Populations of *Eimeria tenella* express resistance to commonly used anticoccidial drugs in southern Nigeria

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**A B S T R A C T**

Coccidiosis is one of the most economically important diseases of poultry. This study determined the preponderance of chicken *Eimeria* in southern Nigeria and assessed the parasite's resistance to three anticoccidial drugs: Amprolium hydrochloride; Amprolium hydrochloride + Sulfadiazine; and Toltrazuril. Multiplex PCR amplification of the SCAR region was used to confirm *Eimeria* preponderance. Resistance was assessed following the inoculation of 2.32 × 10^5 infective oocysts into broilers. Data on weight gain, feed intake, feed conversion and fecal oocyst shed were recorded. At 7 days post inoculation 9 birds per treatment were sacrificed and assessed for macroscopic lesions in four intestinal regions. Percent optimum anticoccidial activity (POAA), Anticoccidial index (ACI) and Anticoccidial sensitivity test (AST) were used to access resistance. The preponderance of *Eimeria* spp. were *E. tenella* (77%), *E. necatrix* (55%), *E. acervulina* (44%) and *E. mitis* (11%), with multi-species infection occurring in 55% of samples assessed. Fecal oocyst shedding was low (P < 0.05) in the medicated groups. Lesions in the cecal region were present in all infected groups regardless of treatment and accounted for 27.8% of lesion scores by severity and 37.5% of lesion scores by frequency. Overall, lesion scores were less (P < 0.05) in birds of the medicated groups compared with the infected-unmedicated group. The high preponderance of *E. tenella* in the field, and the occurrence of cecal lesions – caused mainly by *E. tenella* – despite drug administration, indicate resistance in populations of this species in our isolate. Based on the POAA, ACI and AST values, the *Eimeria* isolate showed reduced sensitivity to toltrazuril.

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**1. Introduction**

Poultry coccidiosis caused by the Apicomplexan parasite *Eimeria*, is one of the world's most economically important diseases of poultry birds. *Eimeria* primarily targets the tissues of the intestinal epithelium which consequently results in a decline in growth and feed utilization in poultry[1,2]. The disease is therefore associated with high production losses, high morbidity and mortality rates of above 50% [3,4]. In broilers, mild or subclinical infections are also important as even minor intestinal lesions can significantly impede feed efficiency and profitability [5].

The control of poultry coccidiosis can often be achieved by a combination of several strategies including; the use of anticoccidial drugs, delivery of live *Eimeria* vaccines, good husbandry practice and optimum biosecurity standards [6,7]. Inclusion of anticoccidial drugs in the diet of poultry birds is arguably the most widespread means of controlling poultry coccidiosis [8,9]. Usage rates upward of 70% have previously been reported in poultry farms across the USA and Europe [10], and up to 100% in Nigeria [11,12]. This is particularly so because modern poultry systems are characterized by high stocking density that encourages parasite accumulation and transmission [13] and anticoccidial drugs are a convenient, and affordable method for keeping parasite challenge at a minimum [14]. In Nigeria, anticoccidial drug use is very popular, particularly because the majority (over 60%) of our poultry farms are characterized by poor hygiene conditions and low biosecurity levels [15]. These drugs seem to be indispensable for the sustainability of poultry production systems.

Unfortunately, as with the problems encountered with the widespread use of antimicrobials in veterinary and public health, the extensive use of chemotherapeutic drugs unavoidably leads to the development of drug resistance among *Eimeria* species [16]. Heavy drug dependence could have profound impact on the biology of *Eimeria* species in Nigeria with regards to the development of drug resistance, which can limit the effectiveness of such products [17].

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Following personal conversations with some local veterinarians in Rivers state, it was established that the anticoccidials Toltrazuril, Amprolium hydrochloride and Amprolium hydrochloride + Sulphaquinoxaline Sodium, are the drugs of choice for the control of chicken coccidiosis in the state. The presence of resistant *Eimeria* spp. in Rivers state are a threat to local poultry production and could compromise local control efforts. This study was therefore embarked upon to assess drug resistance of *Eimeria* spp. to these three drugs using preponderance studies and drug sensitivity trials.

2. Materials and methods

2.1. Ethical approval

The sampling protocol and the use of animals for experimental studies applied here were approved by the University of Port Harcourt Research Ethics Review Committee with ethics approval number UPH/CEREMAD/REC/04.

2.2. Field study

2.2.1. Sampling location

Port Harcourt is the capital of Rivers State and the third largest city in southern Nigeria (Fig. 1). It is a diverse city located in the Niger Delta region (within latitude 4°49'27"N 7°21'E) and is economically significant as the centre of Nigeria's oil industry. Port Harcourt metropolis is home to two local government areas (LGA): Port Harcourt LGA and Obio-akpor LGA. Nine major Live Bird Markets (LBMs) in Port Harcourt city were selected purposively based on the fact that being a densely populated city, live poultry trade traffic would very likely flow into the city from different parts of the state including the rural, urban as well as suburban areas.

2.2.2. Sample collection and processing

From September to November 2017, field studies were conducted and fresh fecal droppings were randomly collected from across cages where live chickens were housed. Samples from each market were pooled into a bulk sample. Each bulk sample was homogenized (in a blender or by stirring with a rod) and filtered using a 106 µm mesh sieve. *Eimeria* oocysts were harvested using the saturated NaCl flotation method (10 min at 1000 × g). The harvested oocysts were re-suspended in distilled water and washed by centrifugation three times to remove the flotation solution (500 × g for 5 min). The sediment containing the oocysts was transferred into beakers, suspended in 2.5% (w/v) K2Cr2O7 solution and allowed to sporulate at room temperature for seven days with regular stirring. After sporulation, oocysts from each sample were cleaned with sodium hypochlorite (4% active chlorine) and washed with distilled water three times as described before [18].

2.3. Molecular characterisation of chicken *Eimeria*

2.3.1. Genomic DNA extraction

Total genomic DNA extraction from oocysts of *Eimeria* species was done as follows: 20 mL of each oocyst suspension, were centrifuged at 750g for 10 min to pellet the oocysts. Each pellet was re-suspended in the minimum volume residual supernatant and transferred to a 2 mL screw top plastic tube containing glass beads (0.1–0.5 mm; ZR BashingBead™, SA) and covered with sterile phosphate buffered saline (PBS; pH 8.0). The pelleted oocysts were then disrupted using a Mini Beadbeater-8, (Biospec Products Bartlesville, USA) for three minutes. Total genomic DNA (gDNA) was isolated from the smashed oocysts homogenate using a Quick-DNATM extraction kit (ZYMO RESEARCH) following the manufacturers protocol. Briefly, 1200 µL of Genomic Lysis Buffer were added to the filtrate and 800µL of the filtrate plus Lysis Buffer suspension transferred to a Zymo-Spin™ IIC column in a collection tube and centrifuged at 10,000 × g for 60 s. Then 500 µL of genomic DNA Wash Buffer were added to the previous step and centrifuged at 10,000 × g for 60 s. The suspension was then transferred to a 1.5 mL centrifuge tube and 100µL of DNA Elution Buffer added directly to the column matrix and centrifuged at 10,000 × g for 30 s to elute the DNA. Finally, the eluted DNA was transferred into a prepared ZymoSpin™ IV-HRC Spin Filter placed in a 1.5 mL micro-centrifuge tube and

Fig. 1. Map of Nigeria: The study area is in Rivers state (highlighted in green) in the southern region of Nigeria. The sampling locations cover Port Harcourt and Obio/Akpor local government areas (The Cartography Unit, University of Port Harcourt).
2.3.2. Multiplex PCR amplification

The Eimeria species was verified by a multiplex PCR assay as earlier described by Fernandez et al., [19]. Six pairs of species-specific primers were used (Table 2). The PCR amplification was based upon a 25 µL volume consisting of 1 µL genomic DNA template, 0.5 µL of each primer, 12.5 µL Taq DNA polymerase (Sigma, USA) and made up to 25 µL with nuclease free water. The standardized cycling conditions consisted of initial denaturation: 96 °C for 5 min followed by 30 cycles of 95 °C denaturation for 1 min, 59 °C annealing for 2 min; 72 °C extension for 1 min, and a final extension: 72 °C for 7 min.

2.3.3. Agarose gel electrophoresis

Agarose gel was prepared as follows: 1.5% (w/v) Agarose was prepared with 100 mL of distilled water and TBE buffer. The mixture was microwaved for 3 min. The Agarose solution was allowed to cool and 1 µL of ethidium bromide added per 100 mL of agarose solution. The solution was loaded onto a casting tray with well position casting and 1 µL of ethidium bromide added per 100 mL of agarose solution. The Agarose solution was allowed to cool for 3 min. The Agarose gel was placed into the gel electrophoresis unit. The unit was filled with TBE until the gel was completely submerged. DNA samples extracted previously were well loaded with loading dye. A molecular ladder of 100 base pairs was loaded into lane 4 and the other samples loaded into additional wells. Gel was run at 120 V for 25 min. DNA fragments (Bands) were visualized under UV light and images captured using a digital camera.

### Table 2

| S/N | Species          | Primer sequences 5'-3' | Expected amplicon size (bp) |
|-----|------------------|------------------------|-----------------------------|
| 1   | *E. acervulina*   | F: AGTCACCCACACAAATTATGGGAAACATG | 811                         |
|     |                   | R: AGTACGACCAGGAGAAGGATGTTG |                             |
| 2   | *E. tenella*      | F: CGGCCAAAACCAGTGTCAGC | 539                         |
|     |                   | R: CGGCACCAACTGGAATGAGGC |                             |
| 3   | *E. mitis*        | F: AGTCAGCCACAGTGAACATTTTG | 460                         |
|     |                   | R: AGTCAGCCACAGTGAACATTTTG |                             |
| 4   | *E. maxima*       | F: GGGAACCGCAGTGGGGTATG  | 272                         |
|     |                   | R: AGGAAAAGGAAAGGCGGCTCTG |                             |
| 5   | *E. neocatarrhiae*| F: TCTATGGCAGAACTAATTTGCTCTA | 200                         |
|     |                   | R: ACAGAGGCTTAAACCCCGAAGAATTTTGTG |                             |
| 6   | *E. brunetti*     | F: TGGTGCAGGGCAGGGGTCTG | 626                         |
|     |                   | R: TGGTGCAGGGCAGGGGTCTG |                             |

(Adapted from Fernandez et al. [19]).

F: forward primer; R: reverse primer; bp: base pairs.
selected from each group, euthanized, necropsied and lesion scores recorded for the four regions of the intestine as described by Johnson and Reid, [23].

2.4.4. Evaluation of resistance

Drug resistance of *Eimeria* was evaluated using three indexes:

1. The Anticoccidial Index (ACI). $ACI = \frac{\text{rate of relative body weight gain} + \text{survival rate}}{\text{lesion score} + \text{oocyst count}}$. An ACI value of $\geq 160$ indicated sensitivity; a value $< 160$ indicated resistance [24]. Oocyst value was calculated as follows: (OPG output of each group/OPG output of PosCntr group) $\times 100$.

2. The Anticoccidial Sensitivity Test (AST) [25]. $AST = \frac{\text{average lesion score in medicated group}}{\text{average lesion score in infected-unmedicated group}} \times 100\%$. AST $\geq 50\%$ was judged to be sensitive and $< 50\%$ was resistance; and

3. Percent Optimum Anticoccidial Activity (POAA). $POAA = \frac{\text{GSR in medicated group} - \text{GSR in infected-unmedicated group}}{\text{GSR in infected-unmedicated group}} \times 100\%$. GSR (Growth and Survival Ratio) was defined as final body weight divided by initial body weight. POAA $> 50\%$ was judged to be sensitive and $\leq 50\%$ was resistance [5].

The overall assessment of drug resistance of our LBM *Eimeria* isolate was adapted from Lan et al., [26]. Briefly, if 3 of 3 indexes showed resistance, our isolate was considered as severely drug resistant ($+++$). Two of 3 meant moderate drug resistance ($++$), 1 of 3 meant slight drug resistance ($+$) and none meant no drug resistance.

2.5. Statistical analyses

Data were analyzed by the software R 3.1.0 using a Windows XP system. The body weights, feed intake, lesion scores and oocysts shed per gram of feces were analyzed by one-way ANOVA and a post hoc analysis using Tukey's multiple comparison test to identify statistically significant variations. The difference was considered significant if $P < 0.05$. Box plots and bar charts were generated using R 3.1.0.

3. Results

3.1. Preponderance of chicken *Eimeria* spp. in Rivers state

The results of the molecular identification showed that *E. tenella*, *E. necatrix*, *E. acervulina* and *E. mitis* are the predominant *Eimeria* species in Rivers state. *E. tenella* was present at 7 of 9 locations (77%), *E. acervulina* at 5 of 9 (55%), *E. mitis* at 1 of 9 (11%). The primers used allowed for the differentiation of all four *Eimeria* species present in the samples. The different sizes of DNA fragments amplified were displayed on Agarose gel as follows: *E. acervulina* (811 bp), *E. tenella* (539 bp) and *E. necatrix* (310 bp) (Fig. 2). Multi- *Eimeria* species infections were found in 5 of 9 (55%) locations with *Eimeria tenella* being the dominant species.

3.2. Drug sensitivity of chicken *Eimeria* spp. isolate in Rivers state.

3.2.1. Performance results: Body weight gain (BWG), feed intake and feed conversion ratio (FCR)

In all treatments, body weight gain (BWG) of the medicated groups and the uninfected-unmedicated groups (NegCntr) were significantly higher than the infected-unmedicated group (PosCntr) (Table 3). In *Amprolium hydrochloride* and *Amprolium hydrochloride* + *Sulfaquinoxaline* sodium treated groups, BWG was better than PosCntr group. Toltrazuril did not result in a significant increase in BWG. Feed consumption was highest in the NegCntr group and lowest in the PosCntr group (Table 4). The highest weight gain and the lowest feed conversion ratio was recorded in the NegCntr group (Table 5).

3.2.2. Lesion scores

Intestinal macroscopic lesions were completely absent in birds of the NegCntr group, and present in the PosCntr group. Figs. 3 and 4 show the occurrence and specific mean lesion scores for the four regions of the intestine assessed respectively. Lesions in the cecal region were present in all medicated groups as well as the PosCntr group. However, lesion scores of birds in the PosCntr group were significantly lower than the infected-unmedicated groups (NegCntr).

![Fig. 2. Agarose gel electrophoresis showing the amplified SCAR gene of the *Eimeria* spp. Lane OM: *E. acervulina* (811 bp), *E. tenella* (500 bp); Lane CH: *E. acervulina* (811 bp), *E. tenella* (500 bp); Lane CR: *E. acervulina* (811 bp), *E. tenella* (500 bp), *E. necatrix* (200 bp); Lane FG: *E. acervulina* (811 bp), *E. tenella* (500 bp), *E. necatrix* (200 bp); Lane SL: *E. acervulina* (811 bp), *E. tenella* (500 bp), *E. necatrix* (200 bp); Lane RO: *E. acervulina* (811 bp), *E. tenella* (500 bp), *E. mitis* (460), *E. necatrix* (200 bp); Lane RM: *E. acervulina* (811 bp), *E. tenella* (500 bp), *E. mitis* (460), *E. necatrix* (200 bp); Lane L: 1000 bp molecular ladder. Abbreviations: OM: Oil Mill; CH: Choba; SL: Slaughter; M1: Mile 1; RK: Rumuokuta; FG: Fruit garden; CR: Creek road; RO: Rumuokoru; RM, Rumuomasie. bp, base pairs.

![Table 3](Table 3) Average body weight gain (g) of experimental birds between 14 days old and 21 days old.

| Treatment          | Replicate 1       | Replicate 2       | Replicate 3       |
|--------------------|-------------------|-------------------|-------------------|
| Amp                | 161.08 ± 57.98*   | 163.06 ± 21.78*   | 157.23 ± 36.36*   |
| Amp + Sul          | 176.22 ± 42.31*   | 165.27 ± 18.78*   | 173.34 ± 50.24*   |
| Tolft              | 149.34 ± 35.11**  | 167.94 ± 55.75**  | 142.51 ± 50.51**  |
| NegCntr            | 185.56 ± 9.75**   | 182.43 ± 10.43**  | 183.00 ± 17.85**  |
| PosCntr            | 112.27 ± 39.95    | 140.41 ± 21.35    | 131.02 ± 45.10    |

Table 3

Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + Sulfaquinoxaline sodium; NegCntr: uninfected-unmedicated negative control; PosCntr: infected-unmedicated positive control. Data represent mean ± SD ($*P < 0.05$ vs. PosCntr; ns: no significant differences).
regions but not in the cecal region. A combined assessment of the average intestinal lesion scores of birds in our drug sensitivity trial showed that, Amp + Sul group had the least lesion score i.e. most effective, and Tolt group had the highest scores, i.e. least effective. Overall, intestinal lesion scores were significantly less ($P < 0.05$) in birds treated with the anticoccidial drugs (Table 6.) compared with birds in PosCntr group.

### 3.2.3. Fecal oocyst shedding

Oocysts shed in the feces of birds from 5 days post infection (dpi), 6 dpi and 7 dpi in all treatment groups is shown in Figs. 5, 6 and 7 respectively. Fecal oocysts shedding increased with the duration of infection with the least oocysts count on day 5 and the highest oocyst count on day 7. Among the medicated groups, Amp group recorded the least number of fecal oocysts excreted with $1.46 \times 10^5 \pm 0.24$ oocysts shed per gram of feces. Tolt group had the highest with $3.17 \times 10^5 \pm 1.98$ oocysts shed per gram of feces. Overall, fecal oocyst counts in all three medicated groups were significantly less ($P < 0.05$) than that in the PosCntr group (Table 7).

#### 3.2.4. Anticoccidial index (ACI), anticoccidial sensitivity test (AST) and percent optimum anticoccidial activity (POAA).

The results of ACI, AST and POAA are summarized in Tables 8 and 9. No bird mortality was recorded throughout the trial. The ACI values of Amp and Amp + Sul groups (> 160) indicated that these drugs were able to treat the chickens infected with our isolate while maintaining bird productivity. Resistance to toltrazuril was expressed as the value of 150.99. Both POAA and AST values (> 50%) showed that our isolate is sensitive to all three coccidiostats tested, however, sensitivity to toltrazuril was much less. In the present study, an overall drug sensitivity assessment based on a combined analysis of the three indexes revealed slight resistance of *Eimeria* isolate to toltrazuril (Table 10).

### 4. Discussion

*Eimeria* spp. isolates were identified by the multiplex PCR amplification of the SCAR rDNA region using species-specific primers [19]. The preponderance of chicken *Eimeria* species recorded in Rivers state were *E. tenella*: 77%, *E. necatrix*: 55%, *E. acervulina*: 44% and *E. mitis*: 11%. Our results are in agreement with a related study involving the molecular characterization of chicken *Eimeria* in northern Nigeria where preponderance rates of 75% for *E. tenella*, 25% *E. necatrix*, 33% *E. acervulina* and 50% *E. mitis* were reported [27]. Our study further revealed that *E. burnetti* and *E. maxima* were absent from our samples. This result is also in agreement with the study by Jatau et al., [27] where these species were also absent. The absence of *E. maxima* and *E. burnetti* in these two regions is an indication that these species may not play significant roles in the epidemiology of chicken coccidiosis in Nigeria.

### Table 4

| Treatment | Feed consumed (g) |
|-----------|-------------------|
| Amp | 4090.59 ± 86.20 |
| Amp + Sul | 4227.52 ± 135.48 |
| Tolt | 4036.94 ± 76.39 |
| NegCntr | 4389.27 ± 75.06 |
| PosCntr | 3561.93 ± 136.38 |

Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + sulfaquinoxaline sodium; NegCntr: uninfected-unmedicated negative control; PosCntr: infected-unmedicated positive control. Data represent mean ± SD.

### Table 5

| Treatment | Replicate 1 | Replicate 2 | Replicate 3 |
|-----------|-------------|-------------|-------------|
| Amp | 2.76 | 2.78 | 2.95 |
| Amp + Sul | 2.75 | 2.75 | 2.71 |
| Tolt | 3.01 | 2.72 | 3.08 |
| NegCntr | 2.66 | 2.69 | 2.61 |
| PosCntr | 3.40 | 2.80 | 3.14 |

Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + sulfaquinoxaline sodium; NegCntr: uninfected-unmedicated negative control; PosCntr: infected-unmedicated positive control.

Fig. 3. Occurrence of lesions in the four regions of the intestine. Keys: Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + sulfaquinoxaline sodium; PosCntr: infected-unmedicated positive control.
Analysis of our results showed that *E. tenella* was present at every positive sample location and had the highest prevalence (77.8%). The same observation was reported by Jatau *et al.* [27] in northern Nigeria. High preponderance rates such as these are often an indication of drug resistance in this species [10]. *E. tenella* therefore remains highly invasive amongst others and is possibly the most economically important *Eimeria* species causing chicken coccidiosis in southern Nigeria. Similar results have been obtained in Ethiopia [28], India [29] and China [9,26,30] where *E. tenella* was reported as the most prevalent.

The co-occurrence of multiple *Eimeria* species in a given location was also evident in this study. Our finding of the high prevalence of mixed *Eimeria* species infections (55%) is in agreement with related results of 68% and 67% found in northern Nigeria by Jatau *et al.*, [31] and Jatau *et al.*, [27] respectively.

*E. tenella* and *E. necatrix* are considered as highly pathogenic species of *Eimeria* [32,33]. While the presence of *Eimeria* oocysts in chicken feces is not a definitive diagnosis of chicken coccidiosis [28], the circulation of such pathogenic species can contribute to high morbidity and mortality in young chickens particularly when conditions of poor nutrition, poor sanitation and hygiene and co-infection with other pathogens persist [34]. Pathogenic species such as these could compromise poultry productivity in the majority of Nigerian chicken farms, particularly among the medium to small scale farms that are often characterized by poor sanitation and minimal-to-no biosecurity [15]. Based on our result, the species with the least preponderance is *E. mitis*. *E. mitis* is not often associated with severe disease in chickens, however, it can result in the reduction of feed efficiency in chickens [32].

Given the high prevalence of poultry coccidiosis reported in Nigeria, Nigerian poultry farms rely heavily on the use of anticoccidials for the control of coccidiosis [11]. It has been established that such practice as

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**Table 6**

Overall lesion score of birds infected with a mixed *Eimeria* spp. isolate assessed by taking an average of the individual scores for the four intestinal regions.

| Treatment | Lesion score |
|-----------|--------------|
| Amp       | 0.333 ± 0.36* |
| Amp + Sul | 0.305 ± 0.21* |
| Tolt      | 0.944 ± 1.26* |
| NegCntr   | 0            |
| PosCntr   | 2.083 ± 0.25 |

Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + sulfaquinoxaline sodium; NegCntr: uninfected-unmedicated negative control; PosCntr: infected-unmedicated positive control. Data represent mean ± SD (*: *P* < 0.05 vs. PosCntr).
the extensive use of anticoccidial drugs could greatly influence the development of anticoccidial resistance in poultry *Eimeria* [35,36].

In this study, *Eimeria* oocysts were isolated from chicken fecal samples collected at a live bird market in Port Harcourt. By targeting all *Eimeria* species from a live bid market instead of a single poultry farm, our parasite detection rates should be higher [37] and our anticoccidial profile should be more representative of what is happening in the average chicken population in the field [35]. This is particularly so because live birds for sale at the market are often sourced from multiple farms [38], making such places important pathogen accumulation and distribution points [39].

The *Eimeria* oocysts were isolated, propagated, characterized and assessed for sensitivity to three anticoccidial drugs – Amprolium hydrochloride, Amprolium hydrochloride + Sulfadoxine sodium and Toltrazuril. The sensitivity of these drugs was assessed on a prophylactic basis as experimental birds were inoculated with the *Eimeria* spp. isolate two days after drug administration commenced. The isolates were subsequently confirmed to contain *E. tenella*, *E. necatrix*, and *E. acervulina*, as described above. The common assay of including the respective anticoccidial drugs into the diet of infected birds was employed.

The extent to which an anticoccidial drug can result in a marked reduction or in some cases, the complete elimination of intestinal lesions is often explored as an indication of drug sensitivity and/or parasite resistance [5,9]. Also, presence and characteristic nature of lesions in specific areas of the intestine can be used to identify the species expressing resistant traits in a mixed inoculum of *Eimeria* [23]. Analysis of our results showed that all three medicated groups resulted in marked reduction in the occurrence of lesions in the upper, middle and lower intestinal regions (Fig. 3). The putative *Eimeria* species in our isolate that cause lesions in these parts of the intestine are *E. acervulina* and *E. necatrix* [40]. This indicates that populations of *E. acervulina* and *E. necatrix* in our isolate are sensitive to the three anticoccidial drugs tested. On the contrary, cecal lesions due primarily to infection by *E. tenella* [40] were present in all birds inoculated with *Eimeria* spp. isolate despite medication. Cecal lesions accounted for 27.8% of lesion scores by severity and 37.5% of lesion scores by frequency. These results indicated reduced sensitivity of all three anticoccidial drugs to populations of *E. tenella* in our study. Overall, birds in the medicated groups had significantly less severe lesion scores, which is an indication of a healthier gut and a greater chance of recovery from disease [41].

Fecal oocyst shedding was apparent in all groups except the uninfected-unmedicated group. Our results are in agreement with similar studies conducted in China [26], Egypt [42] and Iran [5] where birds continued to shed large amounts of oocysts regardless of anticoccidial medication. Oocysts shed per gram of feces by birds in all three medicated groups was however, significantly less than those in the infected-unmedicated groups. Our results therefore showed that the transmission of coccidiosis was sustained in the presence of drug pressure though the transmission potential (amount of oocysts shed) is reduced. This is very important in the epidemiology of the disease and should be factored in when considering disease control options.

Birds in all three medicated groups had higher body weight gains than those in the infected-unmedicated group. However the difference was statistically significant in Amprolium hydrochloride and Amprolium hydrochloride + Sulfadoxine sodium treated groups only. Our results further showed that the body weight gains and feed efficiency of birds in the uninfected-unmedicated groups were significantly better than those in the medicated groups. These results are in agreement with similar studies [9,26] and therefore indicates that regardless of the prophylactic treatment of birds with anticoccidial drugs, coccidiosis can still reduce production efficiency in infected birds [5]. This highlights the economic importance of chicken coccidiosis.

Three anticoccidial efficacy indexes were adopted. An overall assessment of the drug sensitivity of our field isolate using the ACI, AST and the POAA showed sensitivity to Amprolium hydrochloride and Amprolium hydrochloride + Sulfadoxine sodium and slight resistance to Toltrazuril. Our results are markedly different from a related study in China where severe resistance to Amprolium hydrochloride, Toltrazuril and Sulfadoxine sodium was reported [26]. The
sensitive and < 50% was resistance; score in infected-unmedicated group × 100%. AST: negative control hydrochloride + sulfaquinoxaline sodium; value of (lesion score + oocyst value). An ACI = (rate of relative body weight gain + survival rate) – (lesion score + oocyst value). An ACI value of ≥ 160 indicated sensitivity; a value < 160 indicated resistance. Oocyst value = (OPG output of each group/OPG output of PosCntr group) × 100;

Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + sulfaquinoxaline sodium; NegCntr: uninfected-unmedicated negative control; PosCntr: infected-unmedicated positive control. Data represent mean ± SD (∗: P < 0.05 vs. PosCntr).

| Treatment | Survival rate% | BWG rate % | Oocyst value | AST | ACI |
|-----------|----------------|------------|--------------|-----|-----|
| Amp       | 100            | 87.36      | 13.68        | 84.01 | 173.35 |
| Amp + Sul | 100            | 93.44      | 15.85        | 85.36 | 177.29 |
| Tolt      | 100            | 82.88      | 30.95        | 54.68 | 150.99 |
| NegCntr   | 100            | 100        | 0            | 200  |
| PosCntr   | 100            | 69.64      | 100          | 67.56 |

AST: Anticoccidial sensitivity test = (average lesion score in infected-unmedicated group – average lesion score in medicated group)/average lesion score in infected-unmedicated group × 100%. AST ≥ 50% was judged to be sensitive and < 50% was resistance; ACI: Anticoccidial index = (rate of relative body weight gain + survival rate) – (lesion score + oocyst value). An ACI value of ≥ 160 indicated sensitivity; a value < 160 indicated resistance.

Table 9

| Treatment    | Initial Body Weight (g) | Final Body Weight (g) | GSR     | POAA % |
|--------------|-------------------------|-----------------------|---------|--------|
| Amp          | 213.74 ± 2.44           | 374.20 ± 0.66         | 1.76    | 86.58  |
| Amp + Sul    | 227.25 ± 1.59           | 398.86 ± 4.10         | 1.75    | 88.86  |
| Tolt         | 215.55 ± 14.52          | 369.77 ± 21.97        | 1.50    | 59.49  |
| NegCntr      | 236.69 ± 4.53           | 420.25 ± 3.40         | 1.78    | 100.00 |
| PosCntr      | 217.64 ± 7.15           | 345.54 ± 7.85         | 1.59    | 88.00  |

POAA: Percent Optimum Anticoccidial Activity = (GSR in medicated group – GSR in infected-unmedicated group)/(GSR in uninfected-unmedicated group – GSR in infected-unmedicated group) × 100%, GSR (growth and survival ratio) = final body weight divided by initial body weight. POAA > 50% was judged to be sensitive and ≤50% was resistance; Tolt, toltrazuril; Amp, amprolium hydrochloride; Amp + Sul: Amprolium hydrochloride + sulfaquinoxaline sodium; NegCntr: uninfected-unmedicated negative control; PosCntr: infected-unmedicated positive control.

differences reported in poultry Eimeria drug sensitivity results perhaps express the unique ecological and/ or epidemiological factors that modulate the development of drug resistance between regions.

5. Conclusions

The study aimed to evaluate drug resistance of Eimeria spp. in southern Nigeria using preponderance and drug sensitivity assays. Four species, E. tenella, E. necatrix, E. acervulina and E. mitis were identified with E. tenella being the most dominant species present in 7 of 7 Eimeria positive locations. The higher preponderance of E. tenella was an indication of drug resistance in this species and the drug sensitivity results corroborated this finding as populations of E. tenella causing cecal lesions resulted in varying degrees of pathology in birds despite treatment with anticoccidial drugs. Overall, slight resistance to Toltrazuril was observed within the experimental period.

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