional status and income of many small farmers and those with small land holdings as well as landless farmers has been recognized by various scholars and rural development agencies in the last decades (Letebrhan *et al*., 2015). In Nigeria, backyard poultry constitute about 60% and thus the most important form of poultry production (Akintunde *et al*., 2015; Barde *et al*., 2015).

The poultry industry contributes up to 15% to the country's gross domestic product (GDP) and accounts for 36% of total protein intake of the country (Omokaro, 2015). Poultry diseases remain one of the major threats to boosting poultry production in Nigeria (Akintunde and Adeoti, 2014). These diseases might be bacterial, viral, fungal or parasitic origin. Two major parasitic diseases that affect poultry are coccidiosis which is caused by a protozoan parasite of the genus *Eimeria* and ascaridiosis caused by a nematode parasite of the genus *Ascaridia*.

Coccidiosis is an intestinal parasitic disease caused by an intercellular protozoan parasite of the genus *Eimeria* (Nematollahi *et al*., 2009). Birds of any age are susceptible to coccidiosis but most birds get infected in the early few weeks of life (Chookyonix *et al*., 2009). The prevalence rate of coccidiosis is higher during the raining season, primarily because it is positively influenced by the warm and humid weather, which

characterizes the rainy season period by providing favourable conditions for the growth and development of infective oocysts (Alawa *et al*., 2010).

Ascaridosis, an intestinal parasitic disease, causing economic losses in modern poultry farming (Permin and Ranvig, 2001), is caused by a parasitic nematode, *Ascaridia galli.* The nematode infects fowls of all ages, but the greatest degree of damage is often found in young birds under 12 weeks of age (Hogsette and Butcher, 2003). Bad management practices such as wet litter, which encourages the development and sporulation of oocysts, contaminated drinkers and feeders, bad ventilation and high stocking density can exacerbate the clinical signs of coccidiosis and ascaridosis. Concurrent infections are known to influence the response of the host to the co- infecting parasite, (Dahl et al., 2002; Permin et al., 2006). In these cases, the pattern of pathogenesis became more severe where *Ascaridia* preceded their co-infecting parasites, a situation that led to the conclusion that *Ascaridia galli* had an immune supporting effect (Permin et al., 2006). However, given the high prevalence, pathogenicity and endemicity of both parasites, it was deemed necessary to bridge the gap of information that currently exists. The current study was therefore designed to study the response (influence/ effect) of broiler chickens to single and concurrent experimental infection of *Eimeria* and *Ascaridia galli* group.

Materials and Method

Study Area

The experiment was conducted in the poultry house of the College of Veterinary Medicine in Michael Okpara University of Agriculture Umudike farm.

Experimental Birds

Broiler chicks were obtained from a hatchery at day old and brooded for two weeks before being used for the experiment. The birds were managed on deep litter and duly vaccinated against Newcastle disease and Infectious bursal disease in the course of brooding and experimentation. The birds were treated with an anticoccidial (toltrazuril) to eliminate any *Eimeria* infections present.

Eimeria Isolate

The *Eimeria* used for the study was isolated from caeca of 2 to 4 weeks old broiler chicken clinically sick of coccidiosis. The caeca were collected following post mortem and incised with the contents washed into sieves placed in small bowls. The primary filtrate was poured in 200ml beakers and washed three times to remove the red color of blood by decanting following sedimentation. The sediment was examined to confirm the presence of the oocysts. 20ml of the *Eimeria* suspension was dispensed into five petridishes containing 2ml of the sporulating agent, potassium dichromate (KCrO4) and incubated at room temperature on bench tops in the Veterinary Parasitology Laboratory, MOUAU for five days. Following sporulation, the suspensions were recollected in a 200ml beaker and more water was added to dilute or remove the KCrO4. The suspension was allowed to sediment for 20 minutes before decanting. The caecal *Eimeria* isolates were then put into 50ml Teflon tubes and preserved in the refrigerator (4□C) before it was used for the experimental infections.

Ascaridia galli embryonated eggs

Embryonated *Ascaridia galli* eggs used for the study were obtained from *A. galli* egg cultures. *A. galli* adults were collected from the intestines of spent layers slaughtered at umuahia market of Abia State. Briefly, following slaughter, intestines were collected and the small intestine incised to harvest the adult A. galli. The worms were then rinsed with water and crushed in a pistle and rinsed through a sieve with normal saline into 200ml beaker to remove the residue. It was washed three times with normal saline at the end of which 20ml of the egg suspension was

poured into petri dishes, and placed on bench tops in the Vet. Parasitology Laboratory at room temperature 27-29□C for three weeks. During which period, aliquots of the suspension were checked for evidence of sporulation. Sporulated eggs were washed with fresh normal saline and preserved in 50ml Teflon tubes in the refrigerator at 4□C before they were used.

Experimental Design and Infection

At two weeks of age, the birds were removed from the brooding house to various pens where the study was carried out. The uninfected control group was placed in a pen away from the pens containing infected birds. The grouping and infections are as illustrated on table 1.

Table 1: Groupings and infection protocol

	Group	Age (weeks)	No. of birds	Day of infection		Day of PM
1.	Infection with 1000 embryonated *A.galli* only	2	7	0	0	60
2.	Infection with 12000 sporulated caecal *eimeria* oocysts only	2	7	0	0	60
3.	Infection with both *Eimeria* and *A.galli* at same time	2	7	0	0	60
4.	Infection with *Eimeria* first followed by *A. galli*	2	7	0	8	60
5.	Infection with *A.galli* first followed by *Eimeria*	2	7	8	0	60
6.	Uninfected Control	2	7	-	-	60

Parameters monitored were body weight (every 4 days), Feed consumption (every 2 days), Packed cell volume (PCV) (every 4days), Clinical signs, Eimeria oocyts counts (OPG), Faecal egg counts for Ascaridia(EPG), Post mortem worm counts(worm burden).

Data Analysis:

Data were analysed using descriptive statistics and least significant differences (LSD) of the SPSS package version 20.

Results

A pronounced effect of concurrent infections with *Eimeria* and *Ascaridia galli* was observed on oocyst count per gramme of faeces (OPG), egg count per gramme of faeces (EPG), weight gain, feed consumption, packed cell volume, clinical signs, gross and microscopic lesion, and post mortem worm counts.

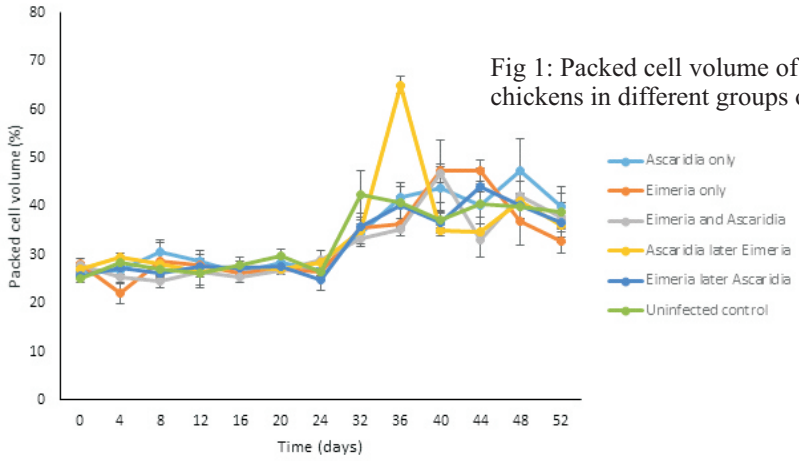

Fig 1: Packed cell volume of the chickens in different groups over a period.

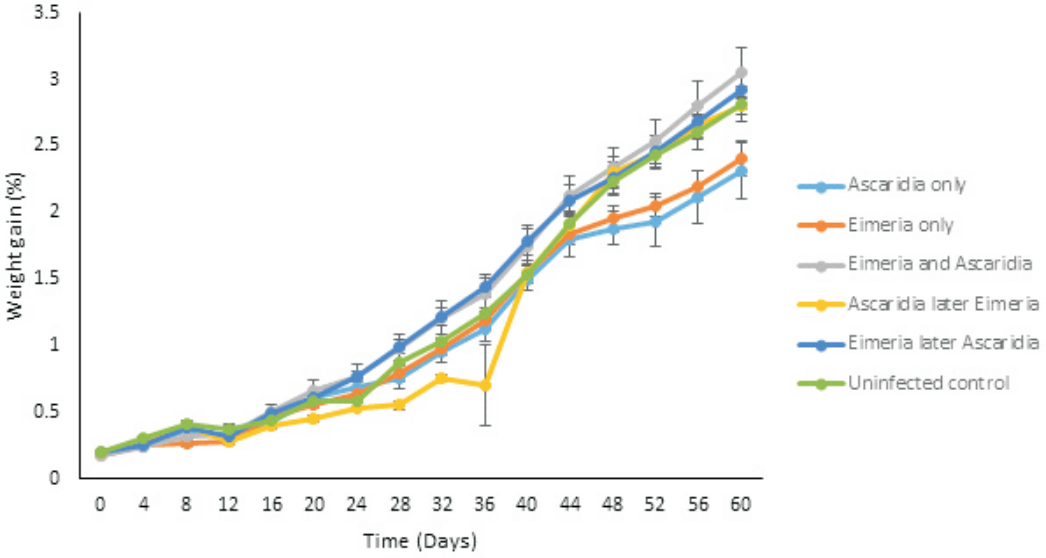

Fig.2: Weight gain from birds in the different groups over time

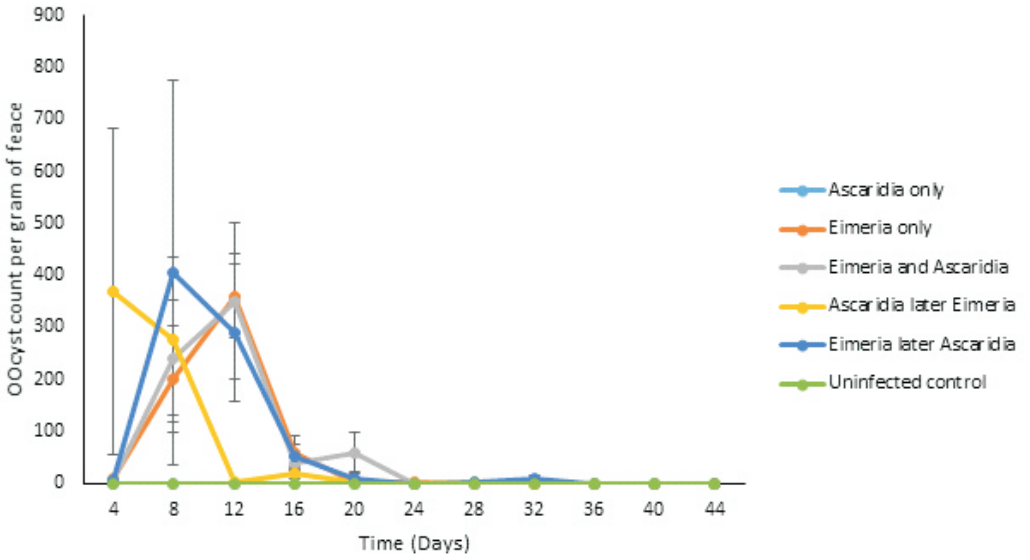

Fig.3: Oocysts per gramme count (OPG) from birds in the different groups over a period of time

Table 1: Ascaridia egg count (EPG) from birds in the different groups over a period of time

	Ascaridia only	*Eimeria* only	*Eimeria* and *Ascaridia* same time	*Ascaridia* later *Eimeria*	*Eimeria* later *Ascaridia*	Uninfected control
Day 40	0	0	0.17 ± 0.17	0	0	0
DAY 44	17.50 ± 10.07	0	28.83 ± 11.68	0	0.86 ± 0.70	0
DAY 48	0	0	0.67 ± 0.67	0	0	0
DAY 49	0.83 ± 0.48	0	1.00 ± 1.00	0	0	0
DAY 51	15.17 ± 14.77	0	0.83 ± 0.40	2.50 ± 1.44	1.71 ± 1.71	0
DAY 53	0.67 ± 0.49	0	2.33 ± 1.61	6.00 ± 3.46	0.29 ± 0.18	0
DAY 55	9.00 ± 8.60	0	1.83 ± 0.79	0	0.57 ± 0.30	0
DAY 57	1.50 ± 0.96	0	0.67 ± 0.42	0.50 ± 0.29	1.43 ± 1.02	0
DAY 60	1.67 ± 0.56	0	0.83 ± 0.40	1.50 ± 0.29	0	0

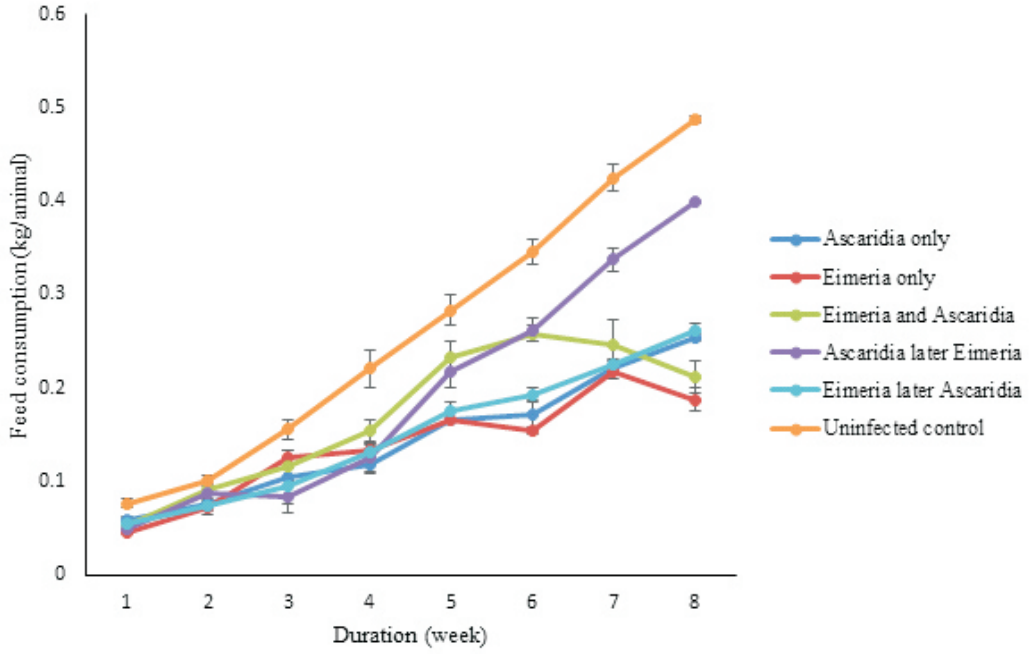

Fig 4: Feed consumption over a period of time for the various groups

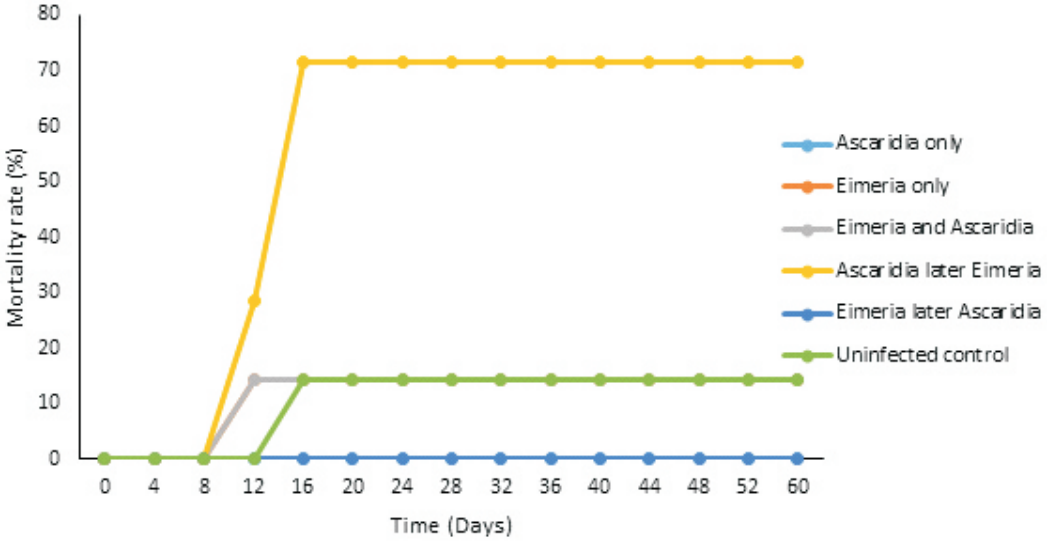

Fig 5: Mortality rate amongst the different groups over a period of time

Gross lesions

The gross pathology following necropsy are summarized by the following features. There were haemorrhages and caecal cores in the caeca of birds infected with *eimeria*. Some birds had a sulphur/orange and watery intestinal contents.

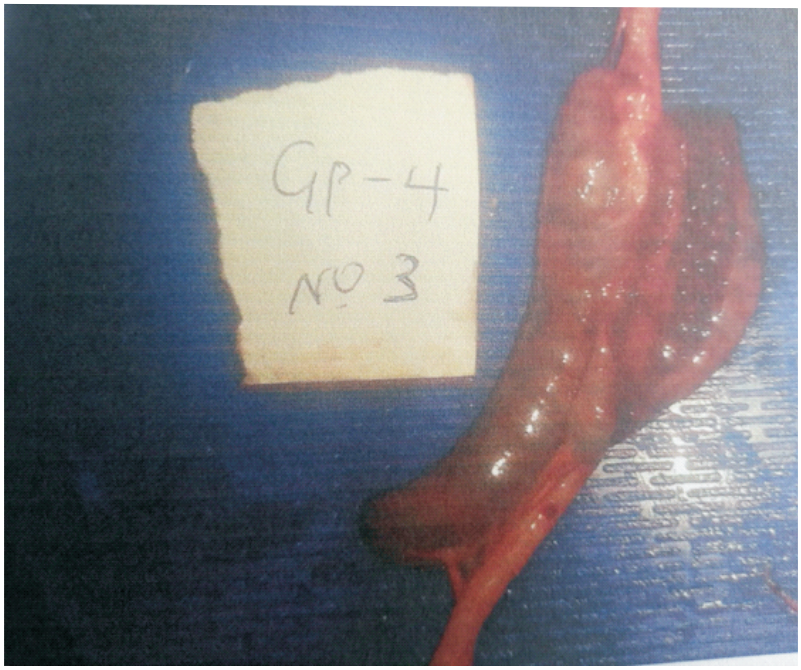

Fig.6: Caeca of a bird infected with *ascaridia* later *eimeria* (Grp4) with haemorrhages and caecal cores

Fig.7: Caeca of a bird infected with sporulated *eimeria* oocysts only (grp 2) with bloody caecal contents.

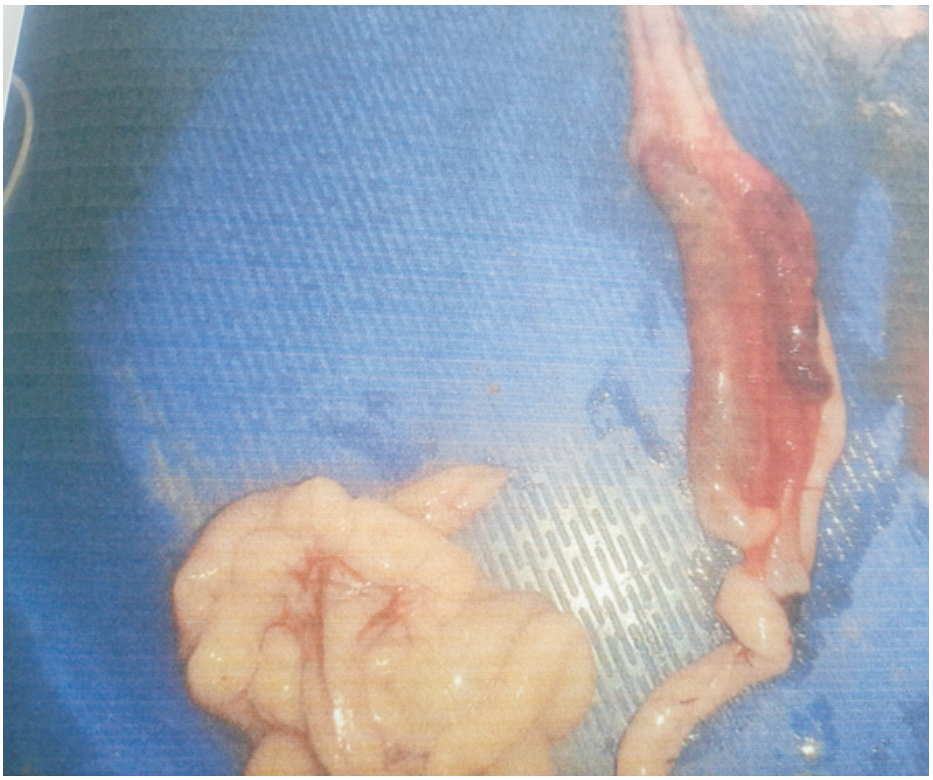

Fig.8: Caeca of an infected bird with Ascaridia and eimeria (grp3) with bloody caecal content.

Histopathology

The caecal mucosa of all the *eimeria*-infected chickens showed various degrees of pathology evidenced by oedema, mononuclear cell infiltration (inflammation) and erosions/ulcerations.

In the case of chicken infected with *ascaridia* and *eimeria* at same time, there was mild oedema, cellular infiltration and mucosal erosions. In those infected with only *eimeria*, erosions of the caecum is seen. There is massive erosions and cellular infiltrations in birds infected first with *ascaridia* and later with *eimeria*. The same lesions as seen with birds infected first with *ascaridia* and later with *eimeria* is seen with those infected with *eimeria* first then *ascaridia* later.

Discussion

A pronounced effect of concurrent infections with *Eimeria* and *Ascaridia galli* was observed on oocyst count per gramme of faeces (OPG), egg count per gramme of faeces (EPG), weight gain, feed consumption, packed cell volume, clinical signs, gross and microscopic lesion, and post mortem worm counts.

The voiding of *Eimeria* oocyts and *A. galli* eggs seen in the faeces of infected birds and the clinical signs are indications that the infections established and cause pathology in the birds. The control group showed no observable clinical signs. Low level infections with A. galli may not cause clinical symptoms as reported by Permin et al (1998b).

The dullness and reduced feed intake may be due to the reduced weight gain or even weight loss in some of the infected groups. The groups infected with single infection of either *Eimeria* or *A. galli* had a reduced weight gain compared to the groups with concurrent infections. Some parasitic diseases notably Ascaridosis and coccidiosis have been associated with reduced appetite (Dalloul and Lillehoj, 2006). However, all the chicken gained weight at the end of the study but the weight gain was higher in the uninfected control than in the infected birds. The bloody diarrhea was a result of haemorrhages and erosions due to *Eimeria* infections and to an extent the enteritis due to *A. galli* infection (McDougald and Reid, 1997; Maikai *et al*., 2007). The reduced PCV of some infected birds would have been as a result of the blood loss due to haemorrhages. This could be attributed to the effects of *Eimeria* oocysts and larval migration of *A. galli* in the process of penetration with resultant destruction of the mucosa of the small intestine and rupture of small blood vessels in the tissue phase of the life cycle of the parasite, which involves some blood loss. This must have resulted to fall of red blood cell which consequently lowered packed cell volume.

The gross lesions tended to be more severe in the *Eimeria* infected birds than the *Ascaridia* infected birds. The *Eimeria* infected birds showed more severe intestinal haemorrhages, bloody faeces and caecal cores while orange or Sulphur-yellow coloured intestinal contents was shown by *Ascaridia* infected birds. The histopathology revealed the pathogenicity, the erosions of the caecal mucosa would have been responsible for bloody diarrhea the infected birds suffered leading to reduced PCV in some of the groups' especially in group 2, 3 and 4. The infiltration of mononuclear cells was in response of the infection or an attempt to arrest the invading parasites. The erosions are in concord with the haemorrhages of caecal cores that were common at post mortem examination and the clinical examination.

Mortalities were common in some of the infected groups being highest in the 5th group. The group 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* infection. *A. galli* likely suppressed the immunity of the birds leading to an increased pathogenic effect of the *Eimeria*. It was reported that concurrent infection of chickens with *P. multocida* and *A. galli* in which the nematode preceded the bacteria organism lead to more severe pathology than when both parasite and the bacteria organism were either given at the same or the *P. multocida* precede the *A. galli* and this was attributed to immunosuppression of the birds by the nematode (Dahl *et al.,* 2002). In the same manner concurrent infections of *E. coli* and *A. galli*, the *A. galli* was suggested to have immune suppressive effect allowing the *E. coli* to establish (Permin *et al*., 2002).

In conclusion, combined infections of *A. galli* followed by *Eimeria* had a severe effect on the birds compared to *Eimeria* followed by *A. galli* and when both parasites were given at the same time. Even a subclinical *A. galli* seems to enhance the establishment of another parasite or bacteria (Dahl *et al*., 20020. Therefore, there is the need to ensure proper protection of birds against these two parasites.

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