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Prevalence and intensity of avian coccidiosis infection and associated risk factors in the Mezam Division, North-West Region, Cameroon: An observational study

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Poultry farming typically suffers significant losses due to a variety of avian diseases. Avian coccidiosis is recognized as the major parasitic disease with the greatest economic impact on poultry industries worldwide. In Cameroon, there is very little data on avian coccidiosis, but it is important to document the situation for better or more appropriate management. The aim of this study was therefore to assess the prevalence and intensity of avian coccidiosis, and associated risk factors in the Bamenda Health District, North-West Region, Cameroon. Stool samples were collected from 15 randomly selected farms. A prospective cross-sectional survey was carried out, and a total of 350 stool samples from chickens were examined microscopically for the presence of oocysts of *Eimeria* species using the formol-ether concentration technique. Questionnaires were also administered to assess risk factors associated with infection by *Eimeria* spp. A total of 152 chickens were infected, giving an overall prevalence of 43.4% (95% CI: 38.3-48.7), and the mean intensity of coccidiosis infection was 10.12 (±SD: 21.372) oocysts/g of stool. The prevalence was highly heterogeneous (p=0.0001) between poultry farms/communities, mostly due to poor farm management practices. Although farmers' knowledge of coccidiosis was fairly good, some of their husbandry practices were poor, increasing birds' exposure to *Eimeria* spp.

Key words: Poultry, avian coccidiosis, *Eimeria* species, Mezam division, Cameroon.

INTRODUCTION

Poultry farming is a rapidly growing economic activity in the livestock sector, especially in low- and middle-income countries. The poultry sector has now become an important economic sector as it constitutes a market share of 42% of domestic meat production (Peter et al., 2012). Chickens are the most important domestic fowl species, adapted globally to various climatic conditions where humans live, and play a significant role in supplying animal-derived protein to improve human nutrition (Chauhan and Roy, 2007). Poultry activity

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usually faces significant losses due to various avian diseases, sometimes leading to uncontrollable epidemics. Poultry mortality due to disease is estimated to be between 20 and 50%, but it can be as high as 80% during epidemic seasons (Engidaw and Getachew, 2018). Among these diseases, coccidiosis is recognized as the major parasitic infection with the greatest economic impact on poultry industries worldwide (Allen and Fetter, 2002). This parasitic infection is caused by protozoans of the genus *Eimeria*, which seriously impair the growth and feed utilization of infected birds, resulting in a loss of productivity (Conway and McKenzie, 2007).

Coccidiosis is endemic in most tropical and subtropical regions, where it is favoured by ecological and management conditions (Obasi et al., 2006). In Cameroon, the domestic fowl mostly used in poultry are chickens, which are mainly kept for the production of meat and eggs for human consumption. Data on coccidiosis is very scarce, but it is important to document the situation for better or more appropriate management. This study, therefore, aimed to assess the endemicity of coccidiosis and associated risk factors in poultry farms in the Mezam Division, North-West Region, Cameroon.

MATERIALS AND METHODS

Study area

The study was carried out in selected poultry farms in the Bamenda municipality, which is the capital of the North-West Region of Cameroon. This region lies within the latitudes 5°94′N and 5°98′N and longitudes 10°15′E and 10°18′E (Acho-Chi, 1998) and covers an area of 17,910 km². It sits along the Cameroon Volcanic Line with a mountainous and undulating topography characterized by abrupt escarpments, towering mountain peaks, deep valleys, and broad alluvial plains. It has two distinct seasons: a short dry season from mid-November to mid-March and a long rainy season from mid-March to mid-November. The average annual rainfall is 2,400 mm, and the average annual temperature is 23°C (Ndoh Nbue et al., 2016; NIC, 2021). With an approximate population of 2 million inhabitants, the North-West Region has an agricultural-based economy with organizations responsible for improving seeds of high-yielding crop varieties, constructing and rehabilitating water schemes, and preserving livestock. Records obtained from MINEPIA reveal that there are over 80 poultry farms registered in their respective cooperatives.

Study design

A prospective cross-sectional survey was conducted from December 2020 to April 2021 in 15 communities/private farms randomly selected in seven Health Areas of the Bamenda Health District (Mezam Division). These farms were chosen for representativeness, based on the significant chicken populations in the area as documented by the authorities of the Regional Delegation of Livestock, Fisheries, and Animal Industries (MINEPIA). Considering the prevalence of coccidiosis (34.0%) in the study area (Tubah Health District, Mezam Division) from a preliminary study conducted in 2014 (Sumo, personal communication), a minimal sample size of 345 individuals was needed to estimate the true prevalence of coccidiosis in the Bamenda Health District (Mezam Division) in 2020-2021 with 5% precision and a 95% confidence interval. All poultry farms containing chickens older than one week were included in the study. Chickens already being administered medication for the prevention of disease or anticoccidial drugs during the sampling period were excluded from the study. Stool samples were collected from all eligible chickens in randomly selected poultry farms for parasitological examinations.

Key informant interviews to assess knowledge and attitudes vis-à-vis of chicken husbandry and risk factors associated with coccidiosis

In addition to the parasitological survey, a structured questionnaire was used to assess the knowledge and attitudes of the farmers regarding infection with *Eimeria* spp. (symptoms, mode and route of contamination, prevention approaches) and chicken husbandry (cleaning frequency of feeders, drinkers, or cages; procedures prior to the introduction of new birds into the poultry; and procedures for disposing of dead birds).

Sample collection and processing

Chickens were closely monitored for the egestion of fresh fecal samples. Fecal samples were collected from freshly passed feces of selected chickens using a separate spatula for each chicken, with the spatula being washed between uses to avoid contamination during the next sample collection. Chickens whose samples were already collected were immediately marked to avoid resampling from the same chicken. Each fecal sample was placed in a pre-labelled 50 ml Falcon tube indicating the age, breed, poultry management, and poultry housing method. These samples were transported to the medical laboratory of the Nkwen District Hospital and immediately stored at 4°C until processed. Once in the laboratory, the formol-ether concentration technique was used to analyze the fecal samples. Briefly, one gram of stool sample was mixed with 9 ml of 10% formalin in a 60 ml plastic screw vial. The mixture was filtered through a tea strainer into a centrifuge tube, and 3 ml of petroleum ether was added. This was mixed vigorously to obtain a homogeneous mixture and then centrifuged for 2 min and 30 s at 3,000 rpm. Four layers were obtained after centrifugation; the first three supernatants were discarded, and the sediment was gently mixed while tapping the tube to re-suspend it. Two drops of this preparation were deposited separately on a pre-labelled glass slide, with Lugol's iodine added to one of the drops and normal saline to the other drop. Cover slips were placed on each drop, and the slide was examined under a light microscope (Optika B-192PL, Italy) at magnifications of 100 and 400X. The identification of parasitic forms of the gastrointestinal tract of poultry was performed using helminthological keys (Soulsby, 1982). The modified McMaster technique (Maff, 1977) was used for the quantitative analysis to determine the intensity of infection, and the results were expressed as the number of oocysts per gram of stool (opg).

Statistical analyses

All relevant data from the study were entered into a Microsoft Excel spreadsheet. Statistical analyses were conducted using Predictive

Table 1. Description of the sampled chickens according to health areas, stocking densities and age groups.

Variable	Number of chicken	%
Health areas		
Mile 2	60	17.1
Mile 3	30	8.6
Mile 4	70	20.0
Mile 5	20	5.7
New Road	40	11.4
Nitob	110	31.4
Ntarikon	20	5.7
Stocking density (chickens/m²)		
< 5	70	20.0
5-10	230	65.7
11-15	50	14.3
Age groups (weeks)		
≤ 4	140	40.0
5 – 9	100	28.6
> 9	110	31.4
Total	350	100

Analytics Software (PASW) Statistics version 18 (SPSS Inc., Chicago, IL, USA). Coccidian prevalence was expressed as the percentage of infected chickens among the total number of individuals examined; with the 95% confidence interval calculated using the Wilson method not corrected for continuity (Wilson, 1927). The intensity of coccidiosis infection was reported as the arithmetic mean of the number of oocysts per gram of stool, with standard deviation used to account for sampling fluctuations. Pearson's Chi-Square and Kruskal-Wallis tests were employed to compare the prevalence and mean intensity of coccidiosis infections across different age groups (≤4 weeks; 5–9 weeks; >9 weeks) and stocking densities (<5 chickens/m²; 5 to 10 chickens/m²; 11 to 15 chickens/m²), respectively. Univariate binary logistic regression was utilized to investigate the association between infection with *Eimeria* spp. and various covariates recorded in the study framework, such as age, sex, seasons, stocking densities, and chicken husbandry practices. The threshold for significance was set at 5% for all statistical analyses.

Could you share more about the specific challenges or unexpected findings encountered during the statistical analysis phase of your study? Understanding these insights could provide valuable context for interpreting the results.

Ethical and administrative considerations

Prior to sample collection, administrative authorization was granted by the Department of Biological Sciences of the University of Bamenda. Access to poultry farmers was facilitated by the MINEPIA Divisional Delegate during the distribution of day-old chicks at the Livestock and Fishery Development Project (LIFIDEP) office. Each poultry farmer signed a consent form before allowing the survey team to work on their farm, and arrangements were made for stool sample collection at their respective poultry farms. Each farmer received the results of the chickens' infection for appropriate treatment.

RESULTS

A total of 350 chickens were sampled from 15 selected poultry farms within the Bamenda municipality. The age of the sampled birds ranged from 2 to 30 weeks with a mean age of 8.26 weeks (±SD: 7.79), with 40.0% of birds belonging to the ≤ 4 weeks age group. Table 1 shows that the most frequent (65.7%) stocking density was 5-10 chickens/m² in the different poultry farms.

Prevalence and intensity of *Eimeria* spp.

Among the 350 chickens from which stool examinations were performed, 152 (43.4%; 95% CI: 38.3 to 48.7) tested positive for *Eimeria* spp. Table 2 summarizes the infection rates of *Eimeria* spp. according to Health Areas, stocking densities, age groups, and seasons. *Eimeria* spp. infections were similar between the rainy and dry seasons (Chi-square: 2.82; df: 1; p = 0.093). However, a significant difference was found in coccidian infection among age groups (Chi-square: 8.41; df: 2; p = 0.015), with chickens aged 5 to 9 weeks showing the highest infection rates.

Table 2. Rate and intensity of avian coccidiosis according to Health Areas, stocking densities, age group and seasons.

Variable	No. chickens examined	No. chickens infected (%; 95% CI)	Mean intensity of infection (SD)
Health areas			
Mile 2	60	30 (50.0; 37.7 - 62.3)	12.57 (27.57)
Mile 3	30	0 (0.0; 0.0 - 11.4)	11.67 (15.55)
Mile 4	70	42 (60.0; 48.3 - 70.7)	11.43 (28.06)
Mile 5	20	10 (50.0; 29.9 - 70.1)	0.50 (2.24)
New Road	40	0 (0.0; 0.0 - 8.8)	3.37 (7.08)
Nitob	110	70 (63.6; 54.3 - 72.0)	11.11 (20.05)
Ntarikon	20	0 (0.0; 0.0 - 16.1)	13.50 (12.11)
Stocking density (chickens/m^2)			
< 5	70	18 (25.7; 16.9 - 37.0)	2.16 (6.46)
5-10	230	120 (52.2; 45.7 - 58.5)	13.87 (25.05)
11-15	50	14 (28.0; 17.5 - 41.7)	4.00 (8.20)
Age groups (weeks)			
≤ 4	140	64 (45.7; 37.7 - 54.0)	8.24 (13.86)
5 - 9	100	52 (52.0; 42.3 - 61.5)	14.76 (26.30)
> 9	110	36 (32.7; 24.7 - 41.9)	8.29 (23.7)
Seasons			
Dry	240	97 (40.4; 34.4 - 46.7)	9.35 (13.18)
Rainy	110	55 (50.0; 40.8 - 59.2)	10.47 (24.23)
Overall	350	152 (43.4; 38.3 - 48.7)	10.12 (21.37)

No: Number of; 95% CI: 95% confidence interval; SD: standard deviation.

Similarly, a significant variation in coccidian infection was observed among farms/communities (Chi-square: 55.31; df: 14; p = 0.0001), with Ndamukong 1 and Atou-Akom having the highest infection rates (Table S1). Regarding Health Areas, the majority of infected birds originated from Nitob (63.6%), followed by Mile 4 (60.0%); Mile 3, New Road, and Ntarinkon showed no infections (Chi-square: 96.61; df: 6; p = 0.0001). The highest prevalence of infection (52.2%) was found among chickens in the stocking density range of 5-10 chickens/m² (Chi-square: 20.95; df: 2; p = 0.0001). The intensities of infection or parasitic loads of *Eimeria* spp. varied between 1 and 200 oocysts per gram of feces (opg) with a mean parasitic load of 10.12 opg (±SD: 21.372). The intensity of coccidiosis infection differed significantly between age groups, with chickens aged 5-9 weeks showing the highest parasitic loads (Chi-square: 9.12; df: 2; p = 0.01). However, no linear correlation was found between the intensity of infection and the age of the chicken (r = 0.019; p = 0.724); the observed trend was convex in form (Figure 1).

A similar convex trend was observed between the parasitic load and stocking density (Figure 2), and no linear association was found between the intensity of coccidiosis infection and the stocking density of the poultry (r = 0.022; p = 0.686). The intensity of infection varied significantly between stocking densities, with farms having 5-10 chickens/m² showing the highest infection rates (Chi-square: 29.49; df: 2; p = 0.0001) (Table 2).

Co-infection with other intestinal parasitic infections

Other intestinal parasites were found during stool sample examinations (Table S1), including helminths (*Ascaridia galli*, *Capillaria* species, *Raillietina* species and *Heterakis gallinarum*) and other protozoa (*Histomonas* and *Blastocystis* species) with an overall prevalence of 10.9% (95% CI: 8.0 - 14.6) and 4.6% (95% CI: 2.8 - 7.3), respectively. The intensity of coccidiosis infection varied with respect to the other intestinal parasitic infections, Chickens harboring helminths being more infected with *Eimeria* spp. whereas those harboring other protozoa were less infected with *Eimeria* spp. (p<0.0001) (Figure 3).

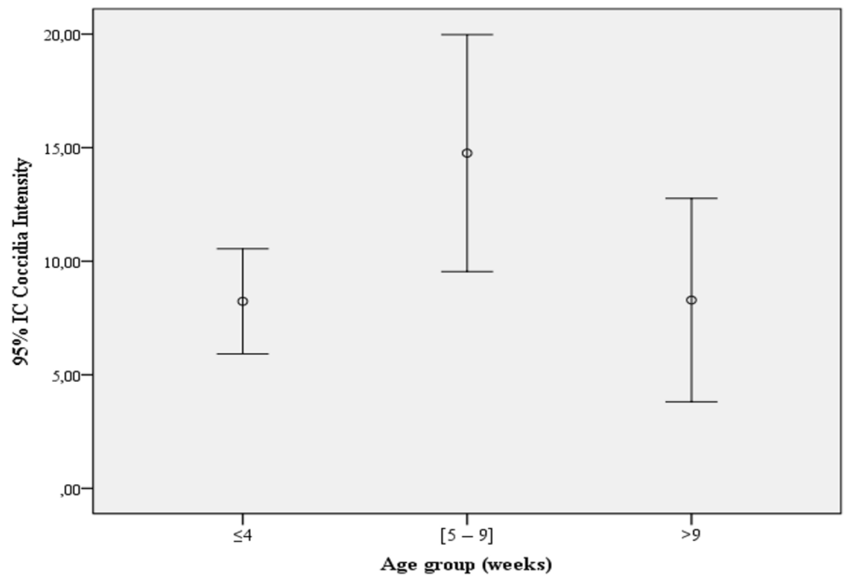

Figure 1. Trend in intensity of *Eimeria* spp. infection according to age groups.

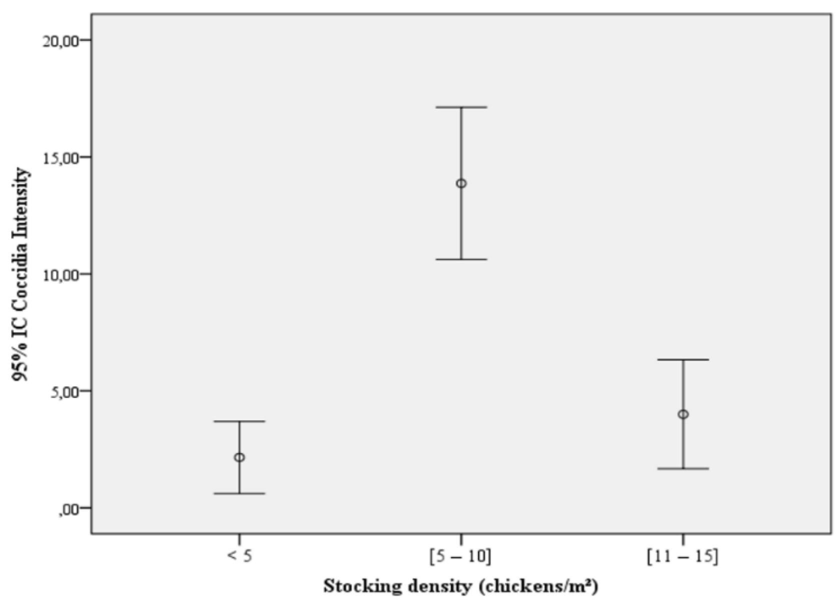

Figure 2. Trend in intensity of *Eimeria* spp. infection according to stocking densities.

Knowledge and attitudes vis-à-vis of chicken husbandry and risk factors associated with coccidiosis

All 15 breeders interviewed stated that they were aware of coccidiosis infection. Among them, 74.3% attributed the contamination of birds to dirty conditions, 14.3% to ingestion of contaminated feces, and 5.7% to contaminated food.

Methods of contamination control included using boots (51.4%), maintaining poor hygiene (20.0%), and food (11.4%), although 17.1% were unsure how *Eimeria* spp. was introduced into their poultry or believed birds were naturally infected at birth. Regarding symptoms, 77.1%

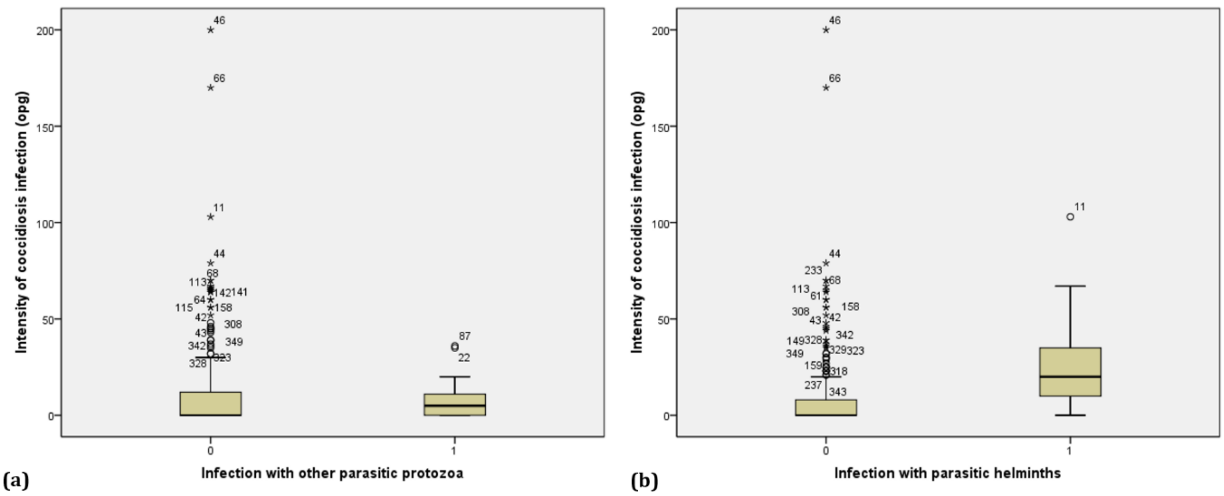

Figure 3. Box plots showing intensity of *Eimeria* spp infection according to infection with other parasitic protozoa (a) or helminths (b).

mentioned watery and bloody diarrhea, 11.4% loss of appetite, and 8.6% weight loss. Medication was cited as the most important preventive measure (51.4%), followed by proper maintenance (dry and good hygiene) of premises.

In terms of husbandry practices, 57.1% of breeders reported cleaning feeders after every batch of chickens, while 17.1% and 25.7% cleaned feeders monthly or daily, respectively. Additionally, 94.3% of breeders cleaned drinkers daily, with a small proportion (5.7%) doing so every two days. Overall, 40.0% of breeders cleaned cages after each batch of chickens, with 8.6% doing so twice a year, 8.6% monthly, 17.1% twice a month, and 25.7% weekly. While none of the breeders housed birds of different age groups in the same pen, 14.3% admitted mixing birds from different age groups within the same poultry.

All breeders reported disinfecting poultry before introducing new chickens, and approximately half (51.4%) also disinfected their boots before entering the poultry area. In handling dead birds, 54.3% of breeders buried them, 28.6% boiled and fed them to dogs, and 11.4% burned them. Almost all breeders (91.4%) acknowledged that stray birds pass around their premises.

The univariate binary logistic regression, which examined the association between coccidiosis infection and various covariates collected in this study, revealed significant links. Cleaning feeders or cages less frequently was significantly associated with coccidiosis infection. Keeping chickens from different age groups in the same poultry was significantly associated with infection by *Eimeria* spp. (OR: 0.457; 95% CI: 0.248-0.842; p=0.012). Additionally, coccidiosis infection showed significant associations with the age of birds, with those aged $\leq$ 4 weeks (OR: 2.227; 95% CI: 1.273 - 3.895; p=0.005) and 5-9 weeks (OR: 1.731; 95% CI: 1.030 to 2.908; p=0.038) being most vulnerable. The presence of stray birds passing around the premises was positively associated with coccidiosis (OR: 2.848; 95% CI: 1.291 - 6.283; p=0.009) (Table 3).

DISCUSSION

This study was conducted in the Bamenda municipality, where poultry farming is a rapidly growing economic activity in the livestock sector, and data on coccidiosis are scarce. To contribute to filling this knowledge gap, a prospective cross-sectional survey was carried out to assess the endemicity of coccidiosis and associated risk factors in poultry farms of the Bamenda Health District (Mezam Division, North West Region). *Eimeria* spp. was found to be widespread in the study area, with an overall prevalence of 43.4% (95% CI: 38.3 to 48.7), indicating a high level of endemicity. These findings are consistent with previous reports from Ethiopia and Algeria (Tadesse and Feyissa, 2016; Debbou-Iouknane et al., 2018).

The prevalence of coccidiosis observed in this study is higher than reported in other studies in Cameroon (33.9%) (Nghonjuyi et al., 2014), Ethiopia (19.5%) (Garbi et al., 2015), and Iran (35.2%) (Hagh-Poor and Garedaghi, 2016), but lower than in studies from Iran (75.0%) (Shirzad, 2011), Ethiopia (72.4%) (Tesfaye and Mekonnen, 2015), and India (72.0%) (Sonune, 2012). This significant variation in disease prevalence may be attributed to factors such as different sampling periods, geographic areas, agroecological conditions, climatic

Table 3. Association between infection with *Eimeria* spp. and the different co-variates collected in the framework of the study.

Variable	Categories	OR (95% CI)	p-value
Frequency of feeders' cleaning	After every batch	1	
	Daily	0.625 (0.317 - 1.232)	0.175
	Monthly	0.225 (0.121 - 0.415)	<0.0001
Frequency of drinkers' cleaning	After two days	1	
	Daily	1.324 (0.537 - 3.267)	0.543
Frequency of cages' cleaning	After every batch	1	
	Weekly	1.007 (0.579 - 1.751)	0.980
	Monthly	0.906 (0.378 - 2.170)	0.825
	Twice a year	3.625 (1.960 - 6.706)	<0.0001
Different age groups in the same poultry	No	1	
	Yes	0.457 (0.248 - 0.842)	0.012
Boots disinfection before entering poultry	No	1	
	Yes	0.837 (0.548 - 1.278)	0.409
Way of disposition of dead birds	Burn	1	
	Burry	1.083 (0.415 - 2.830)	0.870
	Boil and feed dogs	1.000 (0.342 - 2.926)	1.000
	Old birds eaten	0.583 (0.231 - 1.471)	0.253
Stray birds passing around the premises	No	1	
	Yes	2.848 (1.291 - 6.283)	0.009
Seasons	Rainy	1	
	Dry	1.474 (0.936 - 2.321)	0.094
Age groups (weeks)	> 9	1	
	5 – 9	1.731 (1.030 - 2.908)	0.038
	≤ 4	2.227 (1.273 - 3.895)	0.005
Stocking density (chickens/m^2)	< 5	1	
	5-10	0.890 (0.393 - 2.016)	0.780
	11-15	2.805 (1.436 - 5.478)	0.003

OR: Odds ratio; 95% CI: 95% confidence interval.

factors, or varying levels of management practices (Lawal et al., 2016).

The highest prevalence and intensity of coccidiosis infection were observed among chickens aged 5 - 9 weeks. Previous studies have shown that coccidiosis tends to be most endemic among younger birds compared to adults, as resistance to the disease typically increases with age due to the development of the birds' immune systems (Uza et al., 2001; Jordan et al., 2002). An important heterogeneity in coccidiosis infection was observed, likely attributable to inadequate management practices. Notably, in the most heavily infected farms, there was no water bath available for disinfecting shoes at the time of poultry stool sample collection, suggesting that oocysts may have been introduced into the poultry house from the external environment via footwear. Additionally, the highest endemicity of coccidiosis was found in poultry houses with the highest stocking densities

(5-10 chickens/m²), where the close confinement of many birds facilitates parasitic transmission between them (Bekali et al., 2009).

While no linear relationship was found between the intensity of infection and poultry stocking density, a convex trend was observed. This trend may be explained by farmers with limited space being more attentive to poultry care to prevent infection.

This study also revealed a higher prevalence and intensity of infection during the rainy season compared to the dry season, although this difference was not statistically significant (p=0.093). The relatively wet climate and cooler temperatures in the high and mid altitudes of the study area may favor the occurrence of coccidiosis, as reported in Ethiopia (Tesfaye and Mekonnen, 2015).

It was noted that individual birds often harbor more than one parasitic species, consistent with findings from other countries (Thrusfield, 2007). The infection rate of other protozoa tended to decrease with increasing intensity of coccidiosis infection, possibly due to competitive interactions. Unlike helminths, which are extracellular parasites and do not compete directly with *Eimeria* spp. for establishment in the gastrointestinal tract, other protozoa are intracellular and may compete with *Eimeria* spp. for resources.

Although helminth infections typically do not cause significant morbidity and mortality, they can lead to substantial effects such as malabsorption, diarrhea, anemia, and other health issues, particularly in young birds (Ehrenberg and Ault, 2005; Hotez et al., 2007).

All the farmers interviewed in the Mezam Division (North West Region, Cameroon) were already familiar with coccidiosis and demonstrated a generally good understanding of the infection, including its symptoms and transmission pathways. This awareness is likely due to the widespread endemic nature of coccidiosis globally, including in the Mezam Division, where it is recognized as a major parasitic infection with significant economic impact on poultry industries (Allen and Fetter, 2002).

Despite this high level of knowledge among farmers, the infection rate of *Eimeria* spp. was relatively high, primarily attributed to poor practices among breeders such as inadequate hygiene measures and factors like age, breed, body condition, and biosecurity, as previously reported in Ethiopia (Wondimu et al., 2019).

Conclusion

This study revealed that the prevalence of coccidiosis in poultry farms is relatively high and poses a significant threat to poultry producers in the Mezam Division. Although farmers demonstrated a fairly good knowledge of coccidiosis, some of their husbandry practices were poor, leading to increased exposure of birds to *Eimeria* spp. This highlights a clear lack of appropriate control measures against the disease and underscores the need for careful attention among farmers and implementation of effective interventions to manage this economically important parasitic disease in poultry.

These findings are valuable for informing policy and raising awareness among farmers about the importance of adopting and maintaining best husbandry practices to ensure optimal productivity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table S1. Rate of *Eimeria* spp, other protozoa and helminth infections according to farms/communities of origin of chickens.

Farm	No. of birds examined	No. of birds infected with *Eimeria* spp (%)	No. of birds infected with other protozoa (%)	No. of birds infected with helminths (%)
Ndamukong 1	10	0 (0.0)	0 (0.0)	0 (0.0)
Ndamukong 2	30	20 (66.7)	0 (0.0)	1 (3.3)
Sissia	20	10 (50.0)	0 (0.0)	0 (0.0)
Mile 3	30	0 (0.0)	0 (0.0)	0 (0.0)
Alahleh	30	22 (73.3)	0 (0.0)	0 (0.0)
Mandah 1	20	10 (50.0)	2 (10.0)	5 (25.0)
Mandah 2	20	10 (50.0)	0 (0.0)	0 (0.0)
Center Paul	20	10 (50.0)	4 (20.0)	0 (0.0)
Alosimenteng	40	0 (0.0)	0 (0.0)	5 (12.5)
Abangoh	20	20 (100.0)	0 (0.0)	3 (15.0)
Atou-akom	20	10 (50.0)	0 (0.0)	10 (50.0)
Azire	20	10 (50.0)	8 (40.0)	1 (5.0)
Behind Tse	20	10 (50.0)	2 (10.0)	9 (45.0)
Nanga Street	30	20 (66.7)	0 (0.0)	1 (3.3)
Ntarikon	20	0 (0.0)	0 (0.0)	3 (15.0)
Total	350	152 (43.4)	16 (4.6)	38 (10.9)