

Epidemiology of *Eimeria* Infections in Calves in Addis Ababa and Debre Zeit Dairy Farms, Ethiopia

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ABSTRACT

A cross-sectional study was carried out to determine the prevalence, species composition, and associated risk factors with *Eimeria* infection in calves of 40 dairy farms in Addis Ababa city and Debre Zeit town. Of the total 580 calves examined for *Eimeria* infection, 68.1% (395) were infected with 1 or more *Eimeria* species. A total of 11 species of *Eimeria* were identified. The most prevalent was *E. bovis* found in 38.45% of the examined samples. The other species found were *E. zurnii* (18.1%), *E. auburnensis* (17.9%), *E. canadensis* (16.9%), *E. ellipsoidalis* (11.2%), *E. subspherica* (6.7%), *E. cylindrica* (6.4%), *E. alabamensis* (4.7%), *E. wyomingensis* (4.3%), *E. bukidnonensis* (3.1%), and *E. brasiliensis* (1.2%). *Eimeria* oocysts were detected in calves from 15 to 345 days of age. There were no statistically significant ($P > 0.05$) associations between infection by *Eimeria* and the presence of diarrhea in examined calves. Agro-ecology and age of the study calves were observed as

the most important factors associated with occurrence of *Eimeria* infections in calves. There was strong association ($P < 0.001$) between the intensity of infection, age of the calves, and consistency of the feces. The observation of pathogenic species of *Eimeria* (*E. bovis* and *E. zurnii*) in calves in this study suggests that coccidiosis may be contributing to the enteric syndromes affecting calves of the area.

INTRODUCTION

Eimeria infections are one of the most common and important disease of cattle worldwide. Bovine coccidiosis has been observed in almost all areas where cattle are raised and is usually most common and important in calves younger than 1 year. All calves managed under conventional systems are exposed and become infected early in life. Many studies indicated that under natural conditions, mixed species infections are much more common than monospecies infections.¹⁻³ Eimeriosis in cattle is particularly a problem of confined animals kept under intensive husbandry practices. The disease is more common in housed animals than

in those on pastures. In associations with other enteropathogens, coccidia have been indicated as an important cause of diarrhea in calves.²

All age groups of cattle are susceptible to infection, but clinical eimeriosis is most common in young animals. Coccidiosis in cattle commonly occurs as subclinical disease without signs of the disease and involving great economical losses due to reduced appetite, reduced body weight, impaired feed conversion, unthriftiness, diarrhea, dysentery, anemia, and increased susceptibility to other diseases.^{1,4} The development of clinical coccidiosis in cattle mainly depends on factors like species of *Eimeria*, age of infected animal, number of oocysts ingested, presence of concurrent infections, and type of production system and management practices.^{1,5} Compared to clinical coccidiosis, subclinical coccidiosis is economically more important and may account for over 95% of all the losses associated with coccidiosis and can cost cattle ranchers more than US\$400 million per annum. It can also delay growth of calves by as much as 2 months.⁶

A number of epidemiological factors like moisture, temperature, and oxygen tension influence the pattern of the disease.^{7,8} In addition, stress factors like weaning, change of diet, harsh environment, poor nutrition and sanitation, and overcrowding can increase level of infection and incidence of the disease due to stress-induced immunosuppression.^{5,8-12}

More than 13 species of *Eimeria* have been described to infect cattle.^{3,5,10,13} Of these many species, *E. bovis* and *E. zurnii* are considered the most pathogenic species as they are usually associated with clinical coccidiosis under field conditions. Thus determination of prevalence, species composition, associated risk factors, and animal management and husbandry practices is very useful in designing efficient control strategies.^{1,5,14} The prevalence, species composition, and importance of bovine coccidiosis has been documented in various countries of the world; however, it is excluded from reports on animal morbidity and mortal-

ity in Ethiopia. To the authors' knowledge, a single study available on the coccidia of cattle in Ethiopia is the work of Kassa et al¹⁵ who reported outbreak of coccidiosis due to *E. zurnii* and an overall prevalence of 24.9% in a 5-year retrospective laboratory examination in cattle in a study conducted in the Abay Tana settlement dairy farm in Bahr Dar. As a result, there is paucity of information on the occurrence and losses associated with bovine coccidiosis and very little attention has been given to the role of coccidiosis as the cause of disease and production losses in cattle in Ethiopia. Moreover, no attempt was made to determine the prevalence, species composition, and associated risk factors of *Eimeria* infections in cattle.

Therefore, taking into account the significance of the parasite as one of the most important causes of economic losses and the scarcity of information in the country, the present study was designed to determine the prevalence, species composition, and associated risk factors with *Eimeria* infections of calves in Addis Ababa and Debre Zeit dairy farms.

MATERIALS AND METHODS

Study Area and Animals

The study was conducted in Addis Ababa city and Debre Zeit town, both of which are located in central Ethiopia. Addis Ababa, the capital city of Ethiopia, is situated in a highland area with an altitude of 2500 meters above sea level and received an average annual rainfall of 1800 mm during the study period. Debre Zeit is located about 45 km southeast of Addis Ababa at an altitude of 1850 meters above sea level and received an average annual rainfall of 800 mm during the same time. The study was conducted on calves younger than 12 months that were found in 40 farms selected from a total of 154 dairy farms possessing 10 or more cows per farm. A total of 580 fecal samples were collected from the 40 dairy farms.

Study Design

For this study, one-stage cluster sampling method was employed as described by

Thrusfield.¹⁶ A total of 580 calves <12 months of age from a total of 40 dairy farms were selected for the study with 95% confidence interval and 5% desired absolute precision. Farms were selected by stratified random sampling method based on herd size. All calves <12 months of age in the selected farms were included in the study. Each farm was visited once during the study period from September 2004 to March 2005 to collect fecal samples, data on demographic, management, hygiene, and other factors hypothesized to be associated with the risk of infection of calves with *Eimeria* species.

Sample Collection

About 30 g fresh fecal sample was collected per rectum from each calf using sterile disposable plastic gloves. The sample was placed in a labeled clean plastic container and transported in a cool box to the parasitology laboratory on the same day of collection, and preserved at refrigeration temperature until processing within 48 hours of arrival. At the time of sampling, the name of the farm, date of sampling, consistency of the feces (soft, pasty, watery, or normal), and the age, sex, breed, and tag number were recorded for each calf on a recording format.

Parasitological Investigations

Quantitative fecal examination was performed by McMaster technique to determine the number of oocysts per gram of feces (OPG) as per the procedures of Kaufmann.¹⁷ Samples with more than 500 OPG were

mixed thoroughly with 2.5% (w/v) potassium dichromate solution and allowed to sporulate for 10-14 days at room temperature and used for species identification. After sporulation, the fecal mixture was centrifuged and the sediment was processed by the centrifugal flotation procedure using Sheather's sugar solution with specific gravity of 1.27 to recover the oocysts.¹⁸ Identification of *Eimeria* species was based on the morphological features of the sporulated oocysts (size, shape, color, and texture of oocyst wall, presence or absence of micropyle, polar cap, and time of sporulation) with the aid of taxonomic keys.^{10,19,20} Oocysts were measured under ocular eye piece that was calibrated with a micrometer under a 40x objective of a microscope.

Data Analysis

Data collected from study sites were entered into an Excel spreadsheet (Windows) and analyzed with SPSS for Windows (Version 11.5) and STATA for Windows (Version 7) statistical software. The prevalence was calculated for all data as the number of infected individuals divided by the number of sampled individuals x 100. Categorical data were analyzed first with the Chi-square (χ^2) test for independence as a screening process. This test was followed by stepwise multivariate logistic regression to account for confounding variables and interactions. A *t*-test was used to compare mean OPG between 2 groups, whereas analysis of variance was

Table 1. Prevalence of *Eimeria* Infection in Relation to Geographical Zones and Age of Calves.

Factor	Calves Examined, n	Positive, n (%)	OR	95% CI of OR	χ^2	df	P Value
Geographical zone							
Midland (Debre Zeit)	250	57.2	1				
Highland (Addis Ababa)	330	76.4	2.4	1.7-3.5	24.1	1	0.000
Overall	580	68.1					
Age in months							
0-6	381	61.4	1				
>6-12	199	81	2.7	1.8-4.0	22.85	1	0.000

OR =odds ratio, n =number, CI =confidence interval.

Table 2. *t*-Test Analysis of the Association Between Ages of the Calves With Intensity of Infection.

Age in Months	Mean OPG	SD	SEM	Mean Difference	95% CI of the Difference	<i>t</i>	df	<i>P</i> Value
0-6	8463	28574.5	2468.5					
>6-12	1235	1594.9	148.1	7228	1994-12462	2.72	248	0.007

used to compare mean OPG between 3 or more groups. A *P* value of less than 0.05 was required to indicate significance. Odds ratios (ORs) were determined from the coefficients in the logistic regression.

RESULTS

Of the total of 580 fecal samples of calves examined 395 (68.1%) contained oocysts of *Eimeria* (Table 1). All of the 40 surveyed dairy farms (100%) had 1 or more calves shedding *Eimeria* oocysts. A total of 11 species of *Eimeria* were identified. The most prevalent was *E bovis* found in 38.45% of the samples. The other species found were *E zurnii*, *E auburnensis*, *E canadensis*, *E ellipsoidal*, *E subspherica*, *E cylindrica*, *E alabamensis*, *E wyomingensis*, *E bukidnensis*, and *E brasiliensis* present in 18.1%, 17.9%, 16.9%, 11.2%, 6.7%, 6.4%, 4.7%, 4.3%, 3.1%, and 1.2%, respectively. Of the 395 calves positive for *Eimeria*, 45.6% were infected with single species, whereas the remaining 54.4% were infected with 2 to 7 species.

In univariate analysis with Chi-square (χ^2) test for independent samples, herd size, preweaning calf-housing condition, frequency of cleaning calf-rearing houses, and the method of cleaning were not significantly (*P* > 0.05) associated with the risk of infection. There was a statistically significant ($\chi^2 = 24.10$, *P* < 0.001) association between the geographical zones from where samples were taken and the risk of infection. Age of the calves was strongly associated ($\chi^2 =$

22.85, *P* < 0.001) with the risk of infection with *Eimeria* species. Oocysts of *Eimeria* species were recovered in calves over a wide range of ages from 0 to 345 days. The first age at which the oocysts of *Eimeria* species were detected was 15 days. A significant ($\chi^2 = 7.11$, *P* = 0.008) association was also observed between the feeding system of calves (whether in feed troughs or directly on the ground) and the risk of infection with *Eimeria* species. Of the 580 calves sampled, 121 were diarrheic, 124 had soft feces, and 335 were non-diarrheic; the infection with *Eimeria* species was not significantly (OR = 1, *P* > 0.05) associated with diarrhea in examined calves.

The McMaster technique employed to determine the OPG revealed mean and maximum OPG values of 5109 and 267,000, respectively. There was a highly significant (*t* = 2.72, *P* = 0.007) association between age of the calves and the intensity of infection (Table 2). As indicated in Table 3, a highly significant (*F* = 8.075, *P* < 0.001) association was observed between fecal consistency and the intensity of infection. Geographical zone was insignificantly (*P* > 0.05) associated with the intensity of infection. Analysis of all the hypothesized risk factors by stepwise multivariate logistic regression model revealed that the geographical zone where samples were collected and age of the calves were the only factors significantly associated with *Eimeria* infection with *P* values of 0.032 and 0.031, respectively (Table 4).

Table 3. Test for Association Between Fecal Consistency and Intensity of Infection Using One-Way Analysis of Variance.

Fecal Consistency	Calves, n	Mean OPG	SD	SE	Mean Difference	95% Confidence Interval for Mean	<i>P</i> Value
Normal	166	2943	10,686	829		1305.5-4581	
Soft	42	2018	4295	663	925	679-3356	0.795
Diarrhea	42	16,761	45,707	7053	13,818	2518-31,004	0.000

Table 4. Multivariate Logistic Regression Analysis of all the Hypothesized Risk Factors With *Eimeria* Species Infection in Calves.

Risk Factor	Coefficient	SE	z	P > z	95% CI for Coefficient
Geographical zone	0.26	0.04	3.88	0.032	0.023-0.51
Age in months	0.24	0.04	3.98	0.031	0.022-0.45
Feeding system	0.04	0.05	0.77	0.443	-0.06-0.15
Herd size	-0.03	0.03	-1.00	0.319	-0.10-0.03
Preweaning housing	0.001	0.04	0.03	0.979	-0.08-0.09
Frequency of cleaning	-0.03	0.05	-0.62	0.537	-0.14-0.07
Cleaning method	0.03	0.06	0.54	0.587	-0.09-0.15

DISCUSSION

The overall prevalence of *Eimeria* species infection (68.1%) in the present study is higher than previously reported by Kassa et al¹⁵ who found 24.9% *Eimeria* infections in cattle in Tana based on a retrospective laboratory study. This suggests that *Eimeria* infections are more prevalent in central highland Ethiopian dairy farms. It is also higher than previous reports,^{7,21-24} all of which reported an overall prevalence within the range of 19.3% to 59%. The results of the present study, however, are in line with the report of 64.4% in Canada,²⁵ 67.4% in Kenya,²⁶ 68% in Turkey,²⁷ and 70% in South Africa.²⁸ The results are lower than the observations of Ernst et al^{2,29} and Rodriguez-Vivas et al.³⁰ This variation is most likely attributed to the differences in agro-ecology, management, and husbandry practices of the study animals in different countries.

The study showed that *E. bovis* (38.45%) was the most prevalent species followed by *E. zurnii* (18.1%) and *E. auburnensis* (17.9%). These species are the most frequently reported coccidia in outbreaks of coccidiosis throughout the world.^{14,31,32} Even though most of the examined calves were infected with *Eimeria* species, clinical coccidiosis was observed only in a small proportion of the infected calves (16.20%). This observation suggests that most *Eimeria* species in calves in central Ethiopian dairy farms usually result in subclinical infections. This high prevalence of pathogenic species (*E. bovis* and *E. zurnii*) in infected calves and the greater proportions of subclinical

infections could negatively influence animal productivity and cause economic losses from poor feed efficiency, slow weight gain, weight loss, failure of the calves to grow to their full potential, and increased susceptibility to other diseases.^{13,33} Moreover, continuous oocysts shed from subclinically infected calves contaminate the environment of calves or the hair coats and cause severe coccidiosis in highly susceptible new calves that are kept in these areas.^{33,34}

In this study, mixed infections of a single calf host with several *Eimeria* species were commonly observed. The number of *Eimeria* species in mixed infection per examined sample ranged from 2 to 7. This finding is similar to the works of Ernst et al² in the United States and Cornelissen et al²⁴ in The Netherlands, both of which reported 7 species; Arslan and Tuzer²⁷ in Turkey who reported 6 species; and Kennedy and Kralka¹⁰ who reported 5 species in Canada.

The mean (5109) and maximum (267,000) OPG levels observed in this survey were much higher than those reported from other countries. For instance, Kennedy and Kralka¹⁰ reported an average of 25 and a maximum of 109,449 OPG in Canada; Munyua and Ngotho²⁶ reported a maximum of 30,600 OPG in Kenya; and Arslan and Tuzer²⁷ in Turkey reported respective values of mean and maximum OPG of 1280 and 52,000. In the current survey, 10.4% of the infected calves had oocyst counts above 5000 OPG of which the 2 most pathogenic species *E. bovis* and *E. zurnii* composed the largest proportions of 69.23% and 38.5% in

infected calves, respectively. As presented in Table 3, the mean OPG was significantly ($P = 0.023$) higher in diarrheic than non-diarrheic calves. This is in line with the observations of Chibunda et al,¹³ Kennedy,³⁴ and Svensson,³⁵ all of which stated that development of clinical disease depends on the number of oocysts ingested.

Even though the rate of infection increased with the age of examined calves, the intensity of infection decreased with age of calves. As shown in Table 2, the highest intensity of oocysts output was detected in calves 6 months of age and younger than in calves older than 6 months. This suggests the presence of immature immune system in younger calves resulting in more susceptibility to coccidiosis than older calves with immunity from previous exposure, hence more resistant to subsequent reinfections as indicated by Chibunda et al¹³ and Faber et al.³¹

A strongly significant association ($P < 0.001$) was observed between the geographic zones where samples were collected and the risk of infection with *Eimeria* species. The odds of shedding *Eimeria* oocysts were greater by 2.4 times among calves of Addis Ababa dairy farms than calves from Debre Zeit dairy farms (Table 1). This is most likely attributed to the relatively higher rainfall and relative humidity in Addis Ababa, which is a much more conducive climatic condition for the survival, sporulation, and development of the oocysts in Addis Ababa than in Debre Zeit. This observation is in agreement with the work of Rodriguez-Vivas et al³⁰ who reported higher infection rates of *Eimeria* in cattle in high rainfall zones in Mexico.

The age of the calves was also strongly associated ($P < 0.001$) with risk of infection that was reflected by the prevalence *Eimeria* species and appeared to increase with the age of the examined calves. As a result, higher infection rates were observed in calves >6-12 months of age (81%) than calves of 0-6 months of age (61.4%), and the odds of shedding *Eimeria* oocysts among calves of >6-12 months of age was greater

by 2.7 times than the odds of shedding among those calves that were younger than 6 months (Table 1). This is most likely due to the fact that almost all of the study calves older than 6 months were housed as large numbers in overcrowded condition and in physical contact with adult animals that favored higher infection rate from a greater chance of licking each other and ingestion of large number of oocysts. These findings are also in line with earlier studies of Oda and Nishida,²³ Rodriguez-Vivas,³⁰ Pilarczyk et al,⁷ and Kennedy.³⁴

There was also a significant associations ($P < 0.008$) between the likelihood of *Eimeria* infection and whether the calves fed directly on the ground or in feed troughs. As a result, the odds of shedding *Eimeria* oocysts was greater by 1.8 times among calves feeding directly on the ground than the odds of shedding among calves feeding in troughs. This finding is in agreement with the recommendation described by Radostits et al¹² and Kennedy³⁴ to avoid the feeding of calves on the ground as this increases the chance of contamination of the feed with *Eimeria* oocysts.

Further epidemiological investigation on economic significance, prevalence, species composition, and biology of bovine eimeriosis in different agroecology, management systems, seasons, and all age classes of cattle in Ethiopia needs to be pursued.

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