PARASITIC PROTOZOANS (*EIMERIA*, *GIARDIA*, AND *CRYPTOSPORIDIUM*) IN LAMBS WITH DIARRHOEA IN THE VAN PROVINCE (TURKEY)

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Abstract

This study was conducted to investigate the prevalence of parasitic protozoan agents in lambs with diarrhoea in the Van province (Eastern Turkey). Faecal samples from 132 diarrhoeic lambs aged from one day to two months old were collected at 12 different herds from January 2005 to May 2005. The faecal samples were examined by using, native-Lugol, Fulleborn’s salty water technique, and a Modified Ziehl-Neelsen staining technique for *Cryptosporidium*. The parasitological examination revealed that 98 (74.24%) of the lambs were infected with one or more of parasitic protozoan agents. *Eimeria*, *Giardia*, and *Cryptosporidium* were detected in 80 (60.60%), 64 (48.48%), and 18 (13.63%) of the lambs, respectively. According to statistical analysis, *Eimeria* sp. oocysts were significantly higher (P<0.01) in lambs aged between 31 and 60 d (76.81%, 53 of 69) than in those of 16–30 d of age (50%, 21 of 42) and 1-15 d of age (28.57%, 6 of 21). There were significant differences in *Giardia* prevalence when lambs of 1–15 d of age were compared with those 16–30 d of age (P<0.05). Statistical analysis revealed that *Cryptosporidium* sp. oocysts were significantly higher (P<0.01) in lambs aged between 1 and 15 d (66.66%, 14 of 21) than in those of 16–30 d of age (4.76%, 2 of 42) and 31-60 d of age (2.89%, 2 of 69). Moreover, *Giardia* sp. and *Eimeria* sp. showed significant co-occurrence (34.84%). The results obtained confirmed the fact that protozoan infections are widespread in diarrhoeic lambs in this geographical region.

Key words: lambs, diarrhoea, *Eimeria*, *Giardia*, *Cryptosporidium*.

Diarrhoea in lambs is a complex multi-factorial disease involving the animal, environment, nutrition, and infectious agents (bacterial, viral, and parasitic). Despite improvements in management practices and prevention and treatment strategies, diarrhoea is still the most common and costly disease affecting neonatal small ruminants. Coccidiosis, giardiosis, and cryptosporidiosis are protozoan parasitic diseases that are a common cause of diarrhoea in lambs (32).

In lambs, coccidiosis caused by parasites of the genus *Eimeria* can be of a high economic impact, particularly in housed flocks as shown after experimental (31) and natural infections (16). Clinical eimeriosis only occurs if the host is subjected to heavy infection or if its resistance is lowered (35). All age groups are susceptible to infection, but disease outbreaks are usually restricted to younger animals (36). Depending on the rearing conditions, lambs are mostly affected by clinical eimeriosis around six weeks of age, or when the animals are moved to feedlots (14).

According to Yvoré *et al.* (43), the disease appears mostly under stressful conditions, particularly after weaning. Lambs are most susceptible to the problem at one to four months of age, while lambs in their first few weeks of life are resistant to the disease. Exposure to the protozoa during this time confers immunity and resistance to later infections (32).

*Giardia duodenalis* is a well-known enteric protozoan that affects a wide range of domestic and wild animals as well as humans, causing acute, self-limiting, but often severe diarrhoea in young subjects and particularly in immunocompromised individuals (1). *G. duodenalis* has recently emerged as an important parasite in domestic ruminants due to the unexpectedly high levels of infection. In these animals, the typical pattern of infection is a peak of faecal shedding of cysts between five and ten weeks of age (44). *G. duodenalis* is assumed to be a potential pathogen in livestock, causing pasty diarrhoea, weight loss, lethargy, and poor condition (23, 27, 28, 37).
Cryptosporidium is one of the main causes of morbidity and mortality in young livestock, being considered one of the major enteropathogens associated with neonatal diarrhoea in ruminants (11). Barker and Carbonell (5) first described cryptosporidiosis in lambs with diarrhoea in Australia, but no causative role could be ascribed to the organism because of the coincidental infections with pathogenic bacteria. Its role as a primary aetiological agent of diarrhoea in lambs was confirmed in the early 1980s in studies on natural and experimental infections (4, 34). Subsequently, cryptosporidial infection in lambs and goat kids with or without diarrhoea has been reported in many regions in the world and C. parvum is now considered as one of the principal causes of diarrhoeic outbreaks in neonatal lambs (11). Typically, animals between one and five weeks of age are infected with C. parvum, though lambs 5–12-d-old seem to be most susceptible and infection causes mild to severe diarrhoea accompanied by depression, anorexia, weight loss, and shedding of a large number of oocysts (3, 4, 38). The mortality is low in naturally reared suckling lambs, although it increases when the disease is associated with concurrent infections or deficiencies in nutrition or husbandry (11).

For successful treatment and economical control of diarrhoea in lambs, a detailed knowledge about the species causing diarrhoea and time course of infection in a flock is essential. The aim of this study was to estimate the prevalence and age distribution of protozoan agents in lambs with diarrhoea in the Van province.

Material and Method

The study was carried out in the Van province in the eastern part of Turkey. Faecal samples were collected from 132 lambs with diarrhoea, selected at random from 12 herds, from January 2005 to May 2005. The animals were classified into three age groups: 1-15-d-old (group 1), 16-30-d-old (group 2), and 31-60-d-old (group 3). From each animal, a faecal sample was taken from the rectum by using a disposable plastic bag and disposable plastic containers were used in lambs with diarrhoea. The date of sampling, origin, age, and identification number were recorded for each animal. The samples were examined by native–Lugol, Fulleborn’s salty water flotation technique, and Modified Ziehl–Neelsen staining technique for Cryptosporidium (7). Differences in prevalence of Eimeria, Giardia, and Cryptosporidium according to the age range were evaluated with the χ²-square test and Z-test was used to compare infection rates among different age groups.

Results

It was found that 98 (74.24%) of the 132 lambs were infected with one or more protozoan agents (Table 1). The prevalence of protozoa species in the infected lambs was associated with the age (P<0.01). Thirty-four (25.75%) lambs had no parasites. Eimeria sp. was the most commonly detected parasite. Eighty (60.6%) of all lambs had Eimeria oocysts (Table 1). As for the monoinfection with Eimeria sp., 19.69% of the lambs were affected. Giardia sp. cysts and trophozoites were identified in 64 (48.48%) lambs, although eight (6.06%) lambs were infected only with Giardia sp. Cryptosporidium sp. oocysts were identified in the faeces of 18 (13.63%) lambs. As for the infection with Cryptosporidium sp. only, there were affected 1.51% of lambs. Moreover, Giardia sp. and Eimeria sp. showed significant cooccurrence (34.84%) (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Number of infected lambs</th>
<th>Infection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eimeria sp.</td>
<td>80 (26)*</td>
<td>60.6 (19.69)*</td>
</tr>
<tr>
<td>Giardia sp.</td>
<td>64 (8)*</td>
<td>48.48 (6.06)*</td>
</tr>
<tr>
<td>Cryptosporidium sp.</td>
<td>18 (2)*</td>
<td>13.63 (1.51)*</td>
</tr>
<tr>
<td>Eimeria sp. + Giardia sp.</td>
<td>46</td>
<td>34.84</td>
</tr>
<tr>
<td>Cryptosporidium sp. + Giardia sp.</td>
<td>8</td>
<td>4.54</td>
</tr>
<tr>
<td>Eimeria sp. + Cryptosporidium sp.</td>
<td>6</td>
<td>3.03</td>
</tr>
<tr>
<td>Eimeria sp. + Giardia sp. + Cryptosporidium sp.</td>
<td>2</td>
<td>1.51</td>
</tr>
</tbody>
</table>

* Numbers and percentages of lambs with monoinfestation.

Table 2

Prevalence of Eimeria sp., Giardia sp., and Cryptosporidium sp. infections in lambs according to the age

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Number of lambs examined</th>
<th>Number of lambs infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eimeria</td>
<td>Giardia</td>
</tr>
<tr>
<td>1-15</td>
<td>21</td>
<td>6 (28.57)</td>
</tr>
<tr>
<td>16-30</td>
<td>42</td>
<td>21 (50)</td>
</tr>
<tr>
<td>31-60</td>
<td>69</td>
<td>53 (76.81)</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>80 (60.6)</td>
</tr>
</tbody>
</table>

χ²= 60.138*

* P<0.01
In this study, the prevalence of diarrhea in Germany (20), and 17.5% in Zaragoza (Northeastern Spain) (9). The parasitological examination revealed that 98 (74.24%) of 132 lambs examined were infected with one or more parasitic protozoan agents. The results of this study confirmed the fact that protozoal infections were widespread in diarrhoeic lambs and/or more parasitic protozoan agents. The results of this study showed that, protozoal infections were well established, especially when accompanied by dietary changes or situations of stress (14).

Discussion

Eimeria sp., Giardia sp., and Cryptosporidium sp. are commonly identified intestinal pathogens in both diarrhoeic and non-diarrhoeic ruminants (9, 22, 27, 28, 45). The parasitological examination revealed that 98 (74.24%) of 132 lambs examined were infected with one or more parasitic protozoan agents. The results of this study confirmed the fact that, protozoal infections were widespread in diarrhoeic lambs and Eimeria, Giardia, and Cryptosporidium species should be considered as some of the major agents in the aetiology of diarrhoea in lambs. This is in accordance with previous studies in both randomly selected farms and in outbreaks of diarrhoea in ruminants (9, 11, 14, 27, 33, 40).

Eimeria sp. oocysts were identified in 80% of 69 lambs (Table 1). This infection in lambs is relatively common, with prevalence’s ranging from 100% in Antakya (21), to 94.8% in Elazığ (17), 56.7% in Germany (20), and 17.5% in Zaragoza (Northeastern Spain) (9). In this study, the prevalence of Eimeria sp. significantly increased with the age of lambs, reaching its peak at one to two months of age in 53 (76.81%) of 69 lambs examined (Tables 2 and 4), like reported by Causapé et al. (9). According to statistical analysis, the prevalence of Eimeria sp. oocysts was significantly higher in lambs aged between 31 and 60 d (76.81%, 53 of 69) than in those of 16–30 d of age (50%, 21 of 42) and 1-15 d of age (28.57%, 6 of 21) (Table 3). These protozoa are considered to be important in small ruminants of more than four weeks of age and their role in the aetiology of diarrhoea in confined ruminants is well established, especially when accompanied by dietary changes or situations of stress (14).

The overall prevalence (Table 1) of giardiasis was 48.48% in lambs in this study. In some other studies done in various regions of the world, different prevalence rates of the disease in lambs were found. Olson et al. (29), demonstrated 57% prevalence rate in Canada, Giangaspero et al. (15) 3.0% in Abruzzo Region (Central Italy), and Causapé et al. (9) 2.7% in Zaragoza (Northeastern Spain). The prevalence rates of 6.2%, 33%, and 38% were detected in sheep in Granada (Southern Spain), Galicia (NW Spain), and Canada, respectively (8, 12, 29).

The prevalence and clinical significance of this parasitic infection in animals of all ages are not well understood. There have been reports of infection with G. duodenalis in young animals with or without associated clinical disease (23, 25, 39). Nevertheless, Giardia infections in ruminants, although highly prevalent, still raise questions about the clinical significance and the impact on production (37, 41). Causapé et al. (9), reported that infection rates of both Eimeria sp. and G. duodenalis were higher in non-diarrhoeic than in diarrhoeic lambs, except for lambs 22–90-d-old in which the rates were slightly higher. In this study, the prevalence of Giardia sp. was found higher in diarrhoeic lambs over 15 d of age (Table 2). The statistical analysis showed significant differences in Giardia prevalence when lambs of 1–15 d of age were compared with those of 16-30 d of age (Tables 3 and 4).

Concomitant infections, alternatively called mixed infections, are common in the nature, and often involve parasites. Many concomitant protozoal infections in humans and animals were reported (10). In this study, Giardia and Eimeria showed significant cooccurrence (34.84%) and the prevalence of these species in general was higher than that of

### Table 3

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Eimeria</th>
<th>Giardia</th>
<th>Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>6/21-53/69</td>
<td>6/21-34/69</td>
<td>14/21-2/69</td>
</tr>
<tr>
<td>2-3</td>
<td>21/42-53/69</td>
<td>24/42-34/69</td>
<td>2/42-2/69</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01

### Table 4

<table>
<thead>
<tr>
<th>Age groups</th>
<th>E</th>
<th>G</th>
<th>C</th>
<th>C + G</th>
<th>C + E</th>
<th>G + E</th>
<th>C + G + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>0/21-4/42</td>
<td>0/21-5/42</td>
<td>2/21-0/42</td>
<td>6/21-2/42</td>
<td>6/21-0/42</td>
<td>0/21-17/42</td>
<td>0/21-0/42</td>
</tr>
<tr>
<td>1-3</td>
<td>0/21-22/69</td>
<td>0/21-3/69</td>
<td>2/21-0/69</td>
<td>6/21-0/69</td>
<td>6/21-0/69</td>
<td>0/21-29/69</td>
<td>0/21-2/69</td>
</tr>
<tr>
<td>2-3</td>
<td>4/42-22/69</td>
<td>5/42-3/69</td>
<td>0/42-0/69</td>
<td>2/42-0/69</td>
<td>2/42-0/69</td>
<td>17/42-29/69</td>
<td>0/42-2/69</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01. E – Eimeria, G – Giardia, C - Cryptosporidium
Cryptosporidium sp. These two species occupy different microhabitats in the intestine. *Giardia* is the only protozoan species studied here with an entirely extracellular life cycle in the small intestine and its trophozoites are loosely attached to the villi, while *Eimeria* sp. trophozoites are intracellular. Moreover, eight (6.06%) of 132 lambs was only co-infected with *Giardia* sp. and *Cryptosporidium* sp. in the present study. Castro-Hermida et al. (8) reported that concurrent infections by *C. parvum* and *G. duodenalis* were observed in only nine sheep (2%) and eight goats (5%). Likewise, on Ohio farms with calf diarrhea, it was found that five of ten calves with *Cryptosporidium* were also positive for *Giardia* infection by immunofluorescence assay (45). In previous studies, some investigators reported a significant association between *Giardia* and *Cryptosporidium* infections (6, 19, 42). Other workers reported no association between the two organisms in their respective study groups (18). In mixed infections, the burden of one or both the infectious agents may be increased, one or both may be suppressed or one may be increased and the other suppressed (10). Due to the fact that this was a preliminary study involving a small sample size and the intensity of infection by both parasites was not investigated, we cannot as yet, make inferences about the existence of an association between these organisms in our study population. However, it can be said that concurrent infections by *Eimeria* and *Giardia* are observed in lambs with diarrhea in this geographical region.

The overall prevalence of *Cryptosporidium* sp. in lambs in our study was 13.63%. Statistical analysis showed that this infection rate was significantly higher in lambs up to the 15th d of their life (66.66%, 14 of 21) than in those between 16–30 d of age (4.76%, 2 of 42) and 31-60 d of age (2.89%, 2 of 69) (Table 3). These results are consistent with those of other studies reporting a strong correlation between the age and prevalence of this protozoan in domestic ruminants (8, 26, 40). In contrast, in a study of the prevalence of *Cryptosporidium* sp. in asymptomatic adult sheep in Mexico State, there was no correlation between the prevalence of the infection and age (2).

The studies of diarrhoeic outbreaks in lambs in other parts of Turkey reported a 46.5% prevalence of *Cryptosporidium* sp. in Aydin (40), 12% in Elazığ (Eastern Turkey), and 23.3% in İzmir (Western Turkey) (13, 30), whereas studies done in other countries (24, 46) reported infection rates in lambs ranging from 14.7% to 100%.

In conclusion, the results of this study showed that protozoal infections are widespread in diarrhoeic lambs in this geographical region. To our knowledge, this study is the first preliminary investigation of *Eimeria* sp. oocysts, *Cryptosporidium* sp. oocysts, and *Giardia* sp. in the faeces of diarrhoeic lambs in the Van province of Eastern Turkey. *Eimeria* sp. and *Giardia* sp. were clearly very common parasites with prevalence 60.6% and 48.48% in lambs with diarrhea, respectively. The overall prevalence of *Cryptosporidium* infections was generally lower (13.63%) than that of other two protozoans, although it was significantly higher in 1-15-d-old lambs than in older ones and than *Eimeria* and *Giardia* infections in lambs 1-15-d-old. This result confirms that *Cryptosporidium* is involved in the aetiology of lamb neonatal diarrhoea and must be considered as a problem.

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**References**


