STUDY ON THE PREVALENCE OF CRYPTOSPORIDIUM IN CALVES SUFFERING FROM DIARRHEA IN NORTHWESTERN SYRIA

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ABSTRACT

Cryptosporidium is one of the most common causes of diarrhea in calves worldwide, and the risk factors contributing to its control and prevention are extensively studied. This epidemiological study was conducted in an unstable breeding environment. Ninety-six (96) calves suffering from diarrhea were studied from five regions in northwestern Syria. The goal of this study was to assess the prevalence of cryptosporidium in fecal samples and to measure the expected risk and regression for assumed risk factors that may cause diarrhea in calves up to 60 days old. Fecal samples were tested using direct ELISA. The results showed 9.1% (1/11) cases in the western Aleppo countryside, 21.1% (4/19) in the eastern Idlib countryside, 13.6% (3/22) in the Northern Idlib countryside, 2.9% (1/35) in the Jisr Al-Shughur countryside, while no positive case was recorded in the western countryside. This is the first study conducted in the region showing the incidence, regression, and risk rates, and it accentuates the need for a broader study on the effect of certain drugs used in the treatment of diarrhea and its consequences.

Keywords: newborn calves, diarrhea, multiscreen ELISA, Cryptosporidium

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INTRODUCTION

Intestinal and respiratory disorders are the most frequently reported diseases or causes of death in calves (Mötus et al. 2018). Diarrhea in calves is a serious health problem that occurs at the age of less than a month and leads to an incidence rate of up to 20% (Bendali et al. 1999b; Waltner et al. 1986). The mortality rate as a result of diarrhea can range from 1.5% to 8% (Ammar et al. 2014).

Diarrhea is the major cause of economic losses for the dairy and meat industry worldwide (Tajik et al. 2012; Bolieau et al. 2010). The most common causes of the complex diarrheal syndrome are related to group A rotavirus (BRV), Bovine Coronavirus (BoCV), Enterotoxigenic E.Coli (ETEC) (E.Coli K99), and Cryptosporidium Parvum (Radostitis et al. 2007; Garcia et al. 2000). The most common cause of diarrhea in neonatal calves aged 9 to 21 days is due to infection with BRV, BoCV, ETEC (E.Coli K99), Cryptosporidium parvum and Salmonella spp (Izzo et al. 2012; Bartels et al. 2010; Mohteshamuddin et al. 2020). The most common causative agent of diarrhea in newborn calves is cryptosporidium (Cryptosporidium spp). Cryptosporidium is a zoonotic pathogen causing diarrhea in humans, particularly in immunocompromised children and adults. (Nydam et al. 2001; Cho and Yoon 2014; Lombardelli et al. 2019; Conrady et al. 2021).

Cryptosporidia are intestinal protozoans that are parasitic in the intestine of humans and other vertebrates. In cattle, cryptosporidium causes acute or chronic gastrointestinal upset, resulting in mortality, weight loss, and reduced milk production (Brook et al. 2008; Smith et al. 2006). The incidence of cryptosporidium in calves is specifically correlated with their age and the environmental conditions of the barn (Brook et al. 2008). Many factors influence the prevalence of cryptosporidium, including the age of the calves, the general health status of the farm, intake of colostrum, the administrative procedures of the farm, the quality of fodder, the source of drinking water, presence of associated diarrheal cases, and the climate (Ogendo et al. 2017).

The cryptosporidium (C parvum) in calves over four weeks of age has a low probability of causing infection. Few laboratories put cryptosporidium detection tests on the list of routine tests, and very few veterinarians have paid attention to the importance of this matter (Joachim et al. 2003).
Several reports indicated the prevalence of cryptosporidiosis from 6.26 to 39.65% in cattle in various parts of the world (Azami et al. 2007). There are many diagnostic tests for the detection of diarrheal pathogens, such as Direct Electron Microscopy, Enzyme-linked Immunosorbent Assay (ELISA), Rapid Immunoglutination Test (RImgT), Acrylamide Gel Electrophoresis (AGE), Polymerase Chain Reaction (PCR), and Immunofluorescence Test (IFT). ELISA is a preferred technique over other laboratory methods for diagnosing diarrhea in calves due to the low cost, high sample throughput and high sensitivity of the test (Mayameei et al. 2010). According to Bartels et al. 2010 the sensitivity and specificity for Escherichia coli were 90% and 98.5% (using ELISA BIO K99), for rotavirus 96% and 100% (using dsRNA electrophoresis on PAGE, for coronavirus 88.9%, and 98.7% (using ELISA BIO K068) and for C. parvum test are 94.1% and 95.5% using fluctuation technique, respectively.

According to Geurden et al. 2008, the sensitivity and specificity were respectively 68% and 95% for rotavirus ELISA and 33% and 80% for coronavirus ELISA as compared to lateral flow immunochromatography (Izzo et al. 2012). However, the sensitivity and specificity were respectively 100% and 91% for E. coli K99 ELISA when compared to PCR (Izzo et al. 2011) and 94% and 96% for cryptosporidium ELISA for other three diagnostic techniques (another ELISA assay, immunofluorescence assay, and immunochromatography). Cryptosporidium is a protozoan that causes Coccidiosis. It has a similar life cycle to Isospora and its reproduction alternates between sexual and asexual reproduction. Cryptosporidium is widespread in newborn calves, with prevalence rate ranging from 14% to 80%, depending on the age, general health of the calf, and housing conditions (Haschek et al. 2006; Singh et al. 2006; Trotz-Williams et al. 2005; Joachim et al. 2003; Garcia et al. 2000; Lefay et al. 2000; De la Fuente et al. 1999; Lentze et al. 1999; Naciri et al. 1999; Garber et al. 1994). Cryptosporidium in its natural life cycle can develop and multiply in gastrointestinal epithelial cells of infected animals (Shafieyan et al. 2014; Chen et al. 2003). Generally, cryptosporidium infection is fecal-oral by direct or indirect contact with fully formed oocysts shed from infected animals during the infectious phase (Radostits et al. 2007). To date, seven species and two genotypes of cryptosporidium have been identified in cattle (Hunter et al. 2004). Oocysts spread and infect calves in the first three days of life and peak in the first two weeks. Infections can occur in adult cows after three months of age, and symptoms appear after infection within 3-7 days and at least after 4-17 days. Previous studies have shown that the morbidity may reach 100% in calves (De Graaf et al. 1999; O’Handley et al. 1999, Fayer et al. 1998; Xiao and Herd 1994; Argenzio et al. 1990; Harp et al. 1990).

There are two possible mechanisms of cryptosporidium diarrhea: The first involves the destruction of the intestinal epithelia and their microvilli as a result of direct toxic effect of sporozoite infection, which impairs absorption from the intestinal lumen. This mechanism is not supported by most researchers (Gookin, et al., 2002). The second mechanism involves blocking the sodium chloride absorption by increasing secretion of chloride and bicarbonate ions, which impede absorption of sodium from the intestinal lumen. This results in drawing of water from interstitial tissue of intestine into the lumen. This mechanism depends on the secretion of prostaglandins from the epithelial cells in which the sporophytes are enclosed (foster and smith 2009). Regardless of the mechanism or cause of the occurrence of cellular loss and villous atrophy of epithelial cells, the fluid malabsorption due to the movement of sodium combined with chloride or some other nutrients in the top of the villi results in diarrhea, which is offset by an increase in the secretion of negative electrolytes (anions) from crypt cells. In addition, the loss of epithelial cells and atrophy of the mature villi and associated transporters leads to a decrease in the total absorbable surface area of the intestine (Gookin et al. 2002; Argenzio et al. 1994; Argenzio et al. 1993; Argenzio et al. 1990).

MATERIALS AND METHODS

Ninety-six stool samples were collected from diarrheal calves (Friesian, Simmental and local hybrid species) during the period between September 2021, and June 2022. The selection of samples was done by the stratified cluster sampling method. The number of samples was based on the capabilities provided by the commercial kits for examination and the financial resources of the researcher. Due to apparent changes in fecal structure from semi-natural, creamy texture to watery and fecal color change from green-brown to yellow, sampling was performed directly from the rectum using medical single-use gloves placed in sterile plastic containers with tightly closing. Samples are numbered from 1 to 96 and each number is linked to an epidemiological plate (questionnaire) during sample collection, containing the following information about the collected sample: Number of the sample, farm location, farmer reference, case history, the total number of calves with diarrhea in the farm, health status, the kind of floor of the farm, the kind of roof, age and gender, dystocia, timing and amount of colostrum intake, the consistency and color of diarrhea, and the treatment that was used against the disease. The age of calves was between 1 day to 2 months, divided into the following six categories: (1-9 days old), (10-19 days old), (20-29 days old), (30-39 days old), (40-49 days old), (50-59 days old). Samples have been selected by Multi-stage cluster sampling method from 17 villages (Kafir Karmin, Kafir Nasih, Darat Azza, Sarmada, Binnish, Foah, Kafriya, Zardana, Kelli, Harbnoush, Ma’art Misrin, Mishmishan, Balmis, Ain al-Sooda, Kanest Bani Ezz, Salqin, Meles) which are distributed in five regions (western countryside of Aleppo, eastern countryside of Idlib,
Northern countryside of Idlib, western countryside of Idlib, and Jisr Al-Shughur countryside). Clusters (villages) were selected within the five regions of the study area, and infected calves were chosen randomly from those clusters. The five regions selected for the study have geographical diversity comprising of differences in the environment, altitude above sea level, and the topography (plain or mountainous).

The samples were transferred to the laboratory within a maximum period of eight hours to be stored at -20 degree Celsius in the laboratory until use.

Multiple-link ELISA test was used to detect diarrheal pathogens (EASY-DIGEST Rotavirus - Coronavirus - E. coli F5 - Cryptosporidium Diagnosis test for bovines BIO K 151/2 -Bio-X Diagnostics, Belgique). The Antigen Capturing Enzyme-Linked Immunosorbent Assay (Ag-ELISA), is a rapid assay test to determine the causative agent in samples based on the antibody (Monoclonal Antibody) depending on the target antigen (Lequin 2005). In preparation for the Antigen ELISA test, the antibody is attached to a solid surface such as glass or plastic, or a membrane filter. This antibody will adsorb the target antigen if it is present in the sample. There will be different color reactions to verify that the antibody captures the antigen and visualizes the interaction that occurred between them. The antigen can be quantitatively estimated as optical density (OD) measured by a spectrophotometer which is positively related to the amount of antigen. Sensitivity and specificity of the Easy Digest kit was confirmed by comparing its results with those produced by the Digestive BIO K 348 kit of the same company (Bio-X Diagnostics, Belgique 2021) which is given as follows: Cryptosporidium: Sensitivity: 100%, Specificity: 88%.

RESULTS

The results of the ELISA test showed that out of 96 stool samples, there were 9 cases of cryptosporidium infection (9.4%). The positive cases were distributed as follows: 9.1% (1/11) in the western Aleppo countryside, 21.1% (4/19) in the eastern Idlib countryside, 13.6% (3/22) in the Northern Idlib countryside, 2.9% (1/35) in the Jisr Al-Shughur countryside, while no positive case was recorded in the western Idlib countryside.

<table>
<thead>
<tr>
<th>Region</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jisr Al-Shughur</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>Western Aleppo</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Eastern Idlib</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Northern Idlib</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Western Idlib</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Positive%</th>
<th>Negative%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jisr Al-Shughur</td>
<td>3%</td>
<td>97%</td>
</tr>
<tr>
<td>Western Aleppo</td>
<td>9%</td>
<td>91%</td>
</tr>
<tr>
<td>Eastern Idlib</td>
<td>21.10%</td>
<td>78.90%</td>
</tr>
<tr>
<td>Northern Idlib</td>
<td>13.60%</td>
<td>86.40%</td>
</tr>
<tr>
<td>Western Idlib</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>9.40%</td>
<td>90.60%</td>
</tr>
</tbody>
</table>

Graph 1: Distribution of samples in different regions (negative and positive for Cryptosporidium infection)

Statistical Analysis: We used the SPSS V23 for statistical analysis, the Nominal and Ordinal logistic regression method to measure the effect, and the Chi-square to test the independence and Odds Ratio (Risk). The prevalence of cryptosporidium in intensive system farms is 25% (5/20) among the calves. There is a statistically significant correlation between the type of rearing and the infection with cryptosporidium (p < 0.05); Risk Estimate ($R^2 = 7.260$, CI:95% odds: 0.167 (0.040-.695), $P = 0.014$) (Table 1).
Table (1): Crosstabs, number of cases, the total percent of cases by type of breeding, Chi-square test, Nominal logistic regression test (reference-Negative)

<table>
<thead>
<tr>
<th>Type of breeding</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Count</td>
<td>72</td>
<td>4</td>
<td>76</td>
<td>7.26</td>
<td>0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.792</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>75.0%</td>
<td>4.2%</td>
<td>79.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive</td>
<td>Count</td>
<td>15</td>
<td>5</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>15.6%</td>
<td>5.2%</td>
<td>20.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R² : chi-square value  
P value : for R²  
B : beta value for nominal regression  
OR : odds ratio value  
Sig : P value for nominal regression  
a: P < 0.05

There was no correlation (P > 0.05) between cryptosporidium infection and kind of the ceiling of the barn (Graph 2), or the floor of the barn (Graph 3), and the health status in the farm (Graph 4). Five out of nine positive cases (55.5%) were recorded in farms with good health status (Graph 4).

![Graph (2). Crosstab Type of Ceiling * cryptosporidium](image-url)
The study also showed the correlation of positive cases in barns where diarrhea is present in more than one calf (P < 0.05) (R²= 6.874, CI:95% odds: 0.143 (0.028-0.732)) (Table 2).

**Table (2):** Crosstabs, number of cases, the total percent of cases by Calves with diarrhea in the barn, Chi-square test, Nominal logistic regression test (reference-Negative)

<table>
<thead>
<tr>
<th>Calves with diarrhea</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single calf</td>
<td>Count</td>
<td>58</td>
<td>2</td>
<td>60</td>
<td>6.874</td>
<td>0.009*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The study showed that the season and prevalence of cryptosporidium are two independent events (P>0.05), where 5.6% (3/54) cryptosporidium-positive calves were detected in the warm months and 14.3% (6/42) in the cold months (Graph 5).

<table>
<thead>
<tr>
<th>Age of calf (Days)</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9</td>
<td>Count</td>
<td>14</td>
<td>5</td>
<td>19</td>
<td></td>
<td>0.425</td>
<td>0.520</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>14.6%</td>
<td>5.2%</td>
<td>19.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 - 19</td>
<td>Count</td>
<td>10</td>
<td>3</td>
<td>13</td>
<td></td>
<td>1.227</td>
<td>1.4</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>10.4%</td>
<td>3.1%</td>
<td>13.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 - 29</td>
<td>Count</td>
<td>17</td>
<td>0</td>
<td>17</td>
<td></td>
<td>2.037</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>17.7%</td>
<td>0.0%</td>
<td>17.7%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 - 39</td>
<td>Count</td>
<td>22</td>
<td>1</td>
<td>23</td>
<td></td>
<td>3.141</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Crosstabs, number of cases, the total percent of cases by category of Age, Chi-square test, Ordinal logistic regression test (reference-positive).
The prevalence was 9.3% (4/43) positive among female calves while 9.4% (5/53) among male calves. There was no statistical significance for the association between infection rate and calf gender (P>>0.05) (Graph 6).

There is a correlation between the color of diarrhea and the incidence of infection. This appeared through the incidence of infection that appeared within the two colors recorded in the questionnaire: green-brown and yellow, 2% (1/49) and 17% (8/47), respectively (R² = 6.337, CI: 95% odds: 0.102 (0.12- 0.847) (Table 4).

The study showed a strong correlation (P<<0.05) between the consistency of diarrhea and the rate of infection recorded.
Table (5): Crosstabs, number of cases, total percentage of cases by consistency of diarrhea, Chi-square test, Nominal logistic regression test (reference-Negative).

<table>
<thead>
<tr>
<th>Diarrhea consistency</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>like-normal</td>
<td></td>
<td></td>
<td></td>
<td>12.76</td>
<td>0.002⁴</td>
<td>.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Count</td>
<td>43</td>
<td>0</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Total</td>
<td>44.8%</td>
<td>0.0%</td>
<td>44.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>33</td>
<td>9</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Total</td>
<td>34.4%</td>
<td>9.4%</td>
<td>43.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aqueous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Total</td>
<td>11.5%</td>
<td>0.0%</td>
<td>11.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R²: chi-square value
P value: for R²
B: beta value for nominal regression
OR: odds ratio value
Sig: P value for nominal regression
a: P < 0.05

Symptoms accompanying diarrhea were recorded when calves were surveyed at the time of sampling, but they did not have any statistical significance linking them with infection.

The study showed that the highest incidence was recorded on the fifth day after diarrhea 33.3% (1/11), on the sixth day 25% (1/4) and on the fourth day 18.2% (2/11). No positive cases of diarrhea were recorded after the seventh day.

There was a correlation between the infection of calves and their dystocia (P = 0.031) (R²= 0.49, df=1, P = 0.031) (odds: 4.938(1.032-23.617) P = 0.046 ). The incidence of infection was recorded at 27.3% (3/11) in the calves born with a difficult birth, while it was 7.1% (6/85) in the calves born with natural birth Table 6).

Table (6): Crosstabs, number of cases, total percentage of cases by dystocia, Chi-square test, Nominal logistic regression test (reference-Negative).

<table>
<thead>
<tr>
<th>Dystocia</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td>4.684</td>
<td>0.030⁴</td>
<td>1.597</td>
<td>4.938</td>
<td>0.046⁴</td>
</tr>
<tr>
<td>Count</td>
<td>8</td>
<td>3</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Total</td>
<td>8.3%</td>
<td>3.1%</td>
<td>11.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>79</td>
<td>6</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Total</td>
<td>82.3%</td>
<td>6.3%</td>
<td>88.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R²: chi-square value
P value: for R²
B: beta value for nominal regression
OR: odds ratio value
Sig: P value for nominal regression
a: P < 0.05

The recorded incidence cryptosporidium was 50% (3/6) in the calves whose mothers were found infected when the samples were taken (R² = 12.432, CI:95% odds: 14.000 (2.310-84.859) (Table 7).

Table (7): Crosstabs, number of cases, the total percent of cases by mother have diarrhea, Chi-square test, Nominal logistic regression test (reference-Negative).

<table>
<thead>
<tr>
<th>mother have diarrhea</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td>12.432</td>
<td>0.00⁴</td>
<td>2.63</td>
<td>14.00</td>
<td>0.004⁴</td>
</tr>
<tr>
<td>Count</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Total</td>
<td>3.1%</td>
<td>3.1%</td>
<td>6.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>84</td>
<td>6</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R²: chi-square value
P value: for R²
B: beta value for nominal regression
OR: odds ratio value
Sig: P value for nominal regression
a: P < 0.05
There were no cryptosporidium-positive cases of calves that had not taken colostrum, as only three cases were recorded of calves that were not taking colostrum at birth but were fed on substitutes. Delay in administering colostrum was recorded in only 7 cases, with only one sample among those found to be infected with cryptosporidium and there was no correlation (P>>0.005) ($R^2 = 0.320$, $P = 0.571$). There was also no correlation between the amount of colostrum given to the calf and the detection of cryptosporidium infection $p>> 0.05$. (Table 8).

Table (8): Crosstabs, number of cases, the total percent of cases by given colostrum, Chi-square test, Nominal logistic regression test (reference-Negative).

<table>
<thead>
<tr>
<th>Colostrum given</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>$R^2$</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Count</td>
<td>84</td>
<td>9</td>
<td>93</td>
<td>0.320</td>
<td>0.571</td>
<td>15.192</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>87.5%</td>
<td>9.4%</td>
<td>96.9%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Count</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>3.1%</td>
<td>0.0%</td>
<td>3.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2$: chi-square value  
P value: for $R^2$  
B: beta value for nominal regression  
OR: odds ratio value  
Sig: P value for nominal regression  
a: P < 0.05

There was a correlation between the infection detected in the barn and the presence of different ages in it (P<0.05) ($R^2 = 4.089$) (Table 9).

Table (9): Crosstabs, number of cases, the total percent of cases by Ages in barn, Chi-square test, Nominal logistic regression test (reference-Negative).

<table>
<thead>
<tr>
<th>Ages in barn</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>$R^2$</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Count</td>
<td>59</td>
<td>9</td>
<td>68</td>
<td>4.089</td>
<td>0.043³</td>
<td>18.545</td>
<td>113275532</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>61.5%</td>
<td>9.4%</td>
<td>70.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Count</td>
<td>28</td>
<td>0</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>29.2%</td>
<td>0.0%</td>
<td>29.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2$: chi-square value  
P value: for $R^2$  
B: beta value for nominal regression  
OR: odds ratio value  
Sig: P value for nominal regression  
a: P < 0.05

All calves from which the samples appeared positive for infection were treated with a mixture of antibiotics and analgesics 13% (9/65) (P<0.05) ($R^2 = 4.736$) as shown in Table 10.
Out of the 360 calves examined, 67 (18%) calves carrying cryptosporidium have reduced the incidence. 

**DISCUSSION**

Samples were collected from farms that were placed within the research plan. All calves suffering from a change in feces consistency or color were considered having diarrhea, regardless of their health status and/or other accompanying symptoms. The symptoms were recorded within the questionnaire following Svensson's definition of diarrhea, i.e., a change in feces that is present for two days or more with a soft or watery consistency, which may be associated with other components and may be associated with weight loss and poor general condition of the animal (Svensson et al. 2003).

Diarrhea is known by the multiplicity of causes which may be overlapping and are activated as a result of multiple ways of infection. Other factors that may contribute to diarrhea include feeding, environmental factors, and the conditions within the farm. Poor public health measures (biosecurity), lack of colostrum, individual susceptibility to infection, and a combination of these or other factors may lead to the disease and, thus, increase the economic burden on establishments (Alfieri et al. 2006).

The questionnaire included supposed risk factors. It included the location of the farm, climatic factors during the season in which the sample was collected, age, gender, colostrum consumption, quantity and time of intake, as well as the medications used when the sample was taken. Studies by many researchers have shown that there is a strong and significant association between infection with *C parvum* and diarrhea in calves (Haschek et al. 2006; Singh et al. 2006; Trotz et al. 2005; Joachim et al. 2003; Naciri et al. 1999). Various reports have indicated a prevalence of cryptosporidium from 6.26 to 39.65% in livestock in different parts of the world (Joute et al. 2014, Azami et al. 2007). In general, differences exist between studies regarding diarrhea around the world (De la Fuente et al. 1999; Garcia et al. 2000; Uhde et al. 2008). The difference in diarrhea results from different studies may be due to the different diagnostic methods used (Mayameei et al. 2010; Ok et al. 2009).

ELISA is a preferred method over other laboratory methods for diagnosing diarrhea in calves due to the low cost of the test, high sample throughput, and high sensitivity (Mayameei et al. 2010). According to study in the United Arab Emirates, 29 out of 36 fecal samples were positive for cryptosporidium based on the ELISA test (Mohteshamuddin 2020). In the same vein, out of the 28 fecal samples collected from 39 calves, 13 (46.4%) were positive for cryptosporidium in Italy (Lorra et al. 2018). Prevalence of cryptosporidium was 18% in calves aged between 1 to 5 days, while the infection rate was 52% among calves aged 9 to 21 days (Al Mawly et al. 2015). The prevalence of farm-wide cryptosporidium in New Zealand dairy farms was 52% using ELISA or microscopy. The researchers suggested that the false positive by ELISA was caused by some species whose fragments were not amplified by PCR, other than parvum species, or that there was a deterioration in the samples that were refrigerated for a long time This indicates reliability of the ELISA test (Al Mawly et al. 2015). The incidence of 3.92% of the total of 51 calves infected with diarrhea in Turkey (Altug et al. 2013) was lower than the percentage that appeared in our study. The number of samples may be different, and geographical environment or other factors might have reduced the incidence.

The incidence of cryptosporidium in southwestern France was 15.6% (Bendali et al. 1999a), which is fairly close to our study. Out of the 360 calves examined, 67 (18%) calves carrying cryptosporidium oocysts were identified in feces samples in northwestern Ethiopia (Ayele et al. 2018). According to Brandão's study in Brazil, the number of infections (6/9) with *cryptosporidium parvum* which is much higher percentage of infections compared to our study (Brandão et al. 2007). The difference between the two studies could be attributed to the geographical variance or due to

**Table (10):** Crosstabs, number of cases, the total percent of cases by kind of Treatment, Chi-square test, Nominal logistic regression test (reference-Negative).

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>R²</th>
<th>P value</th>
<th>B</th>
<th>OR</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics &amp; analgesics</td>
<td>Count</td>
<td>% of Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>58.3%</td>
<td>9</td>
<td>67.7%</td>
<td>4.736</td>
<td>0.03a</td>
<td>18.59</td>
<td>11934</td>
</tr>
<tr>
<td>No treatment</td>
<td>Count</td>
<td>% of Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>32.3%</td>
<td>0</td>
<td>32.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R² : chi-square value
P value : for R²
B : beta value for nominal regression
OR : odds ratio value
Sig : P value for nominal regression
a: P < 0.05
the presence of another cause such as simultaneous infection. Fonte has shown that simultaneous infection in fecal samples of calves infected with diarrhea, rotavirus and with cryptosporidium were 87%, while it was 11.1% in samples with coronaviruses with cryptosporidium (Fuente et al. 1999). Oocysts of cryptosporidium spread and infections occur in calves in the first three days and peak in the first two weeks of life. Infections occur in adult cows after three months of age and symptoms appear after infection within 3-7 days. Some studies indicate an incidence of up to 100% in calves (de Graaf et al. 1999; O’Handley et al. 1999; Fayer et al. 1998; Xiao and Herd 1994; Argenzio et al. 1990, Harp et al. 1990). Oocysts are not shed during the first three days of a calf’s life (Fayer et al. 1998). Our study shows a correlation between the age group of the calf and the incidence of infection. The total number of calves on each farm did not exceed 3 calves, except in some farms that were considered intensive breeding farms. There is no correlation between studied variables and the presence of diarrhea-causing factors at the farm level, while Odd's preference for shedding cryptosporidium Oocysts, cofactors, and mixed infections was greater in calves aged 9 to 21 days than calves 1 to 5 days old (Al Mawly et al. 2015). The occurrence of severe diarrhea in calves and shedding of oocysts differs from one farm to another which leads to some questions about the confirmation of cryptosporidium as the primary infection (Griffiths et al. 1994). Calves that shed oocysts are at greater risk of diarrhea than uninfected calves. This strong correlation suggests an association between the number of oocysts and the occurrence of diarrhea in calves (Trotz et al. 2005). According to Trotz and Singh, the secretion of diarrheal agents can be recorded in healthy and sick calves showing diarrhea (Trotz et al. 2005; Singh et al. 2006). Our study did not find any infection in feces samples that creamy consistency-like-normal texture.

Cryptosporidium is associated with diarrhea in calves up to 14 days of age (Bartels 2010). Through results, we have shown that abnormal mucous-watery texture feces are prevalent in the positive calves, especially at a young age. It may be due to milk content (dam's nutrition) or causative factors against which the calf may not have antibodies (the immune system is weak and incomplete). The variable distribution of infection with age and different resistance as age increases is related to the development of immunity over time (Ayele et al. 2018). This may be one of the reasons for the unfair management practices of farmers such as lack of care for mother cow's vaccinations, unbalanced nutrition during pregnancy, drying periods, giving colostrum, and milk substitutes in large farms. Climate instability (especially cold) may also be considered a factor for intestinal infections. Factors of farm management are influenced by region, general health status, and biosecurity, in addition to environmental conditions and geographical location (climatic factors) (Venu et al. 2013; Ok et al. 2009). According to the results of the study, there was no correlation between cryptosporidium infection and time sampling, i.e., cold months or warm months, which are two independent events (P > 0.05). The prevalence of infections was recorded as 5.6% (3/54) in warm months and 14.3 % (6/42) in cold months. This can be due to factors other than temperature, or it may be due to the environmental privacy of each region.

The highest incidence of diarrhea was recorded in calves during cold months. The age factor is one of the most critical risk factors that increases the incidence of diarrhea during the first month of life (Mansfeld et al. 2007; Scott et al. 2004; Gutzwiller 2002; Bendali et al. 1999a; Frank and Kaneene 1993). The interest in health care in most of the study barns was almost equal in the use of sterilizers and available vaccines. This was especially true for most of individual breeding systems in barns (private breeding), where the farmer has to pay sufficient attention to the administration of the small farm because it is very close to his home. There was a strong correlation between positive cryptosporidium cases and the general health status of calves and farms. Increased prevalence of infected calves may be attributed to polluted and muddy health conditions, which create appropriate conditions and factors to save and store oocysts on a farm or animal itself, fodder, and water sources containing oocysts. It is a source of infection and increases the risk of infection in farms. (Ayele et al. 2018; Abebe et al. 2008).

The incidence of cryptosporidium in calves is specific and correlated to the barn’s age, floor, and environmental condition (Ogendo et al. 2017, Brook et al. 2008). There was no correlation between the health status of the farm, its floor, and positive cryptosporidium, as most of the floors in the farms sampled were concrete.

According to reports related to cryptosporidium, the incidence of infection was higher in calves in the United Kingdom. This difference may be attributed to the difference in the geographical area, study design, methods of diagnosis, production, and management systems, in addition to the segregation factor during the year in which the study was conducted (Venu et al. 2013).

The results of (Gulliksen et al. 2009) agree with (Frank and Kaneene 1993) that wintertime poses the most significant risk factor for diarrhea but those results contradict the findings of a Swedish study (Svensson et al. 2003, 2006) which are consistent with our current study. Maddox et al. 2006 found that levels of oocyst shedding of cryptosporidium are higher during wintertime compared to summer which agrees with the study by Hamnes et al. 2006. Moreover, cows in Norway that give birth during wintertime produce lower quality colostrum than those that give birth during other seasons of the year (Gulliksen et al. 2008). The increase in the size of the herd leads to wider use of advanced techniques and mechanization, which in turn causes a decrease in the time available for the farmer to inspect the herd. It becomes challenging for the farmers to conduct the individual daily examination of the animals. This may
explain the high mortality rate in calves in the pre-weaning stage due to diarrhea and dehydration (Lucchelli et al. 1992). The prevalence of infection in intensive system farms was 25% (5/20), (p<0.05) Risk Estimate (R2:7.260, CI:95% odds:0.310(0.147-0.653)) The rate of infection was higher in the intensive system than in the home individual system. This higher infection rate may be due to overcrowding, which is usually a risk factor for the emergence of pathological infections. Overcrowding of animals in the farm may lead to an increase in the virulence of the causative agent due to the ease of securing a new host thereby increasing the rate of shedding oocysts and spreading them in the herd.

Colostrum is a crucial indicator and a factor contributing to the occurrence of infection (Ayele et al. 2018). Colostrum containing low levels of immunoglobulin C (IgG) is a risk factor for increasing the likelihood of diarrhea in calves born during the winter compared with calves born in the rest of the seasons. In addition, no variables involving colostrum intake were affected as a risk factor for cryptosporidium diarrhea (Gulliksen et al. 2008). Our study included the subject of colostrum intake, timing, administration, and quantity, in calves (not infected with cryptosporidium) that had not taken colostrum, and only three cases were recorded in calves that were not being feed colostrum at birth but were fed on substitutes milk. Delay giving colostrum was recorded with only 7 cases, only one sample of them was infected with cryptosporidium and we did not see any correlation (P >> 0.005). There was also no correlation between the amount of colostrum given to the calf and the detection of cryptosporidium infection. In previous studies of diarrhea due to viral causes, calf gender, and dystocia were identified as risk factors. It has been suggested that the larger size of male calves at birth compared to females causes dystocia and thus results in a deficiency in the absorption of colostrum (Ammar et al. 2014; Clement et al. 1995). Our study results demonstrate a correlation between the cryptosporidium infection and dystocia (P = 0.031) (R2 = 0.49, df:1, F: 8.22, P = 0.031) but there was no statistically significant association between dystocia and the gender of the infected calf (P >> 0.05).

Bartels found that cryptosporidium shedding increased 3.2 times on farms that routinely used antibiotics for diarrheal calves. The result of the research prompted veterinarians and owners to consider cryptosporidium as one of the possible causes of diarrhea to reduce the use of antibiotics and to include a request for cryptosporidium test in diagnostic protocols when the fecal samples are sent for analysis (Bartels et al. 2010).

Our study shows that the samples that were treated with antibiotics had a higher rate of infection than the samples that were not given any drugs, or they were given astringent drugs or the milk was diluted from them as an initial treatment.

Conclusion: The results from our study demonstrate that the incidence of cryptosporidium is significant. It is necessary to include the detection test of cryptosporidium within the protocols for early detection of causes of diarrhea and to reduce the excessive use of antibiotics. Rapid use of antibiotics without thorough diagnosis has more harms than benefits. This practice may lead to animal fatigue and inability to control the cryptosporidium. There exists a need to put strategic plans in intensive breeding to follow up on changes in the color and texture of feces of the newborn in intensive system farms. It is recommended to expand the research on diarrheal causes, especially bacterial causes, using fast, high-reliability, and inexpensive techniques for breeders and comparing their results with the results of traditional bacterial culture.

Statements and Declarations
Conflict of interests: The authors declare no conflict of interest.
Competing interests: The authors declare no competing financial or non-financial interests.
Authors contributions: HFA and FAD contributed to the study conception and design. Material preparation and samples collection were performed by HFA. Processing of the samples and investigation was done by HFA and FAD. Data analysis was performed by HFA and FAD. Original draft preparation (lead) and writing was done by HFA. GM supported in the original draft writing. Draft review and editing were done by GM.
Availability of Data and material: All data generated or analyzed during this study are included in this published article. Raw data are available from the corresponding author upon reasonable request.
Ethics Approval: None of the animals involved in this study were caught or restrained for sample collection, and all samples were obtained in a non-invasive way. Hence, there was no need for ethical permission.

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