Occurrence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in sheep and goats reared under dairy husbandry systems in Greece

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Abstract – *Giardia duodenalis* and *Cryptosporidium* spp. are gastro-intestinal protozoa known to infect small ruminants. Both protozoa are also considered as a potential public health concern. The objective of this study was to determine their prevalence in lambs and goat kids kept under common Mediterranean dairy husbandry systems and to identify the species and genotypes infecting these small ruminants. In total, 684 faecal samples (429 from lambs and 255 from goat kids) were collected on 21 farms in Greece and examined using a quantitative immunofluorescence assay. *G. duodenalis* was detected in 37.3% of the lambs and 40.4% of the goat kids. On all but one of the farms *G. duodenalis* was detected. Most samples were typed as a mono-infection with *G. duodenalis* assemblage E, both on the β-giardin gene and the triose phosphate isomerase gene. Only 10% of samples were typed as mixed assemblage A and E infections. The prevalence of *Cryptosporidium* spp. was 5.1% in lambs and 7.1% in goat kids. In total, 8 out of the 14 farms with a sheep flock and 7 out of the 14 farms with a goat flock were positive. *Cryptosporidium parvum* (subtype IId), *C. ubiquitum* and *C. xiaoi* were identified, the latter especially in goat kids. In conclusion, the results of the present study illustrate that *G. duodenalis* and *Cryptosporidium* spp. occur frequently on both sheep and goats farms. The prevalence of zoonotic genotypes or species was low, indicating a limited but existing risk for zoonotic infections.

Key words: *Giardia*, *Cryptosporidium*, Goat, Sheep, Dairy, Prevalence, Genotyping.

Résumé – Préalence et caractérisation moléculaire de *Giardia duodenalis* et Cryptosporidium spp. chez les ovin et les caprins élevés dans les systèmes laitiers en Grèce. *Giardia duodenalis* et Cryptosporidium spp. sont des protozoaires gastro-intestinaux connus pour infecter les petits ruminants. Les deux protozoaires sont également considérés comme un risque potentiel pour la santé publique. L’objectif de cette étude était de déterminer la prévalence chez les agneaux et les chevreaux élevés dans les systèmes méditerranéens communs d’élevage laitier et d’identifier les espèces et les génotypes infectant ces petits ruminants. Au total, 684 échantillons fécaux (429 provenant d’agneaux et 255 de chevreaux) ont été collectés dans 21 exploitations en Grèce et examinés à l’aide d’un test quantitatif d’immunofluorescence. *G. duodenalis* a été détecté chez 37.3 % des agneaux et 40.4 % des chevreaux, dans toutes les fermes sauf une. La plupart des échantillons ont été typés comme une mono-infection par l’assemblage E de *G. duodenalis*, en se fondant à la fois sur l’identification du gène de la β-giardine ou du gène de l’isomérase de triose phosphate. Seuls 10 % des échantillons ont été identifiés comme une combinaison d’infections par les assemblages A et E. La prévalence de Cryptosporidium spp. était de 5.1 % chez les agneaux et de 7.1 % chez les chevreaux. Au total, 8 des 14 fermes ovin es et 7 des 14 fermes caprines étaient positives. Cryptosporidium parvum (sous-type IId), *C. ubiquitum* et *C. xiaoi* ont été identifiés, ce dernier en particulier chez les chevreaux. En conclusion, les résultats de cette étude montrent que *G. duodenalis* et Cryptosporidium spp. sont fréquemment présents chez les ovin et les caprins. La prévalence des génotypes ou espèces zoonotiques était faible, ce qui indique un risque limité bien que présent pour des infections zoonotiques.

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Introduction

Giardia duodenalis and Cryptosporidium spp. are gastrointestinal protozoa that affect a wide range of mammals. Both parasites have a direct life cycle and are known to cause enteritis. In small ruminants, mainly young lambs and goat kids are infected. The prevalence of both parasites in small ruminants varies considerably between studies worldwide [13]. The most common clinical symptoms associated with G. duodenalis are the excretion of malodorous, loose to diarrhoeic faeces and impaired weight gain, whereas Cryptosporidium spp. infection can lead to severe diarrhoea, depression, anorexia and weight loss [1, 12]. Mortality has been associated with cryptosporidiosis, especially in animals with concurrent infections.

Since the initial claims on its potential public health relevance, eight genotypes or so-called assemblages have been identified within G. duodenalis [25]. Assemblage A and B are considered to be zoonotic genotypes, affecting both humans and small ruminants [1, 12, 16, 23], whereas Assemblage E is considered to be specific to hoofed livestock and has been found to be the most prevalent assemblage in lambs and goat kids [8, 12]. The genus Cryptosporidium consists of 20 species and more than 40 genotypes. In sheep, C. parvum, C. xiaoii and C. ubiquitum are most frequently identified [9, 12, 27, 29, 35, 41] whereas mainly C. parvum and to a lesser extent C. xiaoii has been reported in goats [9, 12, 35]. Due to the potential clinical and zoonotic relevance, there is a need to better understand the presence and abundance of both parasites in sheep and goat flocks.

The aim of this study was to estimate the prevalence of G. duodenalis and Cryptosporidium spp. infection in sheep and goat dairy farms in Greece. The small ruminant sector is very important in the Mediterranean basin from an economic, social and ecological point of view [5], especially in Greece with approximately 15 million sheep and goats which are kept traditionally for milk under low-input systems [20]. The most commonly applied farming systems practised in Greece can be categorized as extensive farming [43], and there is limited information on prevalence and especially molecular characterization of both parasites in these systems. The prevalence rates reported range from 33.3% to 49.6% for G. duodenalis and from 4.4% to 55% for Cryptosporidium spp. in sheep and goats [21, 32].

Materials and methods

Study design

The study was designed as a cross-sectional study in a high sheep and goat density area in Greece (i.e. the island of Crete where more than 1.5 million animals are kept). The farms enrolled in this survey were selected according to the following criteria: (a) type of animal on the farm (sheep, goats, or mixed sheep and goats), and (b) management practices applied (“intensive management system” where stocking rates are high and the young animals are reared indoors until weaning (30–40 days), and “extensive management system” where stocking rates are lower and young animals are reared with their mothers mostly on pasture). Each farm was visited on a single occasion in a 6-month period, and only animals between the age of 1 day and 10 weeks were considered for inclusion in the study. Sample size was calculated based on the number of expected births as an indicator of the number of animals on the farms: of the lambs, 5% of the expected births on each farm were sampled and of the goat kids, 10% of the expected births were sampled. Data on the type of water supply for the animals (public network supply, a private drill hole or natural well), the age of lambs or goat kids and the presence of diarrhoea were recorded.

Parasitological examination

The faecal samples were examined in the laboratory using a quantitative immunofluorescence assay (IFA; Merifluor Cryptosporidium/Giardia kit; Meridian Diagnostics Inc.), as follows: 1 g of faeces was suspended in tap water and sieved three times through a layer of surgical gauze to withhold large debris. Sedimentation for at least 30 min was followed by discarding the supernatant. The remaining sediment was centrifuged at 3000 rpm for 5 min. The sediment was re-suspended in 1 mL of tap water. After thorough vortexing, an aliquot of 20 μL was applied onto an IFA slide. The samples, including a negative and positive control sample, were left to dry completely. After staining and incubating slides in a dark humidified chamber (for 30 min at room temperature), the entire slide was examined at 400× magnification under a fluorescence microscope. A sample was considered positive if at least one, clearly recognizable Cryptosporidium oocyst or Giardia cyst was identified. The number of (oo)cysts per gram of faeces was obtained by multiplying the total number of (oo)cysts on the slide by 50.

Molecular characterization

Positive isolates for both parasites were selected for DNA extraction, using the QiAamp Stool Mini Kit (Qiagen), according to the manufacturer’s instructions, incorporating an extended initial step of five freeze-thaw cycles (freezing in liquid nitrogen for 5 min and heating at 95 °C for 5 min) in the protocol to maximize (oo)cyst lesion. The selection of the positive isolates aimed to include at least one positive sample per farm.

For the amplification of the Cryptosporidium spp., the 18S ribosomal DNA (18S rDNA) gene PCR protocol was used (previously described in [40]), as well as a PCR targeting the 70 kDa heat shock protein (HSP70, described in [28]). Subgenotyping of the C. parvum positive samples was performed using the 60 kDa glycoprotein (gp60) gene [34]. G. duodenalis positive samples were characterized using the β-giardin gene [24], and the triose phosphate isomerase (TPI) gene [11]. Amplification products were visualized on 1.5% agarose gels with ethidium bromide. A positive and negative (PCR water) control sample was included in each PCR. PCR products were purified using the Qiagreen purification kit (Qiagen) and fully sequenced using the BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were analysed on a 3100 Genetic Analyzer (Applied Biosystems) and assembled with SeqMan II (DNASTAR, Madison,
Results

A total of 21 farms with either a sheep ($n = 7$) or goat ($n = 7$) flock, or a mixed sheep and goat flock ($n = 7$) were visited. On the majority of the farms (14/21) animals were reared under the intensive management system, whereas on 7 farms, goats and sheep were reared under the extensive management system (Table 1). In total, 684 faecal samples were examined, of which 429 samples were from lambs and 255 from goat kids. Mean lamb age was 5 weeks (range 4–8 weeks) and mean goat kid age was 6 weeks (range 4–9 weeks).

The prevalence of *G. duodenalis* was 37.3% ($n = 160/429$) in lambs and 40.4% ($n = 103/255$) in goat kids. In total, 8 out of the 14 farms (57.1%) with a sheep flock, and 7 out of the 14 farms (50.0%) with a goat flock, were positive with a minimum of 1 positive sample on each positive farm.

Intensity of *G. duodenalis* cyst excretion ranged from 50 to 800,000 cysts per gram (cpg) of faeces in lambs with an average of 48,989 cpg, and from 50 to 900,000 cpg for goat kids with an average of 94,053 cpg. The excretion level for *Cryptosporidium* oocysts was low in lambs with an average of 9143 oocysts per gram of faeces (range 200–31,900 oopg), yet was high in goat kids with an average excretion of 47,744 oopg (range 200–551,000 oopg).

A minimum of 1 positive sample was selected for DNA extraction per farm for *G. duodenalis* and *Cryptosporidium* spp., respectively. In total, 71 samples yielded a positive amplification for *G. duodenalis* (Table 2). The majority of the samples were typed as a mono-infection with the ruminant-specific assemblage E, both on the β-giardin gene and the TPI gene. Only a limited number of samples were typed as mixed assemblage A and E infections, both in lambs and in goat kids.

Table 1. Overview of the 21 farms included in the study, with the number of sheep and/or goats on the farms (N) and the type of management system (Sampled = number of animals sampled on each farm).

| Farm identification number | Management system | Sheep | Goats |
|----------------------------|-------------------|-------|-------|
| Farm identification number | Number of animals | Sampled | Number of animals | Sampled |
| 1                          | Intensive         | 203   | 17    |
| 7                          | Intensive         | 500   | 38    |
| 10                         | Intensive         | 450   | 29    |
| 11                         | Extensive         | 550   | 41    |
| 14                         | Extensive         | 170   | 13    |
| 16                         | Extensive         | 100   | 13    |
| 18                         | Extensive         | 750   | 34    |
| 2                          | Intensive         | 656   | 49    |
| 19                         | Extensive/intensive | 400   | 24    |
| 4                          | Intensive         | 301   | 23    |
| 9                          | Intensive         | 500   | 38    |
| 3                          | Intensive         | 500   | 16    |
| 8                          | Intensive         | 680   | 51    |
| 12                         | Extensive         | 570   | 43    |
| 24                         | Extensive         | 0     | 0     |
| 22                         | Intensive         | 0     | 0     |
| 25                         | Intensive         | 0     | 0     |
| 27                         | Intensive         | 0     | 0     |
| 23                         | Intensive         | 0     | 0     |
| 26                         | Intensive         | 0     | 0     |
| 21                         | Intensive         | 0     | 0     |
| Total                      |                   | 429   | 255   |

Table 2. Results for molecular identification of *Giardia duodenalis* positive samples in goat kids and lambs, based on the B-giardin (= beta-giardin) or triose phosphate isomerase (TPI) gene (NA = no amplification; A = assemblage A; E = assemblage E; A + E = mixed infection with assemblage A and E).

| Animal species | Number of samples | B-giardin | TPI |
|----------------|-------------------|-----------|-----|
|                |                   | A | E | NA | A | E | A + E | NA |
| Goat kids      | 30                 | 1 | 28 | 1 | 1 | 26 | 3 | 0 |
| Lambs          | 41                 | 1 | 35 | 5 | 1 | 35 | 3 | 2 |
| Total          | 71                 | 2 | 63 | 6 | 2 | 61 | 6 | 2 |

WI, USA). Sequences were compared with known sequences by BLAST-analysis against the NCBI database.
Table 3. Results for molecular identification of Cryptosporidium positive samples in goat kids and lambs.

| Animal species | Number of samples | C. xiaoi | C. ubiquitum | C. parvum |
|----------------|-------------------|----------|--------------|-----------|
| Goat kids      | 14                | 7        | 5            | 2 (IId)   |
| Lambs          | 10                | 0        | 3            | 7 (IId)   |
| Total          | 24                | 7        | 8            | 9         |

For Cryptosporidium, 24 samples yielded a positive amplicon (Table 3). Three different Cryptosporidium species (C. parvum, C. ubiquitum and C. xiaoi) were identified, although C. xiaoi was not identified in lambs. The C. parvum positive samples were typed as subtype IId on the gp60 gene (IIdA4G2T14 and IIdA4G3T13).

Discussion

The present study illustrates that G. duodenalis is highly prevalent in both lambs and goat kids, as the parasite was detected in all but one of the sheep flocks and in all goat flocks. The high farm and animal prevalence is in line with previous studies in small ruminants in Europe [4, 13, 18]. The high G. duodenalis prevalence and the potential association with production losses [31, 44] require an appropriate level of awareness of this infection on those farms in terms of disease management and prevention of infection. In contrast to G. duodenalis, the prevalence of Cryptosporidium was lower than anticipated in both lambs and goat kids, probably due to the age range of the animals included in the present study. Nevertheless, the farm prevalence on the sheep (57.1%) and goat (50.0%) farms does suggest that Cryptosporidium is widespread and is a potential threat to the small ruminant population. In previous studies in Greece, Cryptosporidium has been associated with large outbreaks of diarrhoea in both sheep and goat flocks [14, 15, 37], similar to other major small ruminant rearing countries [6, 33].

Both for G. duodenalis and for Cryptosporidium spp. a potential public health threat has been suspected, based on the high prevalence of these parasites in small ruminants and on extrapolation of molecular insights from other animal species, such as cattle or companion animals. However, recent molecular data seem to suggest that small ruminants are mostly infected with non-zoonotic genotypes of G. duodenalis [12, 18, 38, 39, 46, 47] and Cryptosporidium spp. [38]. On the other hand, potentially zoonotic genotypes or species such as G. duodenalis assemblage A and B [1, 2, 12, 19, 26, 30, 41], C. parvum [3, 6, 12, 22, 46], C. hominis [17], C. meleagris [42] and C. ubiquitum [10, 12, 45] have been reported in small ruminants. Furthermore, the identification of potentially zoonotic genotypes does not necessarily imply that transmission occurs. Recently, host-associated populations of C. parvum have been described using a multi-locus genotyping (MLG) approach, and C. parvum populations found in goats were even found to differ from bovine and sheep MLGs [7]. Whether this is a true host-specific phenomenon or just a matter of the level of isolation and opportunities for out-crossing is still to be discussed. Nevertheless, the contradicting molecular findings illustrate the difficulty of evaluating a potential public health threat based solely on genetic data without considering the epidemiological background and transmission of infection. Direct transmission of Cryptosporidium infection through bottle feeding or petting of animals on educational farms has been described before [38], but is probably an occasional route of infection. Although there is no direct evidence of transmission of Cryptosporidium and G. duodenalis infections from small ruminants to the human population via contaminated water, it is considered a threat. Furthermore, the detection of both parasites in outbreaks and in water screening is not routine practice in most countries, and large waterborne outbreaks might go unnoticed. In a recent study in Spain, the prevalence of Cryptosporidium and Giardia in water was significantly higher in the inland area, with higher concentration of livestock and fewer water treatment plants [4], illustrating that a variety of factors define the odds for infection. In the specific study area on the island of Crete, only a limited number of water basins are used over the island to produce drinking water for the local population and for the tourist population in the summer. The pastures surrounding the drinking water basins are all grazed by small ruminants. Whether these conditions lead to a substantial public health threat will need to be evaluated further in a longitudinal study, including sampling of water.

In goats, a large proportion of the Cryptosporidium positive samples were typed as C. xiaoi, both on 18S and HSP-70. This is in agreement with previous studies in Spain [6] and France [36], yet contradicts the initial claim that C. xiaoi infections are largely restricted to sheep [9]. In the current study, C. xiaoi was found in goats from three different farms, of which 2 farms maintained a goat-only flock and 1 farm managed a mixed flock. This illustrates that, although the introduction in the goat flocks might be due to contact with infected sheep, a C. xiaoi infection is easily maintained in goats. As advocated by Fayer and Santin [9], further epidemiological data will be needed to confirm whether the reports of C. xiaoi in goats are incidental or a regularly observed finding.

In conclusion, a high animal and farm prevalence of G. duodenalis, and a high farm prevalence of Cryptosporidium spp. were detected in both lambs and goat kids. Although mainly non-zoonotic species were identified in the present study, the frequent contact and proximity of grazing grounds to the natural water sources used to produce drinking water in the study area warrant further investigation of the public health relevance of these infections.

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"Goat-parasite interactions: from knowledge to control".
References

1. Aloisi F, Filippini G, Antenucci P, Legri E, Pezzotti G, Caccio S-M, Pozio E. 2006. Severe weight loss in lambs infected with *Giardia duodenalis* assemblage B. Veterinary Parasitology, 142, 154–158.

2. Berrilli F, D’Alfonso R, Giangaspero A, Marangi M, Brandonisio O, Kaboré Y, Glé C, Cianfanelli C, Lauro R, Di Cave D. 2012. *Giardia duodenalis* genotypes and Cryptosporidium species in humans and domestic animals in Côte d’Ivoire: occurrence and evidence for environmental contamination. Transactions of the Royal Society of Tropical Medicine and Hygiene, 106, 191–195.

3. Caccio SM, Sannella AR, Mariano V, Valentini S, Berti F, Tosi F, Pozio E. 2013. A rare *Cryptosporidium parvum* genotype associated with infection of lambs and zoonotic transmission in Italy. Veterinary Parasitology, 191, 128–131.

4. Castro-Hermida JA, García-Presedo I, Almeida A, González-Warleta M, Correia Da Costa JM, Mezo M. 2011. *Cryptosporidium* spp. and *Giardia duodenalis* in two areas of Galicia (NW Spain). Science of the Total Environment, 409, 2451–2459.

5. Cave D. 2012. *Giardia* and *Giardia duodenalis* assemblage A and E infections in calves. International Journal for Parasitology, 38, 259–264.

6. D’Avino D, Caccio SM, Pozio E, Caccio SM. 2012. Evidence of host-associated genotypes and *Giardia* assemblages in diarrhoeic goat kids (Capra hircus) in Spain. Veterinary Parasitology, 172, 132–134.

7. Drumo R, Widmer G, Morrison LJ, Tait A, Grelloni V, D’Avino D, Ivoire: occurrence and evidence for environmental contamination. Transactions of the Royal Society of Tropical Medicine and Hygiene, 106, 191–195.

8. De Rancourt M, Fois N, Lavin MP, Tchakerian E, Vallerand F. 2006. Mediterranean sheep and goat production: an uncertain future. Small Ruminant Research, 62, 167–179.

9. Fayer R. 2012. Multilocus genotyping of *Giardia duodenalis* in lambs from Spain reveals a high heterogeneity. Research in Veterinary Science, 93, 836–842.

10. Fiuza VR, Cosendey RI, Fraza˜o-Teixeira E, Santí±n M, Fayer R. 2006. Mediterranean sheep and goat production: an uncertain future. Small Ruminant Research, 62, 167–179.

11. Giles J, Chalmers R, Galmieri P, de Oliveira FC. 2011. Molecular characterization of *Cryptosporidium xiaoi* in diarrhoeic goat kids (Capra hircus) in Spain. Veterinary Parasitology, 172, 132–134.

12. Drumo R, Widmer G, Morrison LJ, Tait A, Grelloni V, D’Avino D, Caccio SM. 2012. Evidence of host-associated populations of *Cryptosporidium parvum* in Italy. Applied Environmental Microbiology, 78, 3523–3529.

13. G. duodenalis* identification of a new *Giardia* assemblage in diarrhoeic goat kids (Capra hircus) in Spain. Veterinary Parasitology, 172, 132–134.

14. Fayer R, Santin M. 2009. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: *Cryptosporidiidae*) in sheep (*Ovis aries*). Veterinary Parasitology, 164, 192–200.

15. Fiuza VR, Cosendey RI, Frazão-Teixeira E, Santin M, Fayer R, de Oliveira FC. 2011. Molecular characterization of *Giardia intestinalis* in Brazilian sheep. Veterinary Parasitology, 175, 360–362.

16. Geurden T, Geldhof P, Levecke B, Martens C, Berkvens D, Casaert S, Vercruysse J, Claerebout E. 2008. Mixed *Giardia duodenalis* assemblage A and E infections in calves. International Journal for Parasitology, 38, 259–264.

17. Geurden T, Casaert S, Vercruysse J, Claerebout E. 2008. Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in lambs and goat kids in Belgium. Veterinary Parasitology, 155, 142–145.

18. Geurden T, Vercruysse J, Claerebout E. 2010. Is *Giardia* a significant pathogen in production animals? Experimental Parasitology, 124, 98–106.

19. Giadinis ND, Panousis NK, Papadopoulos E, Antoniadou-Sotiriadou K, Karatzias H. 2006. The effects of halofuginone in cases of cryptosporidiosis in newborn lambs and kids in Greece. Proceedings of the 10th Greek Veterinary Congress, Athens. p. 195–196.

20. Giadinis N, Papadopoulos E, Panousis N, Papazahariadou M, Lafi SQ, Karatzias H. 2007. Effect of halofuginone lactate on treatment and prevention of lamb cryptosporidiosis: an extensive field trial. Journal of Veterinary Pharmacology and Therapeutics, 30, 578–582.

21. Giangaspero A, Paolelli B, Iorio R, Traversa D. 2005. Prevalence and molecular characterization of *Giardia duodenalis* from sheep in central Italy. Journal of Veterinary Parasitology, 96, 32–37.

22. Giangaspero A, Paoletti B, Iorio R, Traversa D. 2005. Prevalence and molecular characterization of *Giardia duodenalis* from sheep in central Italy. Veterinary Parasitology, 96, 32–37.

23. Giles J, Chalmers R, Galmier P, de Oliveira P. 2011. *Giardia* and *Giardia duodenalis* assemblage A and E infections in calves. Veterinary Parasitology, 172, 132–134.

24. Lalle M, Jimenez-Cardosa E, Caccio SM, Pozio E. 2005. Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay. Journal of Parasitology, 91, 203–205.

25. Lasek-Nesselquist E, Welch DM, Sogin ML. 2010. The identification of a new *Giardia* assemblage in marine vertebrates and a preliminary analysis of *G. duodenalis* population biology in marine systems. International Journal for Parasitology, 40, 1063–1074.

26. Lim YA, Mahdy MA, Tan TK, Goh XT, Jex AR, Nolan MJ, Sharma RS, Gasser RB. 2013. First molecular characterization of *Giardia duodenalis* from goats in Malaysia. Molecular and Cellular Probes, 27, 28–31.

27. MClauchlin J, Amar C, Pedraza-Diaz S, Nichols GL. 2000. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: Results of genotyping *Cryptosporidium* spp. in 1,705 faecal samples from humans and 105 faecal samples from livestock animals. Journal of Clinical Microbiology, 38, 3984–3990.

28. Morgan UM, Monis PT, Xiao L, Limor J, Sulaiman I, Raidal S, O’Donoghue P, Gasser R, Murray A, Fayer R, Blagburn BL, Lal AA, Thompson RC. 2001. Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. International Journal for Parasitology, 31, 289–296.
29. Mueller-Doblies D, Giles M, Elwin K, Smith RP, Clifton-Hadley FC, Chalmers RM. 2008. Distribution of Cryptosporidium species in sheep in the UK. Veterinary Parasitology, 154, 214–219.

30. Nolan MJ, Jex AR, Pangasa A, Young ND, Campbell AJ, Stevens M, Gasser RB. 2010. Analysis of nucleotide variation within the triose-phosphate isomerase gene of Giardia duodenalis from sheep and its zoonotic implications. Electrophoresis, 31, 287–298.

31. Olson ME, McAllister TA, Deselliers L, Morck DW, Cheng KJ, Buret AG, Ceri H. 1995. Effects of giardiasis on production in a domestic ruminant (lamb) model. American Journal of Veterinary Research, 56, 1470–1474.

32. Panousis N, Diakou A, Giadinis N, Papadopoulos E, Karatzias H, Haralampidis S. 2008. Prevalence of Cryptosporidium infection in sheep flocks with a history of lambs’ diarrhoea. Revue de Médecine Vétérinaire, 159, 528–531.

33. Paraud C, Pors I, Chartier C. 2010. Evaluation of oral tilmicosin efficacy against severe cryptosporidiosis in neonatal kids under field conditions. Veterinary Parasitology, 170, 149–152.

34. Peng MM, Matos O, Gatei W, Das P, Stantic-Pavlinic M, Bern C, Sulaiman IM, Glaberman S, Lal AA, Xiao L. 2001. A comparison of Cryptosporidium subgenotypes from several geographic regions. Journal of Eukaryotic Microbiology Supplement, 48, 285–318.

35. Quilez J, Torres E, Chalmers RM, Hadfield SJ, Cacho E, Sanchez-Acedo C. 2008. Cryptosporidium Genotypes and subtypes in lambs and goat kids in Spain. Applied and Environmental Microbiology, 74, 6026–6031.

36. Rieux A, Paraud C, Pors I, Chartier C. 2013. Molecular characterization of Cryptosporidium spp. in pre-weaned kids in a dairy goat farm in western France. Veterinary Parasitology, 192, 268–272.

37. Robertson LJ. 2009. Giardia and Cryptosporidium infections in sheep and goats: a review of the potential for transmission to humans via environmental contamination. Epidemiology and Infection, 137, 913–921.

38. Robertson LJ, Gjerde BK, Hansen EF. 2010. The zoonotic potential of Giardia and Cryptosporidium in Norwegian sheep: a longitudinal investigation of 6 flocks of lambs. Veterinary Parasitology, 171, 140–145.

39. Ruiz A, Foronda P, Gonzalez JF, Guedes A, Abreu-Acosta N, Molina JM, Valladares B. 2008. Occurrence and genotype characterization of Giardia duodenalis in goat kids from the Canary Islands, Spain. Veterinary Parasitology, 154, 137–141.

40. Ryan UM, Bath C, Rovertson I, Read C, Elliot A, McInnes L, Traub R, Besier B. 2005. Sheep may not be an important zoonotic reservoir for Cryptosporidium and Giardia Parasites. Applied Environmental Microbiology, 71, 4992–4997.

41. Santin M, Trout JM, Fayer R. 2007. Prevalence and molecular characterization of Cryptosporidium and Giardia species and genotypes in sheep in Maryland. Veterinary Parasitology, 146, 17–24.

42. Silverlås C, Mattsson JG, Insulander M, Lebbad M. 2012. Zoonotic transmission of Cryptosporidium meleagridis on an organic Swedish farm. International Journal for Parasitology, 42, 963–967.

43. Stefanakis A, Volanis M, Zoiopoulos P, Hadjigeorgiou I. 2007. Assessing the potential benefits of technical intervention in evolving the semi-intensive dairy-sheep farms in Crete. Small Ruminant Research, 72, 66–72.

44. Sweeny JP, Robertson ID, Ryan UM, Jacobson C, Woodward RG. 2011. Impacts of naturally acquired protozoa and strongylid nematode infections on growth and faecal attributes in lambs. Veterinary Parasitology, 184, 298–308.

45. Wang Y, Feng Y, Cui B, Jian F, Ning C, Wang R, Zhang L, Xiao L. 2010. Cervine genotype is the major Cryptosporidium genotype in sheep in China. Parasitology Research, 106, 341–347.

46. Yang R, Jacobson C, Gordon C, Ryan U. 2009. Prevalence and molecular characterisation of Cryptosporidium and Giardia species in pre-weaned sheep in Australia. Veterinary Parasitology, 161, 19–24.

47. Zhang W, Zhang X, Wang R, Liu A, Shen Y, Ling H, Cao J, Yang F, Zhang X, Zhang L. 2012. Genetic characterizations of Giardia duodenalis in sheep and goats in Heilongjiang Province, China and possibility of zoonotic transmission. PLoS Neglected Tropical Diseases, 6, e1826.
