

# Prevalence of *Cryptosporidium* infection in sheep flocks with a history of lambs' diarrhoea

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## SUMMARY

The aim of the present study was the investigation of 1) the possible interference of *Cryptosporidium* spp. in the cases of lambs' diarrhoea during the first 3-14 days of life and 2) the prevalence of this parasite in older animals, serving as reservoirs.

Towards this end, faecal samples from 523 dairy sheep of local breeds from 31 flocks with a history of lambs' diarrhoea were examined (Ziehl – Neelsen method). Sampled animals were divided into 3 groups as follows: group A: lambs younger than 14 days (all sampled), group B: lambs older than 14 days and up to 30 days old (50% sampled) and group C: ewes >1.5 years old (10% sampled).

152 out of the 523 (29.06%) sheep were infected. In each group the percentages were: Group A: 114/207 (55.07%), Group B: 12/79 (15.18%) and Group C: 26/237 (10.97%).

Only in 2 flocks oocysts of *Cryptosporidium* spp. were not found in any of the sampled sheep in the different age groups. More precisely, the range of infected animals in each of the 31 flocks in total was 0-42%, while in each group was as follows: Group A: 0%-100%, Group B: 0%-22.2% and Group C: 0%-30%.

Since *Cryptosporidium* spp. was prevalent in dairy sheep flocks, mainly in neonatal lambs, it is concluded that this parasite plays an important role in the diarrhoeic syndrome of lambs in Greece.

**Keywords:** dairy sheep, *Cryptosporidium* spp., prevalence, Greece.

## RÉSUMÉ

### Prévalence de l'infestation à *Cryptosporidium* dans les troupeaux d'ovins dans lesquels les agneaux présentent des diarrhées

Le but de cette étude était de mettre en évidence :

1- une possible interférence entre *Cryptosporidium* spp. dans les cas de diarrhées des agneaux survenant dans les 3 à 14 premiers jours de vie.

2- la prévalence de ce parasite chez les animaux plus âgés qui servent de réservoirs.

Des prélèvements faecaux ont été effectués sur 523 ovins laitiers de race locale provenant de 31 troupeaux avec des antécédents de diarrhée chez les agneaux et ils ont été examinés après coloration de Ziehl-Neelsen. Les échantillons recueillis ont été répartis en trois groupes :

Groupe A : agneaux de moins de 14 jours (tous prélevés), Groupe B : agneaux de plus de 14 jours et jusqu'à 30 jours (prélèvements sur 50 % des animaux), Groupe C : brebis > 1 an et demi (prélèvements sur 10 % des animaux). 152 animaux sur 523 (29,06 %) se montrèrent infectés. Dans chaque groupe les pourcentages furent : Groupe A : 114/207 (55,07 %), Groupe B : 12/79 (15,18 %), Groupe C : 26/237 (10,97 %). Seuls deux troupeaux se révélèrent indemnes d'oocystes de *Cryptosporidium* spp. dans tous les groupes d'âge.

Au total le nombre d'infestés dans les 31 troupeaux variait de 0 à 42 % avec la répartition suivante : Groupe A : 0 à 100 %, Groupe B : 0 à 22,2 %, Groupe C : 0 à 30 %.

Ainsi *Cryptosporidium* spp. était présent dans les troupeaux d'ovins laitiers et principalement chez les agneaux nouveau-nés. La conclusion est que ce parasite joue un rôle important dans le syndrome diarrhéique des agneaux en Grèce.

**Mots-clés :** Ovins laitiers, *Cryptosporidium* spp., prévalence, Grèce.

## Introduction

Cryptosporidiosis is a world wide distributed disease caused by the protozoan parasite *Cryptosporidium parvum* [28, 34], which is much smaller than the *Eimeria* spp., has less host specificity and can infect lambs, kids, calves, other animal species and man [9, 24]. Risk of infection and morbidity are greater in neonatal animals (mainly 4-10 days old) [8]. The infection follows mainly the faecal-oral route but the oocysts can also be induced indirectly by contaminated materials [7, 23]. Sporulated oocysts of *C. parvum* are readily infective to neonatal lambs. Public health concerns are real as the organism causes usually self-limiting diarrhoea or an influenza-like illness that is usually resolved in 2-3 weeks in immunocompetent human patients, but life-threatening disease in those

with immunodeficiency [10, 35]. However, according to recent findings the zoonotic implication of sheep cryptosporidiosis is disputed [31]. Cryptosporidiosis causes economic losses due to mortality, retarded growth of the animals, drugs cost, veterinary assistance and an increase in labour of the staff [9].

The small intestine enterocytes are primarily infected by *C. parvum* and villous atrophy, villous fusion and inflammation of intestinal crypts ensue. This pathology contributes to the clinical signs of diarrhoea via malabsorption, maldigestion and osmotic effects [29]. Initially the affected lambs are alert and nurse, but as diarrhoea persists for 5-7 days, it may lead to dehydration, inappetence, abdominal tension, lethargy and death, although diarrhoea is usually self-limiting [8, 22]. It is

characteristic that diarrhoea due to cryptosporidiosis usually does not respond to conventional antibacterial drugs [11, 15]. Death is not common at the initial stages of the disease, but as the parasitic burden increases, there may be deaths [2, 7].

Damage of the small intestine microvilli predisposes to combined infections with *Escherichia coli* and Rotaviruses, mainly, and, less often, with Coronaviruses and *Salmonella* spp. [29]. This combination of enteric pathogens worsens the clinical signs and prognosis, complicates treatment, results in higher mortality and predisposes to malnutrition. In these severe mixed infections, lambs may die within 2-3 days of the onset of diarrhoea [8].

Neonatal lambs' diarrhoea is very common and of major concern in Greece. Nevertheless, to date there is lack of reports concerning the contribution of cryptosporidiosis in lambs' diarrhoeic syndrome, which is of great significance for the clinician. The only 3 relative studies that exist have been conducted years ago, while the number of samples examined was rather limited [14, 18, 27].

The main aim of the present study was the investigation of the prevalence of *Cryptosporidium* infection in diarrhoeic neonatal dairy lambs in Greece. Another goal was the estimation of the prevalence of this parasite in dairy ewes, serving as reservoir.

## Materials and Methods

Faecal samples from 523 dairy sheep of local breeds from 31 flocks of Central Macedonia were tested. The flocks included into the study consisted of 150-200 adult sheep each of local dairy crossbreeds, kept under the semi-intensive feeding system, which is the most common in the country. All the flocks suffered from neonatal diarrhoea syndrome were visited once during the lambing season (January to March 2007). The selected flocks had not received any anti-cryptosporidial medication prior to sampling. The sampled animals were divided into 3 groups as follows:

Group A: consisted of diarrhoeic lambs younger than 14 days, in each of the 31 flocks.

Group B: consisted of clinically healthy lambs aged 15-30 days old with a history of diarrhoea. The diarrhoea started under their first 12 days of life and lasted from 4-7 days. In each flock 50% of these lambs were sampled.

Group C: consisted of clinically healthy ewes (>1.5 years old). In each flock 10% of them were sampled.

Faecal samples were collected with rectal swabs from each lamb and directly from the rectum of each ewe into plastic gloves, transported to the laboratory of Parasitology and Parasitic Diseases and processed within one or two days of collection. Each sample was passed through a wire mesh screen with an aperture of 0.15 mm and concentrated by means of centrifugation. Smears of the sediment were stained according to the acid-fast Ziehl-Neelsen technique [13]. The entire surface of the slide was examined under 100x magnification for *Cryptosporidium* spp. oocyst identification. No additional examination was carried out for any other causes

of diarrhoea.

The statistical analysis was performed using the statistical program SPSS version 12.0 for Windows. Chi-Square test was used to determine whether the prevalence of cryptosporidiosis was significantly different among groups. Also Analysis of Variance (ANOVA) was performed using flock as a factor. A value of  $P \leq 0.05$  was considered significant.

## Results

The results showed that, in total, 152 out of 523 (29.06%) sheep were found to be infected. More detailed, in each group the prevalence of *Cryptosporidium* spp. is presented in Table 1.

	Group A	Group B	Group C
Infected	114 (55.07 %)	12 (15.18 %)	26 (10.97 %)
Not-infected	93 (44.93 %)	67 (84.82 %)	211 (89.03 %)
Total	207	79	237

TABLE I : Prevalence of *Cryptosporidium* spp. in different groups of sheep within the examined flocks.

ANOVA did not give any significant difference according to flock as a factor. The variance of prevalence within each flock was similar ( $P > 0.05$ ). Only in 2 flocks oocysts of *Cryptosporidium* spp. were not found in any of the sampled sheep in the different age groups. More precisely, the range of infected animals in each of the 31 flocks in total was 0-42%, while in each group was as follows: Group A: 0%-100%, Group B: 0%-22.2% and Group C: 0%-30%.

The percent composition of the infected sheep was as follows: 75% (114/152) were lambs of group A, 7.89% (12/152) lambs of group B and 17.11% (26/152) ewes of group C.

The prevalence of *Cryptosporidium* infection was significantly higher in group A compared with groups B and C ( $P \leq 0.05$ ).

Furthermore, despite the fact that no quantitative method was applied, it is worth noted that a high number of oocysts (approximately >20 oocysts/field) was recorder in group A. In the rest of the groups, the number of oocysts was lower (<2 oocysts/field).

## Discussion

The main objective of the present study was the investigation of the prevalence of *Cryptosporidium* infection in diarrhoeic neonatal dairy lambs in Greece. The results showed that oocysts were not found in all sampled flocks and in all groups of sheep. A significant high prevalence (55.07%) of *Cryptosporidium* spp. oocysts shed by diarrhoeic lambs aged less than 14 days and a low prevalence (15.18%) in healthy lambs aged 15-30 days old with history of diarrhoea. Studies for *Cryptosporidium* infection prevalence in different sheep populations have been conducted from other researchers. In Trinidad and Tobago, 25.5% of diarrhoeic lambs aged 1-90 days old were *Cryptosporidium* spp. positive, while from non-

diarrhoeic lambs positive were only the 11.4% [16]. In Poland fecal examination in 60 randomly selected healthy lambs aged >3 months old and 99 adult sheep revealed a higher percent of infection in lambs than in adult sheep [17]. CAUSAPE *et al* [7] found that lambs aged 1-14 days old were about 2.3 times more likely to be infected than lambs aged >15 days old, while only a small percent of ewes (7.8%) excreted a small number of *Cryptosporidium spp.* oocysts. Also, in the same study the majority of positive lambs (86.3%) were diarrhoeic. In Mexico, ALONSO-FRESAN *et al* [1] found a similar percentage in asymptomatic lambs and healthy adult sheep, although the exact age of lambs is not reported. In Turkey, SEVINC *et al* [32] found a small percentage of infection in lambs aged up to 60 days old, either with oocyst determination or with faecal antibody determination (ELISA test). In Switzerland, faecal examination of a small number of diarrhoeic lambs revealed that *Cryptosporidium spp.* is common [26]. Also, *Cryptosporidium spp.* was rather common in diarrhoeic lambs in Serbia [19].

It is accepted that for cryptosporidial infections an age-related resistance to infection exists. Infection is more frequent during the first 2 weeks of life and a marked decrease in the severity of symptoms has been observed as the age at infection increased [7, 12 25].

The high prevalence of *Cryptosporidium* infection revealed by the present study is the first proof that *Cryptosporidium spp.* is a causative agent for the diarrhoea syndrome of newborn dairy lambs in Greece. This finding adds vital information to the clinicians, who should always take into consideration cryptosporidiosis in the differential diagnosis of neonatal lamb diarrhoea. The results reported here also illustrate that scouring among less than 15 days old dairy lambs is significantly associated with *Cryptosporidium spp.* infection. Cryptosporidiosis is considered a common cause of diarrhoea in neonatal lambs aged 4-15 days old and many trials have been conducted for its treatment and prevention [5, 6, 20, 21, 36].

From work conducted in cattle, it is well known that this parasite is one of the pathogens most commonly found in scouring calves and that it may be detected alone or with other enteropathogens, bacteria and viruses [4, 30]. In lambs there have not been conducted many studies for the epidemiology of lamb diarrhoea syndrome and its association with cryptosporidial infections [3, 26, 33]. Although this combined infection was not evaluated here it will be very interesting such an evaluation to be performed in Greece too.

Another goal of the present study was the estimation of the prevalence of *Cryptosporidium spp.* in dairy ewes. The prevalence of *Cryptosporidium spp.* oocysts in ewes was significantly lower than newborn diarrhoeic lambs but not significantly lower than healthy lambs aged 15-30 days old. The results showed that 10.97% of the ewes shed oocysts of *Cryptosporidium spp.* in their faeces with the excretion not being evident in all sampled flocks. All the ewes shed oocysts were healthy and showed no signs of diarrhoea. This finding implies that ewes can serve as reservoirs of the infection in the flocks. In the study of ORTEGA-MORA *et al* [25] in Spain it was estimated that an asymptomatic ewe can excrete

20,000-444,000 oocysts per day. The minimum infective dose in gnotobiotic lambs was found to be 1 oocyst per lamb, while the average 5 oocysts per lamb [BLEWETT *et al*: cited by 9]. So, it can be concluded that an asymptomatic ewe could shed between 4,000-110,000 infective doses per day [25].

The percent of infected ewes could be higher if an immunologic (IFAT, ELISA) or PCR technique was used, because they are considered more accurate than parasitological examination [25, 31, 32], although MAJEWSKA *et al* [17] didn't find difference in the susceptibility of the 2 techniques. Also, the productive stage of the animals is important, as increased oocyst shedding has been found in ewes in the periparturient period [25].

In conclusion, this study is the first proof of *Cryptosporidium* infection involvement in neonatal dairy lamb diarrhoea in Greece. *Cryptosporidium spp.* was prevalent in dairy flocks and in connection to the large number of oocysts shed by diarrhoeic animals it is stated that this parasite plays an important role in the diarrhoeic syndrome of lambs in Greece. Moreover, the prevalence of *Cryptosporidium spp.* oocysts found in healthy dairy ewes implies that they can serve as reservoirs of the infection.

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