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Original article

Prevalence of calves coccidiosis in Jimma town dairy farms, South-Western Ethiopia

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ABSTRACT

A cross- sectional study was conducted from October 2009 to March 2010 on Jimma town dairy farms to determine the prevalence of Calves coccidian and assess associated risk factors. Faecal samples were collected once from a total of 385 calves range from 5-365 days old and examined for the oocysts of Eimeria species by centrifugal faecal flotation technique using concentrated sucrose solution. Out result revealed that overall prevalence of 198(51.42%) Eimeria species. Of the 53 dairies sampled, almost all of the farms had calves shedding Eimeria oocysts. The species of Eimeria circulating in the farms was presumed to be based on morphology of the oocysts and certain epidemiological features of the Eimeria, a total of 8 species were identified namely Eimeria bovis 94(24.4%), Eimeria zuernii 58(15.1%), Eimeria auburnensis 56(14.6%), Eimeria Canadensis 53(13.8%), Eimeria ellipsoidalis 28(7.3%), Eimeria subspherica 22(5.7%), Eimeria cylindrical 20(5.2%) and Eimeria alabamensis 16(4.2%) in descending order of their relative prevalence. Eimeria species were not found to be statistically associated with faecal consistency, breed and sex (P>0.05). Among the risk factors studied, hygiene status and age of the calves were the most important factors associated with the possibility of infection with diseases. The mean and maximum Eimeria oocysts per gram of faeces (OPG) determined by using McMaster technique was 100 and 24,000, respectively. Coccidiosis is a common and important cause of

morbidity and economical loss in calves in study areas. Hence appropriate disease prevention and control measure is paramount importance to reduce these impacts.

1. Introduction

The future of any dairy production depends, among other things on successful program of raising calves and heifers for replacement. On the other hand, the health and management of replacement animals are important components of total herd profitability. The productivity of the herd can be negatively impacted by impaired growth of calves, decreased milk production of animals that experienced chronic illness as baby calves, spread of infectious diseases from calves to adult cows, increased veterinary costs and the limited opportunity for genetic selection due to high mortality of replacement animals. Amongst all animals present on a dairy farm, the highest morbidity rates generally occur in baby calves prior to weaning (McGuire and Ruegg, 2004).

The most important disease problems in the young calf are diarrhea, pneumonia and Coccidiosis (Heinrichs and Radostits, 2001). Coccidia is the common health problem particularly in neonatal calf that is responsible for the greatest economic losses in this age group for both dairy and beef calves and acute diarrhea accounts for approximately 75% of the mortality losses of dairy calves less than 3 weeks age (Heinrichs and Radostits, 2001). The most important pathogens associated with calf diarrhea worldwide are rotavirus, corona virus, Salmonella species, and protozoan parasites Eimeria and Cryptosporidium species (Heinrichs and Radostits, 2001).

Coccidiosis is important protozoa disease caused by apicomplexan parasites of the genus Eimeria. There are many coccidia organisms in the environment. However, two coccidia organisms are the chief culprits for disease in cattle: Eimeria zuernii and Eimeria bovis. In severe cases, these organisms damage the intestines by destroying intestinal cells and tissues, which interferes with the animal's ability to absorb nutrients that is most frequently in calves 6 to 12 months, although occasionally occurs in yearlings and adults. The result is a marked reduction in feed efficiency and weight gain, bloody diarrhea, resulting in dehydration (Kennedy, 2001: Pilarczyk and Bailicka – Ramisz, 2004: Dedrickson, 2002; Kirkpatrick and Selk, 2003), which is actually characterized by two different syndromes: subclinical and clinical.

Compared to clinical, subclinical coccidiosis is more important and may account for over 95% of all the losses associated with coccidiosis (Richey, 2004; Dedrickson, 2002). These silent, subclinical infections cause substantial economic losses due to poorer feed efficiency, slow weight gain, weight loss, longer heifer development periods and affected cattle may never fully recover from the effects of slower growth, which robs you of profits and increased susceptibility to other disease causing agents (DHM, 1998). This is a series disease in cattle, sheep, goats, pigs, cats and horses; it is less often diagnosed but can result in clinical illness (Cynthia, 2008).

In Ethiopia, although coccidiosis is an important cause of calf morbidity and mortality, studies done to quantify the magnitude of the problem and to determine the underlying economical losses are scant and scarce. The available scant data also refer to only bacterial and viral causes of economical losses and no attention has been given to the role of coccidiosis as causes of disease and production losses in cattle, although these disease are of foremost importance in most parts of the world in view of their economic percussions not only due to the high mortality they sometimes cause, but also because of failure of young stock to grow and gain weight to their full potential, reduction in body weight, and medication and labor costs. On coccidiosis no information is available in Jimma area; taking these facts in to account a cross- sectional study was undertaken to determine the prevalence of calve coccidiosis and assess associated risk factors in dairy farms found in Jimma town.

2. Materials and methods

2.1. Description of the study area

This study was carried out in dairy farms found in Jimma town, the capital of Jimma zone in Oromia National Regional state, Ethiopia. Jimma Town (7013' to 8056' N, 35052' to 37037'E) is 352 Km southwest of Addis Ababa. It lies at an elevation of 880 to 3360 meters above sea level, and experiences hot and humid weather conditions. The

most rain fall pattern of the area is characterized by small rainy season from February to May and high rainy season from June to September. The dry season extends from October to January. The study area receives mean annually rainfall of about 1637mm.

2.2. Study design and population

Cross-sectional study was conducted from October 2009- March 2010 to determine the prevalence and assess the risk factors of Coccidiosis in calves. The study on population consisted of male and female calves of cross and local breeds with less than one year of age belonging to fifty three dairy farms or all dairy farms in Jimma town which were purposively selected based on their number of calves.

2.3. Sample size determination

The sample size required for this study was calculated based on sample size determination method for simple random sampling of infinite population after Thrusfield, (2007) as follows:

$$N= \frac{1.96^2 \text{ pex } (1\text{-pex})}{d^2}$$

Where, n=required sample size:
Pexp= expected prevalence
d= desires absolute precision
Since no previous study was undertaken in the study area, the expected prevalence was considered to be 50%. Accordingly, with 5% absolute precision at 95% confidence level, the number of calves required to determine the prevalence was found to be 384.

2.4. Sampling methods or sample collection

Before sample collection Age, sex, breed, hygienic status and faecal consistency of the calves less than one year's registered. Then after, a fresh fecal sample of about 20gm was collected from the rectum of each calf using sterile disposable plastic gloves. The sample was placed in a labeled clean glass bottle container and transported to the parasitology laboratory on the same day and was kept at 4°C in a refrigerator until processing within 48 hours of arrival.

2.5. Laboratory investigation

Qualitative fecal examination was conducted using flotation technique for the detection of the oocysts of Eimeria using concentrated sucrose solution (Sheather's sucrose solution) with specific gravity of 1.27 as described by Hendrix (1998) as follows: Approximately 2- 3g of faeces was mixed with enough water in a plastic cup using tongue depressor to make a semisolid suspension. The mixture (faeces and water) was strained through a tea strainer over a second plastic cup pressing out the liquid with tongue depressor. The contents of the second plastic cup was transferred in to a 15-ml centrifuge tube which then placed in to the centrifuge and spun for 3 minutes at 2000 revolutions per minute (rpm), processing 6 samples at a time. Then the supernatant, which contains fats and dissolved pigments, was decanted and the sediment was resuspended using a stirring action with a wooden applicator stick after adding the flotation solution to ½ incur from the top of the tubes. The test tubes were inverted six times after inserting a rubber stopper to mix the solution thoroughly with the sediment. The tubes were then filled with flotation solution until a reverse meniscus was present; cover slips were added and centrifuged in variable- angled (not a fixed – angled) centrifuge for 5 minutes. After centrifugation, the cover slip was lifted straight up and placed with its adherent film of sugar solution on a glass slide. The slides were examined under the compound microscope using the 10x objective for the identification of oocysts. Eimeria oocysts were detected in the sample with the correct morphology, i.e. optical properties, structure, size and shape.

2.6. Quantitative faecal examination

This was performed in positive sample to determine the number of oocysts of Eimeria per gram of faeces (OPG). The method used for this purpose was the well – known McMaster technique as described by (Kaufman, 1996) as follows: 3g of faeces was weighed in a plastic cup and mixed with 42 ml of water. The mixture was homogenized and poured through a 250 um aperture sieve. The filtrate was agitated and a 15ml (1gm) suspension

was drawn and filled in to a 15ml centrifuge tube. The sample was centrifuged at 2000rpm for 2minutes, processing 6 samples at a time. The supernatant was poured off and concentrated sugar solution added to the sediment which then agitated adding the sugar solution to the previous level. Then the tube was inverted 4 times and both chambers of McMaster slide were filled quickly with fluid removed from the tube with pipette. The slide was scanned under 10x magnification and the oocysts per gram of faeces (OPG).

2.7. Data Analysis: The recorded raw data were entered in to Microsoft excel data base system to be analyzed using statistical program for social science version 16 and stata for windows (version8) statistical soft ware's. Descriptive statistics was computed. Prevalence of Coccidiosis was calculated as the number calves found infected with coccidia, expressed as the percentage of the total number of calves Thrusfield, (2007) Categorical data were analyzed first with Pearson's chi-square (2) was used to evaluate the association between the prevalence of Coccidiosis and different factors. For analysis of continuous data t-test for independence sample was used to compare means of two groups' whereas ANOVA was used to compare means of three or more groups. P-value less than 0.05 (at 5% level of significance) were considered significant in all analysis.

Determination of Age of Calves: The age of calves was determined according to Pace and Wakeman, (2003) as well as by collecting information from the dairy farm owners and were then conveniently categorized in to two groups: Birth up to 6 months, 6-12 months.

Assessment of hygienic status of calves: the hygienic status of calf pens and the calves themselves were assessed based on housing system (ventilation, draughts, group pens, heavy stocking), sanitation of bedding (soiled bedding) and body parts of the calves Curt A. and Gooch, (2005) and was conveniently categorized as poor and good.

3. Results and discussion

Out of 385 fecal samples examined, 198 were positive for Eimeria oocysts and hence the overall prevalence was found to be (51.42%) and out of the 53 dairy farms surveyed for Coccidiosis, virtually all (99%) had one or more calves shedding Eimeria oocysts. The overall Prevalence (51.42%) of eimeria species infection recorded in this study was found to higher than the previous reported prevalence; 20% by Kebadu (1998) in the Debre Zeit, 24.9%% by Kassa et al., (1985) in Bahir Dar and 46% in Netherlands (Cornelissen et al., 1995), 34.1% in Saudi Arabia (Kasim and Al- Shawa, 1985), but lower than reports from the Central Ethiopia (68.1% by Rahmeto, 2008), and 67.4% in a neighboring, Kenya (Munyua and Ngotho, 1990), 68% in Turkey (Arslan and Tuzer, 1998)), and 64.2% in Canada (Kennedy and Kralka, 1987), 86.3%) in USA (Ernst et al., 1987) and 87.8% in Mexico (Rodriguez-Vivas et al., 1996). Comparable findings were also reported in some countries: 49.6% in Poland (Pilarczyk et al., 2000), 59% in Japan (Hasbullah et al., 1990; Oda and Nishida, 1990) and 52% in South Africa (Matjila and Penzhorn, 2002).

With regard to identification of species Eimeria, a total of 8 species were identified based up on the characteristics of the oocysts of cattle as described by Levine (1985) and Soulsby (1982). This result suggests that Eimeria species are abundant in Jimma town dairy farms. Eimeria bovis (24.42%) is the most prevalent species investigated during the survey followed by E. Zurnii (15.1%) and E. aubernensis (14.62%) (Table 1). This is within the same range as reported for calves in different countries: 11 species were reported in Ethiopia (Rahmeto, 2005), seven species were reported in USA (Ernst et al., 1987) and Netherlands (Cornelissen et al., 1995), six species in Turkey (Arslan and Tuzer, 1998) and five species in Canada (Kennedy and Kralka, 1987). The finding consistent with most of the studies performed throughout of the world and with what was described in literatures. Although a high proportion of calves were infected with highly pathogenic E. bovis and E. zurnii, but clinical coccidiosis was observed in small proportion calves positive for Eimeria spp. This finding is in agreement with Radostits et al. (1994) and a number of other authors, who stated that most animals in a group become infected but only a minority (10-15%) development of immunity after a course of infection with a particular Eimeria species (Harper and Maas, 1996). Also is in line with the hypotheses in literatures, which state that the development of clinical disease depends on the number of oocysts ingested (Kennedy, 2001: LAV, 1996).

Of the 198 calf's positive for Eimeria, 30.8% were infected with single species, while the rest 69.2% were found to be infected with 2-5 species. Multiple infections in a single host with several Eimeria species were common findings in this study. The maximum number of species per sample ranged from 2 to 5 as shown in Table 2.

Table 1
Eimeria species identified and their respective prevalence in descending order.

Types of Eimeria	Total animals examined	No of positive	Prevalence
E.Bovis	385	94	24.4
E.Zurni	385	58	15.1
E. Aubernensis	385	56	14.6
E. Canadensis	385	53	13.8
E. Ellipsoidalis	385	28	7.3
E. Subspherical	385	22	5.7
E. Cylinricqal	385	20	5.2
E. Alabamensis	385	16	4.2

Table 2
Numbers of Eimeria found per sample in infected calves.

Nos of Eimeria Species	No of Calves consist d/t Oocysts	Relative Occurrences
One	61	30.8
Two	114	57.8
Three	14	7.1
Four	7	3.5
Five	2	1.0
Total	198	100

According to sex, the prevalence was a bit higher (30.1%) in female calves than in male ones. However, the sex of the animals was not statistical significantly associated ($P > 0.05$) with the infection (Table 3). Absence of statistically significant difference between the sexes of the study animals might suggest that both sexes of the animals at this age have almost equal chance of being infected with coccidiosis. The finding is in agreement with; previous studies done on adult cattle reported higher prevalence was reported in female (Priti, et al, 2008: Lassen, et al, 2009: Tauseef et al, 2011). Nevertheless, this could be attributed to the physiological stress loaded on female animals in relation to pregnancies and giving birth as compared to males (Radostits, et al, 2007).

According to the Age categories, higher prevalence (33.3%) recorded in those calves with aged from 6-12 month old age categories. The first age at which the Oocyst of Eimeria species, recovered were 15 days. The prevalence of Eimeria species infection has showed statistically significance difference ($P<0.05$) between the two age groups of the sampled calves (Table 3). This finding was consistent with those of other studies (Kennedy, 2001; Pilarczyk et al., 2000; Rodriguez – Vivas, 1996; Oda and Nishida, 1990), who stated that, the disease is seen most frequently in calves that are 6 to 12 months of age. As observed during the study period, almost all of the calves older than 6 months were housed communally in overcrowded condition and in physical contact with adult animals, giving more chance for the animals for licking each other and their facilitating the transmission of Eimeria oocyst to the calves.

The breed of the animals was not statistical significant association ($p>0.05$) with the risk of infection with the parasites. Absence of statistically significant difference between the breed might be due to both breed were confined in the house and have almost equal likelihood of being infected with coccidiosis. The finding in line with different hypothesis, 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 (Ernst, et al, 1987).

Of the 385 calves sampled 165 were diarrheic of which (24.9%) were infected with the parasite, the result revealed that the disease is prevalent in diarrheic animals as compare with animals defecate semisolid faces and dry faces (Table 4). The finding is in agreement with different literature stated that, Coccidia is the common health problem particularly in neonatal calf that is responsible for the greatest economic losses in young age group for both dairy and beef calves by causing acute diarrhea accounts for approximately 75% of the mortality losses of

dairy calves of young age (Heinrichs and Radostits, 2001). In associations with other enteropathogens, coccidia have been indicated as an important cause of diarrhea in calves (Ernst, et al, 1987).

Table 3
Results of analysis of different risk factors for Eimeria species.

Risk factors	No examines	Positive (n %)	95%CI	P	X	Df
Age in months						
<6	189	70(18.2)	0.0038-0.0063	0.002	9.366	1
6-12	196	128(33.3)	0.0038-0.0063			
Sex						
Male	172	82(21.3)	0.0037-0.0062	0.185	1.75	1
Female	213	116(30.1)	0.0037-0.0062			
Breed						
Cross	250	129(33.5)	0.0037-0.0063	0.93	0.008	1
Local	135	69(17.9)				

According to the hygienic status of the farms, there was a statistically significant association (P<0.05) among the infection with coccidiosis and the hygienic status of the farms. Accordingly, calves belonging to the farms with poor hygiene and medium showed significantly higher prevalence than calves belonging to the farms with relatively better hygiene (Table 4). This condition is in line with the idea of Radostits et al., 1994; Ernst and Benz, 1986; Urquhart et al., 1996, who stated unhygienic conditions is a major risk factor that precipitates massive intake of Eimeria oocysts and leads to development of clinical coccidiosis. It is consistent with the recommendation in the literature of not feeding cattle especially calves on the ground because this increases the possibility of contamination of the feed with the manure containing Eimeria oocsyt (Radostits et al., 1994; Kennedy, 2001).

Table 5
T-test analysis of the association between faecal consistency, hygiene status and the risk of infection with Eimeria.

Description	No examined	Positive (n %)	95%CI	P	X2	Df
Faecal consistency						
Diarrhea	165	96(24.9)	0.05026-0.6580	0.56	5.76	2
Semisolid	179	85(22.1)	0.3998-0.5506			
Dry	41	17(4.4)	0.2631-0.5789			
Hygiene status						
Poor hygiene	252	147(38.2)	.5197-0.6448			
Medium	103	29(7.5)	0.1973-0.3787	0.00	32.9	2
Good	30	22(5.7)	0.5411-0.8772			

According quantities Faecal Examination by using Mc master technique to determine the number of Eimeria oocysts per gram of faeces (OPG) reveled minimum and maximum OPG value of 100 and 2400 respectively (Table 5). This finding was in middle among, which reported from different country. Rahmeto (2005) reported the mean 5109 and maximum 267000 Oocyst excretion levels OPG in the Central Ethiopia, Kennedy, and Kralka (1987) reported an average of 25 and a maximum of 109,449 OPG in Canada. Munyua and Ngotho (1990) reported a maximum of 30,600 OPG in Kenya. In Turkish study, a mean and maximum OPG of 1280 and 52,000, respectively has been reported (Arslan and Tuzer, 1998). The maximum OPG belong to 45 days old diarrheic calf. In the present survey, 42.42% of the infected calves have Oocyst counts above 5000 OPG and 57.57% has Oocyst counts below 5000 OPG. Similar results were also reported by other authors (Matjila and Penzhorn, 2002; Munyua and Ngotho, 1990). A count of 5000 Oocysts of faeces is considered high enough by some investigators to warrant the judgment that the animal has the disease (Ernst and Benz, 1981; Rebhun, 1995).

4. Conclusion and recommendations

This study has clearly demonstrated that Eimeria Species are prevalent in Jimma dairy farms. Based on oocyst morphology and epidemiological features, the species of Eimeria circulating in the dairy farms surveyed was presumed to be a total of 8 species were identified of which E. bovis was the most prevalent followed by E. zuernii. In spite of the fact that most of the calves sampled were found to be infected with Eimeria species, clinical coccidiosis was observed in small proportion of the infected calves. Therefore, it is concluded that most Eimeria infections in calves on Jimma town dairy farms, even infected with E. bovis and E. zuernii, result in subclinical infections, they cause should not be underestimated as these infections can still negatively influence animal productivity.

Table 5
Grouped frequency distribution of Eimeria spp oocyst counted per gram of faeces.

OPG	NO calves	Relatives occurrences
0-1000	22	11.11
1001-2000	36	18.18
2001-3000	30	15.2
3001-4000	21	10.6
4001-5000	5	2.5
5001-6000	9	4.5
6001-7000	11	5.6
7001-8000	6	3.1
8001-9000	7	3.5
9001-10000	12	6.1
1001-11000	4	2.0
11001-12000	7	3.5
12001-13000	6	3.0
13001-14000	4	2.0
14001-15000	1	0.5
15001-16000	1	0.5
16001-17000	1	0.5
17001-18000	2	1.0
18001-19000	1	0.5
19001-20000	4	2.0
20001-21000	2	1.0
21001-22000	3	1.5
22001-23000	2	1.0
23001-24000	1	0.5

In the final analysis, this study made it possible to identify the hygiene of calf rearing houses and age of the calves were the most important risk factors that strongly influenced the detection of Eimeria oocysts. The present observations indicate that infections in bovine due to Eimeria species infections are more important in calves above six months of age. Therefore optimum hygienic conditions should be maintained on the farms, the calves should be watched regularly and receive appropriate treatment. Feeding of calves directly on the ground, keeping of calves in close contact with adult animals and mixing of calves of different age's size should be avoided as this may result in spread of the disease to the calves.

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