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Original papers

Prevalence and associated risk factors of *Eimeria* species in rabbits (*Oryctolagus cuniculus*) in Ilorin, Kwara State, Nigeria

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ABSTRACT. Coccidiosis remains a serious concern of great economic impact on domestic animals including rabbits. This study was conducted to determine the prevalence, species diversity, and associated risk factors of rabbit coccidiosis in Ilorin, Kwara State Nigeria. Fecal samples were examined using the floatation technique (positive samples were sporulated for identification of the different *Eimeria* species). 169 out of 215 (78.6%,) rabbits were found to be infected with *Eimeria* species. A total of seven *Eimeria* species (*Eimeria coecicola*, *Eimeria irresidua*, *Eimeria perforans*, *Eimeria magna*, *Eimeria intestinalis*, *Eimeria stiedai* and *Eimeria flavescens*) were identified. Of these, *Eimeria coecicola* was the most prevalent (48/215; 22.3%), while *E. flavescens* (8/215; 3.7%) was the least prevalent. Weaners (80.0%) were more infected than bunnies (79.1%) and adult (77.5%). Females had a higher infection rate (79.4%) than males (77.4%). Californian breed were more infected (84.9%) compared to Chinchila (83.7%), Dutch breed (80.9%), and New Zealand White (63.6%). Rabbits kept in the deep litter housing system had higher prevalence rate (95.2%) compared to those raised in the battery cage system (71.9%). Coccidiosis was more prevalent during the wet season than the dry season. This study concludes that *Eimeria* infections of rabbit is endemic in Ilorin, Kwara State with breeds and housing type been significant risk factors associated with the infection. This study provides the baseline information as the first report on the different *Eimeria* species affecting rabbits in Nigeria.

Keywords: prevalence, *Eimeria*, rabbit

Introduction

Rabbit meat is used as a good source of animal protein since it has low cholesterol and fat contents, making it preferable to other meat [1]. In addition to this commercial value, these animals are used as very important models for medical research and as pets, while some breeds are reared for fur production as well as for medical purposes [2]. Therefore, rabbit production became one of the important animal resources in most parts of the world [2].

Rabbit coccidiosis is a common and widespread protozoal infection in commercial operations and is responsible for major pathogenicity and even death [3]. Coccidiosis is caused by intercellular protozoan parasites of the genus *Eimeria* and can cause significant mortality in domestic rabbits [4]. Although mortality can result from heavy infection by these parasites as the majority of infections result in morbidity due to lower weight gain and diarrhoea [5]. Coccidiosis is associated with major economic losses in rabbit farming including morbidity and mortality, growth retardation and condemnation of affected livers in cases of hepatic coccidiosis [6]. Coccidiosis remains one of the most important infectious causes of digestive disorders in fattening rabbits in most parts of the world [7]. The condition in domestic rabbits occurs either in the intestines (intestinal coccidiosis) or liver (hepatic coccidiosis), and mainly in young rabbits [8–10], and rabbits housed in poor environmental sanitation and poor

hygienic practices [11]. Each species is highly host, organ and tissue specific, and the species that affect rabbits are rarely of zoonotic danger to humans [12].

Little has been done about *Eimeria* infections of rabbits in Nigeria, and the scanty information available is almost entirely old as these works were done in 1990's by Okewole et al. [13] and Biu and Nwosu [14]. Other studies about *Eimeria* infections of rabbits in Nigeria is either limited to an *Eimeria* species [15] or the study was not specific on the *Eimeria* species detected [16].

This present investigation was therefore undertaken to provide baseline information on the prevalence, risk factors and species diversity of rabbit *Eimeria* infection in Ilorin, North-central Nigeria. Our findings will also provide the foremost information on the species of *Eimeria* affecting rabbits in Nigeria as this has never been reported before to the best of our knowledge.

Materials and Methods

Study area. The study was carried out in Ilorin, Kwara State, Nigeria. Ilorin is the capital of Kwara State and the State is located between latitude 8°05'N and 10°15'N, longitude 2°73'E and 6°13'E. It is located in the middle belt (north-central) within the forest savanna region of Nigeria. The state is bordered in the west by Benin Republic, in the east by Kogi State, and the south by Oyo, Osun and Ekiti States. Kwara State population is about 3 million people and it covers a total area of 34,500 km^2 comprising rainforest in the south and wooded savannah in the larger part of the State. It has 16 local government areas. The State has two seasons, the dry and wet season, with heavier rainfall in September and October. The state has relative annual rainfall of between 112.8–146.9 cm, relative annual temperature of 22.1–33.3°C, and relative humidity of 49.6% [17,18].

Study design and sampling procedure. The study was conducted between October 2016 and September 2017 in seven randomly selected farms of the few rabbit farms in Ilorin Kwara State. Rabbits from both small and large scale capacities were randomly sampled. Two hundred and fifteen (215) fecal samples were collected from rabbits (*Oryctolagus cuniculus*) with their age, sex, breed, housing system and date of sampling taken into cognizance. Fecal samples were collected from the rectum or fresh from the ground where sampling from the rectum was not possible. The fecal samples were then placed into sterile sample bottles and labeled. The samples were immediately transported in cool box to the Parasitology Laboratory of the Faculty of Veterinary Medicine, University of Ilorin, Nigeria, for further processing.

Laboratory procedures. Fecal samples were examined for the presence of *Eimeria* oocysts using the floatation technique as described by Abdel-baki and Al-quraishy [2]. Positive samples were sporulated as described by Balicka-Ramisz et al. [19] with little modifications. Briefly, fecal samples were emulsified and then placed in Petri dishes, sprinkled with water to make it damp (not wet). About 2.5% potassium dichromate solution was then added to the sample and allowed to stand for 5–7 days at room temperature to permit the coccidian oocysts to sporulate. After sporulation, flotation technique was again used to examine the sporulated oocyst. The morphological features of the sporulated oocysts including shape, shape index, size, inner and outer wall, micropyle and residium were noted and the identity of the oocysts was determined by the keys previously described by Pellerdy [20], Catchpole and Norton [21] and Levine [22].

Statistical analysis. The descriptive statistics was conducted using percentages and tabulations. The univariate analysis (χ^2, chi-square) test and odds ratios with 95% confidence interval were used to determine the association between each epidemiological factor and *Eimeria* infection. The odds ratios were calculated with respect to a reference category as indicated in the respective tables. Multivariable unconditional logistic regression was used to determine predictors for *Eimeria* infection controlling for other covariates at $P<0.20$ and biologically plausible factors. We tested for collinearity among predictors using the Chi square test for binomial variables. We also tested for interactions between selected variables. A forward step-wise method was used. All statistical tests were conducted using statistical package for social sciences (SPSS) version 22 (SPSS Inc., Chicago). Significant level was set at $P<0.05$.

Results

Prevalence and *Eimeria* species diversity

Oocysts of *Eimeria* species was detected in 169 out of 215 (78.6%, 95% CI 72.7 – 83.7) rabbits fecal samples examined (Table 1).

Seven species of *Eimeria* were detected to affect

Table 1. Prevalence and *Eimeria* species diversity in rabbits in Ilorin, Kwara State, Nigeria

Eimeria species	No. of infected animals (%)	95% CI
Eimeria coecicola	48 (22.3)	17.1–28.3
Eimeria irresidua	35 (16.3)	11.8–21.7
Eimeria perforans	30 (14.0)	9.8–19.1
Eimeria magna	19 (8.8)	5.6–13.2
Eimeria intestinalis	15 (7.0)	4.1–11.0
Eimeria stiedai	14 (6.5)	3.8–10.4
Eimeria flavescens	8 (3.7)	1.7–6.9
Total	169 (78.6)	72.7–83.7

CI = Confidence Interval

rabbits in Ilorin, Kwara State (Fig. 1). *Eimeria coecicola* was the most prevalent representing 22.3% of the sampled population, while *E. flavescens* was the least prevalent (8/215, 3.7%). The prevalence of the other species ranged from 6.5% (*E. stiedai*) to 16.3% (*E. irresidua*) (Table 1).

Risk factors associated with *Eimeria* infections

Table 2 summarized the univariate association of *Eimeria* infection in relation with some epidemiological variables. The Californian breed had the highest prevalence of 84.85% (OR=1.32), this was closely followed by Chinchila breed (83.67%, OR=1.21). The New Zealand White breed was least prevalent (63.64%). *Eimeria* infection was 1.32 times more likely to occur in the Californian and 1.21 times more likely in Chinchila breed compared to the Dutch breed. However, prevalence

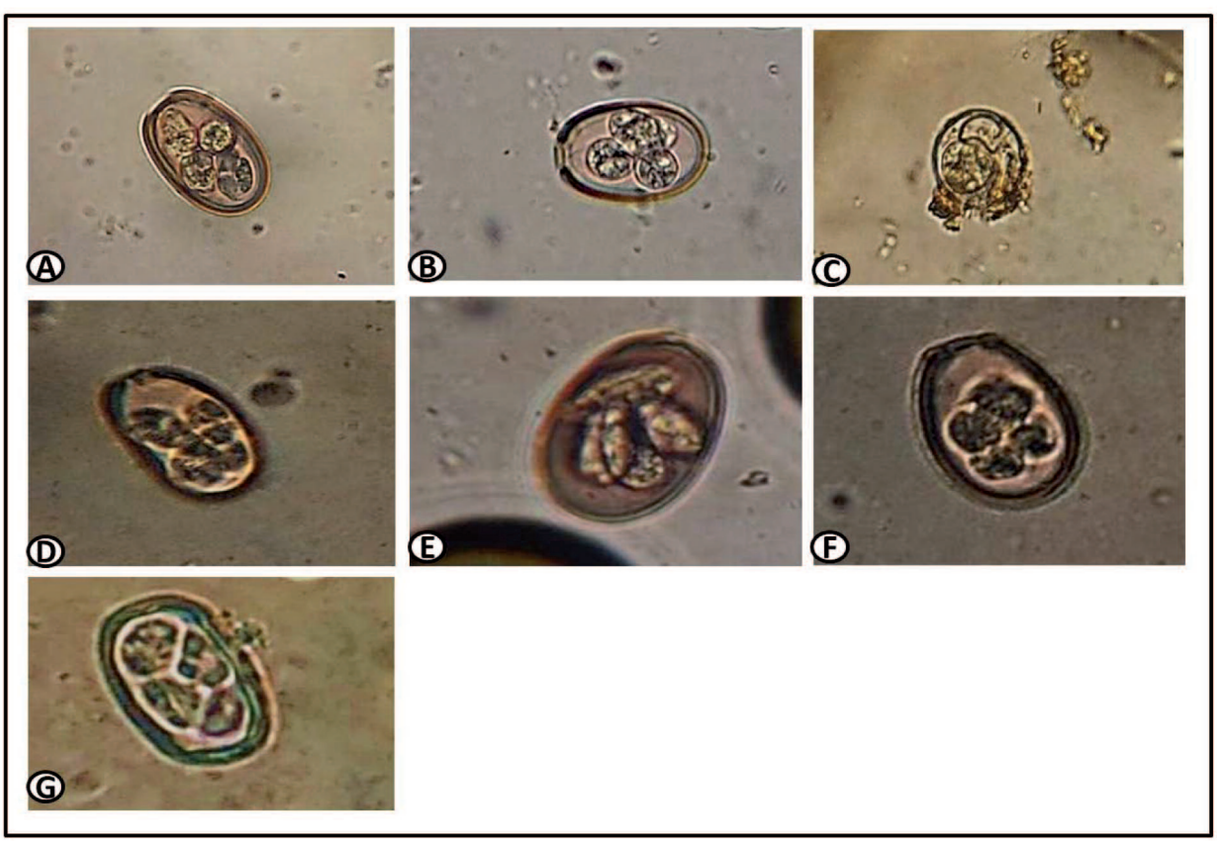

Fig. 1. Identified *Eimeria* species. A. *Eimeria coecicola*; B. *Eimeria flavescens*; C. *Eimeria perforans*; D. *Eimeria intestinalis*; E. *Eimeria stieda*; F. *Eimeria magna*; G. *Eimeria irresidua* (×40 magnification).

Table 2. Univariate association between epidemiological variables and *Eimeria* infection among rabbits in Ilorin, Kwara State

Risk factors	N	Positive (%)	OR (95% CI)	*P* value
Breed				
Californian	33	28 (84.85)	1.32 (0.46–4.34)	0.83
Chinchila	49	41 (83.67)	1.21 (0.48–3.20)	0.70
New Zealand White	44	28 (63.64)	0.42 (0.18–0.94)	0.04*
Dutch [a]	89	72 (80.90)	1.00	
Age				
Bunny	43	34 (79.07)	1.10 (0.47–2.74)	0.84
Weaner	70	56 (80.00)	1.16 (0.55–2.51)	0.70
Adult [a]	102	79 (77.45)	1.00	
Sex				
Female	131	104 (79.39)	1.13 (0.57–2.19)	0.73
Male [a]	84	65 (77.38)	1.00	
Housing type				
Deep litter	62	59 (95.16)	7.69 (2.29–25.84)	<0.01*
Battery cage [a]	153	110 (71.90)	1.00	
Season				
Wet	122	97 (79.51)	1.13 (0.58–2.19)	0.71
Dry [a]	93	72 (77.42)	1.00	

[a] Reference category, N=number of rabbits in each category, * Significant, OR = Odds Ratio, CI = Confidence Interval

of *Eimeria* infection in New Zealand White rabbits was significantly lower than Dutch rabbits (OR=0.42; 95% CI 0.18–0.94) (P<0.05; = 4.67). Growers had the highest prevalence (80.00%), followed by bunnies (79.07%, OR=1.16) and adults (77.45%, OR=1.10) (P>0.05). The prevalence of *Eimeria* was higher in female (104/131; 79.39%) than male (65/84; 77.38%, OR=1.13) (P>0.05 = 0.12).

Rabbits housed in deep litter system had higher prevalence (59/62, 95.16%) than those housed in the battery cage housing type (110/153, 71.90%). The infection with *Eimeria* species was 7.69 times (P<0.05) likely to occur in rabbits raised in deep litter compared to those raised in battery cage. *Eimeria* infection was more prevalent during the wet season (97/122; 79.51%) than to the dry season (72/93; 77.42%), (OR=1.13) (P>0.05).

The multivariate model revealed that breed and housing type had significant association with *Eimeria* infection (Table 3). The Chinchila, Californian and New Zealand White breeds were 1.59, 1.51 and 0.39 times respectively, more likely to be positive for *Eimeria* infection compared to the

Table 3. Multivariate association between risk factors and *Eimeria* infection among rabbits in Ilorin, Kwara State

	β	SE	*P*	OR	95% CI	
					Lower	Upper
Breed						
Californian	-0.41	0.57	0.47	1.51	0.49	4.63
Chinchila	-0.46	0.48	0.34	1.59	0.62	4.11
New Zealand White	0.94	0.44	0.03*	0.39	0.16	0.93
Dutch [a]				1.00		
Housing type						
Deep litter	2.21	0.63	<0.01*	9.08	2.64	31.23
Battery cage [a]				1.00		

[a] Reference category, * Significant, OR = Odds Ratio, CI = Confidence Interval, β = Regression coefficient, SE = Standard Error

Dutch breed. Rabbits raised in deep litter type of housing were at higher risk of infection (9.08) compared to their counterparts raised in the battery cage housing type.

Discussion

Coccidiosis is associated with major economic losses in rabbit farming including morbidity and mortality, growth retardation and condemnation of affected livers in cases of hepatic coccidiosis [6] and remains one of the most important infectious causes of digestive disorders in rabbits [7]. These reasons sparked the interest for this present study as this appears to be the first in terms of *Eimeria* species diversity and its associated risk factors in this animal subject in Nigeria.

This study demonstrates as in other parts of Nigeria that rabbits habour *Eimeria* species [14–16]. The result of this study revealed that, various *Eimeria* species were found in Ilorin, Kwara State cutting across sexes, ages, breeds, housing types, and seasons. The interplay and significance of various risk factors such as sex, age, breed, housing type, and season were also elucidated.

An overall prevalence of 78.6% was recorded in this study. Comparatively with other studies done within Nigeria, the overall prevalence is higher than the 38.0% reported by Musungong and Fakae [15] in their study done in Nsukka, and the 66.7% reported in Jos by Dogo et al. [16]. In relation to other studies done outside of Nigeria, our finding was higher than the 75.0% reported in Saudi Arabia [2], 72.5% reported in Baghdad [1], 41.0% in China [23] and 31.0% in Iran [24] but lower than the 90.2% reported in Kenya [25] and 90.0% reported in Saudi Arabia [26]. A similar prevalence rate as reported in our study was recorded in China [27]. The variation in the overall prevalence of this study with other studies within Nigeria and in other countries may be due to different factors such as: study location, meteorology, agro-ecology, environmental conditions, sample size, sampling technique, period of sampling, and study design [25,26]. The high prevalence reported in our study could be linked to poor sanitary practice, large population of young rabbits (bunnies and weaners) sampled, coprophagy, which is a peculiar habit of rabbits to gain access to vitamins and proteins synthesized in the large intestine, which could keep infection for a longer time in the flock and could promote the spread of coccidiosis in the population, and it is considered a possible important route in the establishment of coccidiosis in healthy rabbit from the infected ones [20,28]. In addition, the adult infected rabbits, which are usually symptomless, act as potential carriers within the free environment and transmit a continuous low-grade dose of oocysts to other rabbits particularly the younger ones [29,30].

Californian breed had the highest prevalence rate followed by Chinchila and the Dutch breed, with the New Zealand White having the significantly lowest prevalent rate. This may be attributed to breed dichotomy as resistance to diseases conditions vary greatly from one breed to another, as well as variation in immune response [31], which may be responsible for our finding.

It was observed that bunnies and weaners had a higher prevalence than adults. The highest prevalence recorded in weaners supports earlier reports that it could be attributed to several factors surrounding weaner rabbits; first, naive rabbits are more susceptible to infection from adult carriers especially after weaning [9,10], Secondly, weaning stress has been reported to lower the immunity of rabbits to infections [10] and thirdly ingestion of coccidian parasites along with contaminated solid feed during weaning period when the ingestion of solid feed begins may raise the intensity of infection for the weaners [10].

We discovered a higher infection rate in females compared to males. Similarly, Khider et al. [1] reported a higher prevalence of *Eimeria* species in female compared to male in their study conducted in Baghdad. Pregnancy, parturition and nursing of the young are stressors that can affect the immune system negatively, which may be the reason behind the higher prevalence recorded in female.

Higher prevalence of infection was recorded in deep litter housing type compared to that of battery cage. This may not be unconnected with the fact that rabbits in deep litter come in contact with their faeces and so, have access to sporulated oocysts and the possibility of the high prevalence we observed [25].

The study recorded higher prevalence during the wet season compared to dry season. This finding is in line with that reported by Laha et al. [32]. This may be due to high humidity seen during the wet season which is necessary for sporulation of oocyst which is the infective stage. Harcourt-Browns [28] stated that oocysts can survive for a long time in the humid environment but they are susceptible to dry conditions.

In our study, *E. coecicola* was the most prevalent *Eimeria* species, followed by *E. irresidua*, *E. perforans*, *E. magna*, *E. intestinalis*, *E. stiedai* and *E. flavescens*, respectively. Among the *Eimeria* species detected, only *E. stiedai* affects the liver, while others affect the intestine [4,25,33]. Based on their clinical symptoms, *E. coecicola* and *E. perforans* are grouped as the non-pathogenic species, while *E. irresidua*, *E. magna*, *E. intestinalis*, *E. stiedai* and *E. flavescens* are grouped as the pathogenic species [4,19,33]. The fact that the non-pathogenic species were of higher prevalence than the pathogenic species, may have resulted to the very low to negligible mortality rate observed in the sampled rabbit farms.

Highest prevalence with *Eimeria coecicola* in rabbits was also recorded in Riyadh, Saudi Arabia [2] and Iberian Peninsula, Southwest Europe [34]. Also, high prevalence of *E. irresidua* has been reported among rabbits in Northwest China [27] and Sichuan province, Southwest China [33].

Although the rabbits in the present study had no clinical manifestations of either intestinal or hepatic coccidiosis, presence of highly pathogenic species indicates that any weather alterations such as sudden heavy rain fall or occurrence of immune deficient diseases may act as risk factors for establishing rabbit coccidiosis in this area. It has also been established that the adult symptomless infected rabbits, transmit a continuous low-grade dose of oocysts to younger rabbits; these low dose of infected oocysts acts similarly to an attenuated organism in vaccines and activates the immune system of the newly infected rabbits and consequently they become protected by this acquired immunity [20,30].

In conclusion, this happens to be the first study to demonstrate the different species of *Eimeria* that infects domestic rabbits (*Oryctolagus cuniculus*) in Nigeria. Seven species of *Eimeria* was detected to affect rabbits in Ilorin, Kwara State. It also provides an insight into the prevalence and associated risk factors that are at interplay in the endemicity of rabbit coccidia infection in the study area. The study also demonstrates that rabbit coccidiosis is endemic in Ilorin with breed and housing being the risk factors playing significant roles in its endemicity. There is need for constant veterinary surveillance so as to prevent economic losses in the rabbit breeding industry as a result of coccidiosis.

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