Cryptosporidiosis is a widespread parasitic disease caused by protozoan parasites belonging to the genus Cryptosporidium [22], which develop and multiply in the epithelial cells of the gastrointestinal or respiratory tracts of a wide range of vertebrate hosts including humans [23]. There are 13 valid species of Cryptosporidium based on morphological criteria, host specificity and DNA-based studies [26]; however, only two of them, i.e., Cryptosporidium parvum and Cryptosporidium andersoni (formerly known as C. muris) are important in cattle [7, 13, 23]. Apart from these, a recently identified C. bovis, was found to be the predominant species infecting 2-11-month-old calves but was not associated with overt disease [19]. C. parvum and C. andersoni can be differentiated on the basis of oocyst morphology and site of infection [15]. C. parvum infects the distal small intestine, nearly spherical in shape and has got an average size of 5 x 4.5 µm [23] while C. andersoni parasitizes the glands of abomasum, ellipsoidal in shape and measures on average 7.4 x 5.5 µm [13].

Transmission of cryptosporidiosis, both within and between host species including humans, is by the fecal-oral spread of the environmentally resistant oocysts, which are fully sporulated and infective when excreted in feces [20]. Of the species infecting cattle, only C. parvum is pathogenic and causes diarrhea in calves [7]. C. andersoni is much less prevalent.

A cross-sectional study was undertaken from September 2004 to March 2005 on 40 dairy farms selected from two study areas located in central Ethiopia to determine the prevalence, species, and the risk factors associated with Cryptosporidium infection. Single fecal samples were collected from a total of 580 calves < 12 months of age and examined for the oocysts of Cryptosporidium by centrifugal fecal flotation technique using concentrated sucrose solution. Modified acid-fast staining also was performed to increase the optical contrast and to stain confusing yeasts differentially. The overall point prevalence of the infection was 17.6%. Of the 40 dairies sampled, 26 (65%) had one or more calves shedding Cryptosporidium oocysts. In this study the species of Cryptosporidium circulating in the farms was presumed to be Cryptosporidium andersonii based on morphology of the oocysts and certain epidemiological features of the parasite. The infection was not found to be statistically associated with diarrhea (P>0.05). The parasite was detected in a wide age range of calves, i.e. from 21 to 345-days old calves. Among the several risk factors studied, the hygiene of the calf-rearing houses was the most important factor found to be associated with the likelihood of infection (P<0.05).

Keywords: Cryptosporidium, Dairy calves, Prevalence, Risk factors, Species.
and was only found in weaned calves or adult cattle [4, 6, 12, 24] and the infection is considered to be clinically mild, affecting weight gain [3] and milk production [9].

Since the first report of its presence in cattle by PANCIERA et al [17], Cryptosporidium has been the object of many prevalence studies worldwide. However, most of the published studies are from North America, Europe and Japan, and little is known about the prevalence of the disease in African countries including Ethiopia. The only documented report of Cryptosporidium infection in Ethiopia is that of Wudu [25], who reported 6.7% prevalence in calves after a longitudinal study conducted to determine the cause of calf morbidity and mortality. However, this study did not provide full account on the epidemiology of the parasite and the species encountered as well.

Therefore, taking in to account the scarcity of data about the parasite in the country and its importance as one of the major causes of calf diarrhea in different parts of the world, a cross-sectional study was undertaken with the objectives to determine the prevalence, species and potential risk factors of Cryptosporidium infection in Ethiopian dairy farms.

Material and Methods

STUDY AREA AND POPULATION

Two study areas namely Addis Ababa city and Debre Zeit town were selected for the study. Addis Ababa, which is the capital city of the Federal Democratic Republic of Ethiopia, is situated in a high land area with an altitude of 2500 meters above sea level and has got an average annual rainfall of 1800 mm. Debre Zeit is a town located about 45 km south east of Addis Ababa at an altitude of 1850 meter above sea level and has an average annual rainfall of 800 mm. The target population of this survey is all dairy farms found in Addis Ababa and Debre Zeit, which consists of 154 dairy farms possessing 10 and above cows per farm. The study population constituted all calves less than 12 months of age in the dairy farms. Before sampling the farms were stratified into three categories based on the total number of cattle they consist as small (<100), medium (100-200) and large (>200).

STUDY DESIGN AND SAMPLE SIZE DETERMINATION

A cross-sectional study was designed to address the above-mentioned objectives. The sampling method employed was a one-stage cluster sampling [21] and a total of 40 dairy farms and 580 calves < 12 months of age were selected for the study with 95% confidence interval and 5% desired absolute precision. The farms were selected proportionally from the three herd size categories by random sampling technique and all the calves < 12 months in the selected farms were included in the study. Each farm was visited once during the study period from September 2004 to March 2005 to collect fecal samples and to conduct questionnaire survey in order to collect data on demographic, management, hygiene and other factors hypothesized to be associated with the risk of infection with Cryptosporidium in dairy herds.

SAMPLE COLLECTION

A fresh fecal sample of approximately 30 gm was collected from each calf by retrieval per rectum, using disposable plastic gloves new for each calf. The sample was placed in a separate disposable plastic container and transported in a cool box to the laboratory on the same day of collection, and preserved at refrigeration temperature until processing within 48 hours of arrival. At the time of sampling, the name of the farm, date of sampling, consistency of the feces (soft, pasty, watery or normal), and the age, sex, breed, and tag no of the calves were recorded for each calf on a recording format.

LABORATORY INVESTIGATION

A centrifugal fecal flotation technique using Sheather’s sugar solution with specific gravity of 1.27 was applied to detect the oocysts of Cryptosporidium [11]. A modified Kinyoun acid-fast staining was also done to increase the optical contrast and to stain confusing yeasts differentially [5].

DATA ANALYSIS

The data collected from the two study sites were entered into Excel spreadsheet (Windows) and analyzed with SPSS for Windows (Version 11.5) and STATA for Windows (Version 7) statistical soft wares. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individuals sampled X 100. Categorical data were analyzed first with the Chi-square (χ²) test for independence as a screening process. This test was followed by stepwise multivariate logistic regression, to account for confounding variables and interactions. A P value < 0.05 was required for significance. Odds ratios (OR) were determined from the coefficients in the logistic regression.

Results

During the study period a total of 580 fecal samples were examined of which 102 (17.60%) were positive for Cryptosporidium oocysts. Of the 40 dairy farms surveyed, 26 (65%) had at least one calf shedding Cryptosporidium oocysts. The point prevalence of Cryptosporidium infection on the basis of various factors and the results of statistical analysis were presented in Tables (I, II and III).

The species of Cryptosporidium identified in this study was presumed to be C. andersoni based on the morphology of the oocysts and the epidemiology of the parasite. The oocysts were ellipsoid in shape with sporozoites, pink-tinged in Sheather’s sugar solution and measured 7 x 5.4 (6.8 x 5.2 - 5.6) mm in size.

Discussion

The species of Cryptosporidium identified in this study was presumed to be C. andersoni based on morphometrics
and observation of the epidemiology of the parasite. The oocysts were ellipsoidal in shape, pink-tinged in Sheather’s sugar solution and measured 7 x 5.4 (6.8 x 5.2 - 5.6) mm in size. All these morphological characters fit the description of *C. andersoni* (*C. muris*) given by several authors overseas [8, 13, 24]. The size measurements reported by these authors and others is 7.4 x 5.5 (6.8 x 5.2 - 5.6) µm. However, further molecular characterization is required to confirm the present species. *C. parvum*, which is the major pathogenic species and the common cause of calf diarrhea in different parts of the world, was not found in this study. The absence of *C. parvum* has also been reported by ABRAHAM et al [1], who did the first formal study on agents associated with neonatal diarrhea in Ethiopian dairy farms and reported the presence of coronavirus, rotavirus, and ETEC from diarrheic calves up to 8 weeks of life.

When compared to the few published reports of *C. andersoni* (*C. muris*) overseas, the present finding is closely similar with the prevalence reported in adult cattle (16%) in Scotland [6]. In USA, relatively a lower prevalence (1.03 to 1.1%) was reported in calves [4, 24] but a wide range of prevalence (0.5 to 31%) was reported in adults and unreported age group [3, 4]. In calves period prevalence of 47% was reported in Brazil [8] and 11.1 to 92.9% in Czech Republic [12], while 0 to 40% was reported in cows in Canada [18] following a longitudinal study.

Age of the calves was not significantly associated (*P > 0.05*) with the likelihood of *Cryptosporidium* infection, although a higher infection rate was observed in calves 6-12 months of age (20.6%) than calves 0-6 months of age (16%) (Table I). The oocysts were recovered in calves over a broad age range i.e. from 21 to 345 days. Therefore, this study suggests that infection with the present *Cryptosporidium* spp. is important in all ages of calves above 3 weeks. In contrast to this study, a significantly higher infection rate with *C. andersoni* was reported in calves over 6 months of age by WADE et al [24]. In the present study, *Cryptosporidium* oocysts were detected in calves aged as early as 3 weeks of age and this was found to be much lower than the first age reported by other studies involving *C. andersoni* viz. 6 weeks [8], 7 weeks [24] and 9 weeks [12]. However, oocyst excretion with *C. parvum* infection has been described in calves as early as 3 days of age [24].

The hygiene of the calf-rearing houses strongly influenced (*P < 0.001*) the detection of *Cryptosporidium* oocysts in dairy calves. Calves in poorly cleaned farms were 5.2 times more likely to be infected with the parasite than calves in well-cleaned farms (Table I). The farms regarded as poor hygienic were dirty and muddy, and presumably this has created a favorable condition for the persistence of *Cryptosporidium* oocysts on the farms. The association of infection with hygienic deficiencies has also been reported by other studies [14].

### Table 1: Point prevalence of *Cryptosporidium* infection displayed on the basis of different factors and results of univariate analysis of the hypothesized risk factors by using chi-square (*χ²*).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. exam</th>
<th>No. Pos (%)</th>
<th>OR</th>
<th>95% CI of OR</th>
<th>P</th>
<th>χ²</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midland (D. Zeit)</td>
<td>250</td>
<td>14</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highland (A. Ababa)</td>
<td>330</td>
<td>20.3</td>
<td>1.6</td>
<td>1 - 2.45</td>
<td>0.048</td>
<td>3.90</td>
<td>1</td>
</tr>
<tr>
<td><strong>Age in months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-6</td>
<td>381</td>
<td>16</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6-12</td>
<td>199</td>
<td>20.6</td>
<td>1.4</td>
<td>0.88 - 2.11</td>
<td>0.168</td>
<td>1.90</td>
<td>1</td>
</tr>
<tr>
<td><strong>Feeding system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>In feed troughs</td>
<td>436</td>
<td>15</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On the ground</td>
<td>144</td>
<td>26</td>
<td>2</td>
<td>1.3 - 3.12</td>
<td>0.003</td>
<td>8.70</td>
<td>1</td>
</tr>
<tr>
<td><strong>Hygiene of calf-rearing houses</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Good</td>
<td>240</td>
<td>13.6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>104</td>
<td>28</td>
<td>2.5</td>
<td>1.5 - 4.1</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>229</td>
<td>45</td>
<td>5.2</td>
<td>2.4 - 11.2</td>
<td>0.000</td>
<td>26.92</td>
<td>2</td>
</tr>
<tr>
<td><strong>Herd size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>231</td>
<td>14.3</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>136</td>
<td>14.7</td>
<td>1.03</td>
<td>0.57 - 1.90</td>
<td>0.912</td>
<td></td>
<td></td>
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<tr>
<td>Small</td>
<td>213</td>
<td>23.0</td>
<td>1.80</td>
<td>1.1 - 2.92</td>
<td>0.019</td>
<td>6.83</td>
<td>2</td>
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<td><strong>Preweaning housing</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Individual pen</td>
<td>353</td>
<td>14.45</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group pen</td>
<td>165</td>
<td>20</td>
<td>1.5</td>
<td>0.91 - 2.40</td>
<td>0.112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tethered in cow-barn</td>
<td>56</td>
<td>32.14</td>
<td>2.8</td>
<td>1.50 - 5.29</td>
<td>0.001</td>
<td>11.15</td>
<td>2</td>
</tr>
</tbody>
</table>

Although small herd size farms had a significantly greater percentage of positive calves (23%) than had the large (14.3%) and medium (14.7%) sized farms in univariate screening analysis, this association was no longer observed in the multivariate model (Table III). In further analysis of the questionnaire data, it was observed that out of the total farms regarded as poor hygienic, 94% were small herd-size farms of which 75% were positive for Cryptosporidium oocysts. The effect of small herd size, therefore, resulted from the confounding influence of hygiene, and did not truly contribute to the risk of the infection. In contrast to the present finding, an association between large herd size and the risk of infection with Cryptosporidium has been observed in several studies overseas, whereby increased density of animals favors infection of greater number of calves which in turn, contaminate their surroundings [10, 14].

There was a significant association between the feeding system of calves and the risk of infection in initial screening test, where calves feeding directly on the ground were 2 times more likely to be infected with Cryptosporidium than calves feeding in feed troughs (Table I). However, this was not shown in multivariate analysis. The study area where samples were taken from and preweaning calf housing condition also were not significantly associated with the risk of infection in multivariate analysis, although they were significant in univariate analysis.

In the present study, no significant association (OR = 1, P>0.05) was found between diarrhea and infection with the presumed C. andersoni, although 7.44% of the diarrheic calves were observed to shed oocysts (Table II). The odds of shedding Cryptosporidium oocysts were increased by 3.23 times among non-diarrheic calves when compared to the odds of shedding among diarrheic (20.6% vs. 7.44%). A similar finding was reported in calves of British Columbia [16] and USA [24]. It was also reported by Anderson [2] that although at later ages calves appear to be more heavily infected, they do not show severe clinical symptoms.

In final analysis of all the hypothesized risk factors with multiple (multivariate) logistic regression, it was observed that only the hygiene of calf-rearing houses was found to be significantly associated with the likelihood of infection with the presumed C. andersoni. Therefore, this finding strongly suggests that sanitation is the mainstay for the control of Cryptosporidium infection among dairy calves.

Acknowledgements

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