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*Original article*

# IMMUNISATION OF CHICKENS WITH COMMERCIAL ANTICOCCIDIAL VACCINES IMMUCOX® AND LIVACOX® SHOWED VARIED PROTECTION AGAINST A VIRULENT *EIMERIA TENELLA* LOCAL ISOLATE AND HOUGHTON STRAIN

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**Summary**

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*Coccidia* is an ubiquitous intestinal protozoan of poultry that invade the mucosa and induce epithelial cell necrosis and inflammation. To assess the pathogenicity of two commercial anticoccidial vaccines against a local isolate of *Eimeria tenella*, ninety (90) day-old dominant black chicks were assigned to 9 groups of 10 birds each. Immunisation was done at 5 days of age by oral gavage. Infection was done with $1.7\times10^4$ *E. tenella* of either Houghton strain (H-strain) or local isolate (L-isolate) at 4 weeks of age. Increased pathogenicity of the local isolate was observed, as it produced a more severe gross pathologic lesion score than the Houghton strain in unvaccinated but infected groups of chickens 6 days post-infection (dpi). The high virulence and pathogenicity of the local *E. tenella* was responsible for the differences in the severity of clinical signs, gross and microscopic lesions observed between the H-strain and L-isolate infected groups. The histopathology showed that the vaccinated groups, infected with the H-strain of *E. tenella*, did not show presence of oocysts or schizonts by 6 dpi. The successful immunogenicity and effectiveness of these two vaccines as measured by the histopathologic lesions and the presence of oocysts in the enterocytes of the caecal crypts of these chickens were reduced in vaccinated and L-isolate infected groups. The findings in the L-isolate groups were setbacks to the use of live attenuated and non-attenuated anticoccidial vaccines for control of coccidiosis and in particular, caecal coccidiosis in chickens reared in environments dominated by virulent *E. tenella*.

**Key words**: anticoccidial, chickens, coccidiosis, immunisation, pathology

## INTRODUCTION

Coccidiosis in chickens is caused by a protozoal parasite of the Phylum Apicomplexa belonging to the genus *Eimeria* (Brown-Jordan *et al*., 2018). These coccidia organisms are ubiquitous intestinal protozoans of poultry and are known to cause acute intestinal disorders (Bussière *et al*., 2018). *Eimeria* species invade the mucosa and damage the epithelial lining of the infected intestinal cells (Cheng *et al*., 2018), thereby inducing epithelial cell necrosis and inflammation. Conventional disease control strategies have relied on prophylactic medication (Cheng *et al*., 2018). Due to the continuous emergence of drug-resistant parasites in the field and the increasing incidence of broiler condemnations due to coccidia, novel approaches are a handy alternative to reduce economic losses (Min *et al*., 2004). The first viable alternative to anticoccidial agents is the use of live vaccines, with a precocious cycle or not, but because the antigenicity of coccidia strains varies geographically (Fitz-Coy & Edgar, 1992; Martin *et al*., 1997), it is important to screen live vaccines against local populations before administering them to large flocks. This is because immunovariant strains can be isolated and substituted to provide region-specific, autogenous vaccines (Danforth, 1998). All live vaccines against coccidiosis contain oocysts of either virulent strains (unmodified, wild type) or attenuated populations that have been derived in the laboratory. The effects of *Eimeria* infection can take the form of devastating flock mortality (McDougald & Fuller, 2005), malabsorption, inefficient feed utilisation, impaired growth rate in broilers, and a temporary reduction of egg production in layers (Williams, 1998). In each type of bird to be immunised, live anti-coccidial vaccines for poultry including adjuvants, preservatives, or suspending agents, should ideally: (a) induce protective immunity against economically important species of *Eimeria;* (b) be safe for the target host species, non-target animals and humans; (c) not represent an environmental hazard; (d) comprise parasites of normal or low virulence, the latter trait being demonstrably stable during propagations through the host; (e) comprise parasites that remain viable during storage under optimal conditions for a reasonable period of time (f); be administered by a commercially practical method to ensure that as many birds as possible receive a similar dose; (g) have no adverse effects upon final performance or other production criteria; (h) be compatible with other poultry vaccines; (i) be free from viral, bacterial, mycoplasmal, fungal, and chemical contaminants; (j) be cost-effective when compared with other methods of coccidiosis control; (k) include drug-sensitive lines, so that they may interbreed with drug-resistant field populations, and thus reduce the overall local resistance (Chapman *et al*., 2013). Immucox® is a non-attenuated vaccine which comprises wild-type strains of *Eimeria* oocysts, with numbers calculated so that if administered at the correct dose, pathogenic effects should not be observed. Livacox T® and Livacox D® are attenuated vaccines which comprise strains that have been selected so that they have reduced or no pathogenicity (Shirley, 1993). Five species of the seven *Eimeria* species of chickens induce gross pathological lesions and four of these are the most important in terms of global disease burden and economic impact (*E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella*) (Williams, 1998). Reduced lesion scores and oocyst shedding from challenged, vaccinated birds compared with unvaccinated control birds

challenged with *Eimeria* species have been used as measures of protection against coccidiosis (Williams & Catchpole, 2000; Shivaramaiah *et al*., 2014). Also, in the case of vaccinated birds, lesions may appear because of challenge with *Eimeria* species, but may also be associated with pathophysiological changes and with the development of protective immunity (Byrnes *et al*., 1993; Shivaramaiah *et al*., 2014).

The objective of this study was therefore to screen live vaccines against local populations of *E. tenella* before administering them to large flocks by immunising chickens with commercially available anticoccidial vaccines in Nigeria: multivalent live attenuated Livacox® (Biopharm) and live non-attenuated Immucox® (Vetech), and then assess the pathogenicity, gross morphological, and microscopic findings of the local isolates and standard strain (Houghton) homologous to the vaccine strain of *E. tenella*.

## MATERIALS AND METHODS

### *Experimental animals and design*

Ninety (90) day-old Black cockerels were purchased and fed *ad libitum* on a proprietary chick's ration without coccidiostat additives and also given access to water *ad libitum*. They were later separated into nine experimental groups (1–9; of 10 chicks each) with different experimental regimens (Table 1).

The experiment comprised two phases: 1st phase – vaccination of birds and 2nd phase – subsequent challenge with *Eimeria tenella*.

Adequate measures were taken to minimise pain or discomfort. The procedures were carried out in accordance with the guidelines laid down by the International Animal Ethics Committee, Faculty of Veterinary Medicine, University of Ilorin ethical committee laws and regulations and Animal Use and Care Committee Guidelines for Care and Use of Animals at the National Veterinary Research Institute, Nigeria.

### *Test vaccines and immunisation*

Two commercially available anti-coccidial vaccines – Livacox® (Biopharm, Czech Republic) and Immucox® (Vetech, Ontario, Canada) were used to immunise the chickens. Immucox® (consisting of *E. acervulina*, *E. tenella*, *E. maxima* and *E. necatrix* with or without *E. brunetti*), composed of several virulent species, is a live non-attenuated vaccine. Livacox® Q (consisting of *E. acervulina*, *E. tenella*, *E. maxima* and *E. necatrix*) is a live attenuated vaccine.

**Table 1.** Experimental grouping of chickens and the experimental regimen

| Experimental groups | Experimental regimen |
|---|---|
| 1 | Unimmunised, uninfected |
| 2 | Unimmunised, infected with Houghton strain of *E. tenella* |
| 3 | Unimmunised, infected with local isolate of *E. tenella* |
| 4 | Livacox® immunised, infected with Houghton strain of *E. tenella* |
| 5 | Livacox® immunised, infected with local isolate of *E. tenella* |
| 6 | Immucox® immunised, infected with Houghton strain of *E. tenella* |
| 7 | Immucox® immunised, infected with local isolate of *E. tenella* |
| 8 | Livacox® immunised, uninfected |
| 9 | Immucox® immunised, uninfected |

Livacox® Q and Immucox® vaccines were used to immunise birds in groups 4 to 9. These vaccines were administered according to manufacturers' instructions. Livacox® and Immucox® were administered on day 5 by oral gavage; directly delivering the reconstituted vaccines into the crop of the chicks, so as to ensure adequate immunisation of each chick.

*Immunisation against infectious bursal disease and newcastle disease vaccination*

Two hundred doses of the infectious bursal disease (Gumboro) vaccine produced by the National Veterinary Research Institute (NVRI), Vom Nigeria, were reconstituted in 2 litres of chlorine-free drinking water and administered to the birds in calculated volume for 90 birds at day 11.

The Newcastle disease (*LaSota* strain) vaccine (200 doses) produced by NVRI, Vom Nigeria, was reconstituted in 2 litres of chlorine-free drinking water and administered to the birds in calculated volume for 90 birds at day 21.

*Molecular identification of the local E. tenella isolate*

*Eimeria tenella* was molecularly confirmed from a field (local) isolate of *Eimeria tenella* recovered from a case of caecal coccidiosis that occurred in a poultry flock whose carcasses were submitted for post mortem examination. Genomic DNA was extracted from this local *Eimeria tenella* using a random amplified polymorphic DNA-sequence characterising the amplified regions (RAPD-SCAR) marker-based multiplex-polymerase chain reaction (PCR) to identify the isolate as *Eimeria tenella*, previously described (Ogedengbe *et al*., 2009; Akanbi & Victor, 2020). Oocysts samples containing pure lines of *Eimeria tenella* were therefore used for the infection study.

*Challenge parasites (Eimeria tenella)*

The molecularly confirmed field (local) isolate of *Eimeria tenella* was used for the challenge of chickens in groups 3, 5, and 7.

The Houghton strain of *Eimeria tenella* used was generously donated by the former Director of the Institute for Animal Health (IAH), Compton UK, Professor M.E Shirley, and Dr Blake as previously described (Akanbi & Victor, 2009; 2020).

*Challenge procedures*

Sporulated local isolate of *Eimeria tenella* oocysts and Houghton strain were used to challenge the birds at 4 weeks of age. Inoculation (infection with the oocysts) in each case was given orally by gavage, and the dosage given was $1.7\times10^4$ sporulated oocysts per bird at 4 weeks post-anticoccidial vaccination as described earlier (Akanbi & Victor, 2020). Following the challenge, the chickens in the challenged-only groups, vaccinated and challenged groups were monitored daily for 6 days post-infection. Also, the vaccine control group was monitored in a separate enclosure to prevent accidental infection.

*Gross pathology and lesion scoring*

Gross examination of all carcasses and especially the intestine and caeca of chickens from all experimental groups were carried out. A standardised criterion for determining the pathogenicity of coccidial species using a scoring scale of 0 to + 4, according to Johnson & Reid (1970) with descriptions of the gross pathologic changes for each score was used. Scores ranged from 0 to 4, with 0 being the absence of lesions and 4 being the presence of the most severe lesions. Gross lesion score criteria for *E. tenella* (Johnson & Reid, 1970), were defined as followed: score 0, absence of lesions; while score 1,

grossly, corresponded to caeca with petechiation, scattered lesion, while the wall of the caeca and content were normal. In score 2, the caeca had petechiation, lesions were more numerous, while the wall of the caeca was thickened and the content – normal with some blood. In score 3, the caeca were bloody, lesions – coalescing, the wall of the caeca – greatly thickened with bloody content, fibrin clots or caecal cores with little or no faecal debris. In score 4, the caeca were bloody, with a bluish-black colouration, coalescent lesions, wall of the caeca was greatly swollen and thickened with bloody, caseous clots, and no faecal debris.

*Histopathology*

For histopathological examination, tissue samples (intestine and caeca) were harvested from chickens in all experimental groups immediately after sacrifice, fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 µm thickness, stained with haematoxylin and eosin (H&E) stain, cleared in xylene and mounted in a mountant as previously described by Gotep *et al*. (2016). Additionally, tissue sections were stained with a special stain, periodic acid Schiff (PAS) to demonstrate *E. tenella* parasites.

*Statistical analysis*

The Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA) for Windows version 22.0 was used in conducting the statistical analysis. Statistical analysis was conducted using ANOVA. The Least Significant Difference (LSD) was used as the *post hoc* test. Statistical significance levels were set at 5% ($P<0.05$). The chart illustrating the gross lesion scores was drawn using Microsoft Excel spreadsheet 2018 (Microsoft Inc., Seattle-WA, USA).

## RESULTS

*Challenge outcome*

The challenge with $1.7\times10^4$ *E. tenella* of either H-strain or L-isolate at 4 weeks of age was successful as evidenced by the establishment of caecal coccidiosis resulting in varying degrees of clinical signs of generalised pallor and moderate bloody diarrhoea in the infected groups 2–7.

There was no clinical sign of coccidiosis in groups 1, 8, and 9. Group 2 chickens, unimmunised and challenged with H-strain of *E. tenella*, were generally pale and emaciated, and had moderate bloody diarrhoea. Group 3 chickens, unimmunised and challenged with L-isolate of *E. tenella* were generally severely pale, emaciated, and passed out marked bloody diarrhoea. Group 4 chickens, immunised with Livacox® and challenged with H-strain of *E. tenella* had slight pallor, were slightly emaciated, and passed out slight bloody diarrhoea. Group 5, immunised with Livacox® and challenged with L-isolate of *E. tenella* showed moderate pallor, slight emaciation, and passed out moderate bloody diarrhoea. Group 6, immunised with Immucox® and challenged with H-strain of *E. tenella* showed slight pallor, were slightly emaciated, and passed out slight bloody diarrhoea. Group 7, immunised with Immucox® and challenged with L-isolate of *E. tenella* showed moderate pallor, slight emaciation with severe bloody diarrhoea (Table 2).

*Gross pathology and lesion score*

There were no visible gross lesions in the caeca of the unimmunised, uninfected chickens in group 1. The unimmunised but H-strain *E. tenella* infected group 2, showed moderate caecal muscular contraction and enlargement with severe enteritis. In group 3 chickens, unimmunised but infected with the L-isolate, there were

**Table 2.** Gross and histopathology properties of chickens immunised with commercial anticoccidial vaccines and challenged with *Eimeria tenella*

| Groups | Immunisation and infection status | Gross pathology | Histopathology |
|---|---|---|---|
| 1 | Unimmunised, uninfected | NVL in the intestine and especially in the caeca | No microscopic evidence of *Eimeria tenella* |
| 2 | Unimmunised, Houghton infected | Moderate caeca muscular contraction and enlargement | Severe enteritis, severe expansion of the circular muscle of the caeca and markedly numerous intra-enterocytic Eimerian protozoan developmental forms |
| 3 | Unimmunised, local isolate infected | Severe caecal muscular contraction, severe enlargement of the caeca and severe intraluminal haemorrhage, caecal core and diphtheritic membrane | Moderate enteritis, moderate expansion of the circular muscle of the intestine and numerous intra-enterocytic Eimerian protozoan developmental forms |
| 4 | Livacox® immunised, Houghton infected | Mild caecal lesion | Moderated enteritis with haemorrhage, with no microscopic evidence of *Eimeria tenella* |
| 5 | Livacox® immunised, local isolate infected | Mild caecal lesion | Moderated enteritis with haemorrhage, with microscopic evidence of *Eimeria tenella* |
| 6 | Immucox® immunised, Houghton infected | Mild caecal lesion | Mild enteritis, and intra-enterocytic *Eimeria tenella* developmental forms |
| 7 | Immucox® immunised, local isolate infected | Moderate enlargement of the caeca and moderate intraluminal haemorrhage, caecal core and diphtheritic membrane | Mild enteritis, and occasional cystic destruction, severe intra-enterocytic Eimerian protozoan developmental forms |
| 8 | Livacox® immunised, un-infected | NVL in the intestine and especially in the caeca | No microscopic evidence of *Eimeria tenella* |
| 9 | Immucox® immunised, uninfected | NVL in the intestine and especially in the caeca | No microscopic evidence of *Eimeria tenella* |

severe caecal muscular contraction, severe enlargement of caeca, and severe intraluminal haemorrhage with caeca; core, diphtheritic membrane, and moderate enteritis. In the Livacox® immunised and H-strain infected (group 4) chickens, there were no visible lesions in the caeca. Group 5 chickens, immunised with Livacox® and infected with L-isolate of *E. tenella*, showed no visible lesion in the caeca while group 6, immunised with Immucox® and infected with Houghton strain of *E. tenella* showed no gross lesion. Group 7 chickens immunised with Immucox® and infected with L-isolate showed moderate enlargement of the caeca and moderate intraluminal haemorrhage, caeca core, and diphtheritic membrane with mild enteritis, and occasional cystic destruction. Groups 8 and 9 chickens immunised with Livacox® and Immucox® respectively, showed no visible gross lesions.

The group 1 chickens, unimmunised, uninfected (absolute control) had no gross lesion and were therefore scored 0, while group 2, unimmunised but Houghton infected had moderate lesions, hence scored 2. Group 3, unimmunised but infected with local isolate (*E. tenella* local isolate control) had the most markedly severe lesions and were scored 4. In groups 4 and 5 (Livacox® immunised; Houghton and local *E. tenella* infected respectively) chickens, caeca had mild gross lesions and were both scored 1 each. Group 6 chickens showed mild caecal lesions and were scored 1. Group 7 chickens, immunised with Immucox® and infected with L-isolate of *E. tenella* showed severe lesion and were scored 3. Detailed pathological findings including those of groups 8 and 9 are listed in Table 2, Fig. 1 and 2.

*Histopathology*

Group 1 unimmunised uninfected chickens had no microscopic lesion in caeca. Both the circular and horizontal muscles were well apposed with no infiltrating cells and the submucosa glands were intact. In group 2, unimmunised and H-strain infected, caeca had a markedly se-

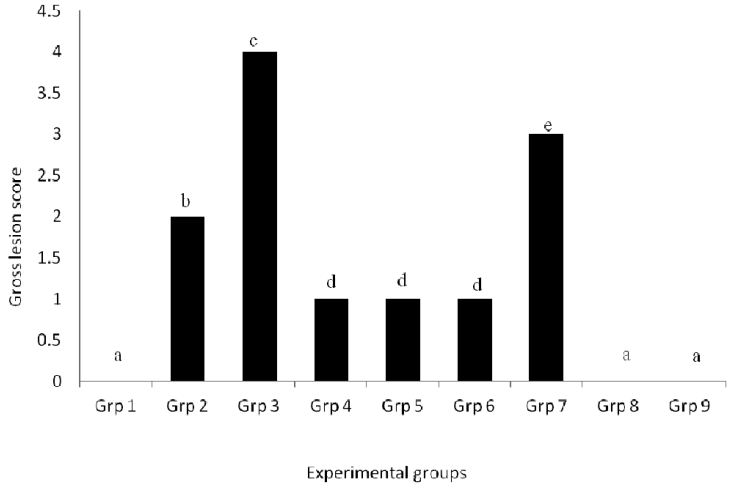


**Fig. 1.** Gross lesion score of experimental groups immunised with commercial anticoccidial vaccines and challenged with *Eimeria tenella*. Different superscripts (a,b,c,d,e) indicate significant statistical difference ($P<0.05$) between the gross lesion scores of groups.

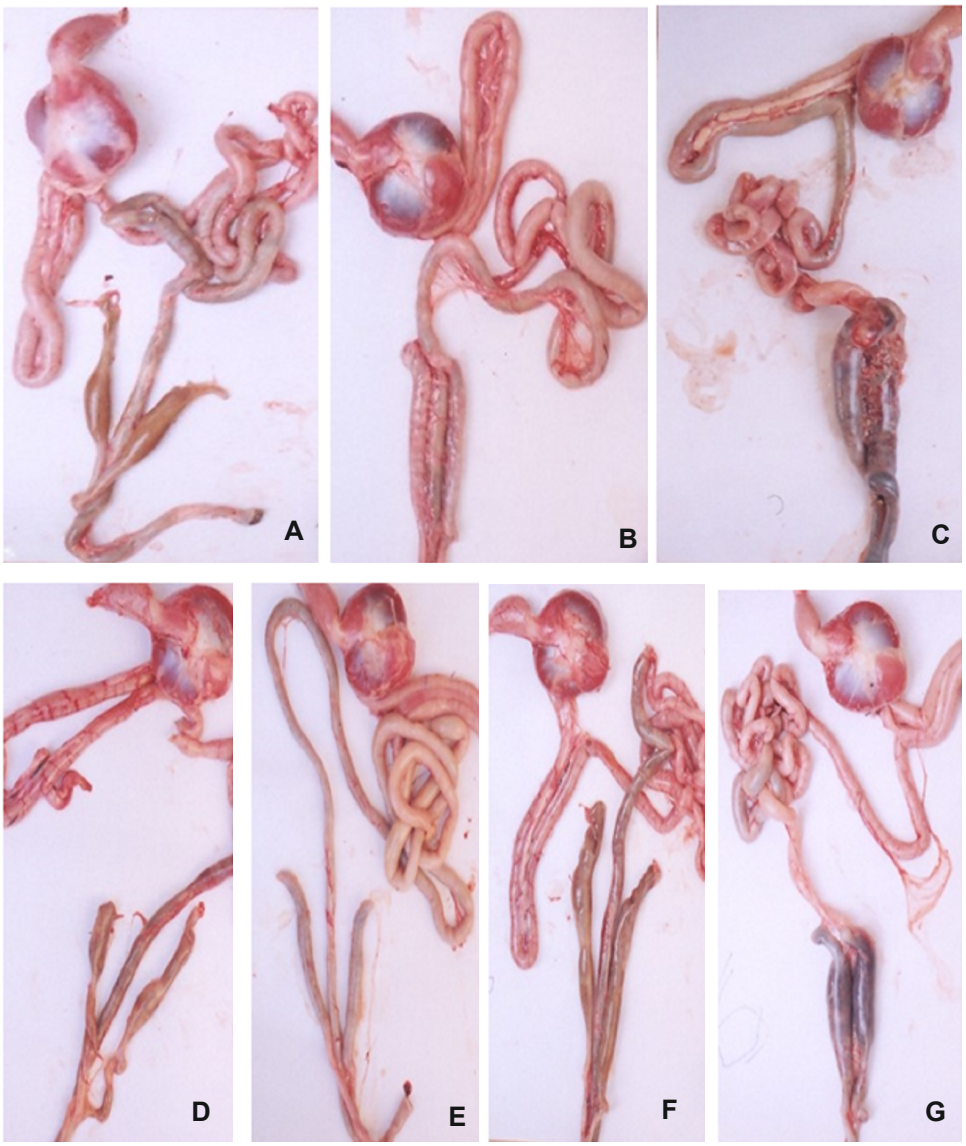


**Fig. 2.** Gross pathological lesions of experimental groups immunised with commercial anticoccidial vaccines and challenged with *Eimeria tenella*. **A.** Groups 1, 8 and 9: no visible gross lesion in the caeca; **B.** Group 2: moderate caecal muscular contraction and enlargement with severe enteritis; **C.** Group 3: severe caeca muscular contraction, severe enlargement of the caeca and severe intraluminal haemorrhage; **D.** Group 4: no visible lesion in the caeca; **E.** Group 5: no visible lesion in the caeca; **F.** Group 6: no gross lesion; **G.** Group 7: moderate enlargement of the caeca and moderate intraluminal haemorrhage.

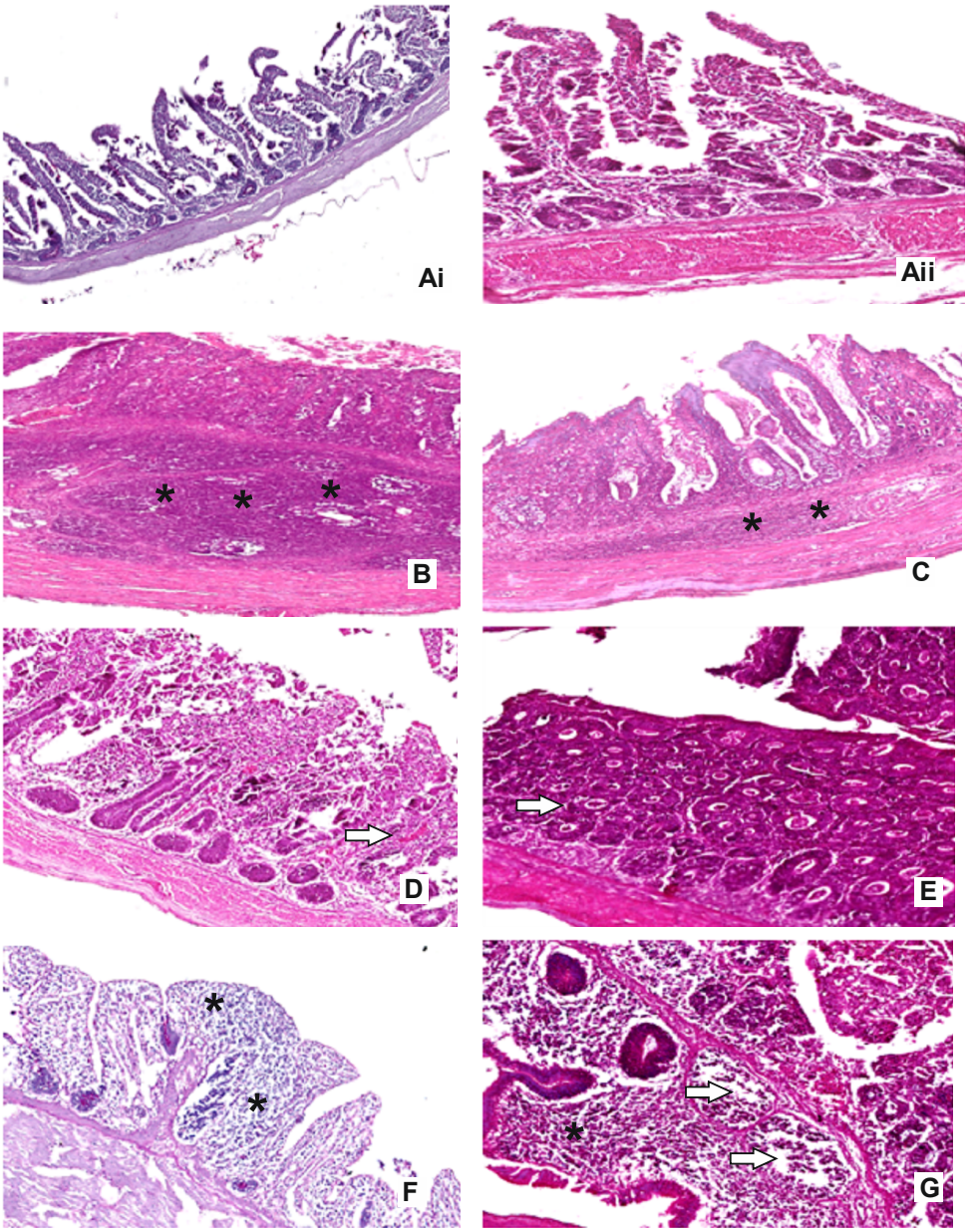


**Fig. 3.** Histopathalogical examinations of caecal sections showing pathological changes (H&E staining, ×100 magnification). **Ai, Aii.** Groups 1, 8 and 9: no lesion in the caeca; **B.** Group 2: marked severe expansion of the musculature (asterisks) by developmental stages of *E. tenella*; **C.** Group 3: muscular caecal moderate expansion by inflammatory cellular infiltrates and numerous intra-enterocytic Eimerian protozoans (asterisks); **D.** Group 4: moderate enteritis, haemorrhage (arrow) without developmental form of *E. tenella* parasites; **E.** Group 5: moderate enteritis (arrow); **F.** Group 6: moderate enteritis (asterisks), muscular oedema, congestion of submucosal blood vessels; **G.** Group 7: severe enteritis (asterisk) and cryptic oedema and necrosis (arrows).

vere expansion of the musculature by the developmental stages of *Eimeria tenella*, and inflammatory cells separating the lamina propria from the underlying muscular layer. These numerous intra-enterocytic eimerian protozoan developmental forms which included oocysts, schizonts, and developing merozoites were located within the cytoplasms of several enterocytes of the submucosal glands and the villi. Only a few intact submucosal glands could be identified at high magnification. There were intercryptic haemorrhages and severe eosinophilic and mononuclear cellular infiltrations. In group 3 chickens, unimmunised but infected with L-isolate, the circular muscle in the caeca showed moderate expansion by inflammatory eosinophilic and mononuclear cellular infiltrates and microscopic numerous intra-enterocytic eimerian protozoan developmental forms within the cytoplasms of crypts and enterocytes of villi. At higher magnification, oocysts, schizonts, and developing merozoites were seen in the submucosal glands and in the enterocytes of villi admixed haemorrhagic areas. In the Livacox® immunised and H-strain infected (group 4) chickens, there were moderate enteritis with haemorrhage with/without any development form of *E. tenella* parasites. A few crypts were destroyed with many intact crypts, admixed intercryptic inflammatory polymorphonuclear cellular infiltrates. There were occasional cryptic necrosis with intraluminal desquamation of enterocytes and oocysts debris with submucosal oedema and inflammatory eosinophilic and mononuclear cellular infiltrations. Group 5 chickens, immunised with Livacox® and infected with L-isolate of *E. tenella*, showed moderate enteritis with haemorrhage and at higher magnification with PAS staining, several caecal crypts were seen to have moderate numbers of *E. tenella* oocysts in their enterocytes' cytoplasms. Cryptic necrosis with intraluminal desquamation of enterocyte and oocysts debris with submucosal oedema were seen. Group 6 (immunised with Immucox® and infected with H-strain of *E. tenella*) showed only microscopic, moderate enteritis, muscular oedema, congestion of submucosa blood vessels. Group 7 chickens (immunised with Immucox® and infected with L-isolate of *E. tenella*) showed severe enteritis and moderately diffuse cryptic intra-enterocytic *Eimeria* protozoan oocysts and occasional cryptic destruction, with submucosal oedema and inflammatory polymorphonuclear cellular infiltration. Groups 8 and 9 chickens immunised with Livacox® and Immucox® respectively showed no histopathologic lesions (Table 2, Fig. 3 and 4).

## DISCUSSION

*Eimeria tenella* is the most pathogenic and one of the least immunogenic of all chicken *Eimeria* species (Bedrnik *et al*., 1989; Venkatas & Adeleke, 2019), therefore, as a test of the immunogenicity, anticoccidial vaccines must be capable of controlling *E. tenella*. *Eimeria tenella* is also considered to be the most important as a consequence of its occurrence, fecundity, and pathogenicity (Shirley *et al*., 2005; Blake & Tomley, 2014; Jatau *et al*., 2016). This coccidial species has also been found to be the most common species of *Eimeria* affecting poultry in Nigeria (Jatau *et al*., 2016; Ojimelukwe *et al*., 2018; Ola-Fadunsin *et al*., 2018). Isolates of *E. tenella* used in pathogenicity test have been found to be very pathogenic unlike the Houghton strain of *E. tenella* which has been adjudged to be less pathogenic than field *E. tenella* isolates, but was once fully pathogenic before it was cloned (Chapman & Shirley, 2003)

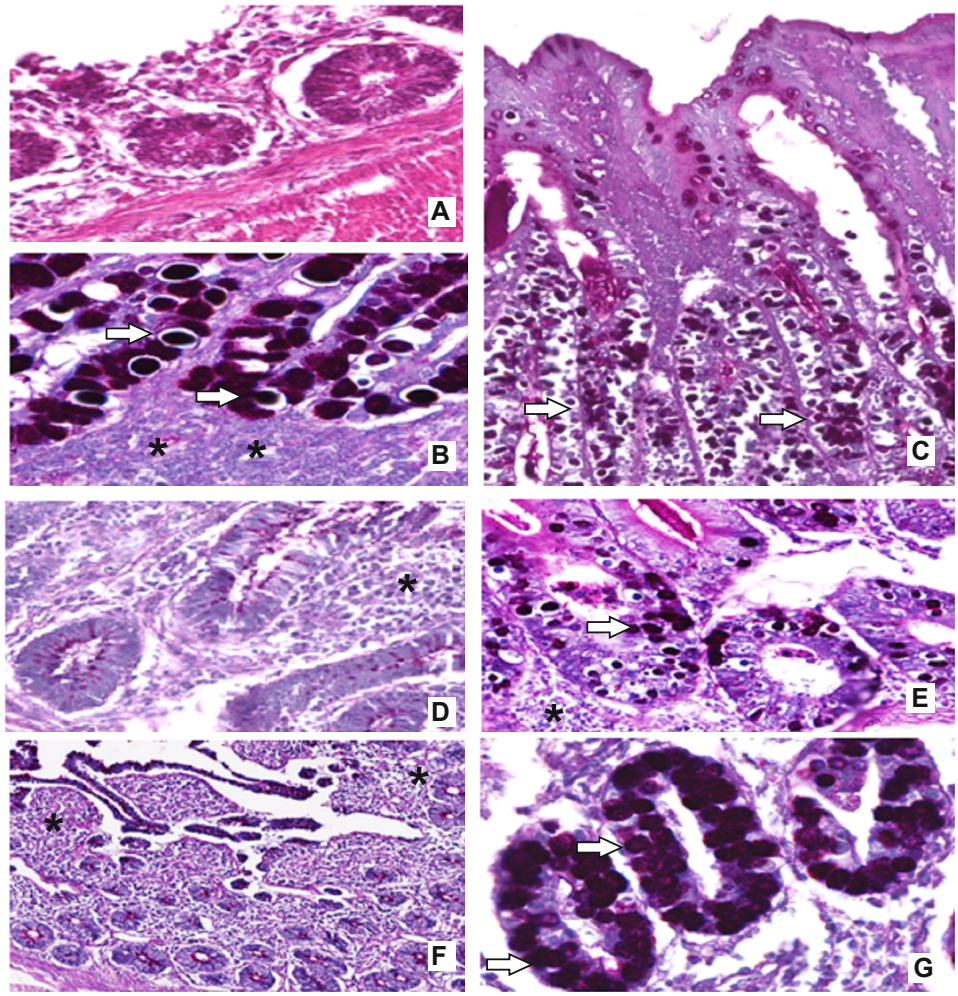


**Fig. 4.** Histopathological examinations of caecal sections showing the intra-enterocytic eimerian protozoan – H&E stain (A), Periodic Acid Schiff (PAS) stain (B–G), all at ×400 magnification. **A.** Groups 1, 8 and 9: no microscopic evidence of *Eimeria tenella*; **B**. Group 2: marked severe expansion (asterisks) of the musculature by developmental stages of *E. tenella* oocysts, schizonts and developing merozoites (arrows) admixed inflammatory cells; **C.** Group 3: muscular caecal moderate expansion by developmental stages of *E. tenella* oocysts, schizonts and developing merozoites (arrows) admixed inflammatory cells; **D.** Group 4: crypts destruction, inflammatory polymorphonuclear cellular infiltrates, cryptic necrosis (asterisk), intralumina desquamation of enterocytes and oocysts debri; **E.** Group 5: caecal crypts with occasional *E. tenella* oocyts (arrow), cryptic necrosis (asterisk), intralumina desquamation and submucosal oedema**; F.** Group 6: presence of inflammatory polymorphonuclear cellular infiltrates (asterisks); **G**. Group 7: moderate diffuse cryptic intra-enterocytic *Eimeria* protozoan oocysts (arrows), with inflammatory polymorphonuclear cellular infiltrates.

although Shirley *et al*. (1989) reported that the Houghton strain had no significant difference in its pathogenicity. Pathogenicity trait has been attributed to be influenced by environmental factors and this may be responsible for the variations in pathogenicity reported for H- strain in different laboratories (Chapman & Shirley, 2003). For example, 20,000 oocysts of H-strain of *E. tenella* were found to cause mortality in White Link chickens (Joyner & Norton, 1969), although a higher dose of 96,000 oocysts did not cause any mortality in male Rhode Island red chickens (Williams, 1973). Increased pathogenicity of the local isolate was observed in this present study as it produced a more severe gross pathologic lesion than the Houghton strain in the unvaccinated but infected group of chickens. The caecal coccidiosis lesion score for the local isolate was also the highest (score +4) and twice as high (+2) as that of the H-strain at the same dose of inocula of 17,000 oocysts. This gross lesion score is similar to the findings of McDonald *et al*. (1986) in Light Sussex chickens in the UK, where a lesion score of 1.57 and 1.95 were reported for H-strain *E. tenella* dose inoculum of 5,000 or 50,000. In these unimmunised, Houghton and local *E. tenella* challenged groups 2 and 3, gross findings of petechiation on the mucosa of the caeca with thickened, eroded, and necrotic caecal epithelia showed that the local isolate of *E. tenella* was more pathogenic hence caused more gross lesions than the Houghton strain. This may be due to geographical variation in the antigenicity of coccidia strains (Fitz-Coy & Edgar, 1992; Martin *et al*., 1997) and the wild-type nature of the local isolate which releases more merozoites from schizonts that causes haemorrhage and enterocyte damage as they were released to infect more caecal epithelial cells. Although it may be inferred that the experimental dose of 17,000 oocysts of H- strain of *E. tenella* in Dominant black cockerel chicks of D109 strain caused a higher gross lesion score than it was previously reported, the role of environmental effect still needs to be investigated. The high pathogenicity and virulence of this local *E. tenella* was responsible for the differences in the severity of clinical signs, gross and microscopic lesions observed between the Houghton (group 2) and local isolate (group 3) infected groups. The result of gross lesion score of +1 for Livacox® immunised and *E. tenella* infected groups in this experiment were at variance with the +2 finding of Anwar *et al*. (2008). Lesion score is an indication of the degree of intestinal/caecal damage (Chapman *et al*., 2013) because it represents the rupture of the enterocytes (Chasser *et al*., 2020). Lesion scores have also been shown to be affected by the type of *Eimeria* isolate, resulting in scores variability (Barrios *et al*., 2017; El-Sherry *et al*., 2019). Clinical signs of coccidiosis were severe in unvaccinated but infected groups 2 and 3, while chickens from vaccinated groups 4, 5, 6, and 7 showed moderate signs of pallor, emaciation, and bloody diarrhoea. Grossly, the muscle of caeca from chickens infected with *E. tenella* showed increased contraction when compared to the caecal muscle of uninfected control chickens as previously observed by Witlock & Fetterer (1983). This increase was more severe in the unvaccinated local isolate group which showed increased severity of the caecal lesion. This contraction has been attributed to the release of acetylcholine (ACH) which has an effect on the muscles of the caeca. The absence of signs of clinical coccidiosis in groups 1, 8, and 9 demonstrated the success of the procedures adopted to prevent contamination by extraneous *Eimeria* in ac-

cordance with the findings of Crouch *et al*. (2003). The success of this procedure was also evident as there were no gross and microscopic findings in the caeca of the absolute control (group 1) and in the Livacox® and Immucox® vaccinated unchallenged controls (groups 8 and 9) respectively. The histopathology clearly showed that the vaccinated groups, infected with Houghton strain of *E. tenella*, did not show the presences of oocysts or schizonts, apart from some cellular infiltration into the submucosa and lamina propria, which may be as a result of the infection but with subsequent depression of the development of oocysts and schizonts by the cellular response as a result of immunity provided by the vaccines. The decreased damage to the caecal mucosa in the immunised chickens has been attributed to the involvement of immune effector mechanisms that leads to attrition or inhibition of the development of early stages of the parasite's life cycle such as sporozoites or first-generation merogonic stages (Kopko, 1998). The immunogenicity of Livacox® and Immucox® was determined by gross and histopathology lesion scores in vaccinated and unvaccinated chickens following virulent challenge. The success and the effectiveness of the immunisation of the chickens by these vaccines, Livacox® and Immucox® were established in the vaccinated and Houghton strain of *Eimeria tenella* infected groups 4 (Livacox®) and 6 (Immucox®) based on the microscopic examination of the caeca of these groups. In these groups, there was no evidence of the development of *Eimeria tenella* in any of the caeca crypts although cellular inflammatory response was evident in the inter-cryptic spaces and within the lamina propria, depicting an immunological response to the presence of infection with the challenged parasite. The successful immunogenicity and effectiveness of these two vaccines were reduced in the vaccinated and local isolate of *Eimeria tenella* infected groups 5 (Livacox®) and 7 (Immucox®) based on the histopathologic lesions and the presence of oocysts in the enterocytes of the crypts of the caeca of these chickens. For Livacox® immunised groups, it was expected that at 4 weeks (28 days) post-immunisation, immunity against coccidiosis should have developed, and be active for at least 2 weeks by the time the experimental infection took place compared to the onset of natural infection period in commercial production. Also, the period needed for coccidia to make sufficient numbers of oocysts to cause disease (Anwar *et al*., 2008) was shortened in this experimental infection. All of these measures however did not prove sufficient enough to prevent the development of oocysts in the cryptic cells of this local *E. tenella* similar to the observation of Anwar *et al*. (2008). This inability of Livacox® to prevent the disease caused by the local *E. tenella* opposes to the findings of Conway *et al*. (1990) in the sense that it caused disease. The presence of oocysts in the cytoplasm of cryptic enterocytes led to necrosis of cells, acute cellular response, release of acute-phase proteins that stimulate fever, shock, and acute inflammation (Min *et al*., 2004). All these led to chronic inflammation, maldigestion, malabsorption, decreased weight gain, and perhaps death in the local *E. tenella* isolate infected chickens. These may be due to the challenging development caused by the use of live attenuated and non-attenuated anticoccidial vaccines in the control of coccidiosis and in particular caecal coccidiosis in chickens reared in an environment dominated by virulent *E. tenella*. This challenge in the control of coccidiosis is in addition to the growth effect in the use of

anticoccidial vaccines (Danforth, 1998; Shapiro, 2001; Crouch *et al*., 2003), economic and regulatory constraints to the continuous use of medication (Chapman, 1999; Allen & Fetterer, 2002), together with the disadvantages of drug use which include withdrawal periods and development of drug resistance (Dalloul & Lillehoj, 2005) in modern intensive poultry production. Another challenge to the use of the present compositions of anticoccidial vaccines (Fatoba & Adeleke, 2018), is the emerging threat to be posed by the identification of three new genetic variants of *Eimeria*, known as operational taxonomic units (OTUs, x, y, z), which have been detected in Nigeria (Jatau *et al*., 2016), Australia (Godwin & Morgan, 2015), and several other regions (Fornace *et al*., 2013; Clark *et al*., 2016).

In view of these pieces of evidence supporting the use of vaccines as a viable (Min et al., 2004), effective and safe alternative to control of coccidiosis (Anwar *et al*., 2008), this experiment is therefore necessary and important to screen these vaccines against local populations before administering them to large flocks, because the antigenicity of coccidia strains has been known to vary geographically (Fitz-Coy & Edgar, 1992; Martin *et al*., 1997). Based on the findings of this experiment, it is therefore important that this local isolate of *Eimeria tenella*, which is immunovariant from the vaccine's strains of *E. tenella* can be used to substitute the *E. tenella* in these commercial vaccines in order to provide a region-specific, autogenous vaccine, as previously suggested (Danforth, 1998).

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