Short communication

Identification of major protozoal enteropathogens causing calf diarrhea in dairy farms in and around Holeta Town, Oromia Special Zone, Ethiopia

Diriba Girmaye[1], Akinaw Wagari[1], and Teshita Edaso Beriso[2]*

[1]School of Veterinary Medicine, Wollega University, Ethiopia, P.O. Box 395

[2]School of Veterinary Medicine, Wolaita Sodo University, Ethiopia, P.O. Box 138

Corresponding author. E-mail: teshitae2602@gmail.com

Abstract

Calf diarrhea can be caused by a variety of pathogens, including bacteria, viruses, protozoa, and intestinal parasites. *Giardia*, *Eimeria*, and *Cryptosporidium* are the most significant protozoan parasites and are all individually and collectively infectious. A case-series study was carried out in and around Holeta dairy farms, Oromia Special Zone, Ethiopia, from November 2017 to April 2018 to identify the main protozoan enteropathogens from diarrheic calves. Samples were purposely collected from three dairy farms: Serkalem Dairy Farm (SDF), Holeta Agricultural Research Centre (HARC), and Ada Berga Agricultural Research Centre (ABARC). A total of 93 fecal samples were taken from diarrheic calves of up to 4 months of age. Protozoan enteropathogens were identified using flotation and modified Ziehl Neelsen staining methods. *Cryptosporidium*, *Eimeria*, and *Giardia* were detected in 44 (47.3%), 50 (53.8%), and 34 (36.6%) of the diarrheic fecal samples examined, respectively. The findings indicated that there were 17 (18.27%) cases of *Cryptosporidium*, *Eimeria*, and *Giardia* as mixed infections, 16 (17.2%) cases of *Cryptosporidium* and *Eimeria*, 5 (5.4%) cases of *Cryptosporidium* and *Giardia*, and 4 (4.3%) cases of *Eimeria* and *Giardia*, compared to 13 (13.97%), 6 (6.45%), and 5 (5.4%) cases of *Cryptosporidium*, *Eimeria* and *Giardia* as single infections, respectively. The selected farms had significant prevalence levels of three common protozoa: *Eimeria*, *Giardia*, and *Cryptosporidium*. Further molecular research is required to identify the species and genotype levels of protozoal enteropathogens and related risk factors.

Keywords: Calf diarrhea; Protozoal Enteropathogens; Holeta; Dairy farms.

Introduction

Neonatal calf diarrhea is defined as a clinical syndrome with a complex multifactorial etiology that causes enormous economic losses to dairy producers (Atwa *et al*., 2012). *Cryptosporidium*, *Eimeria*, and *Giardia* are common protozoal parasites that cause calf diarrhea. They are among the most important intestinal pathogens of domestic and wild animals worldwide, contributing to significant morbidity and mortality in calves (Savioli *et al*., 2006). In addition, *Cryptosporidium* and *Giardia* are probably the most common protozoal agents of human gastrointestinal diseases worldwide, leading to a significant health burden in both the developing and developed worlds (Cacciò *et al*., 2005). In Ethiopia, *Eimeria* is among the most common diarrhea-causing protozoan enteropathogens in calves and causes severe calf morbidity and mortality (Eyuel, 2016).

Cryptosporidium is another important protozoan parasite that causes morbidity in neonatal calves. Cattle are commonly infected by *Cryptosporidium parvum*, *C. bovis, C. ryanae,* and *C. andersoni*. The most important *Cryptosporidium* species causing economic losses is *C. parvum* (Chalmers *et al*., 2011). It is an obligate intracellular parasite that affects epithelial cells covering the luminal surfaces of the respiratory and digestive systems of a wide range of hosts. It frequently causes diarrhea in newborn calves (Coklin *et al*., 2007).

Giardia is one of the most significant zoonotic protozoan parasites that affect a variety of domestic animals and humans globally (Cacciò *et al*., 2005). It is commonly found alone or in combination with other pathogens as a cause of calf diarrhea, which can have economic significance.

Calf mortality represents an irrefutable and irrevocable financial source of economic loss. Thus, identification of the factors that can alter a calf's risk of death is an essential prerequisite for avoiding excessive calf mortality (Wudu *et al*., 2008). Understanding the significant causes and predisposing factors implicated in calf diarrhea (Lorino *et al*., 2005) is crucial for developing preventative strategies and reducing losses during the first few months of life. This understanding will ultimately enhance productivity per livestock unit. Protozoal enteropathogens have not been studied in dairy farms in and around Holeta, although major enteropathogens pose significant risks to dairy farms.

This study aimed to identify the major protozoal enteropathogens in diarrheic calves in dairy farms in and around Holeta town.

Materials and methods

Study area

The study was conducted in dairy farms in and around Holeta, Oromia Special Zone, Ethiopia. Holeta is located 45 km west of Addis Ababa, at 09°02' N latitude and 38°34 E longitude. The climatic condition of the area is predominantly temperate, and it receives a mean annual rainfall ranging from 84.5 mm to 89.7 mm. The altitude is 2400 m.a.s.l. The annual temperature ranges between 18°C and 24°C (Tiki and Addis, 2011).

Study population

Animals that were included in this study were crossbred dairy calves of both sexes and up to 4 months of age that were clinically affected with diarrhea, exhibited signs of systemic disease, and defecated pasty-watery feces. The ages of diarrheal calves were divided into three age groups: <4 weeks, 5-8 weeks, and greater than nine weeks (Lee *et al*., 2007) by taking into account the age group of the calves housed on farms. The breed of animals found in the farms was a crossbreed of 50% (50% Holstein Friesian and 50% Borana) and 75% (75% Holstein Friesian and 25% Borana).

Study design, sampling technique, and sample size determination

A case-series study was conducted on three dairy farms. The farms (SDF, HARC, and ABARC) and calves were selected purposively based on the availability of clinical cases and the willingness of the farm owners. The total number of calves on the three selected farms was 396, and during this study period, 93 available diarrheic cases were selected.

Sample collection and examination

Sample collection from the study animals was performed according to the methods described by Quinn *et al*. (1999). Fecal samples were collected directly from the rectum of untreated diarrheic calves, preferably soon after the onset of diarrhea, by using sterile wide-mouth screw-capped bottles for parasitological

examination. The sample bottles were labeled with calf identification. Then, samples were transported in an icebox with an ice pack to the Parasitology Laboratory of the National Biotechnology Institute in Holeta and processed within 24 hours. Laboratory analyses of the fecal samples were conducted using a flotation technique. For each sample, 5 ml of feces was processed using sugar solution as the flotation medium to recover *Cryptosporidium* oocysts, *Eimeria* oocysts, and *Giardia* cysts. In addition, the modified Ziehl-Neelsen staining technique was conducted for *Cryptosporidium* oocysts. The sample was considered positive for the respective parasites when *Giardia* cysts, *Cryptosporidium* oocysts, and *Eimeria* oocysts were detected in the specimen.

Data management

The collected data were edited, filtered, coded, and entered into Microsoft Excel® 2007. The data were then exported to SPSS software version 20.0 for appropriate statistical analysis. The proportion of each enteropathogen in the total diarrheic calves was determined by using descriptive statistics.

Results

Cryptosporidium, *Eimeria*, and *Giardia* were found in 44 (47.3%), 50 (53.8%), and 34 (36.6%) of the 93 diarrheic faecal samples that were investigated, respectively. Mixed enteropathogens were found in 18.27% of the cases for *Cryptosporidium*, *Eimeria*, and *Giardia*, 17.2% for *Cryptosporidium* and *Eimeria*, 5.4% for *Cryptosporidium* and *Giardia*, and 4.3% for *Eimeria* and *Giardia*. *Eimeria*, *Cryptosporidium*, and *Giardia* infection rates for single cases were 13.57%, 6.45%, and 5.4%, respectively. Age categories less than four weeks had a higher prevalence than other age groups, and crossbreeds of 75% level were more infected with *Cryptosporidium* infection than crossbreeds of 50% (Table 1).

Table 1. Prevalence of *Cryptosporidium* detection with different variables

Variable	Categories	No of Cases	Positive isolate (%)
Age (weeks)	<4	42	28 (66.7)
	5-8	29	8 (27.6)
	>9	22	8 (36.4)
Breed (cross)	50%	46	13 (28.3)
	75%	47	31 (66.0)
Sex	Male	45	19 (42.2)
	Female	48	25 (52.1)
Birth weight (kg)	16-24	37	20 (54.1)
	25-33	41	16 (39.0)
	34-42	15	8 (53.3)

Calves with birth weight between 16–24 kg and <4 weeks old had a higher proportion of *Eimeria* infection than other birth weight and age groups, respectively (Table 2).

Table 2. Prevalence of *Eimeria* detection with different variables

Variable	Categories	No of Cases	Positive isolate (%)
Age (weeks)	<4	42	30 (71.4)
	5-8	29	10 (34.5)
	> 9	22	10 (45.5)
Breed(cross)	50%	46	20 (43.5)
	75%	47	30 (63.8)
Sex	Male	45	25 (55.6)
	female	48	25 (52.2)
Birth weight	16-24	37	24 (64.9)
	25-33	41	19 (46.3)
	34-42	15	7 (46.7)

When compared to males and other age groups, female calves and calves less than four weeks old had a greater prevalence of *Giardia* infection (Table 3).

Table 3. Prevalence of *Giardia* detection with different variables

Variable	Categories	No of Case	Positive isolate (%)
Age (weeks)	<4	42	24 (57.1)
	5-8	29	5 (17.2)
	>9	22	5 (22.7)
Breed(cross)	50%	46	12 (26.1)
	75%	47	22 (46.8)
Sex	Male	45	15 (33.3)
	female	48	19 (39.6)
Birth weight	16-24	37	12 (32.4)
	25-33	41	15 (36.6)
	34-42	15	7 (46.7)

Discussion

Cryptosporidium, *Eimeria*, and *Giardia* were protozoal enteropathogens isolated at the genus level in the study area. The present findings showed that mixed infections with more than one protozoal genus of enteropathogens were found in diarrheic calves at higher rates (18.27% of the cases for *Cryptosporidium*, *Eimeria*, and *Giardia*) than single infections (*Eimeria*, *Cryptosporidium*, and *Giardia* infection rates for single cases were 13.57%, 6.45%, and 5.4%, respectively). This result was in line with earlier reports in which mixed infections were detected in 64.3% of younger calves, and a single *Cryptosporidium* infection was detected in 52.6% of diarrheic calves (De la Fuente *et al*., 1999). However, this finding disagreed with the report by Hailu *et al*. (2020) in southern Ethiopia, which found that the prevalence of *Cryptosporidium, Giardia,* and mixed infections was 13.03%, 9.7%, and 3.3%, respectively, where mixed infection was lower than single infection. It is suggested that the presence of more than one enteropathogen might be one of the factors determining an infection in a clinical or subclinical disease. Co-infection between pathogens can affect susceptibility to other pathogens and, therefore, can alter host immune responsiveness (Radostits *et al*., 2007).

The present findings revealed that the occurrence of calf diarrhea due to *Cryptosporidium*, *Eimeria*, and *Giardia* infection was more prevalent in calves with an age group of < 4 weeks. This finding is in agreement with the reports in Ethiopia (Wudu, 2004; Yeshwas, 2015; Hailu *et al*., 2020) that the proportion of diarrhea in younger calves is higher than that in older calves. This might be due to several factors, such as early contamination soon after birth by contact with their dams and a contaminated environment (Castro-Hermida *et al*.,

2002). This result indicated that calves from 75% crossbreed level had a higher proportion of *Cryptosporidium, Eimeria, and Giardia* infections. This might be due to calves becoming susceptible as the percentage of exotic breeds' blood levels increases due to environmental and genetic factors (Radostits *et al*., 2007).

Cryptosporidium infection in the present study was 47.3%, which was higher than the previous reports from Ethiopia that ranged from 13.57%–27.8% (Abebe *et al*., 2008; Alemayehu *et al*., 2013; Ayele *et al*., 2018; Manyazewal *et al*., 2018; Hailu *et al*., 2020). On the other hand, there were relatively higher reports of *Cryptosporidium infection*, with a prevalence of 64% in Debre Zeit (Wudu *et al*., 2008), 63.9% in and around Addis Ababa (Demissie, 2007), and 51.79% in Holeta, Debre Zeit, and Muke Turi, Ethiopia (Yenehiwot, 2008) than the current finding. This variation is most likely attributed to the differences in management and husbandry practices of the study animals in different study areas, regions, and countries (Radostits *et al*., 2007).

The present study also showed a 53.8% prevalence of *Eimeria*. This finding was lower than previous reports, with a prevalence of 68.1% in Addis Ababa and Debre Zeit (Abebe *et al*., 2008). In contrast, the current finding was higher than the report in Eastern Ethiopia, with a rate of 22.7% (Dawid *et al*., 2012) and 25.4% in Egypt (Toaleb *et al*., 2011). This variation might be due to husbandry practices, the rate of exposure of calves to oocysts, environmental temperature, humidity, sunlight, and stressors on the calves (Ward *et al*., 1979).

In this study, the detection rate of *Giardia* was 36.6%. This finding was lower than the detection rate of 6.45% in southern Ethiopia (Hailu *et al*., 2020). This variation might be due to differences in research methodology between studies. In addition to the differences inherent to the research methodology employed, several authors highlighted that the differences in management and hygienic-sanitary conditions between the farms could affect the infection rate (Geurden *et al*., 2010). In this study, the use of only one diagnostic technique (microscopy), small sample sizes, and failure to identify the species level were identified as limitations of the current research.

In the present study, major protozoal enteropathogens were isolated from selected dairy farms. *Cryptosporidium*, *Eimeria*, and *Giardia* were identified as common protozoal causative agents of calf diarrhea. Infections were more prevalent in the first few weeks of the age group and 75% of crossbred calves. In most cases, mixed infections of protozoal parasites were isolated. Further

molecular studies should be conducted to identify protozoal enteropathogens at the species and genotype levels. The general health status of the calves should be well maintained, with good hygiene soon after birth to keep calves free of infections due to such pathogens.

Ethical clearance

The Research Animal Ethics and Welfare Committee of Wollega University provided the ethical clearance certificate for this study (Reference RCITT-VP035/01/03/2021). The purpose of the study was explained to farm owners, and the best practices for veterinary care were followed.

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