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DETECTION AND MORPHOLOGICAL IDENTIFICATION OF *EIMERIA* SPECIES IN MIGRATORY BIRDS AND CHICKENS IN SOME POULTRY FARMS IN KADUNA NORTH LGA OF KADUNA STATE

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ABSTRACT

Poultry is an important source of protein and income for several households in Nigeria. However, the poultry industry is threatened by outbreaks of infectious diseases which may spread through the interaction of wild or migratory birds with poultry. The study aimed to detect and morphologically identify *Eimeria* species from faecal samples of chickens, pigeons and African fire finches collected from different poultry farms in Kaduna metropolis with a view to determining the role of migratory birds in the transmission of poultry coccidiosis. Two hundred and nine (209) faecal samples were freshly collected from 91 chickens, 61 pigeons, and 57 African fire finches and examined for the presence of *Eimeria* oocysts using Wisconsin faecal flotation technique; species identification was based on morphological characteristics of the sporulated oocyst. *Eimeria* oocyst were detected in all the sampling locations, seven species of *Eimeria* were identified namely *E. tenella*, *E. brunetti*, *E. acervulina*, *E. necatrix*, *E. tropicalis*, *E. labbeana*, and *E. columbae*. *Eimeria brunetti* was identified in both chickens and pigeons, while *E. tenella*, *E. acervulina*, and *E. necatrix* were identified in only chicken. On the other hand, *E. tropicalis*, *E. labbeana*, and *E. columbae* were found only in pigeons. The presence of *Eimeria* species in chickens, especially the acutely pathogenic *E. tenella* poses a significant challenge to poultry production. Furthermore, the infection among pigeons could serve as carriers of the protozoan from one farm to another. The results showed that African fire finches visiting poultry farms may not pose any threat in the transmission of the infection.

Keywords: *Eimeria* species, Migratory birds, Morphological identification, Poultry, Pigeons

INTRODUCTION

Infectious diseases of chicken have historically been one of the biggest obstacles to increasing poultry production in Nigeria (Laseinde, 2002; Etuk *et al*., 2004; Akintunde and Adeoti, 2014). Due to the high occurrence of these diseases in chickens caused by the tropical environmental factors that the farmer works in, they are particularly important (Seifert, 2006; Adewole, 2012). One of the most serious poultry disease is coccidiosis which affects commercial chicken production throughout the world by an estimated US$ 1.5 billion annually (Arabkhazaeli *et al*., 2011; 2013; 2014). It is a serious parasitic illness of chickens having a significant financial impact on Nigeria's poultry businesses (Etuk *et al*., 2004; Musa *et al*., 2010; Usman *et al*., 2011).

Although, coccidiosis is a worldwide problem, it is most frequently found in young animals, particularly hens that are caged or kept in small spaces infected with oocysts. Coccidia is host specific, and there is no cross-immunity between species of coccidia. Finding a large chicken flock that is unaffected by *Eimeria* is relatively rare (Williams, 1999). Wild birds are not normally hosts for these infections because of their stringent host specificity. By consuming or drinking tainted water, litter, food, or other substances containing coccidian oocysts, the illness is passed from bird to bird (Owai and Gloria, 2010; Patrick and Mgbere, 2010;). Recent research has emphasized the role of common house fly in the mechanical dissemination of the coccidian parasites, which puts even birds in new homes at risk of outbreaks. In contrast, tools, staff, rats, and wild birds have also been blamed for the spread of coccidiosis (Abdu *et al*., 2008; Musa *et al*., 2010).

Seven different species of *Eimeria* have been known to infect chicken, with *E. Tenella*, *E.maxima*, and *E. acervulina* which have traditionally been thought to be the most economically significant species (Thenmozhi *et al*., 2014). In coccidiosis co-infection with different *Eimeria* species is frequent (Haug *et al*., 2008; Jenkins *et al*., 2008) which not only increases pathogenicity but also results in incorrect diagnoses. A total of sixteen *Eimeria* species from the family Columbidae have been identified in pigeons. However, many *Eimeria* species from columbidae have inadequate descriptions and lack of measurements, due to these reasons, it has been challenging to confirm the existence of new species of *Eimeria*. Duszynski *et al*. (2000) has reported that just two species which are *E. labbeana* and *E. columbarium* likely exist in pigeons.

During the commercial, industrial, or domestic production of birds, contact between wild and domestic birds is thought to play a role in the spread of a variety of diseases. Large amounts of food provided by poultry houses aid in the expansion of the population of these wild birds (Angelo *et al*., 2011). According to Alexander (2000), interaction with wild birds is a factor in transmission because they often display low levels of virulence for domestic birds.

Estrildidae is the family that includes fire finches (order Passeriformes). The Senegal fire finch (*Logonosticta senegala*), which measures 8 centimeters (3 inches) in length and is commonly seen in gardens and scrublands, is possibly the most widespread and tamest bird in Africa. Both are red-rumped, with the male being primarily light red and the female being brown (Anonymous, 2017)

There have been a few reports of *Eimeria* species in passerine birds, even though isospora species commonly infect passerine birds (Berto *et al*., 2011). Pigeons can also contract infections (Aleksandra and Pilarczyk, 2014). They are home to several infectious stages of several intestinal parasite illnesses, such as coccidiosis (Kommu *et al*., 2016).

In a previous study conducted by Adeyemi *et al*., 2020**,** identification by morphometry of the oocysts (both sporulated and unsporulated) was carried out by measurement of 50 oocysts using calibrated ocular micrometer at 400x magnification. They were categorized in accordance with Haug *et al*. (2008) into three groups: Acervulina-Mitis (AM) group (small oocysts, <18.8µm; tentatively Eimeria acervulina and/or Eimeria mitis); *Necatrix*, *Tenella* and *Praecox* (*NTP*) group (medium sized oocysts, 18.9 µm to 23.8 µm; tentatively Eimeria necatrix, Eimeria tenella and/or Eimeria praecox) or a *Brunetti-Maxima* (*BM*) group (large ovoid oocysts, >23.9µm; tentatively Eimeria brunetti and/or Eimeria maxima).

This study was aimed at detection and morphological identification of *Eimeria* species from faecal samples of chicken, pigeons and African fire finches collected from different farms in Kaduna metropolis with a view to determining the role of migratory birds in the transmission of poultry coccidiosis in the area.

MATERIALS AND METHODS

Study Area

Kaduna metropolitan is the capital of Kaduna State located in the North-central part of Nigeria. It is located on Latitude 10.9° and 10.15°N and 7.5° and 7.9° E, it covers a land mass area of 46,053 square kilometers with an estimated population of 8.9 million people (NPC, 2006). The climate of the area is tropical continental with annual rainfall from April to October while the dry season is from November to March.

Kaduna State consists of twenty-three (23) L.G. As out of which the Kaduna North L.G. A is among the L.G. As situated in the metropolis. Kaduna State is bordered by Zamfara, Katsina and Kano to the north; Plateau and Bauchi to the east; Nasarawa to the south and Niger to the west. The FCT also boarders Kaduna to the southeast (Anonymous, 2022).

Study Sites

This study was carried out in poultry farms that were randomly selected in Kaduna North L.G.A of Kaduna State. The farms selected for this study were based on the rearing system practiced either intensive or free range system. Four farms were selected, two in Mando and two in NDA Ribadu Cantonement. These farms were denoted letter A, B, C and D respectively.

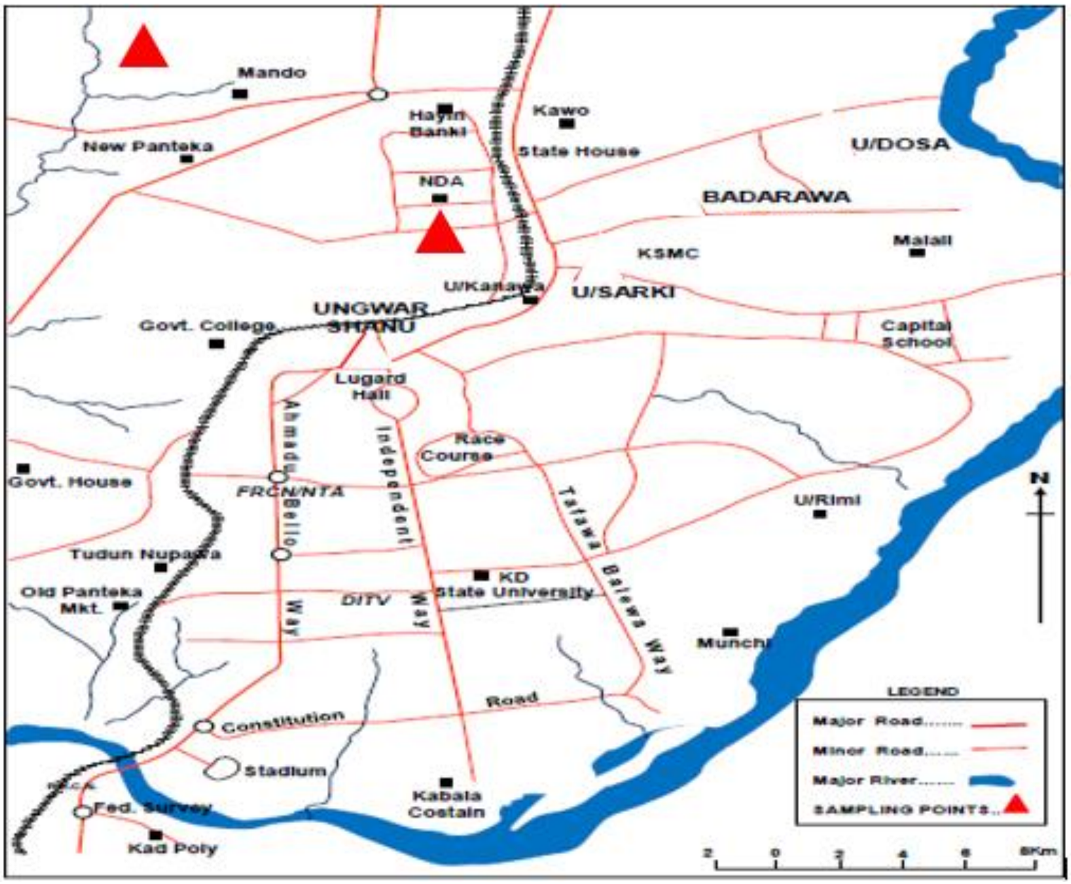

Figure 3.1: Map of Kaduna North L.G.A showing study sites (Mando and NDA).
Source: (KASU GIS, 2023)

Informed Consent

All participating poultry farms whose consent were obtained were enrolled.

Sample Size Determination

The sample size was determined using 14.2 % prevalence of coccidiosis as reported by Mohammed and Sunday (2015).

$$N = \frac{Z^2 p(1-p)}{d^2}$$

Where,
N= sample size
Z= Standard deviation (1.96)
p= prevalence (14.2%, Mohammed and Sunday, 2015)
q= 1-p
d= precision (allowable error) = 5% (0.05)

$$N = \frac{1.96^2 x\ 0.142\ (1-0.142)}{0.05^2}$$

N= 187.2

Sample Collection

Faecal samples of chicken were collected from a total of 4 poultry farms, the faecal samples were preserved in clean sample bottles containing 2.5% potassium dichromate solution. Faecal sample collection of the wild birds (African fire finches and speckled pigeons) were carried out by capturing birds using bird traps. Trapped birds were caged and then clean white plastic bags were laid under the cage to collect the faecal samples. These samples were transported immediately to the laboratory for examination and identification of the species of the *Eimeria* parasites, samples of the litter, the feed and water were also collected from each of the selected poultry farms for examination.

Sample Analysis

Faecal Sample Examination

Each sample was examined in the laboratory using the Wisconsin faecal flotation technique, described by Conway and Mckenzie (2007) for the detection of the parasite oocysts. In a rubber tube, 3 g of each faecal sample was dissolved in 5 ml of the sugar-salt flotation media, these was thoroughly mixed with a glass rod. Thereafter, the faecal mixture was sieved into a test tube using a muslin towel. The floating medium was then transferred into test tube, causing the oocysts to float to the top. The test tube was covered with a cover slip, which was left in place for five minutes to allow oocysts attach to the cover slip. The cover slip was carefully removed from the test tube and placed on a clean microscope slide. The resulting faecal preparation on the slide was then examined under a light microscope at X10 and X40 magnifications for parasite detection. Where present, the morphology features, such as size (length and width), shape and thickness of the oocysts, were noted. *Eimeria* oocysts detected were identified using the keys provided by Taylor *et al*., (2007).

Sporulation Procedure

Water was used to homogenize and filter faecal samples containing unsporulated oocysts. Thereafter, 2.5 % aqueous potassium dichromate solution was used to resuspend the filtrate. This was carried out to ensure that there was enough moisture and to eliminate any other microorganisms that might be in the faeces and competing with the oocysts for nutrition and oxygen. To enable the sporulation procedure, the samples were placed into rubber tubes and stored at room temperature. To allow the unsporulated oocysts to sporulate, some of the samples were let to stand for between 24 and 48 hours and others for up to 7 days. To determine whether sporulated oocysts are present in each sample, it was examined under a microscope at various points in time. A calibrated ocular microscope operating at X40 magnification was used to determine the morphology and size of the sporulated oocysts as described by Conway and McKenzie (2007).

Morphometric Identification of the *Eimeria* Species

The identification of the different species of *Eimeria* was carried out based on the shape and size of sporulated oocysts. The *Eimeria* species were identified based on the identification key provided by McDougald (2003).

Examination of Feeds, Water and litter Samples

The sample of the poultry feeds, litter and water of the poultry farm was taken from the surrounding and was examined for the presence of the oocysts of the *eimeria* parasites. The feeds, litter and water samples were examined to ensure that the parasites are not from the surrounding of the poultry farms.

Data Analysis

Percentage prevalence and frequencies, variation and intensity of *Eimeria* species among the chickens, African fire finches and speckled pigeons were calculated. Data obtained was analyzed using SPSS Statistical Software (Version 22). Chi-square test was used to establish the statistical difference.

RESULTS

The morphological characteristics of the different *Eimeria* species detected is presented in Table 1. Seven species of *Eimeria* were morphologically identified in samples collected from migratory birds (pigeons) and chickens across the four poultry farms in Kaduna North LGA of Kaduna State, namely; *E. tenella*, *E. brunetti*, *E. acervulina*, *E. necatrix*, *E. tropicalis*, *E. labbeana*, and *E. columbae*. *Eimeria brunetti* was identified in both chicken and pigeons, while

E. tenella, *E. acervulina*, *E. necatrix* were identified in only chickens. On the other hand, *E. tropicalis*, *E. labbeana*, and *E. columbae* were found only in pigeons. The unsporulated oocyst of *E. tenella* was characterized by an ovoid shape with smooth wall, having double contoured lines and a micropyle. It measured 21.6 x 18.7 µm in size with a sporulation time of 7 days (Plate Ia and Plate II). *Eimeria brunetti* oocyst shared similar morphological features with *E. tenella* in having an ovoid shaped appearance with double contoured lines and smooth wall and a sporulation time of 7 days. However, it lacked a micropyle and appeared bigger in size with dimensions of 25.1 x 20.7 µm (Plate Ib).

Oocysts of *E. acervulina* were characterized by an ovoid shaped appearance, smooth wall with double-contoured lines; however, they lacked micropyle (Plate Ic). They shared the same sporulation time of 7 days as other *Eimeria* species identified in the birds and measured 18.5 x 16.7 µm. The oocyst of *E. necatrix* measured 20.35 x 18.5 µm in size, with a sporulation time of 7 days. Morphologically, oocysts of *E. necatrix* were characterized by presence of double- contoured lines, smooth wall, and oblong-to-ovoid shape (Plate Id). *Eimeria tropicalis* had characteristic thin wall with double contoured lines; micropyle was absent (Plate Ie), measured 22.2 x 21.4 µm in size, with a sporulation time of 7 days. The presence of thick wall, double contoured lines, and the absence of micropyle characterized the oocyst of *E. labbeana* (Plate If). They measured 20.4 x 18.5 µm in size and sporulated in 7 days. *Eimeria columbae* oocyst measured 18.5 x 18.2 µm in size, sporulated in 7 days, and had thin wall, double contoured lines, and micropyle (Plate Ig).

Table 1*:* Distribution of Different *Eimeria* Species Identified from Selected Poultry Farms in Kaduna North

Birds	Suspected *Eimeria* species Identified	Oocyst size (µm)	Sporulation time (Days)	Characteristics morphology of unsporulated Oocyst
Chickens	*E. tenella*	21.60 x 18.7	7	Double-contoured lines, smooth wall, microphyle, ovoid
	E. necatrix	20.35 x 18.5	7	Double-contoured lines, smooth wall, microphyle, oblong ovoid

	E.acervulina	18.50 x 16.7	7	Double-contoured lines, smooth wall, ovoid
	E. brunetti	25.10 x 20.7	7	Double-contoured lines, smooth wall, ovoid
Pigeons	*E. columbae*	18.50 x 18.2	7	Thin wall, Double-contoured lines, microphyle present, oval
	E. tropicalis	22.20 x 21.4	7	Thin wall, Double-contoured lines, microphyle absent, spherical
	E. labbeana	20.40 x18.5	7	Double-contoured lines, thick wall, microphyle absent, sub-spherical
African fire finches	N.D	N.D	N.D	N.D

Legend: *E* = *Eimeria* N.D = Not Detected

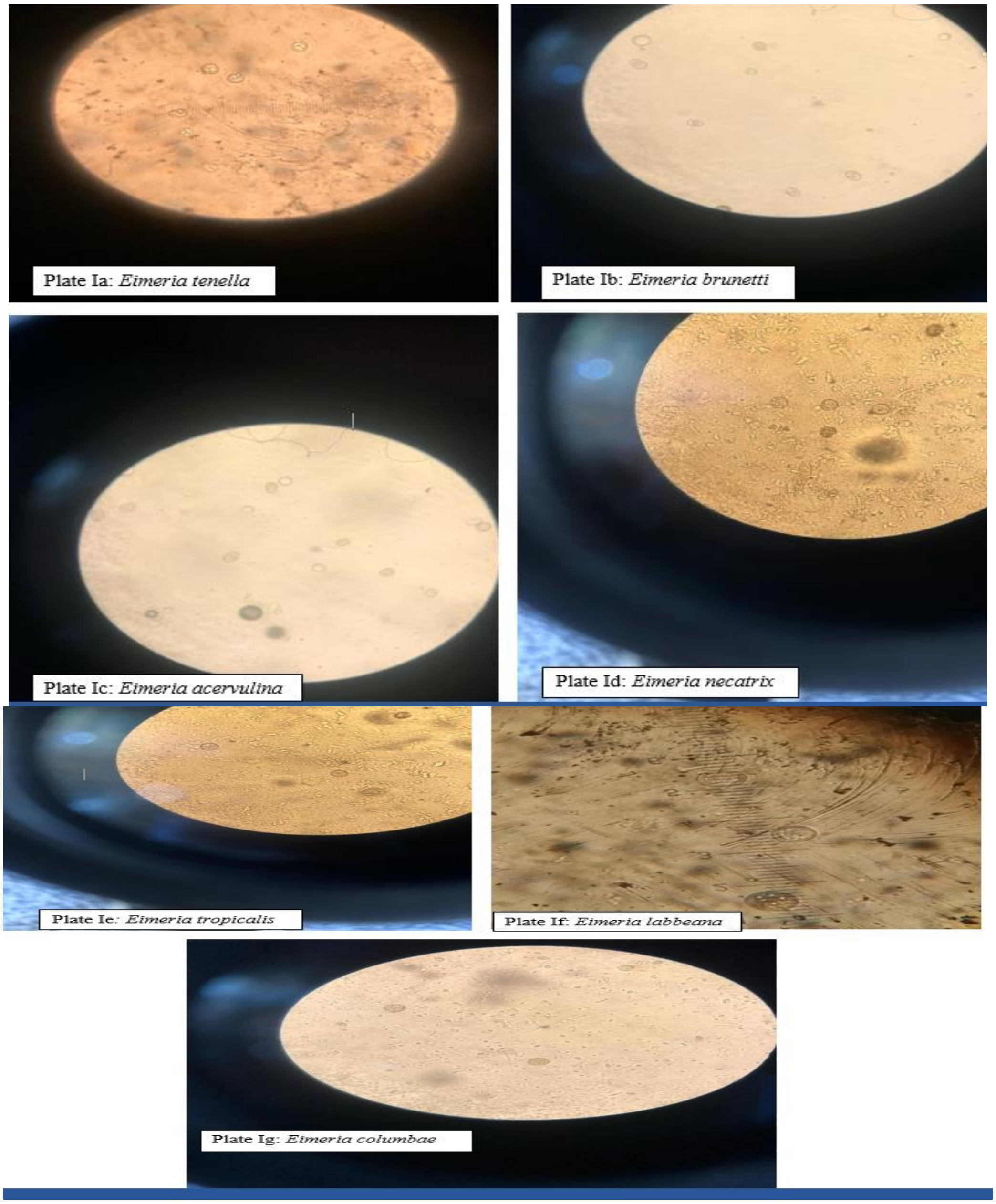

Plate 1a -1g: Morphological Examination of Sporulated Oocysts of Seven *Eimeria* Species Identified in Chickens and Pigeons

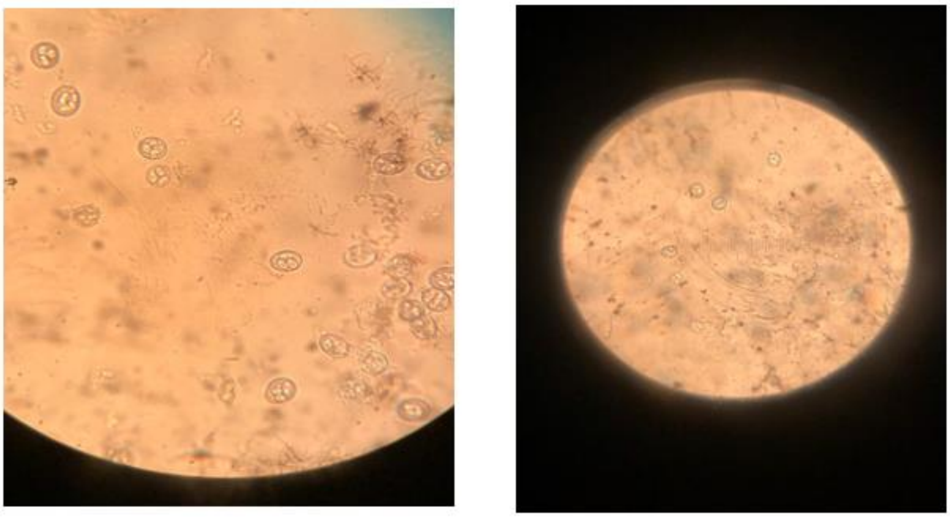

Plate 2: Photomicrograph of Sporulated Oocysts

Prevalence of single and mixed infection of *Eimeria* species is presented in Table 2. In both chicken and pigeons, cases of single and mixed infections were identified. Furthermore, incidences of single infections were higher than mixed infections in both species of birds; however, the differences were not significant ($p > 0.05$). In chicken, 75% of *Eimeria* infections were single infections while 25% were cases of mixed infection. Similarly, single infections among chickens accounted for 63.6% of all infections among the species of bird, while 36.4% were cases of mixed infections. On the basis of relative susceptibility to single or mixed *Eimeria* infections, pigeons appeared to be most susceptible followed by chicken: 66.7% of all cases of single infections and 53.8% of all cases of mixed infections were recorded in pigeons, while in chicken single and mixed infections accounted for 33.3 and 46.2 percent, respectively. The data across the four farms shows that African fire finch were not (or are least) susceptible to *Eimeria* infections as no positive cases was recorded among the finches that were examined in the course of the study.

Table 2: Prevalence of Single and Mixed Infection of *Eimeria* Species in Chicken, Pigeons, and African Fire Finches in the Selected Poultry Farms in Kaduna North L.G.A of Kaduna state.

Types of birds	No. of infected	Single infection	Mixed infection
		No. (%)	No. (%)
Chicken	8	6 (75.0)	2 (25.0)
Pigeons	11	7 (63.6)	4 (36.4)
African fire finch	0	0 (0.0)	0 (0.0)
Total	19	13 (68.4)	6 (31.6)

$\chi^2_{(1)} = 0.277$; $p = 0.599$

Identification of *Eimeria* Species Based on Single Infection is presented in Table 3. Ten oocysts were measured from each of the samples that were positive. Then the average of these ten oocysts from each of these positive samples were calculated in order to identify the *Eimeria* species based on single infection (Table 3). For sample C25, after the average of ten oocysts were calculated, the length and width of the species measured 22.1 x 18.5 µm respectively, having an ovoid shape, from this measurement and shape, the likely species is *E. tenella.* For sample C22, after the average of ten oocysts were calculated, the length and width of the species measured 21.5 x 18.5 µm respectively, having an oblong ovoid shape, from this measurement and shape, the likely species is *E. necatrix*. For sample P6, after the average of ten oocysts were calculated, the length and width of the species measured 20.7 x 19.8 µm respectively, having a spherical shape, from this measurement and shape, the likely species is *E. tropicalis.* For sample P9, after the average of ten oocysts were calculated, the length and width of the species measured 21.5 x 21.3 µm respectively, having a spherical shape, from this measurement and shape, the likely species is *E. tropicalis*. For sample P21, after the average of ten oocysts were calculated, the length and width of the species measured 20.2 x 18.3 µm respectively, having a spherical shape, from this measurement and shape, the likely species is *E. tropicalis*. For sample P25, after the average of ten oocysts were calculated, the length and width of the species measured 21.8 x 19.4 µm respectively, having a spherical shape, from this measurement and shape, the likely species is *E. tropicalis*.

Table 3: Suspected *Eimeria* Parasite Single Infection of Chickens, African fire finch and Pigeons Based on the Average of Ten Measured Oocysts from each Positive Samples.

Positive samples	Average size of oocyst (µm) length and width	Shape	Suspected *Eimeria* species
C25	22.1 x 18.5	Ovoid	*E. tenella*
C22	21.5 x 18.5	Oblong ovoid	*E. necatrix*
P6	20.7 x 19.8	Spherical	*E. tropicalis*

P 9	21.5 x 21.3	Spherical	*E. tropicalis*
P21	20.2 x 18.3	Spherical	*E. tropicalis*
P25	21.8 x 19.4	Spherical	*E. tropicalis*

DISCUSSION

The present study demonstrated a high preponderance of two species of migratory birds, namely pigeons and the African fire finches, on the selected poultry farms within Kaduna North LGA. The poultry industry is threatened by outbreaks of infectious diseases which may spread through interaction of wild or migratory birds with poultry as reported in this study (Assam *et al*., 2020; Bello *et al*., 2022). In a related study, Bello *et al*. (2022) identified factors that increase poultry-wild bird interaction including spillage of poultry feed, drying of feed materials outdoors, presence of trees, shades, and roosting sites, etc.

The isolation of seven *eimeria* species in the faecal samples collected in this study was in agreement with the findings of Ola-Fadunsin *et al*. (2019) and Idriss *et al*. (2019), it also agrees with the reports of Olarenwaju and Agbor (2014), Shamim *et al*. (2015) and Ola-Fadunsin *et al*. (2019) who identified eight species of *Eimeria* among poultry birds in Kwara State, North-Central Nigeria. Idriss *et al*. (2019) had also reported eight species of *Eimeria* among local breeds of chicken in Geidam Local Government Area of Yobe State, North Eastern Nigeria. Olarenwaju and Agbor (2014) identified only three species of *Eimeria* in their survey of coccidiosis among birds sold at Gwagwalada main market, Abuja Nigeria. However, it is in contrast with the work of Shamim *et al*. (2015) as they identified only two species of *Eimeria* in broilers raised under traditional management system the Mirpur district in Azad Kashmir, Pakistan.

There are variations in the species discovered in this study and those discovered by other authors. Some of the species identified in this study were *E. tenella*, *E. brunetti*, *E. acervulina*, *E. necatrix*, *E. tropicalis*, *E. labbeana*, and *E. columbae*. The discovery of *E. tropicalis*, *E. labbeana*, and *E. columbae* in pigeons in the present study could be due to the migratory nature of the birds, this differed from the work of Idriss *et al*. (2019) who reported no species among the pigeons. However, they reported *Eimeria maxima*, *E. praecox*, *E. mitis*, and *E. mivati* among the chickens. Olarenwaju and Agbor (2014) reported the presence of *E. tenella*, *E. acervulina* and *E. maxima* which were also detected in the present study, except for *E. maxima*. According to Ombugadu *et al*. (2019) *E. tenella*, *E. necatrix*, *E. bruneti*, *E. acervulina*, *E. mitis* and *E. praecox* were the primary causative agents of the infection in Nigeria, with *Eimeria tenella* implicated in caecal coccidiosis while chronic intestinal coccidiosis has been associated with *E. acervulina* and *E. maxima* (Ombugadu *et al*., 2019).

The present results further showed that across the four sampling location, *Eimeria* was found only in chicken and pigeons but none in the African Fire Finches. This raises the question of differential susceptibility of avian species to *Eimeria* infection. This protozoan parasite has been variously reported in poultry birds (Olarenwaju and Agbor, 2014; Shamim *et al*., 2015; Chalchisa and Deressa, 2016; Mohammed and Mahmuda, 2017; Idowu *et al*., 2019; Idriss *et al*., 2019; Ola-Fadunsin *et al*., 2019; Rashid *et al*., 2019). There have also been reports of *Eimeria* in pigeons (Mohammed *et al*., 2017; Ramesh *et al*., 2018; Al-Agouri *et al*., 2021).

Furthermore, the present results identified cases of single and mixed infection of *Eimeria* species among the birds examined, with cases of single infections higher than mixed infection, this could be because a guideline was followed during the microscopy, where if 10 or more oocysts of the same sizes are seen, then this is considered as single infection whereas an oocyst count of different sizes of more than 10 was considered a mixed infection. However, Idriss *et al*. (2019) remarked that infection with single species is rare. The non-infection of the African fire finches could be because they have developed resistance to the protozoan parasite. Their presence in farms may not pose any threat in the spread of this parasite from farm to farm. If this later species of birds is not susceptible to the infection, it becomes important to conduct further studies to, first, validate this observation, and secondly identify the inherent factor(s) in the species that may be responsible for its resistance to the protozoan infection. Such finding may play a role in the development of vaccines against the infection. It is recommended that further study be carried out to identify the exact species of these parasite by the use of PCR and also sequencing.

Appendix 1: *Eimeria* Parasite Co-infection in the Positive Samples of Chickens, African Fire Finches and Speckled Pigeons Based on the Mean Length and Width Measured.

Positive samples	Mean Length x width of suspected species of *Eimeria* oocysts seen (µm)	Combination of suspected *Eimeria* species in each positive sample
C25		
First species	22.2 x 18.5	*E. tenella*
Second species	24.35 x 19.11	*E. brunetti*
Third species	18.5 x 14.8	*E. acervulina*
Fourth species	20.35 x 19.42	*E. tenella*
C22		
First species	22.2 x 18.5	*E. tenella*
Second species	20.35 x 18.5	*E. necatrix*
Third species	18.5 x 18.5	*E. acervulina*
P6		
First species	22.2 x 21.7	*E. tropicalis*
Second species	20.35 x 18.5	*E. labbeana*
Third species	18.5 x 18.5	*E. columbae*

P9		
First species	22.2 x 22.0	*E. tropicalis*
Second species	18.5 x 18.5	*E. columbae*
P21		
First species	18.5 x 17.6	*E. columbae*
Second species	22.2 x 20.5	*E. tropicalis*
Third species	20.3 x 18.5	*E. labbeana*
P 25		
First species	22.2 x 18.5	*E. labbeana*
Second species	20.35 x 18.5	*E. labbeana*
Third species	22.2 x 22.2	*E. tropicalis*
Fourth species	22.2 x 20.35	*E. tropicalis*
Fifth species	18.5 x 18.5	*E. labbeana*
Sixth species	25.9 x 22.2	*E. brunetti*

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