



Calves gastrointestinal nematodes and Eimeria prevalence and associated risk factors in dairy farms, southern Ethiopia

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ABSTRACT

Dairy production plays a vital role in livestock farming in Ethiopia. Nevertheless, the productivity of the sector has been impacted negatively by the morbidity and mortality of replacement animals. A Cross sectional study was therefore, aimed at estimating the prevalence of *Nematode* and *Eimeria* infection in calves in Hawassa, Shashemene and Arsi Negelle, southern Ethiopia. To this end a flotation technique was used to recover *Nematode* egg and *Eimeria* oocyst from rectally collected faeces. The overall prevalence of gastrointestinal parasitic infection, *Nematode* and *Eimeria* species collectively, was 43.9% (95% CI=38.6-49.4). The estimated proportion of *Nematode*, *Eimeria* and mixed infection was 35.8%, 21.5% and 13.3%, respectively. Among the potential factors considered faecal consistency, age and study area were found to increase recovery of *Nematode* egg and *Eimeria* oocyst in faeces ($p < 0.05$). Area wise, the prevalence has been noted to be higher at Arsi Negelle followed by Hawassa and Shashemene ($p < 0.05$). Besides, younger and diarrheic calves were found more infected by *Nematode* and *Eimeria* species than their adult and non-diarrheic counterpart. Based on their morphological appearance, Strongyle type (20.3%), Trichuris (4.2%) and Ascaris (16.3%) eggs, *Eimeria* oocysts (21.5%) were observed. In the light of this finding the authors would like to advise the need for strategic intervention.

Research article

INTRODUCTION

There is increasing demand for dairy products in urban and peri-urban areas, which indicates the importance of dairying in Ethiopia (Tegegn et al., 2013). Ethiopia's cattle population is estimated at 60.4 million. Calves accounted nearly for about 18% (CSA, 2018). The sustainability of any dairy

production depends on the successful program of raising calves for replacement (Bath et al., 1985). In the modern societies, dairy farming has transformed into a business, where the owners have to look at improving the efficiency of the productions. One of the principal means of action to improve profit margins is to control and reduce costs.

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This can be achieved by improving dairy animal health, especially the replacement calves. The costs of young stock diseases are difficult to quantify but are linked to a reduced growth and increased mortality rate. Hence, the pre-weaning period represents a time of significant losses in the dairy industry (Breen et al., 2012).

Health management of replacement animals is crucial for farm profitability. Its productivity can be impacted negatively by the high mortality of replacement animals. The highest morbidity and mortality rates on dairy generally occur before weaning (Breen et al., 2012; Constable et al., 2017; Radostits, 2001). In many dairy farms today, the young stocks are often observed less frequently than adult cows by both veterinary advisors and farm staff, resulting in delayed disease detection and treatment; veterinary attention becomes focused on diseased individuals as they arise rather than working towards producing groups of healthy calves (Breen et al., 2012).

Gastrointestinal *nematodes* and *Eimeria* species are the most important agents causing disease in calves (Bruhn et al., 2012). Calves are most vulnerable to gastrointestinal parasites in their first grazing season, although yearlings and, less often, adults are sometimes affected (Constable et al., 2017). Gastrointestinal parasitic infections, nematode and *Eimeria* species, play a key role in the economic losses in that they cause low productivity, delayed growth, declined weight gain and death of the animal, and significant expenses of treatment (Höglund et al., 2018; Höglund et al., 2013; Höglund et al., 2001; Sutherland, and Scott, 2010). Even if various studies undergone on cattle helminthosis and *Eimeria* infections, only limited information

available. So this research output added to the existing information to build-up the data base. The aim of this study was to estimate the prevalence of *Nematode* and *Eimeria* species infections in calves and to identify potential risk factors associated with these infections.

MATERIALS AND METHODS

Study areas and animals

The study areas were Arsi-Negelle, Shashemene and Hawassa which are located in southern part of Ethiopia. The altitudes of the study areas range from 1,500 to 2,300 meters above sea level (masl). The annual rainfall varies, measuring 800–1,300 mm in Hawassa, 500–1,091 mm in Arsi Negelle, and 800–1,300 mm in Shashemene. The mean annual minimum and maximum temperatures for Hawassa, Arsi-Negelle and Shashemene were 12.1°C and 26.4°C, 12.6 °C and 27.3 °C, and 14 °C and 27 °C, respectively (National Meteorological Agency, 2017). Small scale dairy farming (i.e. urban and per-urban) was the characteristic feature of all the study areas. In the selected dairy farms, all calves (N=330) aged 12 months or younger were included in the study. The age of the calves was determined through interviews with the farm owners.

Study design and sample size

A cross-sectional study design was employed to estimate the prevalence of GIT *Nematode* and *Eimeria* in calves of the dairy farms. The study was conducted from January 2018 to September 2018. Lists of dairy farms were prepared in collaboration with Arsi-Negelle, Shashemene and Hawassa district animal health service

Office. Then, all farms having greater than or equal to three cows were purposively selected, making a total of 92 dairy farms with three or more calves. The sample size was computed by considering the gastrointestinal parasites prevalence, 61%, reported by Telila et al. (2014) and taking in to account of 95% CI and 5% absolute desired precision (Thrusfield, 2018). Accordingly, the computed sample size was 365. But during the study period 330 calves were found in all the selected farms. Hence, all the available calves were considered for the study.

Study methods

Data on farm size, management practices, and the presence of other animal species were gathered from farm owners using a pretested questionnaire. Subsequently, approximately 15 gm of fresh fecal samples were collected directly from the rectum of selected calves using disposable arm-length gloves. The samples were placed in screw-cap universal bottles and labeled with unique codes matching the corresponding numbers on the data collection sheet. Moreover, other information like farm name, calf age, sex, and faecal consistency (i.e. diarrhea, soft or normal) were recorded on the format prepared for this purpose. Then the samples were placed in cool ice box and transported to the Parasitology laboratory of the Faculty of Veterinary Medicine, Hawassa University. The samples were kept in the refrigerator and examined within 24 hours of the collection. The faecal samples were processed by the flotation technique, and a flotation fluid of saturated sodium chloride solution (Specific gravity=1.2) was used; and the processed

samples were examined under 10x or 40x magnifications for *Nematodes* eggs and *Eimeria* oocysts (Zajac and Conboy, 2012; Foreyt, 2001).

Data analysis

All collected data were entered into a Microsoft Excel spreadsheet, where they were edited, coded, and summarized using descriptive statistics such as means and proportions. Univariable logistic regression analysis was employed to evaluate the association of the putative risk factors considered in this particular study with calves infection by nematodes and *Eimeria* species. Those non-collinear variables ($\text{gamma}=0.20$) with a p-value of 0.25 in the univariable logistic regression analysis were subjected to a multivariable logistic regression analysis. Finally, the model fitness was assessed by the Hosmer–Lemeshow goodness-of-fit test (Dohoo et al., 2009). For the data analysis STATA 14.2 software was used. The study considered a 95% level of confidence and 5% desired level of precision.

RESULTS

Prevalence of gastrointestinal *Nematodes* and *Eimeria*

The overall prevalence of gastrointestinal parasitic infection was 43.9% (95% CI=38.6-49.4) of which *Nematodes* and *Eimeria* species and mixed infections accounted for 118 (35.8%), 71 (21.5%) and 44 (13.3%), respectively. The prevalence of *Nematode* and *Eimeria* species infection varied between the study areas: Shashemene, Hawassa and Arsi Negelle (Table 1).

Table- 1: Animal level prevalence of *Nematodes* and *Eimeria* species infections of calves vs. variables considered for the study

Variables	Variables level	No examined	<i>Nematode</i> species		<i>Eimeria</i> species	
			No (%) positive	95% CI	No (%) positive	95% CI
Study area	Shashemene	114	26 (22.8%)	16.0-31.2	14 (12.3%)	7.4-19.7
	Hawassa	179	74 (41.3%)	34.3-48.7	42 (23.5%)	17.8-30.2
	Arsi Negelle	37	18 (48.6%)	33.0-64.6	15 (40.5%)	25.9-57.1
Age	0-3 months	142	39 (27.5%)	20.7-35.4	24 (16.9%)	11.6-24.0
	4-6 months	113	47 (41.6%)	32.8-50.9	32 (28.3%)	20.7-37.4
	7-12 months	75	32 (42.7%)	31.9-54.2	15 (20.0%)	12.4-30.7
Sex	Female	212	78 (36.8%)	30.5-43.5	48 (22.6%)	17.5-28.8
	Male	118	40 (33.9%)	25.9-43.0	23 (19.5%)	13.3-27.7
Animal composition	Cattle only	269	109 (40.5%)	34.8-46.5	63 (23.4%)	18.7-28.9
	Mixed*	61	9 (14.8%)	7.8-26.2	8 (13.1%)	6.6-24.3
Faecal consistency	Normal	148	44 (29.7%)	22.9-37.6	22 (14.9%)	10.0-21.6
	Semi-formed	150	56 (37.3%)	30.0-45.4	38 (25.3%)	19.0-33.0
	Diarrhea	32	18 (56.3%)	38.7-72.4	11 (34.4%)	19.9-52.4
Total		330	118 (35.8%)	30.7-41.1	71 (21.5%)	17.4-26.3

* Mixed= Mixed with other domestic animals like equine, sheep and/or goats

Logistic regression analysis of potential risk factors for GIT nematode infection

The univariable logistic regression analysis revealed that among the potential factors considered in the study areas, age and faecal consistency were associated with the occurrence of both *Nematode* species (Table 2) and *Eimeria* species in calves (Table 3). All the risk factors, variables, were non-collinear (r is between -0.254 and 0.195), and hence, those risk factors with $p < 0.25$ in univariable logistic regression were subjected to the multivariable analysis.

The odds of acquiring *Nematode* infection by calves in Arsi Negelle and Hawassa were 3.8 and 2.8 times higher as compared to Shashemene, respectively (Table 2). Similarly, the likelihood of shedding *Eimeria* oocysts by calves of Arsi Negelle (Adjusted OR=6.2, $p < 0.001$) and Hawassa (Adjusted OR=2.6, $p = 0.007$) was significantly different from that of calves in Shashemene (Table 3).

Table- 2: Logistic regression analysis of potential risk factors for GIT *Nematode* infection in calves, southern Ethiopia.

Variables	Variables level	No. examined	No. (%) positive	Univariable			Multivariable		
				OR	95% CI	p-value	OR	95% CI	p-value
Study area	Shashemene	114	26 (22.8%)	Rf.	-	-	Rf.	-	-
	Hawassa	179	74 (41.3%)	2.4	1.4-4.0	0.001	2.8	1.6-5.0	0.001
	Arsi Negelle	37	18 (48.6%)	3.2	1.5-7.0	0.003	3.8	1.7-8.7	0.002
Age	0-3 months	142	39 (27.5%)	Rf.	-	-	Rf.	-	-
	4-6 months	113	47 (41.6%)	1.9	1.1-3.2	0.018	2.2	1.2-3.8	0.007
	7-12 months	75	32 (42.7%)	2.0	1.1-3.6	0.024	2.2	1.1-4.0	0.017
Sex	Female	212	78 (36.8%)	Rf.					
	Male	118	40 (33.9%)	1.1	0.7-1.8	0.599			
Faecal consistency	Normal	148	44 (29.7%)	Rf.	-	-	Rf.	-	-
	Semi-formed	150	56 (37.3%)	1.4	0.9-2.3	0.165	1.6	1.0-2.6	0.076
	Diarrhea	32	18 (56.3%)	3.0	1.4-6.6	0.005	5.7	2.4-13.7	0.001
Total		330	118 (35.8%)						

OR = Odds ratio, Rf. = Reference

Table- 3: Logistic regression analysis of potential risk factors for *Eimeria* species infection in calves, southern Ethiopia.

Variable	Category	No. examined	No. (%) positive	Univariable			Multivariable		
				OR	95% CI	p-value	OR	95% CI	p-value
Study area	Shashemene	114	14 (12.3%)	Rf.	-	-	Rf.	-	-
	Hawassa	179	42 (23.5%)	2.2	1.1-4.2	0.019	2.6	1.3-5.3	0.007
	Arsi Negelle	37	15 (40.5%)	4.9	2.1-11.5	0.000	6.2	2.5-15.7	0.001
Age	0-3 months	142	24 (16.9%)	Rf.	-	-	Rf.	-	-
	4-6 months	113	32 (28.3%)	1.9	1.1-3.6	0.030	2.3	1.2-4.4	0.010
	7-12 months	75	15 (20.0%)	1.2	0.6-2.5	0.572	2.3	0.6-2.7	0.527
Sex	Female	212	48 (22.6%)	1.2					
	Male	118	23 (19.5%)	Rf.	0.7-2.1	0.505	-	-	-
Faecal consistency	Normal	148	22 (14.9%)	Rf.	-	-	Rf.	-	-
	Semi-formed	150	38 (25.3%)	2.0	1.1-3.5	0.026	2.1	1.2-3.9	0.016
	Diarrhea	32	11 (34.4%)	3.0	1.3-7.1	0.012	5.3	2.0-13.6	0.001
Total		330	71 (21.5%)						

Based on their morphological characteristics of their eggs (i.e. when examined under 10X

and/or 40X microscopic magnification) four major groups of gastrointestinal nematode parasites were identified (Table 4).

Table- 4: Types of parasites identified based on morphology of their eggs/oocyst

Group of parasites	Proportion (%)	Std. Err	95% CI
Strongyles	20.3%	0.02	16.3-25.0
Trichuris	4.2%	0.01	2.5-7.1
Ascaris	16.3%	0.02	12.7-20.8
Eimeria	21.5%	0.02	17.4-26.3

Among the different reproductive disorders, abortion and retained fetal membrane showed statistically significant association ($p < 0.05$) with

uterine infection that was expressed either in the form of endometritis or metritis (Table 4).

Table-4. Association of uterine infection with the other reproductive disorders of cows as a predisposing factor

Predisposing factors	Number of cows	Cows with uterine infection	Cows without uterine infection	t ²	p-value
RFM	11	7(63.6%)	4(36.4%)	11.9	0.001
Abortion	6	5(83.3%)	1(16.7%)	13.11	0.000
Dystocia	3	1(33.3%)	2(66.7%)	0.16	0.688
Hypocalcemia	3	1(33.3%)	2(66.7%)	0.16	0.688

RFM=Retained Fetal Membrane

DISCUSSION

Calves are vital members of the dairy farms and represent the future replacement stock, and they deserve special attention and great care of health (Chang'a et al., 2010). The overall prevalence of gastrointestinal parasitic infection was 43.9 %, of which *Nematode* and *Eimeria* species infections were accounted for 35.8% and 21.5%, respectively. The prevalence of *Nematode* species observed during this study was in a general agreement with the report from the West Hararghe zone (Tulu and Lelisa, 2016; Kemal and Terefe, 2013). The multivariable logistic

regression analysis showed that among the potential risk factors considered in the study areas age and the study areas were found significantly associated with infection of calves both by *Nematode* and *Eimeria* species (Table 2 and 3). The variation between the study areas might be due to the differences in the prevailing management factors in the areas (Constable et al., 2017). The prevalence of *Nematode* species infection increases as the age of the calves increases. Similarly, Tulu and Lelisa (2016) reported that as the age of calves increased, the exposure time to the infective stage of larvae and oocysts also increased. Relatively the lower *Eimeria* infection during the first three months of age was due to the effect of colostrum

antibodies from infected cows (Fiege et al., 1992). Again the prevalence of *Eimeria* species infection declined after 7 months of age probably as a result of immunity development to re-infection by the same species of *Eimeria* (Senger et al., 1959). Cross protection between species does not occur, and hence, undoubtedly the calves might be infected by other species of *Eimeria* (Bangoura and Bardsley, 2020). The proportion of both *Nematode* (OR=5.7, $p < 0.05$) and *Eimeria* species (OR=5.3, $P < 0.05$) infection was significantly higher in diarrheic calves than calves with normal faeces consistency. In dairy farms diarrhea was most frequently observed disorder in various parts (Tamrat et al., 2020; ychli ska-Buczek et al., 2015; Megersa et al., 2009; Wudu et al., 2008). It is known that some *Nematodes* species and *Eimeria* species known to damage the intestinal epithelial cells leading to inflammation and cause diarrhea (Constable et al., 1017; Ramadan et al., 2015).

In conclusion, gastrointestinal parasitic infections are highly prevalent in the study areas. This study identified that study area, age of the calves and faecal consistency were significantly associated with the prevalence of *Nematode* and *Eimeria* species infection of calves. Therefore, it is advisable to introduce strategic interventions that include regular treatment of the herd including the calves.

CONFLICT OF INTEREST: There is no financial or other relationship that might lead to a conflict of interest.

CONSENT TO PARTICIPATE: Informed consent was obtained from all participants involved in the study.

AUTHORS CONTRIBUTIONS: All authors agreed with the content and to submit for publication. Contributions of each author is as follows: MA data collection; KA design of work, data analysis and comment on draft; EZG design of work, data analysis and comment on draft; VDMLP design and comment on draft; DS design of work, data analysis and draft preparation.

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