Prevalence of calf coccidiosis in Mekelle, northern Ethiopia

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Abstract

A cross-sectional study was conducted from November 2015 to March 2016 with the objectives of estimating the prevalence of coccidiosis, identifying the most known pathogenic coccidia species involved, and assessing the overall knowledge of dairy farm owners and workers on calve coccidiosis and adapted control methods in randomly selected 7 kebeles of Mekelle dairy farms. Two hundred thirty two fecal samples were randomly collected from calves belonging to seventy dairy farms and examined for the presence of oocysts of *Eimeria* by floatation technique using saturated salt solution. For positive samples, solution of 2.5% potassium dichromate was added to the feces containing the oocyst for sporulation and identification of the *Eimeria* species. The risk factors and the overall knowledge on calf coccidiosis and adapted control methods were assessed by two types of questionnaires and a prepared observational format. The overall prevalence of coccidiosis was 72.7% (169/232) and the identified *Eimeria* species were *E. bovis* and *E. zuernii* with the prevalence of 26.6% (45) and 11.2% (19); respectively. There was statistically significant difference ($p< 0.05$) among various age categories with the infection of *Eimeria* species. However, sex and body condition of animals were not significantly associated ($p > 0.05$) with *Eimeria* infection. Questioner survey result of animal health experts and owners showed 33 calves were suspected to be infected with pathogenic *Eimeria* spp. In conclusion, the study revealed that calf coccidiosis is prevalent in dairy farms of Mekelle. Hence, appropriate disease prevention and control program need to be undertaken to reduce its impact.

Keywords: Calve; Coccidiosis; Eimeria; Mekelle; Prevalence; Risk factors

Introduction

Eimeria infections are one of the most common and important diseases 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 one year (Daugschies and Najdrowsk, 2005). About 11 species of *Eimeria* have been identified and documented to cause disease in cattle (Coetzer and Justin, 2004) and the most common pathogenic species in cattle are *E. bovis and E. zuernii* (Maas, 2007). All calves kept under traditional management systems are exposed and become infected early in life (Daugschies and Najdrowsk, 2005). In association with other enteropathogens, coccidia have been indicated as an important cause of diarrhea in calves on the first few months of their age (Abebe *et al.*, 2008).

As coccidiosis spreads from one animal to another by contact with infected feces, it is one of the serious problems for calf rearing industry. The most common clinical manifestations include in-appetence, weakness, loss of weight, diarrhea, depression and anemia. 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 practice (Alula *et al.,* 2013)

The prevalence, species composition, and importance of bovine coccidiosis have been documented in various countries of the world. In Ethiopia, Dawid *et al* (2012) reported that young age and poor hygienic status of the farms were strongly associated with infection of coccidiosis in dairy farms. However, in another study, Alula *et al* (2013), agreed that age was a significant factor but breed, body condition, sex, and management system were not significantly associated with the disease. According to Dawid *et al* (2012), although coccidiosis is an important cause of calf morbidity and mortality in Ethiopia, very little attention has been given to this disease.

Reports on coccidiosis are scarce in various parts of the country. Mekelle is one of the big cities of Ethiopia where urban and periurban dairy farms dwell to provide milk for population of the city. No study has been undertaken to assess the magnitude of this disease in this particular area. Therefore the objectives of this study were to estimate the prevalence of coccidiosis in dairy calves with identification of most known pathogenic coccidia spp. involved and assessing

the overall knowledge of dairy farm owners and workers on calve coccidiosis and adapted control methods.

Materials and methods

Study area

The study was conducted from November 2015 up to March 2016 in Mekelle, northern Ethiopia. Mekelle is the capital city of Tigray Regional State which is located 783 km north of the capital city of Ethiopia. Its geographic location is 13°32'N and 39°33'E. The city presently has 7 sub cities named as Hawelti, Hadnet, Ayder, Semen, Quiha, Kedamay Weyane and Adi-Haqi where the study were implemented (Fig. 1). It has an average altitude of 2200 m.a.s.l with a mean minimum and maximum annual temperatures of 8.7 and 26.8, respectively. Mekelle receives an average of 600 mm of rainfall annually and more than 70% of the rain falls between July and August, followed by long dry season (October to May) (MOM,1998).

Urban agriculture is a common practice in Mekelle. The livestock production practiced in this area includes extensive, intensive and semi intensive management systems. The livestock population of the area includes a total of 36,516 cattle; 8,442 shoats; 800 horses; 200 mule; 3,080 donkey; 100 camel; 53,796 poultry 3000 dogs according to the report of Mekelle Zonal Agricultural Office (MZAO, 2009).

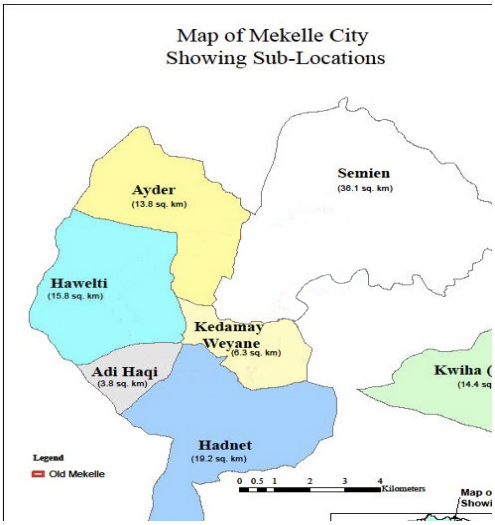

Fig.1. Map of Mekelle city with its sub cities, adapted from Moumie and Tesfu, (2008)

Study ppopulation and study design

Cross sectional study was conducted from November 2015 to March 2016 in Mekelle, northern Ethiopia. The study population included both sexes, cross and local breeds of dairy cattle from birth up to 1 year of age belonging to dairy farms. Fecal samples were collected from all age group calves. Potential risk factors and practiced control measures for coccidiosis and differential diseases were assessed using pretested semi structured questionnaire. The samples were collected from all existing sub cities by allocating proportional number of dairy farms. The questionnaire focused on animal health and production experts and owners or managers or other workers who had past experience in each dairy farm.

Sample size determination

The sample size required for this study was calculated based on sample size determination method previously described (Thrusfield, 2005).

$N=[1.96^2 Pexp\ (1-Pexp)]/\ d^2$

Where, N = required sample size, Pexp = expected prevalence, d= desires absolute precision.

Accordingly, based on the expected prevalence of 22.7% in Dire Dawa selected dairy farms (Dawid *et al.,* 2012*)* with 5% absolute precision at 95% confidence level, the sample size was determined to be 232 calves.

Sampling procedure

For fecal sample collection, all 7 sub cities were selected purposively. From each sub cities, 10 dairy farms were selected randomly and purposely all calves below age of 12 months were sampled based on the potential number of calves of each dairy farm to achieve the sample size (N=232). All calves were selected until required sample size was achieved, since the required age group of study animals was very low in selected dairy farms. With regard to selection of the owners as respondent, a total of 70 were selected purposely from all the randomly selected dairy farms of all sub cities. In addition, a total of 24 professionals working as public and private animal health experts were selected purposely to respond to the specific questions in the prepared questioner.

Determination of age and body condition scoring of calves

The ages of calves were determined by collecting information from the dairy farm owners and then categorized in to birth to <6 months and ≥ 6-12 months. Body condition score (BCS) of the calves was estimated and scored in the range 1-5 based on modified Nicholson and Butterworth, (1986). However, for the purpose of data analysis it was categorized in to three major categories, Poor (BCS: 1-2), Moderate (BCS: 2-3.5), Obese (BCS>3.5) (NAWC, 2005).

Fecal sample collection and laboratory examination

The 232 fresh fecal samples of about 20 gm were collected from the rectum of each calf using sterile disposable plastic gloves. The sample was placed in a label clean glass bottle container and transported to the parasitology laboratory on the same day and were kept at 4°C in a refrigerator until processing within 48 hours of arrival in the laboratory.

The fecal samples were examined with a flotation method using saturated sodium chloride solution (specific gravity: 1.20) (Yu *et al*., 2011). To determine oocyst per gram (OPG) of feces, 3 grams of feces was weighed from each fecal

sample. Then, 42 ml of tap water was added to the 3 gram of fecal samples in a plastic beaker and homogenized by stirring until the sample and water mixture was completely homogenized. It was then filtered and centrifuged and the sediment re-suspended in saturated sodium chloride solution. The sediment was thoroughly mixed with the sodium chloride solution and filled into the chambers of McMaster slide by Pasteur pipette and counted under 10x magnification using microscope. The oocyst was counted to determine parasitic load by McMaster technique as documented by Nolan *et al* (2006).

For a sporulation of pathogenic *Eimeria* oocysts, a solution of 2.5% potassium dichromate was added to each positive fecal sample, which contained most of the *Eimeria* oocyst in a beaker, mixed thoroughly with a wooden applicator and poured into a petri dish. Each petri dish was left on the bench for 10–14 days at room temperature to allow sporulation of *Eimeria* spp. (Alula *et al*., 2013). The *Eimeria* species were determined based on the morphology of oocysts and sporocysts (shape, color, micropyle and its cap, presence or absence of residual, polar granule) and time of sporulation (Yu *et al*., 2011). The size of pathogenic oocysts was measured using calibrated micrometer under a 40x objective of a microscope. The microscopic morphology of *E. bovis* is ovoid, yellow in colour and has micropyle at narrow end and that of *E. zurnii* has spherical shape, colorless and does not contain micropyle. In relation to dimension, *E. bovis* has about 27.7x20.3 microns & *E. zurnii* 17.8 x15.6 microns (Tylor *et al*., 2007)

Assessment of the management practices of dairy calves

All the management practices and potential risks that could influence the presence of coccidiosis were recorded using questionnaires and prepared observational format. The collected data inlcude status of house ventilation, hygiene, housing type, stocking rate, sanitation of bedding, knowledge of farmers on the disease, waste disposal means, disease control methods practiced, situation of contact with other animals, type of feeding and watering methods etc. (Curt, 2005). A total of 15 were selected for pretesting and 70 owners or managers or workers were interviewed randomly on the selected dairy farms. Pretesting questionnaires were not included in the analysis. Regarding the experts, questionnaire was distributed purposely to all experts who had direct contact with the sampled dairy farms. Finally, the prepared observation format was filled during sample collection in all randomly selected dairy farms.

Statistical analysis

Statistical analysis was carried out using STATA Version 11. Descriptive statistics were used to calculate various proportions. Chi-square (χ^2) test and Fisher exact test were used to determine the significant association among different risk factors of calf coccidiosis as appropriate.

Results

Prevalence of coccidiosis and causative *Eimeria* species

Out of 232 fecal samples examined, 169 (72.8%) were found to be positive for *Eimeria* species. The identified pathogenic spp. were *E. bovis* 45 (26.6%) and *E. zuernii* 19 (11.2%) and the remaining were none pathogen *Eimeria* species.

Table 1. Rate of occurrence of pathogenic *Eimeria* species

Eimeria spp.	Number of positive (%)	Range of OPG* of faeces	Mean OPG of feces
E. bovis	45(26.6)	50-12,000	8,325
E. zuernii	19(11.2)	100-8400	6,430
Overall	169(72.8)	50-32,000	18,170

*- oocyst per gram of feces

The species of most pathogenic *Eimeria* were identified on the basis of their morphological characters (Fig 2). The average length x width of the measured species was 16.3x14.1µm for *E. zurnii* and 25x19µm *E. bovis*.

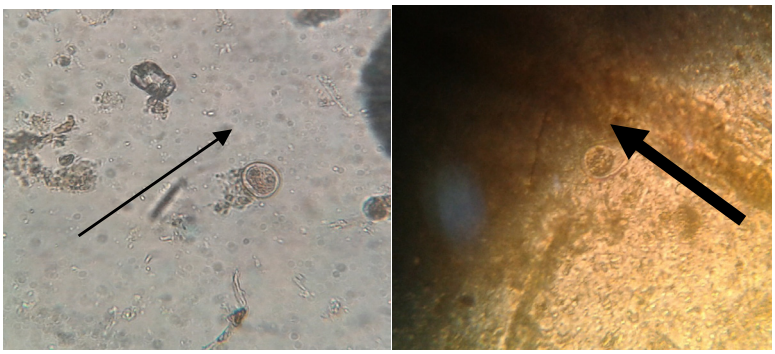

Figure 2. Morphology of *E. zuernii* oocyst (left) and *E. bovis* oocyst (right) X400

The intensity of non-pathogenic and pathogenic *Eimeria* infection

The intensity of infection as measured by number of OPG of feces is an important factor that describes the severity of the infection in particular animal. The maximum overall *Eimeria* oocyst counted was 32,000 OPG. With regards to the pathogenic species, the highest intensity was 12,000 for *E. bovis* and 8400 for *E. zuernii* as shown in Table 1. From the 7 selected sub cities, the highest intensity of both pathogenic *Eimeria* species was found in Adi Haqi sub city as shown in Fig. 3.

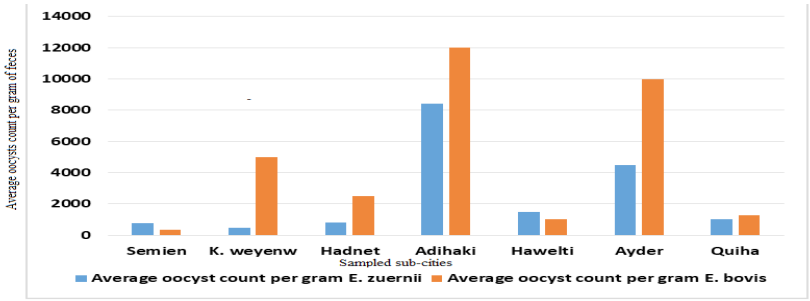

Figure 3. Average intensity of the two pathogenic *Eimeria* species in each sub city

The present study revealed that there is a significant association ($p<0.05$) between the occurrence of coccidiosis and age of calves (Table 2). Higher prevalence was recorded in those calves with age categories of ≥6-12 months). However, there is no significant difference ($p>0.05$) between sex and among animals with different body conditions (Table 2).

Table 2. Prevalence of *Eimeria* species in calves with respect to age, sex and BCS

Variable	Lab. Result in number (%)		X^2(p-value)
	No. (%) Positive	No. (%) Negative	
Age			
<6 months	68(66)	35(34)	4.3626(0.037)
≥6 months	101(78.3)	28(21.7)	
Sex			
Male	24(63.2)	14(36.8)	2.1557(0.142)
Female	145(74.7)	49(25.3)	
BCS			
Thin	16(84.2)	3(15.8)	
Moderate	152(73.1)	58(26.9)	0.257
Fat	0(0)	1(100)	

Identified gaps in farm management practices and knowledge of farmers in relation to coccidiosis in dairy farms

Management practices that could predispose dairy farms to *Eimeria* spp. were recorded. The lack of knowledge of the dairy farm owners about coccidiosis (80%), absence of proper waste disposal (72.9%), contact of calves with other animal (75.7%), poor hygienic practices in feeding and watering (62.9%) were some of management problems that could predispose the dairy farms for coccidiosis (Table 3). All interviewed animal health experts responded that they have seen major clinical signs that look like coccidiosis & have got response after treatment on calves during their practice (Table 3).

Among observational assessment, lack of isolation facility (100%), rough and moist floor (81.4%), free contact of calves with other dairy cattle (68.6%), overcrowding (67.1%) and improper waste disposal (64.3%) were some potential risk factors recorded during the investigation (Table 4).

Table 3. Response on assessment of management and risk factors by owners.

Parameters	Frequency		Percentages	
	Present	Absent	Present	Absent
Ventilation of calf house	33	37	47.1	52.9
Sanitation of dairy farm	30	40	42.9	57.1
Feeding & watering hygiene	26	44	37.1	62.9
Proper waste disposal (liquid & solid wastes)	19	51	27.4	72.9
Contact of calves with adult cattle	53	17	75.7	24.3
Knowledge of the disease	14	56	20	80
Past history of diarrhea & emaciation in farm	24	46	34.3	65.7
Whether treated for diarrhea or not	10	14	41.7	58.3
General control measures against diarrhea and emaciation were taken or not	23	47	32.9	67.1
Colostrum feeding	65	5	92.9	7.1

Table 4. Findings based on researcher observation

Farm assessment indicatives	Frequency		Percentages	
	Yes	No	Yes	No
Over crowding	47	23	67.1	32.0
Rough and moist floor	18	52	25.7	74.3
No partition for different age group animal	57	13	81.4	18.6
Contamination of feed& water with feces	41	29	58.6	41.4
Improper waste disposal	45	25	64.3	35.7
Lack of isolation facility for sick animals	70	0	100	0
Ventilation	28	42	40	60
Free contact of calves with other dairy calves/adult	48	22	68.6	31.4

Discussion

The present study has revealed the presence of pathogenic calf *Eimeria* species parasitizing the gastrointestinal tract of calves under the age of one year in Mekelle, northern part of Ethiopia. Accordingly the overall prevalence of

Eimeria spp. is 72.8%, which is in line with the 68.1% reported from Addis Ababa and Debre Zeit (Abebe *et al*., 2008), 68% (Cicek *et al.*, 2007) and 75% (Nalbantoglu *et al.*, 2008) in Turkey. However lower prevalence was reported in Kombolcha district of South Wollo 31.9% (Alula *et al.*, 2013) and 22.7% in Dire Dawa eastern Ethiopia (Dawid *et al.*, 2012). Variation in prevalence could be mainly due to difference in management.

The overall *Eimeria* intensity based on oocyst per gram of feces (OPG) ranged from 50 to 32,000. This is higher than 50-1500 OPG in study conducted in India (Das *et al*., 2015). This could be due to susceptibility differences, geo-ecological and seasonal variations (Radostits *et al*., 2006). Majority of *Eimeria* positive calves were asymptomatic and infection was diagnosed only based on laboratory examination.

The present study showed prevalence of *E. bovis* (26.6 %) and *E. zuernii* (11.2%) which is lower than the report of *E. bovis* (42.3%) and E. *zuernii* (28.3%), in previous study in Ethiopia (Alula et *al.,* 2013). Das *et al* (2015) reported lower prevalence of *E. bovis* (6.8%) and *E. zuernii* (2.35%).

The mean intensity of the pathogenic *Eimeria* spp. was 12,000 and 8400 OPG for *E. bovis* and *E. zuernii,* respectively which is lower than 58,600 for *E. bovis* and 14,500 for *E. zuernii* in calves in Poland (Klockiewicz *et al.,* 2007). This variation could be due to concentration differences of excreted coccidia oocysts in the sampled feces, individual response differences, breed and management intervention differences to control the disease.

The analysis of risk factor showed that age is significantly associated with prevalence but there was non-significant association of sex and body condition with the prevalence of coccidiosis. Similar findings were also reported by Alula *et al* (2013) and Abebe *et al* (2008). Higher infection rate was observed in calf $\geq$ 6 to 12 months of age than calves of <6 months of age which is similar to the study of Das *et al* (2015) and Gezaleign (2011). This is due to the fact that there was good nursing and feeding colostrum for younger calves. Majority (92.9%) of the respondent responded positively on colostrum feeding which is in line with the the current finding. Feeding colostrum will lead to development of calf immunity and resist coccidia infection. Non-significant effect of body condition and sex could be due to the level of infection, sampled size or most of the affected animals harbor the disease without showing clinical signs as reported by Fraser (2006).

Conclusion

Higher prevalence of coccidiosis in calves was found. The pathogenic *Eimeria* species identified were *E. bovis* and *E. zuernii*. Among the risk factors, age was found to significantly affect prevalence but body condition and sex had no significant influence in prevalence of coccidiosis in calves.

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