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 small-holder 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 loose 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...
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 prevalence 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 test 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, 2008).
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 (≤30m², 30–100 m², ≥100 m²), 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 significance level was used and results were considered significant at p ≤ 0.05. 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 ≤ 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

| 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.
3.2.3. Risk factors of Giardia infection at the farm level

The corresponding sensitivity and specificity were 89.06%, 46.43%, 79.17%, and 65%, respectively. Likewise, the positive predictive value was 68.4%, while 83.6% was the negative predictive value.

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 \((p\leq0.05)\). 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 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 |
| Arsinegele | 37 | 16 | 43.2 | 27.0–65.5 | 37 | 2 | 5.4 | 0.7–18.2 |
| Shashemene | 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 Chi² (2) = 4.3121, \(p= 0.116\).

Table 3

Logistic regression analysis of farm/herd/level risk factors for Cryptosporidium infection in calves of Hawassa, Shashemene and Arsinegele towns, Southern Ethiopia.

| Variable | Category | No. tested | No. pos. | % pos. | Univariate | Multivariate |
|---|---|---|---|---|---|---|
| Study area | Arsinegele | 37 | 21 | 56.8 | 1.0 | - |
| | Shashemene | 37 | 28 | 75.8 | 2.37 | 0.88–6.40 | 0.089 |
| | Hawassa | 18 | 15 | 83.3 | 3.81 | 0.94–15.45 | 0.061 |
| Farm type | Commercial | 54 | 30 | 55.6 | 1.0 | - |
| | Small holder | 38 | 34 | 89.5 | 6.8 | 2.12–21.84 | 0.001 |
| 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 | 12.0 | 5.49–186.54 | 0.000 |
| Herd composition | | | | | |
| | Only cattle | 60 | 43 | 71.7 | 1.32 | 0.53–3.33 | 0.549 |
| | | | | | |
| Grazing | Zero | 76 | 50 | 65.6 | 1.0 | - |
| | Communal | 16 | 14 | 87.5 | 3.64 | 0.77–17.24 | 0.104 |
| Farm size | ≥100 m² | 17 | 6 | 35.3 | 1.0 | - |
| | 30–100 m² | 34 | 23 | 67.7 | 3.83 | 1.12–13.08 | 0.032 |
| | ≤30 m² | 41 | 35 | 85.4 | 10.69 | 2.66–39.99 | 0.000 |
| House floor | Concrete | 71 | 46 | 64.8 | 1.0 | - |
| | Soil | 21 | 18 | 85.7 | 3.26 | 0.87–12.15 | 0.001 |
| 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 | - |
| | Suckling | 51 | 39 | 76.5 | 2.08 | 0.84–5.12 | 0.111 |

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 OR 95% CI p | Multivariate 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 – –                 | – –                     |
|                         | 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 – –                 | – –                     |
|                         | Indoor                           | 277       | 40 (14.4)    | 2.81 0.84–9.45 0.094    | 3.43 0.93–12.57 0.063   |
| Farm size               | ≤30 m²                           | 99        | 10 (10.10)   | 1.0 – –                 | – –                     |
|                         | 30–100 m²                        | 102       | 14 (13.73)   | 1.42 0.60–3.36 0.430    | – –                     |
|                         | ≥100 m²                          | 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 – –                 | – –                     |
| 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 – –                 | – –                     |
|                         | 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 0.85–8.64 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 – –                 | – –                     |
|                         | Soft                             | 150       | 20 (13.13)   | 1.36 0.67–2.78 0.393    | 1.27 0.61–2.68 0.524    |
|                         | Diarrhea                         | 32        | 20 (6.25)    | 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, Shashemene and Arsinegelle towns, Southern Ethiopia.

| Variable                | Category                        | No. tested | No. pos. % pos. | Univariate OR 95% CI p | Multivariate OR 95% CI p |
|-------------------------|---------------------------------|-----------|-----------------|------------------------|-------------------------|
| Study area              | Arsinegelle                      | 18        | 12 11.11       | 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 0.97–20.50 0.054   |
| Farm type               | Smallholder                      | 38        | 3 7.89         | 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 – –                 | – –                     |
|                         | 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 1.39–28.72 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 m²                           | 41        | 4 9.76         | 1.0 – –                 | – –                     |
|                         | 30–100 m²                        | 34        | 10 29.41       | 3.85 1.08–13.70 0.037   | – –                     |
|                         | ≥100 m²                          | 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 – –                 | – –                     |
|                         | 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.
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 X2 (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...
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% per 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 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 m²) was 3.8 times higher when compared to small farm size (<30 m²) (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 diarrheea.

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.

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