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A CROSS-SECTIONAL STUDY ON PREVALENCE OF *Cryptosporidiosis* AND ITS ASSOCIATED RISK FACTORS IN CALVES IN GONDAR AND ITS SUBURBS, NW ETHIOPIA

Mastewal BIRHAN✉, Andargachew MISGANAW and Tilahun GESSESSE

College of Veterinary Medicine and Animal Sciences, Department of Veterinary Paraclinical studies, University of Gondar, Ethiopia
✉*E-mail: Maste675@gmail.com; ORCID: 0000-0002-0984-5582*

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ABSTRACT: Cryptosporidiosis is a common gastrointestinal disorder in humans and animals caused by various *Cryptosporidium* species. The present study was carried out to determine the prevalence of *cryptosporidium oocyst* and its potential risk factors in calves less than one year of age in and around Gondar town. For this purpose, 384 fecal samples (n=384 calves) from different dairy farms were collected and screened by using modified Ziehl-Neelsen staining technique. The overall prevalence of *Cryptosporidium* oocysts was 21.4 % (82/384). The association between different risk factors and prevalence of *Cryptosporidium* oocysts was assessed. There were significant associations (P<0.05) between prevalence of *Cryptosporidium* oocysts and age of calves, fecal consistencies, daily cleaning of the farm and water source. On the other hand, there was no significant association between prevalence of *Cryptosporidium* oocysts and sex, breed, and body condition of the calves and also provision of colostrums to the calves (P>0.05). In conclusion, this study demonstrated host factors and management factors greatly affect the prevalence of Cryptosporididosis in calves. Therefore, the current study reported the role of host factors (age and sex) and management factors (water source and daily cleaning of the farm) needed to be clearly recognized by all stakeholders in order to understand their effects on the disease occurrences as well as in control and prevention of in calves.

Keywords: Calves; Cryptosporidiosis; Dairy farm; Oocyst; Ziehl-Neelsen

INTRODUCTION

The productivity of cattle depends largely on their reproductive performance and the survival of calves (Yeshwas et al., 2014). Calve morbidity and mortality are perennial problems for dairy producers worldwide especially the tropics is not an ideal location for calf rearing as the high temperature and humidity introduce many potential disease problems to milk fed calves which impair appropriate heifer replacement (Gebremedhin, 2014). The neonatal calve mortality in the first month of age is more than 80% of the total mortality in calves. Major causes of mortality of neonatal calves are conditions like diarrhea and pneumonia (Khan et al., 2009).

Calf diarrhea (also known as calf scouring) is a commonly reported disease and a major cause of economic loss to cattle producers (Yong-il and Kyoung-Jin, 2014). Diarrhoea is the most important disease in young calves and accounts for approximately75% of the mortality of dairy calves within the first 3 weeks of age (Walter, 2012).

The pathogens most commonly incriminated in neonatal calf scours include viral (rotavirus and coronavirus), protozoal (*Cryptosporidium parvum, coccidia*) and bacterial pathogens (enterotoxigenic *Escherichia coli* K99 and *Salmonella* species). Calves are at greatest risk of developing diarrhea during the first month of life, and the risk then decreases with age (Izzo et al., 2011).

Cryptosporidiosis is an emerging protozoan disease, caused by *Cryptosporidium* species that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide. The infection encountered after ingestion of the microscopic infective oocysts (Dinka and Berhanu, 2015). These protozoan parasites mainly infect the intestinal tract and rarely the respiratory tract of animals and people (Zkan and Yunus, 2001). Clinical cryptosporidiosis is frequently not diagnosed, yet it has been incriminated as an important cause of diarrhea in neonates. Clinically, the disease is characterized by anorexia and diarrhea, often intermittent, which may result in poor growth rate. The severity of clinical disease may be associated with the animals' immune and nutritional status (Olson et al., 1997).

Cryptosporidium are responsible for most cattle infections (*Cryptosporidium parvum, Cryptosporidium bovis* and *Cryptosporidium andersoni* and with *Cryptosporidium* deer-like genotype). *Cryptosporidium parvum* is known to infect humans worldwide and is recognized as the major zoonotic *Cryptosporidium* species, whereas *C. andersoni* has been

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reported in humans only once. The most prevalent species were *C. parvum* in dairy calves. *C. bovis* oocysts are morphologically indistinguishable from *C. parvum* oocysts. *Cryptosporidium bovis* is a highly prevalent species that infects primarily post-weaned calves (Olson et al., 1997). *Cryptosporidium parvum* is the main species in young calves and is a cause of neonatal diarrhoea. *C. bovis, C. ryanae and C. andersoni* are more common in weaned calves and older cattle, with different prevalence and age distribution (Silverla and Blanco, 2008). The prevalence of *C. parvum* infection in animals is high. Dairy and beef calves are generally considered as presenting the highest risk because of their numbers, distribution, high infection occurrence, and high oocyst excretion levels besides be exposed to contamination environment sources, such as soil and water (Almeida et al., 2010).

Taking into account the high prevalence of *Cryptosporidium* infection in calves and the prevalence and its associated risk factors of *Cryptosporidium* infection in calves in the study area is not found. Therefore, the objectives of this experimental trial were to determine the prevalence of cryptosporidium on calves and to know its associated risk factors.

MATERIALS AND METHODS

Study Area Description

The study was conducted in Gondar town at University of Gondar, located 738 km away north-west of Addis Ababa, the capital city of Ethiopia. The total population of Gondar town is estimated to be 206,987 of which 98,085 are males and 108,902 females (CSA 2008). It is situated with a *latitude and longitude* of 2°36' N, and 37° 28'E, respectively. It has an altitude of about 2133 meter above sea level with an average temperature of 20°C and an average annual rainfall of 1800 mm. The livestock population in the area comprises of cattle (8,202), goat (22,590), sheep (2,695), horse (1,065) and donkey (9,001) (CSA, 2008).

Study Population

The sampling units for the study were dairy calves of up to 1 years of age. This was based on the previous reports that indicated higher occurrence of the disease in these age categories. The association of the disease occurrence was seen in relation with different age category with classification of < 3 months, 3-6 months, >6 months and consistency of feces weather it was diarrheic or not. Risk factors for the study animals were considered during data collection and analyze to assess the effect of these risk factors on the prevalence of cryptosporidium oocyt. Calves from dairy farms in and around Gondar town were constituted in the study population. There were few relatively large dairy farms and a lot of small holder dairy farms in the study area.

Study Design

A cross-sectional study supported with close ended questionnaire survey was carried out to determine the prevalence of Cryptosporidiosis in calves and its associated risk factors in calves. Modified Ziehl-Neelsen staining test on feces collected directly from rectum of calves less than 1 year of ages was performed as laboratory technique to detect the oocyst of the parasite.

Sample Size Determination

Simple Random sampling technique was used to select study farms and all calves that are found in the study farms were sampled. The sample size was determined based on the expected prevalence of 13.6% by Dinka Ayana in central Ethiopia and absolute desired the precision of 5% at confidence level of 95% according to formula provided by Thrusfield (1995). The formula is:

$$N = \frac{(1.96)^2 x\ p\ (1-p)}{D^2}$$

Where N=sample size; P=expected prevalence; D= desired absolute precision

Thus, based on the formula the total sample size were 180, to increase, the precision level these number was increased to 384.

Sample Collection and Processing

In this study, fecal samples were collected directly from the rectum of the study animals using disposable gloves, placed in universal bottle and transport to laboratory for processing on the same day. Farm owners and workers were interviewed with questionnaire which focuses on different aspects, associated with management risk factors including, provision of colostrums, daily cleaning of the farm, Age of the calves, Breeds of the calve, housing condition of calf, water source, and also during farm visit the flooring system of the farm was checked. All the fecal samples were tested for the presence of *Cryptosporidium* spp. oocysts in feces. Samples will detect using the modified Ziehl-Neelsen staining technique as described by Clarke and McIntyre (2001).

Data Analysis

The data obtained was entered and managed using Microsoft Excel 2007 excel spread sheet. Then, it were imported to SPSS version-20 and analyzed by chi-square test to determine the significance of the variation in prevalence rates

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between management factors, host factors and consistency of feces. Prevalence was calculated as the number of calves found positive for cryptosporidium parasite per animals examined. A 95% confidence interval and 5% significance level were used to determine whether there was significant difference in the measured parameters.

RESULT

Cryptosporidiosis is an emerging protozoan disease, caused by *Cryptosporidium* species that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide. According to this study, a prevalence of 21.4% (82/384) was recorded among the studied calves for the positivity of *Cryptosporidium* oocysts.

Prevalence of Cryptosporidium Oocyst with Respect to Calve Factors

According to table 2, the highest prevalence among calves aged less than three months with 47.8% (32/67) followed by calves of 3-6 months age group 24.1% (35/145). On the other hand, the lowest prevalence was recorded in older calves (>6 months) 8.7% (15/172). Thus, significant association (P<0.05) between different age groups and the prevalence of *Cryptosporidium oocyst* were recorded. In addition, a result obtained from study to determine association of the parasite infestation with respect to sexes of calves, showed higher rate of infection in females (22.3%) than males (20.5%). There was no significant association (P>0.05) between both sexes in shedding of *Cryptosporidium* oocyst. As reported in Table 2, no significant association between prevalence of *cryptosporidium* oocyst and body condition (P>0.05) was observed. The study result showed high prevalence of *cryptosporidium* oocyst in calves that had poor body condition (55.3%) followed by medium (20.5%) and the lowest prevalence was documented in calves that had good body condition (6.8%).

Furthermore, prevalence of *Cryptosporidium* in calves were breed considered as risk factor, the result in table 2 showed a relatively lower prevalence rate of (19.8%) in local breed calves than in cross breed calves (22%). Thus, prevalence rate among local and cross breed calves were not significant statistically (P>0.05). The prevalence of *Cryptosporidium* was studied based on fecal consistency and out of 384 calves sampled, almost all (98.4%) was non diarrheic while about 1.56 % of them were diarrheic. The prevalence rates of *Cryptosporidium* were 20.6% and 66.7% in non-diarrheic and diarrheic calves, respectively. Hence, consistency of feces has a direct link with prevalence of cryptosporidium (P<0.05).

Table 2 - Prevalence of *Cryptosporidium oocyst* with respect to calve factors

Calf factors	Categories	Total calves Screened (N)	*Cryptosporidium* Positive calves (%)	P-value
Age	< 3 months	67	32(47.8)	0
	3-6 month	145	35(24.1)	
	>6month	172	15(8.7)	
Breed	Local	114	22(19.3)	0.52
	Cross	270	60(22.2)	
Sex	Female	184	41(22.3)	0.67
	Male	200	41(20.5)	
Body condition	Poor	38	21(55.3)	0.062
	Medium	273	56(20.5)	
	Good	73	5(6.8)	
Consistency of Feces	Diarrheic	6	4(66.7)	0.006
	Non Diarrheic	278	78(20.6)	

Prevalence of Cryptosporidium Oocyst with Respect to Management Habits

As indicated in table 3, no significant association (P>0.05) was found between provision of colostrums to calves and the prevalence of *Cryptosporidium* oocyst. Although, the prevalence was high (26.8%) in calves that denied provision of colostrum than calves supplied a colostrums (19.9%). As per the exploration carried out to see association between prevalence of *cryptosporidium* oocyst and number of daily cleaning of the farm, it showed significant association (P<0.05). In farms experienced a single barn cleaning habit recorded highest prevalence (32.5%) compared to farms cleaning calve barns twice a day (25%) and three times per day (15.1%).

Moreover, water source showed its significance as a potential risk factor for the occurrence of the disease. Thus, higher prevalence (28.6%) of *cryptosporidium* oocyst was recorded in calves used to drink water from river than calves getting drink tap water (18.9%). Hence, source of water for calves to drink and the prevalence of cryptosporidiosis was found to be statistically significant (P<0.05).

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Table 3 - Association of *Cryptosporidium* oocyst with management habits

Management factors	Categories	Total calves N	*Cryptosporidium* positive calves (%)	p-value
Colostrums	Yes	302	60 (19.9)	0.173
	No	82	22 (26.8)	
Daily cleaning of the farm	≤ 1 times/day	77	25(32.5)	0.04
	2 times/day	108	27(25)	
	≥ 3 times/day	199	30(15.1)	
Water source	River	98	28 (28.6)	0.043
	Tap water	286	54(18.9)	

DISCUSSION

The overall prevalence of *Cryptosporidium* in calves was relatively higher (21.4%) than that reported from eastern Ethiopia 17.6% by Rhmato et al. (2007) and from central Ethiopia 13.6% by Ayana and Alemu (2015). On the other hand, the current finding is relatively lower than reports from central Ethiopia 27.8% by Regassa et al. (2013). However, there were close similar result that reported from other countries on the prevalence of Cryptosporidosis in calves with the present study like a prevalence of 21.65% in Iran (Radfar et al., 2006), 21.9% in Brazil (Melissa et al., 2015) and 20% in Malaysia (Nur Hazirah et al., 2016). Furthermore, there were also a variety of results from various studies concerning the prevalence of *Cryptosporidium* in calves worldwide, 86.7% in Tunisia (Soltane et al., 2007) and 83.3% in chain on calves less than one month of age (Zhaohui et al., 2014) and 3.9% in Turkey (Esin et al., 2013). The difference in the prevalence of the disease might be attributed to variation in the season of study time or it could be due to the age of examined animals. It might be also due to hygiene of the farm and sensitivity of the test.

In the current study animal age played a great role for the prevalence of *Cryptosporidium* oocyst. It has been observed that the high prevalence (47.8%) of *Cryptosporidium* oocyst in this study was observed in calves < 3 months followed by calves aged 3-6 months (24.1%) while calves aged greater than 6 months had the lowest prevalence of *Cryptosporidium* oocyst (8.7%). The findings are in agreement with findings obtained by Regassa et al. (2013), who found calves less than 3 months are at higher risk of infection compared to the older ones and studies which stated that there was significant association between the prevalence of *cryptosporidium* and age, by Ayinmode and Fagbemi (2011), Bawm et al. (2014) and Akinkuotu et al. (2014). On the other hand, it was in contrast to the findings of Rhmato Abebe et al. (2007), who stated that, there were no significant association between the prevalence of *cryptosporidium* and age. The contradiction between reports of the studies might be due to different grouping of ages of the calves since different authors used different categories of age. For instance, Rhmato Abebe et al. (2007) grouped calves age <6 monthes and > 6monthes. In addition, this study and Regassa et al. (2013) grouped the age of calves into <3 months, 3-6 months and >6 months. Moreover, it could be due to species of *cryptosporidium oocyst* that found in the study area. The higher prevalence in calves less than 3 months can be attributed to the fact that these age groups are highly susceptible to the disease because of the immature immune system of the animal at this age (Regassa et al., 2013).

The current study reported that there was no significant difference between the prevalence of *Cryptosporidium* oocyst in female and male calves, although the result showed high prevalence (22.3%) in female than (20.5%) male calves. This report was supported by Emanuel and Luuk, (2010), Regassa et al. (2013), and Akinkuotu et al. (2014). There was no significant difference in *Cryptosporidium* infection ratio between males and females when they are breed in the same place and expose to similar condition, because neither males nor females have factors facilitate or impede infection while the other gender lacks them (Al- zubaidi, 2012). In contrast the study disagrees with the report of Ayinmode and Fagbemi (2010) who reported that there is a significant difference existed between infection rates in females and males calves.

The present study also showed breed of the calf was not statistically significant with *oocyst* shedding of *Cryptosporidium,* however, the result indicated that higher prevalence (22.2%) in cross breed than (19.3%) local breed calves. This was in agreement with the report of Nasir et al. (2009). This could be due to cross breed calves had lower immunity than local breeds.

The result of the present study revealed that the consistency of feces had a significant effect on the prevalence of *cryptosporidium* oocyst. This report agreed with the report of Nasir et al. (2009), Sharma and Busang (2012), Esin Guven et al. (2013), Samir et al. (2014) and Danlmadi and Ugbomoiko (2015). On the other hand, the present study contradicted with the work of Melissa Carvalho et al. (2015) who reported that the absence of correlation between the occurrence of diarrhea and positivity for *Cryptosporidium* species can be related to other factors inherent to the animals such as the presence of other gastrointestinal parasites and/or concomitant infections. The difference between this reports might be due age of target population since older calves did not show sever clinical sign even if they were heavily infected.

According to the findings of current study, it was observed that the prevalence of *cryptosporidium* oocyst in calves were associated with daily cleaning of calves' house. The risk for being shedder of oocyt was significantly higher in calves

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that used to clean their calves barns < ones day followed by two times per day, while farms that cleaned their calves house ≥ three times per day showed the lowest prevalence. This observation was in agreement with Rhmato Abebe et al. (2007) and Emanuel and Luuk (2010) who reported that Calves were more likely to shed *Cryptosporidium* species positive oocysts if they were raised at dirty floor houses most likely due to the increased microenvironment for *Cryptosporidium* species. The findings of this study disagreed with the report of Nasir et al. (2009) and Almeida et al. (2010) who indicated that there was no significant association between the prevalence of *cryptosporidium* with hygiene of the house.

In the present study, a significant association was found between water sources and shedding of *cryptosporidium* oocyst. Thus, higher prevalence was observed on calves that drunk water from the river than tap water. This report was supported by Bawm et al. (2014) who stated that there was high significant association between water source and prevalence of *cryptosporidium* oocyst. The higher prevalence on calves that drunk water from the river could be due to higher contamination of river by feces of animas and survival of oocyst for long time in the water.

There was no significant association between prevalence of cryptosporidium oocyst and Colostrums in the study. But the present study showed higher prevalence in calves that had not chance to feed colostrums than that had chance to feed colostrums. This might be due to ingestion of protective factors in the colostrums that can reduce shedding of cryptosporidium oocyst.

CONCLUSION AND RECOMMENDATIONS

Cryptosporidiosis is a significant disease in livestock, affecting mostly neonates. From the present study, it can be concluded that *cryptosporidium* was prevalent in Gondar and its suburbs and its present prevalence was comparable to other parts of Ethiopia. The result showed some of the calves and management factors greatly affect its prevalence. Calves related possible risk factors significantly affect the prevalence *cryptosporidium* oocyst. Likewise, from management habits barn cleaning and water source supply strongly contribute to the high prevalence of *cryptosporidium* oocyst. Based on major findings and the above conclusion the following recommendations were forwarded

- Awareness creation for the farm owners about the potential risks of the Cryptosporidiosis on their farm and possible control programme of the disease should be undertaken.
- A further study prospective in nature, capturing seasonal variations to elucidate the magnitude of the disease, is desirable.
- Further study using molecular technique to identify the species of the parasite must be conducted in order to implement successful control and prevention programmes.

DECLARATIONS

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Andargachew Misganaw (AM) conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. Mastewal Birhan (MB) participated in drafting and reviewing the manuscript and conceived the study, coordinated the overall activity, and reviewed the manuscript. Muluken Yayeh (MY), Amebaye Kinubeh (AK) and Tilahun Gasses (TG) participated in drafting and reviewing the manuscript. Participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Data will be made available up on request of the primary author

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