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Epidemiological Investigation of *Eimeria* Species and Effectiveness of Togolese Medicinal Plants Used Against Chicken Coccidiosis

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ABSTRACT

Eimeria species cause coccidiosis, a poultry disease that occurs worldwide. Infection is linked to decreased feed efficiency and body weight increase. The present study aimed to assess the prevalence of coccidian species in Togolese poultry farms and evaluate the anticoccidial efficacy of three local medicinal plants. From July to September 2023, two hundred and ninety-five fecal samples were randomly collected using a cross-sectional observational study in the maritime region of Togo, specifically in Vo, Lacs, Zio, and Grand-Lomé districts. Data on risk factors were collected through an interview with the poultry farmers. All fecal samples collected were subjected to *Eimeria* oocyst counting using the standard McMaster technique. The anticoccidial activity of the extract of *Azadirachta indica* leaves *Carica papaya* seeds, and *Sarcocephalus latifolius* roots in a completely randomized design was evaluated on 23-day-old male Isa brown chicks infected with 30.10^4 oocysts. Body weight gain, feed efficiency, lesion score, proportion of bloody droppings, anticoccidial index, and excretion of coccidia oocysts were assessed. The results revealed an overall prevalence of 39.66% (117/295) for coccidiosis, with 75% of positive samples having fewer than 10,000 oocysts/g. The logistic regression test indicated that the interval between two anticoccidial prophylaxis applications, age, management, and breed were significant risk factors associated with coccidial infection, with young chicks (≤ 8 weeks) being 5.66 times more susceptible than those older ones (8 weeks) with 0.86 as an odd ratio. Six *Eimeria* species were identified, with *E. maxima* (54.17%), *E. brunetti* (33.33%), and *E. tenella* (25%) being the most common. The anticoccidial efficacy of *Azadirachta indica* leaves, *Carica papaya* seeds *Sarcocephalus latifolius* roots extract, and amprolium was demonstrated by a reduction in lesion scores, bloody diarrhea, and oocysts per gram in feces (OPG) as well as an improvement in body weight, feed conversion ratio, and production efficiency factor when compared to infected and untreated groups. The anticoccidial index was marked in the chickens treated with *Sarcocephalus latifolius* roots extract (170) and amprolium (176). The findings of this large-scale epidemiological study and anticoccidial efficacy tests revealed that these Togolese medicinal plants can be sustainable and cost-effective strategies for coccidiosis control.

Keywords: Anticoccidial drug sensibility, Coccidiosis, Prevalence, Risk factor, Togo

INTRODUCTION

Despite breakthroughs in preventative and control measures through chemotherapy, diet, management, and genetics, coccidiosis remains one of the most costly and pervasive diseases that impact poultry and other domestic species (Tanweer et al., 2014; Kadykalo et al., 2018). It is a gastrointestinal tract (GIT) disease caused by a microscopic protozoan parasite (coccidia) from the genus *Eimeria* in the phylum Apicomplexa (Gilbert et al., 2011).

Eimeria acervulina, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria tenella*, *Eimeria lata*, *Eimeria nagambie*, *Eimeria zaria* have all been identified and classified as domestic species affecting chickens (Abbas et al., 2012; Blake et al., 2021). The oocyst, expelled with the feces sporulates two days later in the environment and remains as the infectious form of the parasite. This sporulation occurs under conditions of poor litter management (wet or water-soaked litter promotes sporulation), contaminated drinkers and feeders, poor ventilation, and high stocking density. Chicks become infected by the ingestion of sporulated oocysts in contaminated water and feed (McDougald, 2003). *Eimeria* invade the intestinal mucosa and damage the epithelium (anorexia, difficulty in digestion and nutrient absorption, dehydration, diarrhea, and blood loss at three to five days post-infection), which can lead to the leakage of serum into the gut and stimulation of mucus production, both of which can provide a rich source of nutrients for opportunistic bacteria (*C. perfringens*) proliferation (Moore, 2016). The proliferation of sporocysts in the epithelial cells of the intestine leads to anorexia, difficulty in digestion and nutrient absorption, dehydration, and blood loss at three to five days post-infection (Hauck et al., 2019). The macroscopic lesions in the digestive tract predispose poultry to many bacterial gastrointestinal diseases, such as *clostridiosis*, *salmonellosis*, and *colibacillosis* (Dakpogan et al., 2018). Immunosuppressive viral diseases, including infectious bursal disease, Marek's disease, and infectious viral anemia of chicks also exacerbate coccidiosis (Lanckriet et al., 2010). According to Blake et al. (2020), the yearly cost of controlling coccidiosis in hens in Brazil, Egypt, Guatemala, India, New Zealand, Nigeria, and the United States for anticoccidial medications (e.g., ionophores and synthetic chemicals) in feed or water is £10.36 billion (recalculating internationally). In West Africa and especially in Togo, the poultry industry has recently experienced growth in terms of meat and egg production according to the Food and Agriculture Organization of the United Nations (FAO, 2015). The estimated poultry population in Togo increased from 18 million in 2014 to more than 25 million in 2017 with the establishment of parent farms and/or day-old chick producers (DSID, 2018). This subsector has played a significant role in poverty alleviation and food security, particularly in rural regions, by providing direct or indirect employment to both male and female communities (Islam et al. 2012). However, poultry production systems are still confronting several infectious diseases, such as coccidiosis, which have a negative impact on their performance and create barriers to maximum production (Sharma et al., 2013; Zhang et al., 2013; Zhuang et al., 2014). Given the unfavorable clinical and economic impact of the disease, special attention should be paid to coccidiosis and regular epidemiological assessment of *Eimeria* species present in poultry farms should be conducted for control and prevention strategies other than expensive synthetic anticoccidials, which may leave residues in poultry-derived food that are harmful to the health of consumers. Researchers recently focused on therapeutic plants and their derivatives, such as papaya, garlic, neem and moringa. They obtain beneficial phytochemicals, substances against induced eimeriosis and innocuous consumers for chicken products (egg and meat) (Wunderlich et al. 2014; Bauri et al., 2015; Muthamilselvan et al. 2016; Thagfan et al. 2017; Dakpogan et al. 2019). Therefore, the purposed of this study was to offer an up-to-date status of coccidiosis in Togo by studying the occurrence of *Eimeria* infection in poultry farms and comparing the anticoccidial activity of *Azadirachta indica*, *Sarcocephalus latifolius*, and *Carica papaya* decoction with synthetic medications in broiler chickens challenged with *Eimeria tenella*.

MATERIALS AND METHODS

Ethical approval

The ethics and scientific committee of the Regional Center of Excellence in Poultry Sciences, University of Lome (CERSA/UL) and the Livestock Directorate (DE) of Togo's Ministry of Agriculture, Livestock, and Rural Development (MAEDR) with reference number 328/DE dated June 16, 2023, had approved the current study.

Study area

The study was carried out in the Maritime Region of Togo, specifically in Vo, Lacs, Zio, and Grand-Lome district. Geographically, Togo is a West African country located approximately between 6°N and 1°E at a minimum altitude of 0m from the Atlantic Ocean and a maximum altitude of 983m from Mount Pic d'Agou. This Southern region of Togo experiences annual temperature variations (from 23°C to 32°C) with an average rainfall of up to 1200 mm per year. The sub-equatorial or Guinean climate characterizes this region with two rainy seasons (March to July or monsoon, and September to October) and two dry seasons (November to February or Harmattan, and July to September). According to FAO (2011), Togo is characterized by a traditional and commercial poultry production system, with the majority (82%) of farms located in the Maritime region (Figure 1).

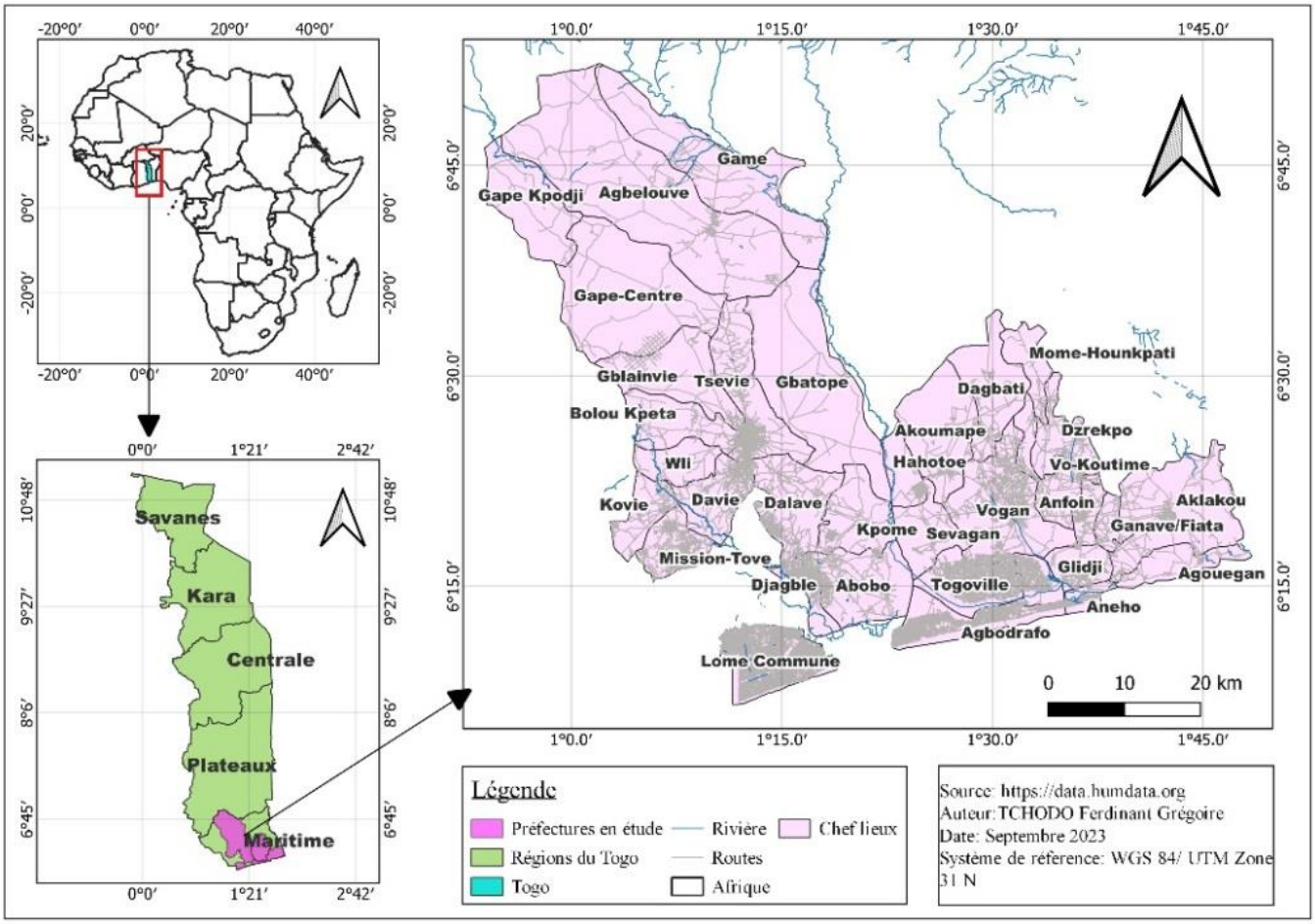


Figure 1. Map of the maritime region, Togo

Epidemiological study design

Sampling procedure

A cross-sectional observational study was conducted in poultry farms in the maritime region to assess the prevalence and intensity of coccidian infection and identify prevalent *Eimeria* oocyst species in the region. The optimal sample size of 42 poultry farms was determined by considering the theoretical coccidiosis prevalence (31%) in a litter-based layer-rearing system (Lunden et al., 2010). To achieve this, a sampling method combining stratified sampling with weighting and convenience sampling within each district of the region was employed.

Sample collection

Two-hundred ninety-five (295) samples of chicken feces were collected from randomly selected poultry farms in the Maritime Region of Togo between July and September 2023. All studied farms have no history of using the coccidiosis vaccination in their programs. The 295 fecal samples were collected from broiler farms, laying hen farms, local chicken farms, and broiler breeder farms. Additionally, the type of farms management consisted of cage (6) litter-based system (260) and free ranged (29). Each sample consisted of freshly egested manure collected from several chickens or a pooled fecal sample from different areas in the poultry house. A survey questionnaire was used to collect information regarding age, farming system, chicken type, environment, and other risk factor-related information through direct interviews/oral conversations. The questionnaire was in French, however, it was translated to farmers by a native speaker. The collected samples were put into screw-cap containers, kept on ice in a cooler and then sent to the Poultry Production Techniques Laboratory at CERSA, University of Lome in Togo for microscopic evaluation.

Parasitological examination

Three grams of each fecal sample were suspended in 42ml of NaCl-saturated water, homogenized, and filtered through a tea strainer to remove coarse elements. Using a

pasteur pipette, 0.15 ml of this mixture was placed in each chamber of the Mac Master slide. After a 5-minute rest, the eggs stuck under the upper glass were observed using a microscope (Olympus, Japan) at a magnification of x40. The number of oocysts was presented as the number of oocysts per gram of feces (OPG; Haug et al., 2008).

Morphology and morphometry oocysts identification

Flores et al. (2022) described the oocyst sporulation process, which was applied in this work. Positive fecal samples (117) were diluted five times with phosphate-buffered saline (PBS, pH 7.4), homogenized using a vortex mixer, and filtered through a mesh sieve. The filtrate was transferred to a polypropylene container and centrifuged for 10 minutes at 1,000 × g. The sediment containing the oocysts was re-suspended with PBS and washed twice using centrifugation (1,000 × g, 10 minutes). After washing, the two ml of sediment was re-suspended with two ml of potassium dichromate solution (2.5%) in small petri dishes. This was done to maintain adequate moisture while also killing other microbes in the samples that competed for oxygen and nutrients with the oocysts. To accomplish sporulation, the samples were incubated in an oven at 28 degrees celsius for 1-3 days with aeration. Microscopy at 100 × magnification was used to evaluate oocyst sporulation. Photographs were acquired for identification using an compound microscope (Olympus, Japan) equipped with an IX73 digital camera. The primary morphological traits were described, according to the key provided by McDougald (2003). Measurements of oocyst size were carried out using a calibrated ocular micrometer.

Anticoccidial activity of Togolese medicinal plants Herb extract and anticoccidial drug

Azadirachta indica leaves, *Carica papaya* seed in the flowering stage, and fresh root of *Sarcocephalus latifolius* were acquired at a local market (Gbossime, Lome in Togo). They were washed and then dried at room temperature (30 °C) for two hours. They were weighed after a period of partial drying. For each 100 g of plant, one liter of boiled water was utilized. Each plant powder was mixed with 100 °C tap water. After 30 minutes, the infusion was filtered, allowed to cool to room temperature (30°C), and given to the chicks. This procedure was performed every morning for the whole five-day therapeutic session (Dakpogan et al., 2019). The infected chicks received the infusion *ad libitum* for five days following infection, which corresponded to the period of oxidant assault generated by the coccidian parasite (Ogwiji et al., 2024), while the uninfected groups received water. The typical anticoccidial chemical was amprolium, which was administered at a dose of 0.6 g per liter of water.

Experimental design

A total of 125-day-old Isa-brown male chicks from a local hatchery were housed in a deep litter carpeted beginning enclosure, under 22 hours of lighting, and held at initially 35 °C with a decline up to 22 day-olds before being divided into the experimental groups. The chicks had unlimited access to feed (Table 1) and water. The main biosecurity precautions were vaccinations against newcastle disease, infectious bronchitis, and infectious bursal disease. The chicks were randomly assigned to five treatment groups. Each treatment group had 25 chicks, with 5 replicates per group and 5 chicks per replication. The experimental groups consisted of the group of chicks treated with *Azadirachta indica* infusion, the group of chicks treated with *Sarcocephalus latifolius* infusion, the group of chicks treated with *Carica papaya* infusion, the group of chicks treated with amprolium, and the group of untreated control chick*s'* roots extracts (infusion), amprolium, and untreated control chicks.

Table 1. Calculated composition of experimental feed during the starter (0-10 days of age) phase

Ingredient (%)	Starter (0-8 weeks)
White maize	57
Wheat bran	4
Roasted soybeans	12
Brewery by product	4
Oyster shell	1.5
Fish meal[1]	16
Broiler concentrate	5
Dl-methionine	0.1
L-lysine	0.2
Sodium chloride	0.2
Chemical nutritional characteristics	
Metabolizable energy (kcal/kg)	2920.99
Crude protein (%)	21.29
Crude fiber (%)	3.30
Lysine	1.21
Methionine ((%)	0.50
Methionine + cysteine (%)	0.76
Calcium	0.92
Total phosphorus	0.58

[1]A commercial fish meal with 60% crude protein made in Senegal and utilized by West African breeders.

Unsporulated *E. tenella* oocysts were obtained from the feces of naturally infected hens at 7 days postinfection

(DPI) in Togo's seaside region. According to El-Ashram and Suo's (2017) procedure, these unsporulated oocysts were first sporulated, purified, and kept in a 2.5% potassium dichromate solution at 25°C for 72 hours. The key to oocyst species identification established by McDougald (1998) was used by observing lesions and utilizing morphologic features and morphometry. The sporulated oocysts were kept at 4°C. Following parasite species confirmation, oocysts were collected, sporulated, and cleaned before the dose was adjusted to 3. 10^4 /mL/chick. All feces produced by each group of chicks in the 24 hours preceding the experimental infection were examined to guarantee the absence of any oocyst. At 23 days old, all chicks were orally gavaged with a one mL distilled water suspension containing 30,000 *E. tenella* sporulated oocysts adjusted using the standard method of McMaster.

Assessment of anticoccidial effectivity of Togolese medicinal plants

The efficacy of herbal extracts was evaluated using bloody diarrhea, survival rate, oocyst excretion, lesion score, body weight increase, and feed conversion ratio. Clinical symptoms and death were reported at each DPI. From the third to seventh day after inoculation, the proportion of blood in feces was assessed by counting bloody excreta twice a day. According to Abbas et al. (2010), the degree of bloody diarrhea was classified into one of four categories ranging from zero to three, based on the average of bloody excreta fragments rounded to the nearest integer. Briefly, the numbers 0, 1, 2, and 3 signified 0, 33, 33-66, and 66-99% of total feces, respectively. The survival rate was calculated by dividing the number of surviving chicks by the total number of chicks. Oocyst excretion was measured and counted between 6 and 14 days after inoculation using the approach outlined by Haug et al. (2008). Lan et al. (2016) cited the method for calculating oocyst value and decrease rate. The lesion scores were determined on the sixth day after infection (Johnson and Reid, 1970). Chick body weight and feed consumption were determined before the startlement of the experiment, as well as after the first and second weeks of infection. Lan et al. (2016) procedure was used to estimate the anticoccidial index (ACI) for each treatment using the formula shown below.

$$ACI = (\text{relative ratio of BWG} + \text{survival rate}) - (\text{lesion scores} + \text{oocyst value}) \quad \text{(Formula 1)}$$

Morisawa et al. (1977) method was followed to assess the anticoccidial index (ACI) values. An ACI > 180 indicated excellent anticoccidial impact, 160-180 indicated marked, 140-160 indicated moderate, 120-140 indicated mild, and < 120 indicated inactive.

Statistical analysis

The collected data were statistically processed using SPSS software version 26 (2018). Prevalence was calculated for all data by dividing the number of positive samples by the total number of samples examined, then multiplied by one hundred. The association between disease prevalence and hypothetical risk factors was evaluated using the chi-square test. Univariate logistic regression was used to calculate odds ratios of associated risk factors. The anticoccidial and performance indicators were expressed as mean ± SEM and a 1-way variance analysis was used to determine differences in those parameters between groups. Using the General Linear Model (GLM) procedure, the bloody diarrhea and lesion scores of each treatment were compared. The significant level was set at $p < 0.05$ using the Tukey test.

RESULTS

The overall prevalence of coccidiosis

The prevalence of chicken coccidiosis in poultry farms in the Maritime Region of Togo showed that the majority of the fecal samples examined were negative (60.34%). Only 39.66% of the 295 collected and examined fecal samples from the poultry farms were positive for chicken *Eimeria* oocysts during the period of July to September 2023.

Prevalence of chicken coccidiosis associated with risk factors

Table 2 shows a significant variable difference ($p < 0.05$) in the prevalence of coccidiosis among different age groups, management types, chicken types, and disease prevention frequency. The prevalence in the age group was (83.16%) in young chickens aged 3 to 8 weeks compared to adults (36.63%) aged over 8 weeks, indicating that young chicks were more susceptible than adults ($p = 0.0192$). No cases of coccidiosis were observed in cage-reared chicks. However, 34.27% of the samples from the litter-reared farms tested positive compared to 97.02% from the free-range farms ($p < 0.0001$). With regards to the breed type, the prevalence was 0 and 27.10% in parent stock and layers compared to broilers and local chickens, which had a prevalence of 57.69% and 93.56%, respectively ($p = 0.0002$). Farms that used drug to prevent the disease every two to three weeks had a lower prevalence (0% and 13.37%) compared to those that prevent the disease every four weeks or beyond (34.78% and 62.37%; $p = 0.0031$).

Table 2. Prevalence of coccidiosis associated with risk factors in maritime region of Togo

Risk factors	Category	No. Samples (%)	Positive (%)	Prevalence (%)	Chi-square ($\chi 2$)	P-value
Age	Adult	276 (93.48)	101	36.63	5.482	0.0192
	Young	19 (6.52)	16	83.16		
Type of management	Cage	6 (2.17)	0	0.00	20.8	< 0.0001
	Litter	260 (88.04)	89	34.27		
	Free range	29 (9.78)	28	97.02		
Type of breed	Lay hen	196 (66.30)	53	27.10	19.65	0.0002
	Broiler	64 (21.74)	37	57.69		
	Local chicken	29 (9.78)	27	93.56		
	Broiler breeder	6 (2.17)	0	0.00		
Frequency of disease prevention	2 weeks	35 (11.96)	0	0.00	13.87	0.0031
	3 weeks	45 (15.22)	6	13.37		
	4 weeks	83 (28.26)	29	34.78		
	Onset of disease	131 (44.57)	82	62.37		

The odds ratio associated with risk factor

Figure 2 illustrates the analysis of risk factors according to the epidemiological status of farms. Chickens aged less than eight weeks were 5.66 times more susceptible ($p = 0.019$) to the presence of *Eimeria* oocysts than those over eight weeks old (0.86). Broiler chickens that received classic anticoccidial preventive drugs with a frequency of ≤ 2 weeks (0.11) were less susceptible ($p = 0.003$) than those with a frequency of more than 2 weeks (1.48). Farmers who formulate their feed were 1.52 times more at risk of disease ($p = 0.040$) than those who used commercial feed (0.21). Depending on the rearing system, chickens raised in a traditional free-range system were 22 times more susceptible ($p < 0.0001$) than those raised in confinement (0.67).

Degree of infection according to the number of oocysts per gram of feces

The degree of *Eimeria* oocyst shedding is presented in Table 3. Out of the 295 samples examined, 117 were positive for *Eimeria* oocysts. Seventy-five percent (75%) of the samples had a low infection level (< 10,000 oocysts/g of feces) compared to 12.5% for moderate infection (10,000-15,000 oocysts/g of feces) and high (12.5%) infection (>15,000 oocysts/g of feces).

Infection level associated with risk factors

Figure 3 illustrates the status of oocyst shedding according to risk factors. The number of oocysts in the samples was < 500/g (29.17%), 500-999/g (8.833%), 1000-9999/g (37.50%), and > 10,000 (25%, Figure 3a). On the other hand, Figure 3b shows that irrespective of the management system (litter-based or free-range farming), only 25% of the samples had high infection (> 10,000 oocysts/g). In contrast, 26% of broilers raised on litter showed an infection > 10,000 compared to a load < 10,000 in broilers raised free-range (Figure 3c).

Distribution of chicken coccidiosis in the Maritime region of Togo

The distribution of the prevalence of chicken coccidiosis was geographically depicted on the map in Figure 4. The prevalence was 20% in the Zio district, 23.08% in the Lacs district, 20% in the Vo district, and 43.86% in the Grand-Lome district. The infection was low (<10,000 oocysts/g) in the farms of the Zio, Vo, and Lacs districts. However, it was moderate (10,000-15,000 oocysts/g) in the farms of the Grand-Lome district.

Presumptive *Eimeria* species identification

All the positive samples analyzed presented oocysts of *Eimeria* spp. In total six different species of *Eimeria* were identified. The most prevalent were the large oocyst with *E. maxima* (54.17%), the medium one with *E. brunetti* (33.33%), and *E. tenella* (25%), followed by *E. acervulina* (8.33%), *E. praecox* (8.33%), and the small oocyst with *E. mitis* (4.17%). Table 4 shows the morphometric features and linear regression of the *Eimeria* species. Except for *E. brunetti*, *E. mitis*, *E. acervuline*, and *E. praecox*, the mean length of all *Eimeria* species was greater than the mean width ($p < 0.05$). The species infection status is presented in Table 5. Species infection ranged from a single species to four different species per sample. The species *E. maxima* was the most prevalent in all farms at over 87%. The prevalence of infection with single species was 25% compared to 75% for mixed species infection (37.5%, 25%, and 12.5% respectively for double, triple, and quadruple infections).

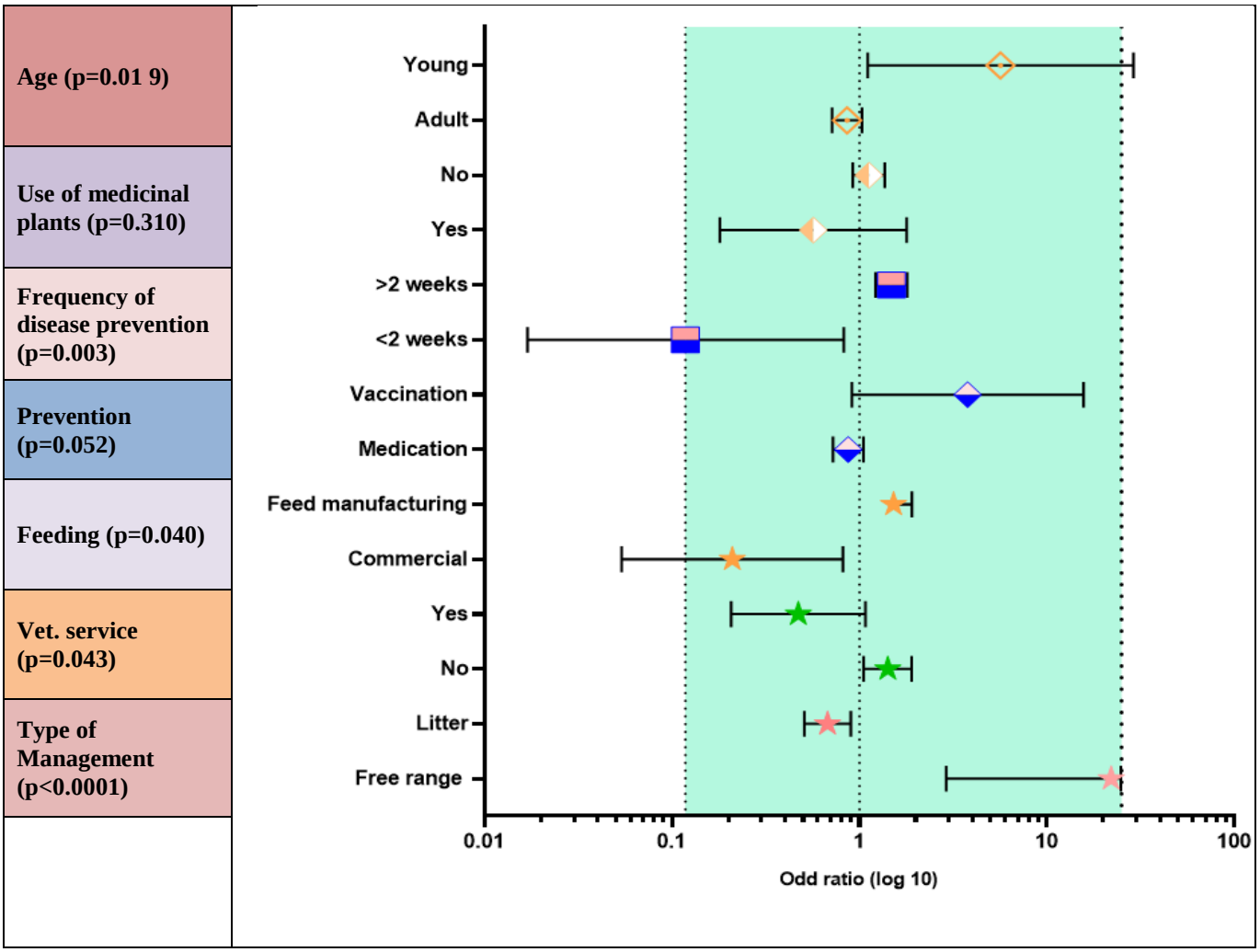
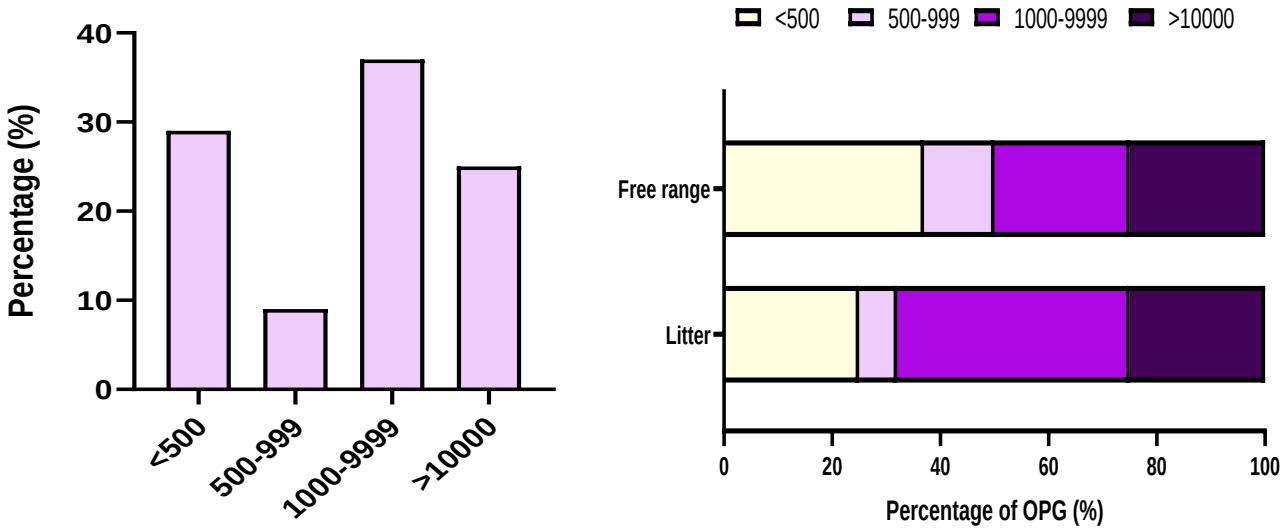


Figure 2. Graphical presentation of potential risk factors associated with coccidiosis in the Maritime region of Togo

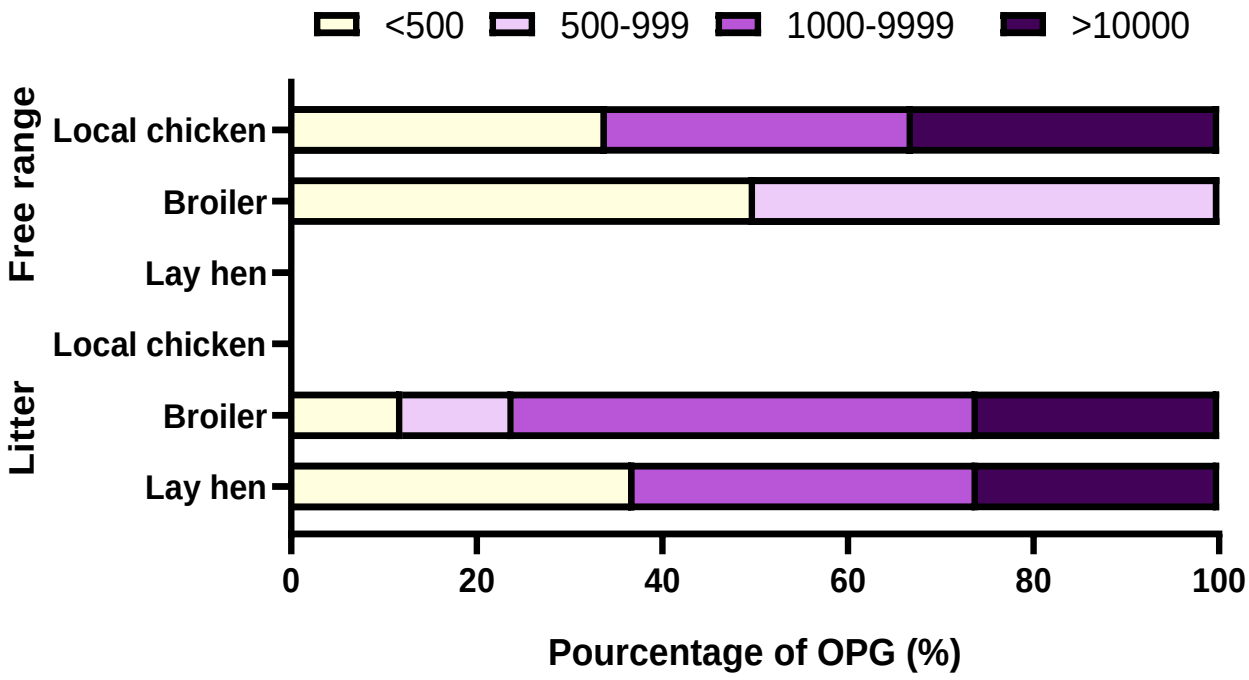
Table 3. Level of infection based on the oocytes per gram count of coccidiosis in the Maritime region of Togo

Oocyst count/g of feces	Degree of infection	No. positive samples	Percentage (%)
< 10 000 oocysts	Low	87	75
10 000-15 000 oocysts	Moderate	15	12.5
> 15 000 oocysts	High	15	12.5



a: Oocyst number/g of feaces

b: Level of infection based on management system



c: Level of infection based on management system and breed

Figure 3. Infection level associated with risk factors of coccidiosis in the Maritime region of Togo

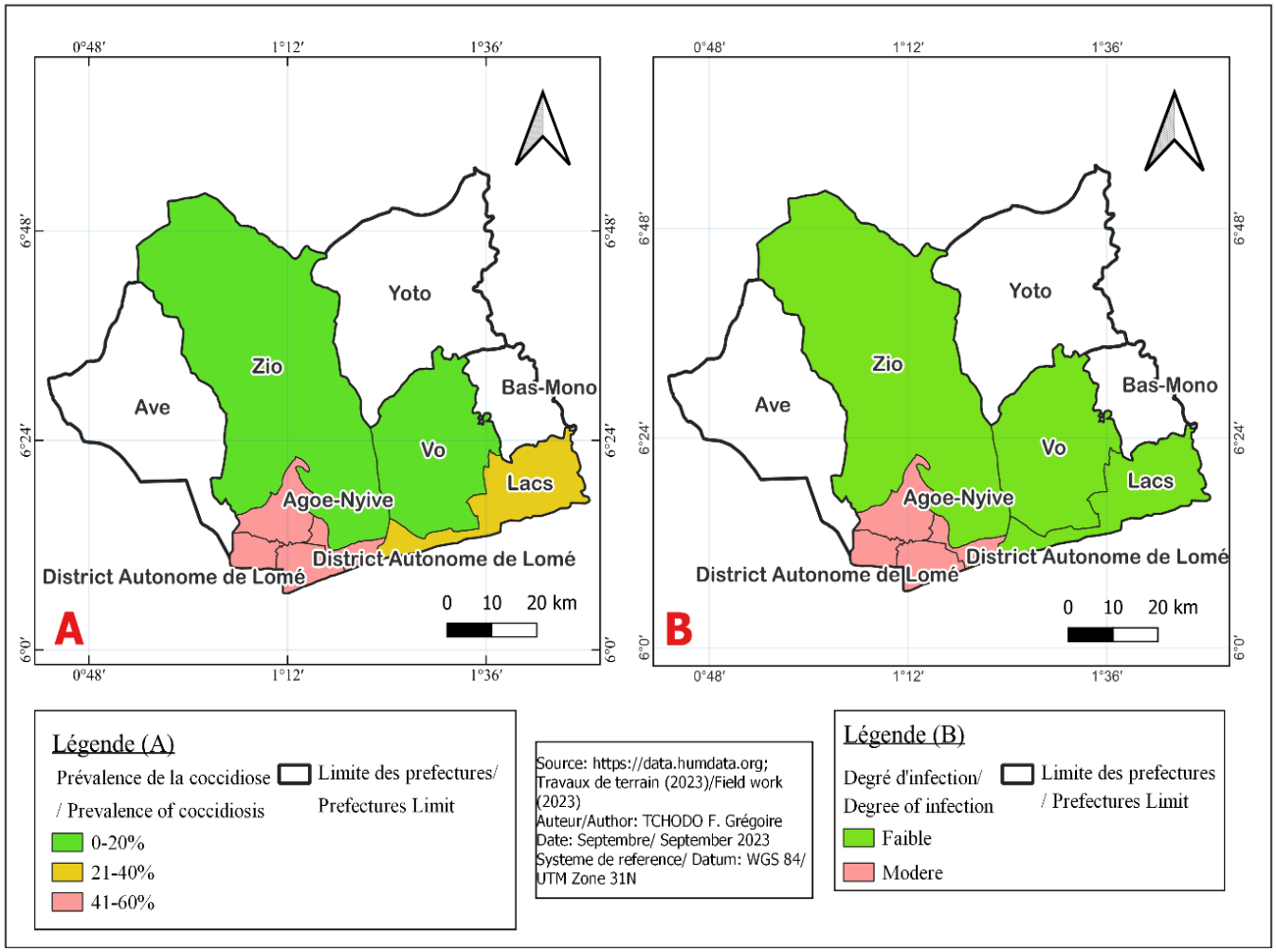


Figure 4. Prevalence of coccidiosis in the Maritime region of Togo. **A**: Prevalence by district; **B**: Degree of infection by study district

Table 4. Morphometry and linear regression of *Eimeria* spp. identified in the maritime of Togo

Species	%[a]	Shape	Oocysts		Linear regression [b]	
			Length (µm)	Width (µm)	R^2	Equation
E. maxima	54.17	Ovoid	31.6(27.0 - 41.1)	21.7(23.3 – 31. 6)	0.609	y = 8.143 + 0.904x
E. brunetti	33.33	Ovoid	25.7(24.0 – 29.9)	19.1(18.5 – 25.8)	0.144	y = 22.070 + 0.246x
E. mitis	4.17	Subspherical	14.5(6.8 – 9.3)	13.8(5.9 – 7.39)	0.205	y = 4.098 + 0.648x
E. praecox	8.33	Ovoid	21.4(20.7 – 22.3)	18.3(17.0 – 19. 5)	0.206	y = 15.542 + 0.322x
E. acervulina	8.33	Ovoid	19.1(18.1 – 19.8)	17.8(15.9 – 18.8)	0.550	y =11.776 + 0.414x
E. tenella	25.00	Ovoid	23.1(22.0 – 28.8)	20.9(18.8 – 24.7)	0.390	y =9.641 + 0.696x

[a] Percentage of oocysts. [b] Linear regression of the width and length of the oocysts

Table 5. Mixed infection of *Eimeria spp* in the Maritime region of Togo

Status of infection	Species	Prevalence (%)
Simple	*E. maxima*	31.25
	E. mitis	6.25
Double	*E. maxima, E. brunetti*	18.75
	E. tenella, E. brunetti	6.25
	E. tenella, E. maxima	31.25
Triple	*E. praecox, E. brunetti, E. tenella*	6.25
	E. brunetti, E. maxima, E. tenella	18.75
	E. acervulina, E. brunetti, E. maxima	6.25
	E. maxima, E. tenella, E. brunetti	6.25
Quadruple	*E. maxima, E. acervulina, E. tenella, E. praecox*	18.75
Total		100

Figure 5. Effect of medicinal plant used against coccidiosis on disease expression (clustering together, ruffled feathers, and bloody droppings), in the different groups of chickens in Togo

Behavior changes and bloody diarrhea

All infected chicks showed clinical signs of coccidiosis, such as clustering, ruffled feathers, and depression (Figure 5). However, no mortality was ever recorded during the study (Table 2). A complete absence of bloody droppings and a lower proportion of bloody dropping was observed in infected chicks treated with amprolium *Sarcocephalus latifolius* and *Azadirachta indica* extract respectively (Table 6).

Lesion scores

Figure 6 (A-D) shows the pathological lesion scores of the caeca of the experimental groups, which ranged from normal to severe (Fig6.A-D). Chicks treated with amprolium and infusion of Sarcocephalus latifolius root showed significant improvement ($p < 0.05$) in cecal morphology and a reduction in length (fig6.A-B). The ceca of the infected unmedicated group showed significant pathologic abnormalities (Figure 6, D), including ceca length shrinkage (atrophy), wall thickening, erosion, and blood clotting. The medicated *Azadirachta indica* group showed considerable improvement ($p < 0.05$) in cecal morphology and reduced length.

Comprehensive evaluation of anticoccidial efficacy

Figure 7 depicts oocysts shed in chicks' feces from 5 days post-infection to 7 days post-infection across all treatment groups. The number of fecal oocysts shed increased with the duration of the infection, with the fewest on day 5 and the most on day 7. Infected treated chicks had considerably decreased *Eimeria tenella* oocyst excretion ($p < 0.05$) compared to the infected untreated control chick group. The lowest oocyst excretions were recorded in the amprolium treated chick group (1.32×10^5)

followed by *Sarcocephalus latifolius* roots extract (1.49 x 10^5) oocysts shed per gram of feces.

Anticoccidial index

The infected unmedicated and infected and treated with *Azadirachta indica* demonstrated passive anticoccidial effects with the lowest Anticoccidial index value (ACI; Figure 8). Amprolium-treated and the *Sarcocephalus latifolius*-treated group demonstrated significant anticoccidial effects, with ACI indices of 176 and 170, respectively. The *Carica papaya* group's ACI index was 135, indicating a mild anticoccidial effect.

Performance

The average daily body weight gain of infected chicks treated with amprolium and medicinal plant extracts was significantly higher ($p < 0.05$) among all the groups (Table 7). The lowest body weight gains in the second week post-inoculation period were observed in infected untreated chicks' groups. The feed conversion ratio did not vary among the experimental groups in the first week. However, in the second week, the lowest feed conversion ratio ($p < 0.05$) was observed in *Sarcocephalus latifolius* treated chicks' group compared to other groups (Table 7).

Table 6. Effect of medicinal plant used against coccidiosis in bloody diarrhea, lesion score, and morbidity in the different groups of chickens in Togo

Groups	Bloody diarrhea	Lesion scores	Survivability (%)	Morbidity (%)
Control	$2.91^{a} \pm 1.97$	$4^{a} \pm 0$	100	100
Amprolium	$0.00^{b} \pm 0.00$	$0.60^{b} \pm 0.40$	100	100
Azadirachta indica	$1.81^{b} \pm 0.18$	$3^{a} \pm 0.34$	100	100
Carica papaya	$2.48^{a} \pm 1.78$	$2.4^{ab} \pm 0.7$	100	100
Sarcocephalus latifolius	$0.61^{b} \pm 0.16$	$1^{b} \pm 0.7$	100	100
P-value	$p < 0.0001$	$p < 0.0001$	-	-

[a, b] Means within columns with different superscript letters differ significantly ($p < 0.05$)

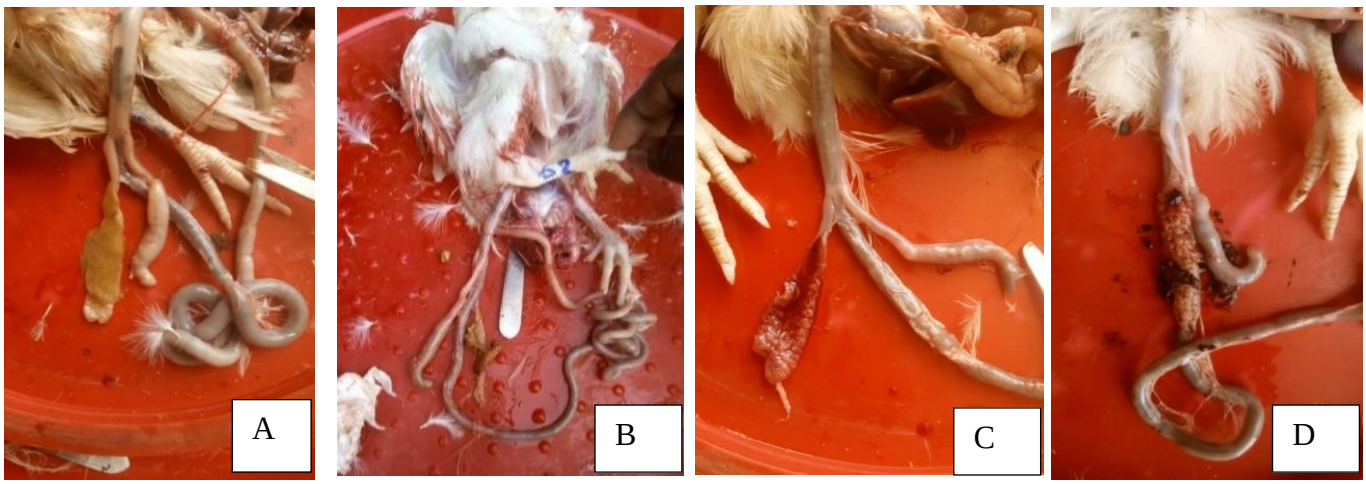


Figure 6. Effect of the medicinal plant used against coccidiosis on lesion scores of chicken's challenges with *Eimeria* tenella oocysts. Caeca was collected from each group on day 6 of the infection. **A, B, C,** and **D** mean scores of 0, 2, 3, and 4 of lesions found respectively.

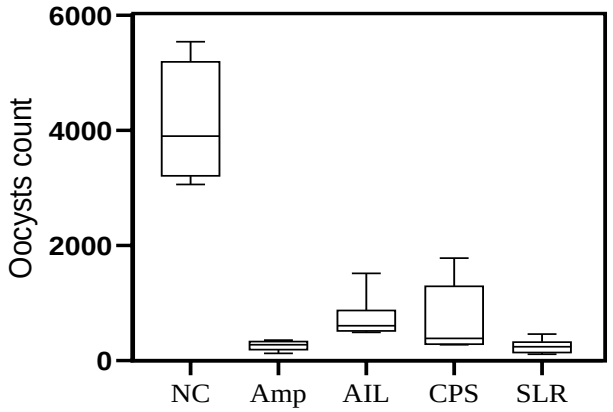


Figure 7. Effect of medicinal plant used against coccidiosis on oocysts shed per gram of feces from 5 days post inoculation (DPI) to 7 DPI. NC, infected unmedicated; Amp, Amprolium group; AIL, CPS, and SLR, infected and treated respectively with extract of *Azadirachta indica* leaves, *Carica papaya* seeds, and *Sarcocephalus latifolius* roots.

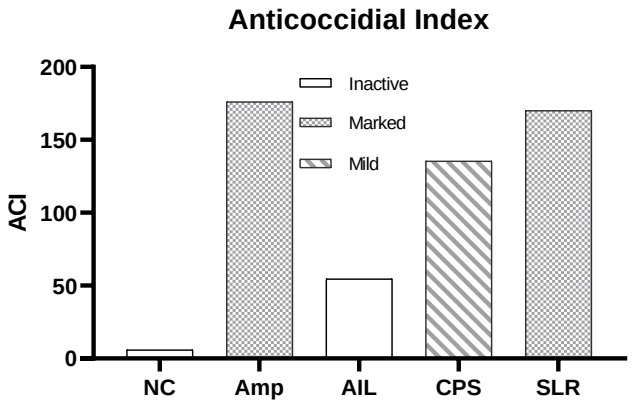


Figure 8. Effect of medicinal plant used against coccidiosis on the anticoccidial index (ACI). NC, infected unmedicated; Amp, Amprolium group; AIL, CPS, and SLR, infected and treated respectively with extract of *Azadirachta indica* leaves, *Carica papaya* seeds, and *Sarcocephalus latifolius* roots.

Table 7. Effect of medicinal plant used against coccidiosis in body weight gain and feed conversion ratio (M ± SE) of infected chicks in Togo

Groups	Body weight gain (g)		Feed conversion ratio	
	D 0 – D 6	D 6 – D 14	D 0 – D 6	D 6 – D 14
Control	$6.61^{a} \pm 0.31$	$7.24^{a} \pm 0.17$	$4.31^{a} \pm 0.09$	$4.09^{a} \pm 0.05$
Amprolium	$10.15^{bc} \pm 0.43$	$11.29^{b} \pm 0.40$	$3.42^{b} \pm 0.27$	$3.61^{ac} \pm 0.22$
Azadirachta indica	$9.55^{b} \pm 0.32$	$11.47^{b} \pm 0.37$	$3.23^{b} \pm 0.11$	$3.52^{bc} \pm 0.07$
Carica papaya	$10.04^{b} \pm 0.31$	$12.03^{bc} \pm 0.17$	$3.38^{b} \pm 0.16$	$3.74^{ac} \pm 0.23$
Sarcocephalus latifolius	$11.03^{c} \pm 0.34$	$12.44^{c} \pm 0.31$	$3.61^{b} \pm 0.23$	$3.46^{bc} \pm 0,14$
p-value	< 0.0001	< 0.0001	0.0042	< 0.0001

[a, b] Means within columns with different superscript letters differ significantly ($p < 0.05$).

DISCUSSION

Chicken coccidiosis is considered one of the most widespread intestinal diseases in poultry production systems, thus holding significant economic importance. Therefore, from July to September 2023, the overall prevalence of chicken coccidiosis in the Maritime Region poultry farm of Togo was estimated at 39.66%. This rate (36.6%) was comparable to that reported by Dakpogan and Salifou (2013) in litter-based, high stocking density layer rearing system in Benin. However, it was greater than the values reported by Adem and Ame (2023) in Ethiopia's Haramaya district (27.01%) and Abera et al. (2016) in poultry farms in Addis Ababa (27.6%). This prevalence was also lower than that seen in Nigeria (42.7%), Algeria (63.26%), and Turkey (54.3%) as reported by Adang and Isah (2016), Debbou-louknane et al. (2018), and Karaer et al. (2012) respectively. This variation in coccidia infection prevalence may be attributed to factors such as epidemiology of coccidia infection, knowledge of the disease, climatic conditions, and geographical environment. One observed practice during sample collection was the perpetual pouring of water onto the litter from drinkers or during water serving, which promotes *Eimeria* accumulation, sporulation, and subsequent infection. This fluctuation in prevalence may also be attributed to the development of immunity against coccidia due to the frequent use of anticoccidial drugs or other preventive measures (Haug et al., 2008). This could explain the low prevalence rate (4%) observed in farms

that frequently use anticoccidial drugs prophylactically at intervals less than three weeks compared to those with a frequency or interval of more than three weeks (34.33%). Logistic regression revealed that coccidia infection was significantly higher in chickens aged less than 8 weeks (66.67%) compared to chickens older than eight weeks (23.67%). Therefore, the risk of young chickens contracting the disease was 5.66 times greater than for adults. This could be explained by an immature immune system in young birds, making them more susceptible to infection even with less pathogenic *Eimeria* strains. The low rate observed in adult chicks reveals that as the chick's age increases, they become immune and resistant to infections. This was consistent with the findings of Adem and Ame (2023), who also found a high prevalence (31.8%) in young chicks (2-8 weeks) compared to adults (22.9%) over 8 weeks old. Similarly, Abera et al. (2016) and Adang and Isah (2016) have shown that coccidia infection was related to the age of chickens in poultry farms in Addis Ababa, Ethiopia, and in traditional chicken farming in the Gombe metropolis in Nigeria. However, these results were inconsistent with those obtained by Abadi et al. (2012), Dakpogan and Salifou (2013), and Bachaya et al. (2015), who reported higher prevalence in adults than in young chickens. In this study, no cases of coccidian infection were noted in samples from cage or battery farming systems. However, the prevalence of coccidiosis was higher in the traditional extensive free-range farming system (88.89%) than in the deep litter or semi-intensive confinement farming system (19.75%). The results revealed that the high prevalence of coccidiosis according to the farming system and poultry type was associated with broiler chickens (100%) and local chickens (85.71%) raised in the traditional extensive free-range farming system compared to those raised in confinement (44.44% for broilers and 0% for local chickens). This lower rate observed in confinement farming could be explained by the particular attention given to chickens in this system with the adoption of prophylactic programs, preventive measures, and adherence to hygiene rules that were lacking in the traditional extensive free-range system. The determination of oocyst per gram (OPG) using the McMaster technique allowed for the assessment of the current degree of coccidian infection in the field and the parasite's reproduction rate in the chicks' intestines. The OPG values in the collected samples varied considerably, with 75% of the samples showing a low OPG (< 10,000 oocysts per gram of feces), 12.5% moderate (10,000-14,999 oocysts/g of feces), and 12.5% high (> 15,000 oocysts/g of feces). This was in line with the results found by Adem and Ame (2023), who recorded over 96% of samples positive for coccidian infection with an OPG < 10,000/g of feces. This suggested a considerable production of oocysts in the field, but the use of anticoccidials and other preventive measures reduced the oocyst number. The morphological identification of prevalent *Eimeria* species in poultry farms in the Maritime Region of Togo revealed six different species with an average mixed infection of 2.125 *Eimeria* species; 75% of positive samples contained 2 to 4 *Eimeria* species in a fecal sample. This high prevalence of mixed-species infection was observed in other studies, indicating the widespread nature of this phenomenon. *E. maxima* (54.17%), *E. brunetti* (33.33%), and *E. tenella* (25%) were the most prevalent species, with *E. maxima* being the most widespread. This high prevalence of *E. maxima* infection can be attributed to its high potential to affect the poultry's small intestine compared to other *Eimeria* species. This was consistent with the results observed in South Korea, Southeast Nigeria, and Romania, respectively by Ola-Fadunsin (2017), and Györke et al. (2013). Furthermore, poultry researchers were interested in finding alternatives to synthetic anticoccidial medications (Dakpogan et al. 2018; Alhotan and Abudabos, 2019; Al-Quraishy et al., 2020; Qaid et al. 2021). The current study also investigated the efficacy of natural products versus the standard synthetic anticoccidial product on Isa-brown male chicks subjected to *Eimeria* oocyst challenge and raised on litter. Anticoccidial medications, such as amprolium 20%, were the most often used ionophores in Togolese poultry farms due to their action on the chicken *Eimeria* parasites. Bloody diarrhea, intestinal lesion scores, anticoccidial index, body weight gain, and oocyst shedding were all evaluated, as well as mortality and morbidity rates. Coccidiosis was well-known for producing severe illness in poultry farms, including bloody diarrhea, by destroying intestinal epithelial cells. The present study discovered typical clinical symptoms of poultry coccidiosis, such as clustering together, ruffled feathers, depression, and anorexia, which were in line with the findings of Qaid et al. (2021). Treatments were considered protective when chicks continue to gain weight and their lesion scores and bloody diarrhea score were zero or at a low number (Allen et al., 2004). Present studies revealed that infected treated chicks had lower lesion scores, a reduced proportion of bloody droppings and a lower excretion of *Eimeria tenella* oocysts. Amprolium and *Sarcocephalus latifolius* had no effect on sick chicks' bloody diarrhea, despite the knowledge that reduced bleeding can protect infected

chicks from secondary infections, inflammatory reactions, and hazardous chemical absorption (Havrlentová et al., 2011). In intensive farming, the spread of oocysts shed in feces poses a risk for coccidiosis (Wondimu et al., 2019). This study found that *Sarcocephalus latifolius* root decoction significantly reduced oocysts/g output compared to amprolium groups, suggesting that *Sarcocephalus latifolius* roots may play a significant role in controlling large-scale avian coccidiosis epidemics in poultry farms, as *Cinnamomum verum* suggested by Qaid et al. (2021). The findings were congruent with those of Dakpogan et al. (2019), who found that *Senna siamea* and *Khaya senegalensis* extract treatment against chicken coccidiosis reduced lesion scores, bloody diarrhea, and oocysts per gram of feces (OPG). Furthermore, Qasem et al. (2020) found that Rumex nervosus leaf extracts reduced the quantity of oocysts in a chick's challenge with *Eimeria* tenella. Li-Yun et al. (2021) discovered that continuous administration of various doses of natural garlic essential oil could significantly reduce clinical symptoms, cecal lesions, and oocyst count. Several studies have shown that the avian cecum was one of the most essential digestive organs, and when *Eimeria* kills the epithelial cells of the cecum, the chicken suffers from malabsorption, resulting in poor body weight gain (Blake and Tomley, 2014; Lan et al., 2016). Then, treatment with *Azadirachta indica* leaves, *Carica papaya* seeds, and *Sarcocephalus latifolius* root extract enhanced the sick chick's body weight increase and feed conversion ratio, which were comparable to the results produced by the traditional anticoccidial medicine. We also utilized a conventional approach to estimate total drug sensitivity (known as the ACI). The current study found that the ACI of *Sarcocephalus latifolius* roots and amprolium were 170.15 and 176.11, respectively, indicating a good anti-coccidiosis effect.

CONCLUSION

The study revealed a notable prevalence of coccidiosis (39.66%) with *E. maxima* being the most prevalent species in the Maritime region of Togo from July to September 2023. Factors, including age, management, breed, and disease prevention frequency were statistically identified as the main risk factors for coccidiosis. Young chickens (< 8 weeks) were found to be more susceptible to infection compared to adults (> 8 weeks) and mixed species infection with multiple *Eimeria* species was common. Furthermore, the results indicated that *Sarcocephalus latifolius* root extract may successfully alleviate clinical symptoms, cecal lesions, and oocyst discharge. At the same time, it was also required to establish adequate dosage and methods to formulate *Sarcocephalus latifolius* root into a new, beneficial, and harmless pharmacological agent for both illness prevention and therapy.

DECLARATIONS

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Authors' contributions

Tchodo Ferdinand Gregoire contributed to the experimental design, data collection, analysis, and manuscript writing. Hervé Brice Dakpogan, Banfitebiyi Gambogou, Ombortime N'nanle, and Benjamin Adjei-Mensah assisted in data collection and text revision. Tona Kokou and Batomayena Bakoma worked on the experiment's design and supervision, as well as manuscript revision. The final manuscript has been read and approved by all authors.

Competing interests

The authors declare no conflicts of interest.

Ethical considerations

The authors have ensured that the work meets the journal's ethical guidelines (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publishing and/or submission, and redundancy) for submission and publication.

Availability of data and materials

The necessary data will be provided by authors according to reasonable requests.

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REFERENCES

Abadi A, Netsanet W, and Haileleul N (2012). Coccidiosis prevailing in parent stocks: A comparative study between growers and adult layers in Kombolcha poultry breeding and Multiplication Center Ethiopia. Global Veterinaria, 8(3): 285-291. Available at: https://www.idosi.org/gv/GV8(3)12/13.pdf

Abbas RZ, Colwell DD, and Gilleard J (2012). Botanicals: An alternative approach for the control of avian coccidiosis. World's Poultry Science Journal, 68(2): 203-215. DOI: https://www.doi.org/ 10.1017/S0043933912000268

Abbas RZ, Iqbal Z, Khan MN, Zafar MA, and Zia MA (2010). Anticoccidial activity of *Curcuma longa* L. in broilers. Brazilian Archives of Biology and Technology, 53(1): 63-67. DOI: https://www.doi.org/10.1590/S1516-89132010000100008

Abera S, Ibrahim NA, and Kabeta T (2016). Prevalence of poultry coccidiosis in Addis Ababa poultry farms. British Journal of Poultry Sciences, 5(2): 21-25. DOI: https://www.doi.org/10.5829/idosi.bjps.2016.21.25

Adang LK and Isah Z (2016). Prevalence of *Eimeria* species in local breed chickens in Gombe metropolis Gombe State Nigeria. International Journal of Biological and Chemical Sciences, 10(6): 2667-2676. DOI: http://www.doi.org/10.4314/ijbcs.v10i6.21

Adem DM and Ame MM (2023). Prevalence of poultry coccidiosis and its associated risk factors in and around Haramaya District Ethiopia. Veterinary Medicine Open Journal, 8(1): 9-17. DOI: https://www.doi.org/10.17140/VMOJ-8-172

Alhotan RA and Abudabos A (2019). Anticoccidial and antioxidant effects of plants derived polyphenol in broilers exposed to induced coccidiosis. Environmental Science and Pollution Research, 26: 14194-14199. DOI: https://www.doi.org/10.1007/s11356-019-04615-2

Allen PC Danforth HD, and Vinyard BL (2004). Development of a protective index to rank efectiveness of multiple treatments within an experiment: Applica - tion to a cross - protection study of several strains of *Eimeria maxima* and a live vaccine. Avian Diseases, 48(2): 370-375. DOI: https://www.doi.org/10.1637/7010

Al-Quraishy SMA, Qasem EM, Al-Shaebi M, Murshed MM, and Dkhil MA (2020). Rumex nervosus changed the oxidative status of chicken caecum infected with *in ovo tenella*. Journal of King Saud University Science, 32: 2207-2211. DOI: https://www.doi.org/10.1016/j.jksus.2020.02.034

Bachaya HA, Abbas RZ, and Raza MA (2015). Existence of coccidiosis and associated risk factors in broiler chickens in Southern Punjab Pakistan. Pakistan Veterinary Journal, 35(1): 81-84. Available at: https://www.pvj.com.pk/pdf-files/35_1/81-84.pdf

Blake DP and Tomley FM (2014). Securing poultry production from the ever-present *Eimeria* challenge. Trends Parasitology, 30(1): 12-19. DOI: http://www.doi.org/10.1016/j.pt.2013.10.003

Blake DP, Knox J, Dehaeck B, Huntington B, Rathinam T, and Ravipati V (2020). Re-calculating the cost of coccidiosis in chickens. Veterinary Research, 51: 115. DOI: https://www.doi.org/10.1186/s13567-020-00837-2

Blake DP, Vrba V Xia D, Jatau ID, Spiro S, Nolan MJ, Underwood G, and Tomley FM (2021). Genetic and biological 439 characterisation of three cryptic *Eimeria* operational taxonomic units that infect chickens (*Gallus gallus* domesticus). International Journal for Parasitology, 51(8): 621-634. DOI: https://www.doi.org/10.1016/j.ijpara.2020.12.004

Bauri RK, Tigga MN, and Kullu SS (2015). A review on use of medicinal plants to control parasites. Indian Journal of Natural Products and Resources, 6(4): 268-277. Available at: https://nopr.niscpr.res.in/bitstream/123456789/33656/1/IJNPR%206(4)%20268-277.pdf

Dakpogan HB, Gandaho CS, Houndonougbo VP, Dossa LH, Houndonougbo MF, and Chrysostome C (2019). Anticoccidial activity of Khaya senegalensis Senna siamea and Chamaecrista rotundifolia in chicken (*Gallus gallus*). International Journal of Biological and Chemical Sciences, 13(4): 2121-2128. DOI: https://www.doi.org/10.4314/ijbcs.v13i4.19

Dakpogan HB, Mensah S, Attindehou S, Chrysostome C, Aboh A, Naciri M, Salifou S, and Mensah GA (2018). Anticocidal activity *of Carica papaya* and *Vernonia amygdalina* extract. International Journal of Biological and Chemical Sciences, 12(5): 2101-2108. DOI: https://www.doi.org/10.4314/ijbcs.v12i5.12

Dakpogan HB and Salifou S (2013). Coccidiosis prevalence and intensity in litter based high stocking density layer rearing system of Benin. Journal of Animal & Plant Sciences, 17(2): 2522-2526. Available at: https://www.m.elewa.org/JAPS/2013/17.2/1.pdf

Debbou-louknane N, Benbarek H, and Ayad A (2018). Prevalence and aetiology of coccidiosis in broiler chickens in Bejaia province Algeria. Onderstepoort Journal of Veterinary Research, 85(1): a1590. DOI: https://www.doi.org/10.4102/ojvr.v85i1.1590

Direction de la Statistique de l'Informatique et de la Documentation (DSID) (2018). Direction de la Statistique de l'Informatique et de la Documentation [Direction of Statistic, Technology information and documentation]: La filière avicole en forte progression [The poultry industry in strong growth]. Available at: https://dgttm.bf/index.php/nous-connaitre/organisation/la-del/91-dgttm/qui-sommes-nous/organisation/175-direction-informatique-statistiques-de-la-documentation#:~:text=Mission%20%3A%20La%20direction%20de%20I,directions%20r%C3%A9gionales%20charg%C3%A9es%20des%20transports

El-Ashram S and Suo X (2017). Electrical cream separator coupled with vacuum filtration for the purification of eimerian oocysts and trichostrongylid eggs. Scientific Reports, 7: 43346. DOI: https://www.doi.org/10.1038/srep43346

Food and agriculture organization of the United Nations (FAO) (2011). Food and agriculture organization of the United Nations. Family poultry communications. International Network for Family Poultry Development, 20(2): 1-57. Available at: https://openknowledge.fao.org/server/api/core/bitstreams/723f123f-32dc-416e-b4ca-af688abf576b/content

Food and agriculture organization of the United Nations (FAO) (2015). Poultry sector Togo. National reviews of livestock from the division of production and animal health of the FAO. No. 9. Rome.

Flores RA, Binh TN, Paula LTC, Tuấn CV, Haung N, Suk K, Woo HK, Byoung-Kuk N, and Wongi M (2022). Epidemiological investigation and drug resistance of *Eimeria* species in Korean chicken farms. BMC Veterinary Research, 18: 277. DOI: https://www.doi.org/10.1186/s12917-022-03369-3

Gilbert ER, Cox CM, and Williams PM (2011). *Eimeria* species and genetic background influence the serum protein profile

of broilers with coccidiosis. Plos One, 6(2): 10.1371 DOI: https://www.doi.org/10.1371/journal.pone.0014636

Györke A, Pop L, and Cozma V (2013). Prevalence and distribution of *Eimeria* species in broiler chicken farms of different capacities. Parasite, 20: 50. DOI: https://www.doi.org/10.1051/parasite/2013052

Hauck R, Carrisosa M, McCrea BA, Dormitorio T, and Macklin KS (2019). Evaluation of next-generation amplicon sequencing to identify *Eimeria* spp. of chickens. Avian Diseases, 63: 577-583. DOI: https://www.doi.org/10.1637/aviandiseases-D-19-00104

Haug A, Gjevre AG, Thebo P, Mattsson JG, and Kaldhusdal M (2008). Coccidial infections in commercial broilers: Epidemiological aspects and comparison of *Eimeria* species identification by morphometric and polymerase chain reaction techniques. Avian Pathology, 37(2): 161-170. DOI: https://www.doi.org/10.1080/03079450801915130

Havrlentová M, Petruláková Z, Burgárová A, Gago F, Hlinková A, and Šturdík E (2011). Cereal b glucans and their significance for the preparation of functional foods- A review. Czech Journal of Food Sciences, 29(1): 1-14. DOI: https://www.doi.org/10.17221/162/2009-CJFS

Islam SKMA, Alauddin M, Hassan MM, Khan SA, Alam MR, Hossain MB, Ahasan ASML, Saifuddin AKM, Sultana S, Tun HM et al. (2012). Biochemical analysis on blood and crop contents of household chickens along with their production and health status in Bangladesh. Pakistan Veterinary Journal, 32(4): 575-578. Available at: http://www.pvj.com.pk/pdf-files/32_4/575-578.pdf

Johnson J and Reid WM (1970). Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology, 28: 30-36. DOI: https://www.doi.org/10.1016/0014-4894(70)90063-9

Kadykalo S, Roberts T, Thompson M, Wilson J, Lang M, and Espeisse O (2018). The value of anticoccidials for sustainable global poultry production. International Journal of Antimicrobial Agents, 51: 304-310. DOI: https://www.doi.org/10.1016/j.ijantimicag.2017.09.004

Karaer Z, Guven E, Akcay A, Kar S, Nalbantoglu S, and Cakmak A (2012). Prevalence of subclinical coccidiosis in broiler farms in Turkey. Tropical Animal Health and Production, 44: 589-594. DOI: https://www.doi.org/10.1007/s11250-011-9940-z

Lan L, Zuo B, Ding H, Huang Y, Chen X, and Du A (2016). Anticoccidial evaluation of a traditional Chinese medicine—Brucea javanica—in broilers. Poultry Science, 95: 811-818. DOI: http://www.doi.org/10.3382/ps/pev441

Lanckriet A, Timbermont L, De Gussem M, Marien M, Vancraeynest D, Haesebrouk F, Ducatelle R, and Van Immerseel F (2010). The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model. Avian Pathology, 39(1): 63-68. DOI: https://www.doi.org/10.1080/03079450903505771

Li-Yun C, Ke-Qian D, Jing X, Yi-fan C, Jian-Zhong X, De-He W, Er-Ying H, Li-Jun X, Hui C, and Rong-Yan Z (2021). Effect of natural garlic essential oil on chickens with artificially infected *Eimeria tenella*. Veterinary Parasitology, 300: 109614. DOI: https://www.doi.org/10.1016/j.vetpar.2021.109614

Lunden A, Thebo P, Gunnarson S, Hooshmand-Rad P, Tauson R, and Uggla A (2010). *Eimeria* infections in litter-based high stocking density systems for loose housed laying hens in Sweden. British Poultry Science, 41(4): 440-447. DOI: https://www.doi.org/10.1080/713654973

McDougald LR (1998). Intestinal protozoa important to poultry. Poultry Science, 77(8): 1156-1158. DOI: https://www.doi.org/10.1093/ps/77.8.1156

McDougald LR (2003). Coccidiosis. In: Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald, and D. E. Swayne (Editors), Diseases of poultry, 11th Edition. Iowa State Press., Ames, Iowa, USA, pp. 974-991.

Moore RJ (2016). Necrotic enteritis predisposing factors in broiler chickens. Avian Pathology, 45: 275-281. DOI: https://www.doi.org/10.1080/03079457.2016.1150587

Morisawa Y, Kataoka M, Kitano N, and Matsuzawa T (1977). Studies on anticoccidial agents. 10. Synthesis and anticoccidial activity of 5-nitronicotinamide and its analogs. Journal of Medicinal Chemistry, 20(1): 129-133. DOI: https://www.doi.org/10.1021/jm00211a027

Muthamilselvan T, Kuo TF, Wu YC, and Yang WC (2016). Herbal remedies for coccidiosis control: a review of plants, compounds, and anticoccidial actions. Evidence-Based Complementary and Alternative Medicine, 2016: 1-19. Available at DOI: http://www.www.doi.org/10.1155/2016/2657981

Ogwiji M, Jatau ID, Natala AJ, Mohany M, Al-Rejaie SS, and Zhu M (2024). Effect of prebiotic, probiotic, and synbiotic products on oxidative status, performance, and parasitological parameters of broiler chickens induced with cecal coccidiosis. Journal of Applied Poultry Research, 33: 100472 DOI: https://www.doi.org/10.1016/j.japr.2024.100472

Ola-Fadunsin SD (2017). Investigations on the occurrence and associated risk factors of avian coccidiosis in Osun State Southwestern Nigeria. Journal of Parasitology Research, 2017: 9264191. DOI: https://www.doi.org/10.1155/2017/9264191

Qaid MM, Saud IA Mahmoud MA, and Maged AA (2021). Anticoccidial effectivity of a traditional medicinal plant Cinnamomum verum in broiler chickens infected with *Eimeria tenella*. Poultry Science, 100: 100902. DOI: https://www.doi.org/10.1016/j.psj.2020.11.071

Qasem MAA, Dkhil MA, Al-Shaebi EM, Murshed M, Mares M, and Al-Quraishy S (2020). Rumex nervosus leaf extracts enhance the regulation of goblet cells and the inflammatory response during infection of chickens with *Eimeria tenella*. Journal of King Saud University - Science, 32: 1818-1823. DOI: https://www.doi.org/10.1016/j.jksus.2020.01.024

Sharma S, Azmi S, Iqbal A, Nasirudullah N, and Mushtaq I (2013). Pathomorphological alterations associated with chicken coccidiosis in Jammu division of India. Journal of Parasitic Diseases, 39: 147-151. DOI: https://www.doi.org/10.1007/s12639 013-0302-9

Tanweer AJ, Saddique U, Bailey C, and Khan R (2014). Antiparasitic effect of wild rue (Peganum harmala L.) against experimentally induced coccidiosis in broiler chicks. Parasitology Research, 113: 2951-2960. DOI: https://www.doi.org/10.1007/s00436-014-3957-y

Thagfan FA, Dkhil MA, and Al-Quraishy S (2017). *In vivo* anticoccidial activity of Salvadora persica root extracts. Pakistan Journal of Zoology, 49(1): 53-57. DOI: http://www.doi.org/10.17582/journal.pjz/2017.49.1.53.57

Wondimu A, Mesfin E, and Bayu Y (2019). Prevalence of poultry coccidiosis and associated risk factors in intensive farming system of Gondar town Ethiopia. Veterinary Medicine International, 2019: 5748690. DOI: https://www.doi.org/10.1155/2019/5748690

Wunderlich F, Al-Quraishy S, Steinbrenner H, Sies H, and Dkhil MA (2014). Towards identifying novel anti-*Eimeria* agents: trace elements, vitamins, and plantbased natural products. Parasitology Research, 113: 3547-3556. DOI: https://www.doi.org/10.1007/s00436-014-4101-8

Zhang JJ, Wang LX, Ruan WK, and An J (2013). Investigation into the prevalence of coccidiosis and maduramycin drug resistance in chickens in China. Veterinary Parasitology, 191(1-2): 29-34. DOI: https://www.doi.org/10.1016/j.vetpar.2012.07.027

Zhuang Q, Wang S, Li J, Liu D, Liu S, Jiang W, and Chen J (2014). A clinical survey of common avian infectious diseases in China. Avian Diseases, 58: 297-302. DOI: https://www.doi.org/10.1637/10709-110113-resNet.1