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Cryptosporidium and *Giardia* infections in dairy calves in southern Ethiopia

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Giardia and *Cryptosporidium* are the most common enteric protozoan parasites causing diarrhea in humans and animals worldwide. This study was conducted with the objectives of estimating prevalence and identifying risk factors for *Cryptosporidium* and *Giardia* infections in dairy calves in selected districts of southern Ethiopia. Fecal samples ($n = 330$) were collected from calves in 92 farms. The monoclonal antibody-based commercial direct immunofluorescent kit was used to test the samples for *Cryptosporidium* oocysts and *Giardia* cysts. A questionnaire survey was also administered to collect data on potential risk factors of infections. The results showed a farm-level prevalence of 69.6% (95% confidence interval [CI]: 59.1–78.7%) for *Cryptosporidium* and 38.04% (95% CI: 28.1–48.8%) for *Giardia*. Likewise, an overall animal level prevalence of 13.0% (95% CI: 9.6–17.2%) for *Cryptosporidium* and 9.7% (95% CI: 6.7–13.4%) for *Giardia* was found. At the farm level, multivariate logistic regression model showed that calves in smallholder farms were 5.3 times more likely to shed *Cryptosporidium* oocysts than calves in commercial farms ($p=0.019$). However, in case of *Giardia*, calves in commercial farms were 5.5 times more likely to shed cysts than calves in smallholder farms ($p=0.037$). Calves with diarrhea were nearly three times more likely to be positive for *Cryptosporidium* oocysts than those with normal feces ($p=0.027$). At the animal level, larger farms and younger calves were associated with *Giardia* cysts shedding, while larger herd size and lose fecal consistency were associated with *Cryptosporidium* oocysts shedding. *Giardia* and *Cryptosporidium* infection are endemic in the studied dairy farms. Therefore, detailed molecular epidemiological studies are essential to identify the role of domestic animals in the transmission of infections to humans and vice versa, and to determine the best options for prevention and control of cryptosporidiosis and giardiasis.

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1. Introduction

Health management of the replacement calves is an important component of farm routines due to the presence of several infectious and contagious agents that can seriously affect calves performance. Thus scholars of the field advise calves health management to be a priority since the first day of life as newborn (Radostits, 2001; Breen et al., 2012). Indeed, such group of animals ensures the sustainability of the farm business with new stock in place for culled individuals. However, in practice young stocks are less frequently observed compared to adult cows resulting in delayed disease detection and treatment (Breen et al., 2012). *Cryptosporidium* and *Giardia* are among the most important intestinal pathogens of domestic and wild animals worldwide contributing to significant morbidity and mortality in calves (Appelbee et al., 2005; Hunter and Thompson, 2005; Savioli et al., 2006). In addition, *Cryptosporidium* and *Giardia* are probably the commonest protozoal agents of human gastrointestinal diseases worldwide leading to significant health burden in both the developing and developed world (Caccio et al., 2005).

The protozoan organisms of the genus *Cryptosporidium* are obligate, intracellular parasites that infect the epithelial cells lining the luminal surfaces of the digestive and respiratory tracts of a wide variety of hosts (Arrowood, 2002) often leading to diarrhea in young calves (Coklin et al., 2007; Constable et al., 2017). *Giardia* is a microscopic flagellate protozoa parasite infecting wild and domesticated vertebrate animals (Olson et al., 1997), while *Cryptosporidium* primarily affects neonatal calves (de Graaf et al., 1999). *Cryptosporidium* and *Giardia* infections cause malabsorption and stunting (Savioli et al., 2006; Wegayehu et al., 2017). Both *Cryptosporidium* and *Giardia* are transmitted through the feco-oral route, either directly or indirectly by ingestion (Caccio et al., 2005; Coklin et al., 2007; Adamska et al., 2012; Constable et al., 2017). The common features of both *Cryptosporidium* and *Giardia* are that they require small doses for infection, cysts/oocysts are infectious upon excretion in feces, are stable/resistant in the environment and their dispersal in the environment contaminate drinking water and food (Caccio et al., 2005).

Giardia infection in ruminant is mostly asymptomatic, but may also be associated with the occurrence of diarrhea and ill thrift in calves. It is commonly found alone or in combination with other pathogens as a cause of calf diarrhea, which can have

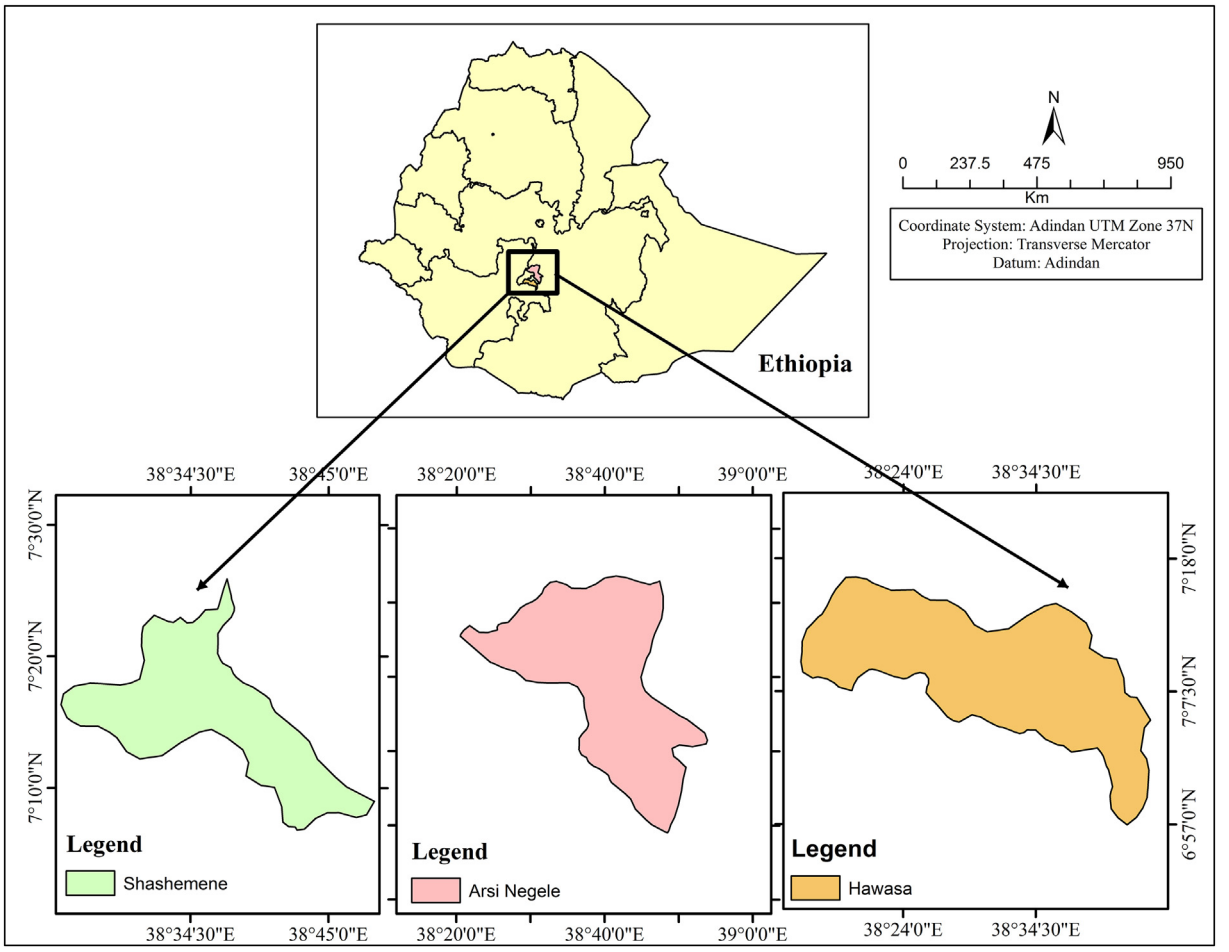

Fig. 1. Map of the study areas.

economic significance (Savioli et al., 2006). Molecular studies showed that *Giardia* is a complex parasite with eight genetic assemblages (A to H). All the subtypes/assemblages are not of zoonotic importance (Wang et al., 2014).

Out of 61 valid genotypes of *Cryptosporidium* spp. 23 of them have been described from a wide range of vertebrates causing asymptomatic or mild-to-severe gastrointestinal disease (Ryan et al., 2014). *C. parvum*, *C. hominis*, and *C. meleagridis* are the most common species in humans of which *C. parvum* (the zoonotic species) and *C. hominis* account for >90% of cases of cryptosporidiosis (Xiao and Ryan, 2004).

Cryptosporidium and *Giardia* infections are responsible for an enormous health burden, especially in developing countries. The annual number of episodes of diarrhea attributed to *Cryptosporidium* infection in Sub-Saharan African countries alone was estimated at 2.9 million in children aged <24 months (Sow et al., 2016). *Cryptosporidium* infection is associated with a greater than two-fold increase in mortality in children aged 12 to 23 months (Kotloff et al., 2013). However, giardiasis causes an estimated 2.8×10^8 cases per annum and it is self-limiting in the majority of patients, severe symptoms of diarrhea and sickness can be persistent and even life-threatening particularly in infants, immunocompromised, and older age (Lane and Lloyd, 2002).

The evidence we have in Ethiopia indicate that *Cryptosporidium* prevalence in calves ranges from 15.8%–27.8% (Abebe et al., 2008; Regassa et al., 2013; Wegayehu et al., 2016a; Ayele et al., 2018; Manyazewal et al., 2018). The corresponding figure for *Giardia* prevalence is reported to be between 7.8% (Wegayehu et al., 2013) and 9.6% (Wegayehu et al., 2016b). In humans, cryptosporidiosis prevalence of 25.9% (Wegayehu et al., 2016b) has been reported. For *Giardia* the prevalence was ranging from 8.6%–55.0% (Endeshaw et al., 2004; Tigabu et al., 2010; Wegayehu et al., 2013; 55.0%; De Lucio et al., 2016). More importantly, *Giardia* and *Cryptosporidium* infections have been noted to be high among children who have close contact with cattle (Wegayehu et al., 2013). The objectives of this study were to estimate the prevalence and identify the associated risk factors for *Cryptosporidium* and *Giardia* infection in calves of dairy cattle in selected districts of southern Ethiopia.

2. Materials and methods

2.1. Study areas

The study was conducted in urban and peri-urban areas of Arsinegelle, Shashemene and Hawassa towns, the southern part of Ethiopia (Fig. 1). The study areas are located at the escarpment of the rift valley. The altitude of the areas ranged from 1500 to 2300 m above sea level (masl). According to the reports of National Meteorological Agency (2017) the mean annual rainfall for the year 2017 was 800–1300 mm, 500–1100 mm, and 800–1300 mm for Hawassa, Arsinegelle, and Shashemene towns, respectively. The mean annual minimum and maximum temperatures for Hawassa, Arsinegelle, and Shashemene were 12.1 °C and 26.4 °C, 12.6 °C and 27.3 °C, and 14 °C and 27 °C, respectively. With the growing urban population and increasing demand for milk, small and large scale farms have been noted to grow both within and at the outskirts of the towns. In this study, all farms with three or more dairy cows were considered to be the sampling population of the study.

2.2. Study design and sample size

This cross-sectional study was conducted from December 2017 to November 2018 to estimate the prevalence and identify associated risk factors of *Cryptosporidium* and *Giardia* infections in calves. The required sample size was calculated using the formula for simple random sampling (Thrusfield, 2018) at a 95% confidence interval ($Z = 1.96$), expected prevalence (P) of 27.8% for *Cryptosporidium* (Regassa et al., 2013) and 9.6% for *Giardia* (Wegayehu et al., 2016b), and 5% precision level (D): $N = Z^2P(1 - P) / D^2$. Accordingly, 308 calves for *Cryptosporidium* and 133 calves for *Giardia* was calculated. However, 330 calves from 92 dairy farms were considered for the study.

In each town, the list of dairy farms was prepared in collaboration with district animal health experts and farms were selected randomly. In selected farms, all calves ≤one year of age were subjected to random sampling. Each dairy farm was visited once for fecal samples collection and face-to-face interviews with animal owners/attendants. The interview was guided using a semistructured questionnaire.

2.3. Fecal sample collection and transportation

A fresh fecal sample of about 15 g was individually collected from the rectum of calves using disposable plastic gloves. Each collected sample was kept into a screw cap universal bottle and labeled with a unique number. The samples were preserved in 10% formalin and transported in an icebox to the National Animal Health Diagnostic and Investigation Center (NAHDIC) at Sebeta for the direct immunofluorescent test.

2.4. Copro-antigenic test

Upon arrival at NAHDIC, the fecal samples were examined using the procedure of the commercial direct immunofluorescent kit (Merifluor®, Meridian Bioscience Inc., Cincinnati, Ohio 45244, USA) used for the simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts. The kit detects the two protozoan parasites rapidly and accurately. The specificity and sensitivity of the test for *Cryptosporidium* was 94% and 97%, respectively. But, both the sensitivity and specificity of the test for *Giardia* were 95%, compared with the traditional acid-fast staining technique (Zimmerman and Needham, 1995; Garcia and Shimizu, 1997,

Merifluor® Cryptosporidium/Giardia Meridian Bioscience, Ohio 45244, USA). About 2 to 3 g of preserved (i.e. in 10% formalin) fecal sample was mixed with fixative, CON-Trate, and then processed and examined as described by PARA-PAK®CON-Trate® (Fecal concentration kit).

2.5. Questionnaire survey

The questionnaire was administered to the farm owners or attendants face to face by the researchers to get data about potential predictors of *Cryptosporidium* and *Giardia* oo(cyst) shedding. The factors included in the study were: study area (Arsinegelle, Shashemane, Hawassa), herd size (≤7, 8–29, ≥30 animals), herd composition (cattle with other animals, only cattle), farm type (commercial, smallholder), farm size (≤30m^2, 30–100 m^2, ≥100 m^2), grazing type (zero, communal), house floor (concrete, soil), source of water (tap water, well), method of milk feeding (bottle, suckling), sex (male, female), age (0–3 months, 4-–6 months, ≥7 months), and breed (local, exotic and cross). The consistency of the feces was assessed immediately after collection through observation and recorded as normal, soft, and diarrhea.

2.6. Data management and analyses

All the data collected during the questionnaire survey and laboratory testing were entered into Microsoft Excel spreadsheets, followed by edition and coding. Then, the coded data was transported to STATA 14 software (Stata Corp. College Station, Texas) for descriptive and analytical statistics. The prevalence of *Cryptosporidium* and *Giardia* infections were computed separately. In this study, farms containing at least one positive calf were considered as positive. The association between the putative risk factors for the occurrence of *Cryptosporidium* and *Giardia* were analyzed first using univariate logistic regression analysis and followed by multivariate logistic regression. A multivariate model was built using non-collinear variables with a univariate $p \leq 0.25$. Finally, the model fitness was assessed by the Hosmer–Lemeshow goodness-of-fit test (Dohoo et al., 2009). The reliability of the fitted model was further evaluated using receiver operating characteristic curve (ROC). In this analysis, confounding factors were detected using a change in Odds ratio (OR) [≥ 20% variation in OR between univariate and multivariate result] in backward stepwise multivariate regression. The 95% confidence level was used and results were considered significant at $p \leq 0.05$.

2.7. Ethical considerations

During the fecal sample collection, animals were handled strictly following the good animal practice or in compliance with animal welfare to minimize pain and suffering. Informed verbal consent was also obtained from the animal owners.

3. Results

3.1. Farm and animal level prevalence of Cryptosporidium and Giardia using direct fluorescent antibody test

At farm level 69.6%, 38.0%, and 12% of the farms were positive for *Cryptosporidium*, *Giardia* and mixed infections, respectively. The overall animal level prevalence of *Cryptosporidium*, *Giardia* and mixed infections were 13.03% 9.7% and 3.3%, respectively (Tables 1 and 2). There is no significant difference between study areas in the prevalence of *Cryptosporidium* infection both at farm and individual animal level (p>0.05).

The prevalence of *Giardia* cyst shedding at farm level was significantly different across the three studies towns (p=0.015), i.e. the highest prevalence was noted for Hawassa town (66.67%) (Table 2).

3.2. Risk factors

3.2.1. Risk factors of Cryptosporidium infection at the farm level

At farm level analysis, farm type, herd size and farm size were significantly associated with *Cryptosporidium* infection in the univariate analysis while farm type was independent predictor in the multivariate analysis (p=0.019) (Table 3).

Table 1
Overall farm and animal level prevalence of *Cryptosporidium* in calves of Hawassa, Arsinegele and Shashemene towns, Southern Ethiopia.

Study area	Herd/farm level*				Animal level**			
	No. Exam.	No. pos.	% prev.	95% CI	No. Exam.	No. pos	% prev.	95% CI
Hawassa	18	15	83.3	58.6–96.4	180	25	13.9	9.2–19.8
Arsinegelle	37	21	56.8	39.5–72.9	37	4	10.8	3.0–25.4
Shashemane	37	28	75.7	58.8–88.2	113	14	12.4	6.9–19.9
Total	92	64	69.6	59.1–78.7	330	43	13.0	9.6–17.2

No. Exam.: number examined; No. pos.: number positive; % prev.: percent prevalence; CI: confidence interval.
* Pearson Chi-square (2) = 5.1312, $p = 0.077$.
** Pearson Chi-square (2) = 0.3189, $p = 0.853$.

Table 2
Overall farm and animal level prevalence of *Giardia* in calves of Hawassa, Arsinegele and Shashemene towns, Southern Ethiopia.

Study area	Herd/farm level*				Animal level**			
	No. Exam.	No. pos.	% prev.	95% CI	No. Exam.	No. pos	% prev.	95% CI
Hawassa	18	12	66.7	40.9–86.7	180	23	12.8	8.3–18.6
Arsinegelle	37	16	43.2	27.0–65.5	37	2	5.4	0.7–18.2
Shashemane	37	7	18.9	7.9–35.2	113	7	6.2	2.54–12.4
Total	92	35	38.0	28.1–48.8	330	32	9.7	6.7–13.4

* Pearson Chi2 (2) = 8.4488, p= 0.015.
** Pearson Chi^2 (2) = 4.3121, $p = 0.116$.

Multicollinearity matrix revealed that farm size and farm type are collinear to each other ($r = 0.56$). Similarly, farm size and herd size are collinear to each other ($r = 0.52$). The farm type was selected for the multivariate model. Herd size was found to be confounder (the change in odds ratio between univariate and multivariate analysis is over 20%, hence was not selected for the final model. Herd composition and water source were not selected for the final model due to lack of independent association (p>0.25) in the univariate analysis. Study area, farm type, grazing, house floor, and milk feeding were the variables selected for entry into the multivariate logistic regression model. The multivariate logistic regression model has Hosmer-Lemeshow X^2 (8) = 3.65, p=0.887, ROC = 0.7695 suggesting that there is no significant difference between the observed and predicted values. The sensitivity, specificity, positive and negative predictive value of the model is 89.06%, 46.43%, 79.17%, and 65%, respectively.

3.2.2. Risk factors of Cryptosporidium infection at the animal level

At animal level, *Cryptosporidium* infection was associated to herd size and feces consistency in the univariate analysis (p≤0.05). Multicollinearity matrix revealed that all independent variables for *Cryptosporidium* infection are non-collinear with each other. So, the following independent variables were selected for the multivariate model: farm type, herd size, grazing, age, and feces consistency. All the other independent variables were excluded from the regression model due to univariate p >0.25. None of the factors investigated were independent predictors of *Cryptosporidium* infection in the final multivariate model (p>0.05) (Table 4).

3.2.3. Risk factors of Giardia infection at the farm level

At farm level analysis, the variable entered into the multivariate model were study area, farm type, herd size, grazing, and milk feeding. Farm size was excluded due to collinearity with farm type ($r = 0.56$). Herd composition and water source were excluded due to univariate $p > 0.25$ (Table 5). The final model revealed a Hosmer-Lemeshow X^2 (8) =11.54, p=0.1728, and ROC = 0.8090. The corresponding sensitivity and specificity were 52%, and 91%, respectively. Likewise, the positive predictive value was 68.4%, while 83.6% was the negative predictive value.

Table 3
Logistic regression analysis of farm/herd/level risk factors for *Cryptosporidium* infection in calves of Hawassa, Shashemane and Arsinegelle towns, Southern Ethiopia.

Variable	Category	No. tested	No. pos.	% pos.	Univariate			Multivariate		
					OR	95% CI	p	OR	95%/CI	p
Study area	Arsinegelle	37	21	56.8	1.0	–	–	1.0	–	–
	Shashemane	37	28	75.8	2.37	0.88–6.40	0.089	1.06	0.32–3.52	0.918
	Hawassa	18	15	83.3	3.81	0.94–15.45	0.061	3.91	0.90–16.99	0.069
Farm type	Commercial	54	30	55.6	1.0	–	–	–	–	–
	Small bolder	38	34	89.5	6.8	2.12–21.84	0.001	5.28	1.31–21.34	0.019
Herd size	≤7 animals	12	3	25.0	1.0	–	–			
	8–29 animals	45	29	64.4	5.44	1.29–23.00	0.021			
	≥30 animals	35	32	91.4	32.0	5.49–186.54	0.000			
Herd composition	Cattle and other (mixed)	32	21	65.6	1.0	–	–			
	Only cattle	60	43	71.7	1.32	0.53–3.33	0.549			
Grazing	Zero	76	50	65.6	1.0	–	–	1.0	–	–
	Communal	16	14	87.5	3.64	0.77–17.24	0.104	1.89	0.31–11.68	0.495
Farm size	≥100 m^2	17	6	35.3	1.0	–	–			
	30–100 m^2	34	23	67.7	3.83	1.12–13.08	0.032			
	≤3 0 m^2	41	35	85.4	10.69	2.86–39.99	0.000			
House floor	Concrete	71	46	64.8	1.0	–	–	1.0	–	–
	Soil	21	18	85.7	3.26	0.87–12.15	0.001	1.49	0.31–0.7.09	0.618
Water source	Pipe	47	31	65.9	1.0	–	–			
	well	45	33	73.3	1.42	0.58–3.47	0.443			
Milk feeding	Bottle	41	25	60.9	1.0	–	–	1.0	–	–
	Suckling	51	39	76.5	2.08	0.84–5.12	0.111	1.39	0.50–3.88	0.527

OR = odds ratio, CI = confidence interval, pos. = positive, No. = number, P = P-value.

Table 4
Animal level logistic regression analysis of potential risk factors for *Cryptosporidium* infection in calves, southern Ethiopia.

Variable	Category	No. exam.	No. pos. (%)	Univariate			Multivariate		
				OR	95% CI	p	OR	95% CI	p
Study area	Arsinegelle	37	4 (10.8)	1.0	–	–	–	–	–
	Shashemene	113	14 (12.4)	1.17	0.36–3.76	0.798	–	–	–
	Hawassa	180	25 (13.9)	1.33	0.43–4.08	0.617	–	–	–
Farm type	Smallholder	67	5 (7.5)	1.0	–	–	1.0	–	–
	Commercial	263	38 (14.5)	2.09	0.79–5.55	0.137	1.25	0.39–4.01	0.703
Herd size	≤7	124	21 (16.9)	3.67	1.05–12.86	0.042	2.98	0.70–12.81	0.141
	8–29	149	19 (12.8)	2.63	0.75–9.26	0.132	1.84	0.46–7.39	0.390
	≥30	57	3 (5.3)	1.0	–	–	1.0	–	–
Herd composition	Cattle only	213	25 (11.7)	1.0	–	–			
	Mixed	117	18 (15.4)	1.37	0.71–2.63	0.348			
Grazing	Outdoor	53	3 (5.7)	1.0	–	–	1.0	–	–
	Indoor	277	40 (14.4)	2.81	0.84–9.45	0.094	3.43	0.93–12.57	0.063
Farm size	≤30 m^2	99	10 (10.10)	1.0	–	–			
	30–100 m^2	102	14 (13.73)	1.42	0.60–3.36	0.430			
	≥100 m^2	129	19 (14.73)	1.54	0.68–3.47	0.301			
House floor	Soil	41	5 (12.2)	1.0					
	Concrete	289	38 (13.2)	1.09	0.40–2.95	0.865			
Water source	Well	133	16 (12.0)	1.0					
	Pipe	197	27 (13.7)	1.16	0.60–2.25	0.658			
Milk feeding	Suckling	118	14 (11.9)	1.0					
	Feeder	212	29 (13.7)	1.18	0.60–2.33	0.639			
Age	0–3 months	144	9 (6.4%)	2.32	–	–	1.0	–	–
	4–6 moths	113	4 (3.5%)	2.41	0.84–6.43	0.105	2.25	0.79–6.40	0.129
	≥7 months	73	–	1.0	0.85–6.84	0.099	2.77	0.95–8.11	0.063
Breed	Local	34	4 (11.76)	1.0	–	–			
	Exotic & cross	296	39 (13.18)	1.14	0.38–3.41	0.817			
Feces consistency	Normal	148	15 (10.14)	1.0	–	–	1.0	–	–
	Soft	150	20 (13.13)	1.36	0.67–2.78	0.393	1.27	0.61–2.68	0.524
	Diarrhea	32	8 (25.0)	2.96	1.13–7.73	0.027	2.44	0.88–6.76	0.088

No. exam. = number examined, OR = odds ratio, CI = confidence interval, pos. = positive, No. = number, p = p-value.

3.2.4. Risk factors of Giardia infection at the animal level

During the animal level risk factor analysis for *Giardia* infection, multicollinearity matrix revealed that all independent variables are non-collinear with each other. The following independent variables were not entered into a multivariate logistic regression model due to a univariate $p > 0.25$: Study area, herd size, herd composition, grazing, milk feeding, breed, sex, and feces

Table 5
Logistic regression analysis of potential risk factors for *Giardia* infection at farm or herd level in calves of Hawassa, Shashemane and Arsinegelle towns, Southern Ethiopia.

Variable	Category	No. tested	No. pos.	% pos.	Univariate			Multivariate		
					OR	95% CI	P	OR	95% CI	P
Study area	Arsinegelle	18	12	11.11	1.0	–	–	1.0	–	–
	Shashemene	37	7	18.92	1.87	0.35–10.06	0.468	3.19	0.50–20.33	0.220
	Hawassa	37	16	43.24	6.10	1.22–30.42	0.028	5.36	0.97–29.50	0.054
Farm type	Smallholder	38	3	7.89	1.0	–	–	1.0	–	–
	Commercial	54	22	40.74	8.02	2.19–29.37	0.002	5.48	1.11–22.02	0.037
Herd size	≤7 animals	35	6	17.14	1.0	–	–	1.0	–	–
	8–29 animals	45	12	26.67	1.76	0.59–5.28	0.315	1.35	0.35–5.19	0.658
	≥30 animals	12	7	58.33	6.77	1.59–28.72	0.010	1.64	0.29–9.09	0.573
Herd comp.	Cattle & other animals	60	14	23.33	1.0	–	–			
	Only cattle	32	11	34.38	1.72	0.67–4.42	0.259			
Grazing	Common (outdoor)	16	2	12.50	1.0	–	–	–	–	–
	Zero (indoor)	76	23	30.26	3.04	0.64–14.46	0.163	1.96	0.31–12.32	0.472
Farm size	≤30 m^2	41	4	9.76	1.0	–	–			
	30–100 m^2	34	10	29.41	3.85	1.08–13.70	0.037			
	≥100 m^2	17	11	64.71	16.96	4.05–71.08	0.000			
House floor	Soil	21	1	4.76						
	Concrete	71	24	33.80	10.21	1.29–80.74	0.001			
Water source	Well	47	11	23.40	1.0					
	Pipe	45	14	31.11	1.48	0.59–3.72	0.407			
Milk feeding	Suckling only	51	10	19.61	1.0			1.0	–	–
	Feeder (jug, bottle)	41	15	36.59	2.37	0.92–6.05	0.072	1.51	0.50–4.58	0.465

Comp. = composition.

Table 6
Animal level logistic regression analysis of potential risk factors for *Giardia* infection in calves, southern Ethiopia.

Variable	Category	No. examined	No pos (%)	Univariate			Multivariate		
				OR	95% CI	p	OR	95% CI	p
Study area	Arsinegelle	37	2 (5.4)	1.0	–	–			
	Shashemene	113	7 (6.2)	1.16	0.23–5.82	0.861			
	Hawassa	180	23 (12.8)	2.56	0.58–11.38	0.216			
Farm type	Smallholder	67	3 (4.5)	1.0	–	–	1.0	–	–
	Commercial	263	29 (11.0)	2.64	0.78–8.96	0.118	1.17	0.25–5.45	0.843
Herd size	<7 animals	124	11 (8.9)	1.0					
	8–29 animals	149	15 (10.1)	1.15	0.51–2.60	0.738			
	≥30 animals	57	6 (10.5)	1.21	0.42–3.45	0.723			
Herd composition	Cattle only	213	20 (9.4)	1.10	0.52–2.34	0.799			
	Mixed	117	12 (10.3)	1.0					
Grazing	Zero	277	26 (9.4)	1.0					
	Outdoor	53	6 (11.3)	1.23	0.48–3.16	0.663			
Farm size	≤30 m^2	99	4 (4.0)	1.0	1.0	–	1.0	–	–
	30–100 m^2	102	12 (11.8)	3.17	0.99–10.18	0.053	2.79	0.72–10.75	0.137
	≥100 m^2	129	16 (12.4)	3.36	1.09–10.40	0.035	3.13	0.83–11.90	0.093
House floor	Soil	41	1 (2.4)	1.0	1.0	–	1.0	–	–
	Concrete	289	31 (10.7)	4.81	0.64–6.19	0.128	3.23	0.36–28.94	0.295
Water source	Pipe	197	16 (8.1)	1.0	1.0	–	1.0	–	–
	Well	133	16 (12.0)	1.55	0.74–3.21	0.242	1.76	0.81–3.84	0.155
Milk feeding	Suckling	212	20 (9.4)	1.09	0.51–2.31	0.829			
	Feeder (jog/bottle)	118	12 (10.2)	1.0	–	–			
Breed	Local	34	2 (5.9)	1.0					
	Exotic or cross	296	30 (10.1)	1.80	0.41–7.91	0.434			
Age	0–3 months	144	21 (14.6)	2.95	0.97–8.93	0.056	3.77	1.21–11.77	0.022
	4–6 months	113	7 (6.2)	1.14	0.32–4.04	0.840	1.40	0.39–5.11	0.608
	≥7 months	73	4 (5.5)	1.0	–	–	–	–	–
Sex	Female	118	9 (7.6)	1.0	–	–			
	Male	212	23 (10.9)	1.47	0.66–3.30	0.346			
Feces consistency	Normal	150	14 (9.3)	1.0	–	–			
	Diarrhea	32	3 (9.4)	1.01	0.27–3.72	0.994			
	Soft	148	15 (10.1)	1.10	0.51–2.36	0.815			

The selection of the best-fitting model for *Giardia* infection showed that farm size and age of the calves were important predictors (Table 7). (Hosmer-Lemeshow X^2 (5) = 2.07, p = 0.8399, ROC = 0.6796).

consistency. While farm type, farm size, house floor, water source, and age were entered into the final model because they were non-collinear and the univariate *p* was <0.25. Accordingly it was noted that calves less than three months of age were 3.77 more likely to shed *Giardia* cysts than calves ≥7 months age. The final model also had Hosmer-Lemeshow X^2 (8) = 17.10, $p = 0.0291$, ROC = 0.6771 (Table 6).

4. Discussion

This study tried to add knowledge towards a better understanding of the prevalence and risk factors of *Cryptosporidium* and *Giardia* infections in southern Ethiopia. The study confirmed that calves in all the study areas shed *Cryptosporidium* oocysts and *Giardia* cysts contributing to the contamination of the environment.

The direct fluorescent antibody test used in this study was selected due to its high sensitivity and specificity. The Merifluor direct fluorescent antibody test used in the present study detects cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts, which is far better than the traditional fecal concentration and acid-fast stain (Garcia and Shimizu, 1997).

The reported farm-level prevalence for *Cryptosporidium* (69.6%) and *Giardia* (38.0%) were in line with earlier study findings in the country (Abebe et al., 2008) where *Cryptosporidium* oocyst shedding in calves was in 65% of farms in central Ethiopia. This entails the presence of one or more infected calves in most of the farms studied. Differently, there was a report of lower overall farm level *Cryptosporidium* prevalence (37.7%) in dairy calves of Addis Ababa and its environs (Manyazewal et al., 2018). Perhaps

Table 7
Best fitting model for predictors of *Giardia* infection in calves, southern Ethiopia.

Variable	Category	OR	95% CI	P
Farm size	≤30 m^2	1.0	–	
	30–100 m^2	3.80	1.16–12.42	−0.028
	>100 m^2	3.82	1.22–11.97	0.021
Age	≥7 months	1.0	–	
	4–6 months	1.21	0.34–4.35	−0.770
	0–3 months	3.44	1.12–10.59	0.031

this may be explained by the different study areas and laboratory technique we used. In general, our findings and earlier reports in the country fall within the global infection prevalence estimate for *Cryptosporidium*, i.e. 15.0–100% as per the report from western Canada (Olson et al., 1997; Gow and Waldner, 2006), USA (Garber et al., 1994; Santin et al., 2004), Norway (Hamnes et al., 2006), Spain (Castro-Hermida et al., 2006) and Denmark (Maddox-Hyttel et al., 2006).

The farm level prevalence of *Giardia* was significantly higher ($p< 0.05$) in calves of Hawassa than in calves of Arsinegelle and Shashemene towns. Although not significant, the farm and animal level prevalence of *Cryptosporidium* was also higher than Arsinegelle and Shashemene towns. This might be due to the presence of abundant moisture and poorly drained floor, which might prolong the survival of the cysts (Barwick et al., 2003). An alternative explanation might be that the water runoff from nearby areas of Hawassa town might contain feces of infected humans and animals containing oocysts and cysts that might contaminate the drinking water sources leading to increased prevalence in calves of Hawassa town. Moreover, such increases in prevalence trend for Giardia was higher in commercial farms than in smallholder farms. This might be associated with compromised farm hygienic status due to logistic and inadequate farm labour as farm size grows with commercialization. Such scenario is known to create persistent infection source via feed and water contamination by infected animals (Constable et al., 2017). Indeed, higher stocking density aggravates the likelihood of transmission between infected and susceptible animals by increasing the contact rate. This has been evidenced with several studies elsewhere (Garber et al., 1994; Mohammed et al., 1999).

The farm-level prevalence of *Giardia* in calves kept in houses where the floor is concrete was significantly high as compared to calves kept in house floor made of soil. This finding in fact is contradictory to what is generally known in the literature, and with limited number of samples we have, it is difficult to explain the finding. The significantly higher farm-level prevalence of *Cryptosporidium* infection in smallholder farms than in commercial farms ($p=0.019$) in the present study might be explained by the space limitation which increases the rate of contact among animals. Rearing animals in smaller areas has been shown to increase the contamination of the rearing areas and the contact of calves to each other (Garber et al., 1994; Mohammed et al., 1999; Constable et al., 2017; Ayele et al., 2018). It has been identified previously that close contact is indeed a risk factor for *C. parvum* infection (Hunter and Thompson, 2005; Savioli et al., 2006).

On animal level, the global estimate for cryptosporidia infection was noted to range from 2.2–93.3% (Huang et al., 2014; Castro-Hermida et al., 2006) and our estimate could signify the wide discrepancy of global prevalence estimate for cryptosporidiosis. The fact holds true for *Giardia* that reveal global prevalence estimate ranging from 5.1% to 100% (Coklin et al.,2007; Toledo et al., 2017).

Locally, the *Cryptosporidium* infection in the present study was lower than the previous reports from Ethiopia that ranged from 17.6%–27.8% (Abebe et al., 2008; Regassa et al., 2013; Ayele et al., 2018; Manyazewal et al., 2018). The current *Giardia* prevalence was in agreement with the previous report from Ethiopia (Wegayehu et al., 2016b). However, as compared to the present findings, Wegayehu et al. (2013) reported a much lower prevalence for both *Giardia duodenalis* (2.3%) and *Cryptosporidium* spp. (7.8%) in cattle of North Shewa Zone, Ethiopia.

In the present study, the infection prevalence of both parasites was higher in pre-weaned calves of 0–3 months age than in any other age group, which is consistent with previous observations (Venu et al., 2013; Delafosse et al., 2015; Ayele et al., 2018; Manyazewal et al., 2018). It has been reported that the maximum *Giardia* cyst excretion was seen in calves of 2–3 weeks of age, which coincides with the depletion of colostral immunity (Kakandelwa, 2015; Toledo et al., 2017). As the age of calves increases, cyst excretion decreases and becomes intermittent (Ghazy et al., 2015). Thus, the high occurrence of *Giardia* in young calves (0–3 months) could be due to the limited functional integrity of the immune system or slow development of specific immunity by the host against the parasite (Kakandelwa, 2015) that makes calves more vulnerable for infection and disease.

The likelihood of shedding *Giardia* cysts in large farm size (>100 m^2) was 3.8 times higher when compared to small farm size (<30 m^2) ($p=0.021$). The plausible explanation for this might be related to the intensive animal management system in farms of large size, which might favor the transmission of *Giardia* cysts (Kakandelwa, 2015). This suggests the importance of reducing an environmental load of *Giardia* cysts and *Cryptosporidium* oocysts through a regular and thorough cleaning which also reduces other pathogens that might exacerbate *Giardia* and *Cryptosporidium* infections.

Fecal consistency resulted as an indicator of the occurrence of *Cryptosporidium* infection as *Cryptosporidium* infection rate was higher in diarrheic than in non-diarrheic calves. This is consistent with the findings of Delafosse et al. (2015), Constable et al. (2017) and Ayele et al. (2018) but contradict the findings of Manyazewal et al. (2018) and Abebe et al. (2008). Despite the ability of these agents to cause the observed loose fecal consistency, it must be considered that we have not investigated further on to the other causes of diarrehea.

The present study is the first one to report the prevalence of the two protozoan parasites in calves of the study areas. However authors would like to recommend cautious interpretation of the findings due to certain limitations including: nature of the study design, intermittent release of the infectious agents and casual association. Moreover, the diagnostic test used in this study could not however, identify whether the parasites recovered were zoonotic or otherwise.

5. Conclusions

The moderate animal level prevalence along with the presence of one or more infected calves in most farms of the study area indicate that giardiasis and cryptosporidiosis are endemic in the study areas. Besides, the increased prevalence in very young calves and the presence of such calves in larger farms could have a higher contribution to environmental contamination with

relatively more frequent oocyst and cyst shedding. To produce more detailed evidence on the zoonotic relevance, there is a need for detailed molecular studies.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

References

Abebe, R., Wossene, A., Kumsa, B., 2008. An epidemiological study of *Cryptosporidium* infection in dairy calves on selected dairy farms of central Ethiopia. Revue Méd. Vét 159 (2), 107–111.
Adamska, M., Leonska-Duniec, A., Sawczuk, M., Macieska, A., Skotarczak, B., 2012. Recovery of *Cryptosporidium* from spiked water and stool samples measured by PCR and real-time PCR. Vet. Med. 57 (5), 224–232.
Appelbee, A.J., Thompson, R.C.A., Olson, M.E., 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife - current status and future needs. Trends Parasitol. 21, 370–376.
Arrowood, M.J., 2002. Vitro cultivation of *Cryptosporidium* species. Clin. Microbiol. Rev. 215 (3), 390–400.
Ayele, A., Seyoum, Z., Leta, S., 2018. *Cryptosporidium* infection in bovine calves: prevalence and potential risk factors in Northwest Ethiopia. BMC Res Notes 11, 105.
Barwick, R., Mohammed, H., White, M., Bryant, R., 2003. Prevalence of *Giardia* spp. and *Cryptosporidium* spp. on dairy farms in South-Eastern New York state. Prev. Vet. Med. 59, 1–11.
Breen, J., Down, P., Kerby, M., Bradlle, A., 2012. Restoring the dairy herd: Rearing youngstock and replacing cows. In: Green, M.R. (Ed.), Dairy Herd Health. CABI Publishing, Boston, MA, pp. 35–72.
Caccio, S.M., Thompson, C.A., McLauchlin, J., Smith, H.V., 2005. Unraveling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol. 21 (9), 430–437.
Castro-Hermida, J.A., González-Losada, Y.A., Ares-Mazás, E., 2006. Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). Vet. Parasitol. 106, 1–10.
Coklin, T., Farber, J., Parrington, L., Dixon, B., 2007. Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp.in dairy cattle in Ontario, Canada. Vet. Parasitol. 150 (4), 297–305.
Constable, D.P., Hinchcliff, W.K., Done, H.S., Grünberg, W., 2017. Veterinary Medicine, a Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 11th edition. ELSEVIER, St. Louis, USA, pp. 397–400.
De Lucio A, Amor-Aramendía A, Bailo B, Saugar JM, Anegagrie M, Arroyo A, López-Quintana, B. Zewdie, D, Ayehubizu, Z, Yizengaw, E, Abera, B, Yimer, M, Mulu, W, Hailu, T, Herrador, Z, Fuentes, I., and Carmena, D. (2016). Prevalence and genetic diversity of *Giardia duodenalis* and *Cryptosporidium* spp. among school children in a rural area of the Amhara Region, North-West Ethiopia. PLoS One 11(7): 0159992.
Delafosse, A., Chartier, C., Dupuy, M.C., Dumoulin, M., Pors, I., Paraud, C., 2015. Cryptosporidium parvum infection and associated risk factors in dairy calves in western France. Prev. Vet. Med. 118, 406–412.
Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research. 2nd ed. Prince Edward Island, AVC, Charlottetown, pp. 239–249.
Endeshaw, T., Mohamod, H.M., Tilahun, W., 2004. *Cryptosporidium parvum* and other intestinal parasites among diarrheal patients referred to EHNRI in Ethiopia. Ethiop. Med. J. 42, 195–198.
Garber, L.P., Salman, M.D., Hurd, H.S., Keefe, T., Schlater, J.L., 1994. Potential risk factors of *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc. 205, 86–90.
Garcia, L.S., Shimizu, R.Y., 1997. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for the detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. J. Clin. Microbiol. 35, 1526–1529.
Ghazy, A., Shafy, S.A., Shaapan, R.M., 2015. Cryptosporidiosis in animals and man: 1. Taxonomic classification, life cycle, epidemiology and zoonotic importance. Asian J. Epidemiol. 8, 48–63.
Gow, S., Waldner, C., 2006. An examination of the prevalence of and risk factors for shedding of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves from western Canadian cow-calf herds. Vet. Parasitol. 137, 50–61.
de Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H., Peeters, J.E., 1999. Areview of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 29, 1269–1287.
Hamnes, I.S., Gjerde, B., Robertson, L., 2006. Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway. Vet. Parasitol. 140, 204–216.
Huang, J., Yue, D., Qi, M., Wang, R., Zhao, J., Li, J., Shi, K., Wang, M., Zhang, L., 2014. Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Ningxia, northwestern China. Vet. Res. 10, 292.
Hunter, P.R., Thompson, R.C.A., 2005. The zoonotic transmission of *Giardia* and *Cryptosporidium*. Int. J. Parasitol. 35, 1181–1190.
Kakandelwa, C., 2015. Prevalence and Risk Factors of *Giardia* Infections in Dairy Cattle Herds in Lusaka and Chilanga Districts of Zambia. The requirements for the degree of Master of Science in. One Health Analytical Epidemiology, Luska (unpublished).
Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., et al., 2013. Burden and etiology of diarrhoeal disease in infants and young children in developing countries (the global enteric multicenter study, GEMS): a prospective, case-control study. Lancet 382 (9888), 209–222.
Lane, S., Lloyd, D., 2002. Current trends in research into the waterborne parasite *Giardia*. Crit. Rev. Microbiol. 28, 123–147.
Maddox-Hyttel, C., Langkjær, R.B., Enemark, H.L., Vigre, H., 2006. *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs—occurrence and management associated risk factors. Vet. Parasitol. 141, 48–59.
Manyazewal, A., Francesca, S., Pal, M., Gezahegn, M., Tesfaye, M., Lucy, M., Teklu, W., Getachew, T., 2018. Prevalence, risk factors and molecular characterization of *Cryptosporidium* infection in cattle in Addis Ababa and its environs, Ethiopia. Vet. Parasitol. Reg Stud Report 13, 79–84.
Mohammed, H.O., Wade, S.E., Schaaf, S., 1999. Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. Vet. Parasitol. 83, 1–13.
National Meteorological Agency, 2017. The climate of City: Hawassa. Retrieved from. http://www.ethiomet.gov.et/climate-of-city/2466/, Accessed date: 28 June 2019.
Olson, M.E., Guselle, N.J., O'Handley, R.M., Swift, M.L., McAllister, T.A., Jelinski, M.D., Morck, D.W., 1997. Giardia and Cryptosporidium in dairy calves in British Columbia. Can. Vet. J. 38, 703–706.
Radostits, O.M., 2001. Herd Health, Food Animal Production Medicine. 3rd edition. W.B. Saunders Company, USA, pp. 333–396.
Regassa, A., Gizaw, O., Abunna, F., Abebe, R., Beyene, D., Megersa, B., Debela, E., Asmare, K., Skierve, E., 2013. Cryptosporidium in calves, lambs, and kids at Haramaya, eastern Ethiopia. Ethiop. Vet. J. 17 (1), 81–94.
Ryan, U., Fayer, R., Xiao, L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141, 1667–1685.
Santin, M., Trout, J.M., Xiao, L., 2004. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet. Parasitol. 122, 103–117.
Savioli, L., Smith, H., Thompson, A., 2006. *Giardia* and *Cryptosporidium* join the neglected diseases initiative. Trends Parasitol. 22, 203–208.

Sow, S.O., Muhsen, K., Nasrin, D., Blackwelder, W.C., Wu, Y., Farag, T.H., et al., 2016. The burden of *Cryptosporidium* diarrheal disease among children <24 months of age in moderate/high mortality regions of Sub-Saharan Africa and South Asia, utilizing data from the Global Enteric Multicenter Study (GEMS). PLoS Negl. Trop. Dis. 10 (5), e0004729.

Thrusfield, M., 2018. Veterinary Epidemiology. Fourth edition. John Wiley and Sons, Inc., USA, pp. 275–284.

Tigabu, E., Petros, B., Endeshaw, T., 2010. Prevalence of giardiasis and cryptosporidiosis among children in relation to water sources in selected village of Pawi special district in Benishangul-Gumuz Region, northwestern Ethiopia. Ethiop. J. Health Dev. 24 (3), 205–213.

Toledo, R.S., Martins, F.D.C., Freire, R.L., 2017. Waterborne *Giardia* and *Cryptosporidium*: contamination of human drinking water by sewage and cattle feces. Vet. Med. 35 (5), 3395–3416.

Venu, R., Latha, B.R., Abdul Basith, S., Sreekumar, C., Dhinakar Raj, G., Raman, M., 2013. Factors influencing the prevalence of *Cryptosporidium* infection in south Indian dairy calves. J. Parasit. Dis. 37 (2), 168–172.

Wang, H., Zhao, G., Ghen, G., Jian, F., Zhang, S, Feng, C., Wang, R., Zhu, J., Dong, H., Hua, J., Wang, M., Zhang, L., 2014. Multilocus genotyping of *Giardia duodenalis* in dairy cattle in Henan, China. PloS one 9. https://doi.org/10.1371/journal.pone.0100453.

Wegayehu, T., Adamu, H., Petros, B., 2013. Prevalence of *Giardia duodenalis* and *Cryptosporidium* species infections among children and cattle in North Shewa Zone, Ethiopia. BMC Infect. Dis. 13, 419.

Wegayehu, T., Karim, M.R., Anberber, M., Adamu, H., Erko, B., Zhang, L., Tilahun, G., 2016a. Prevalence and genetic characterization of *Cryptosporidium* species in dairy calves in central Ethiopia. PLoS One 11 (5), e0154647.

Wegayehu, T., Karim, M.R., Erko, B., Zhang, L., Tilahun, G., 2016b. Multilocus genotyping of *Giardia duodenalis* isolates from calves in Oromia special zone, Central Ethiopia. Infect. Genet. Evol. 43, 281–288.

Wegayehu, T., Karim, M.R., Li, J., Adamu, H., Erko, B., Zhang, L., Tilahun, G., 2017. Prevalence and genetic characterization of *Cryptosporidium* species and *Giardia duodenalis* in lambs in Oromia special zone, central Ethiopia. BMC Vet. Res. 13, 22.

Xiao, L., Ryan, U.M., 2004. Cryptosporidiosis: an update in molecular epidemiology. Curr. Opin. Infect. Dis. 17, 483–490.

Zimmerman, S.K., Needham, C.A., 1995. Comparison of conventional stool concentration and preserved-smear methods with Merifluor *Cryptosporidium*/*Giardia* direct immunofluorescence assay and ProSpecT *Giardia* EZ microplate assay for detection of *Giardia lamblia*. J. Clin. Microbiol. 33, 1942–1943.