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Short communication

Severe weight loss in lambs infected with *Giardia duodenalis* assemblage B

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Abstract

An outbreak of giardiasis was observed in a sheep farm in Central Italy. Infected lambs (30–90 days of age) showed a malabsorption syndrome, decreased weight gain and impairment in feed efficiency. The most relevant clinical sign was the excretion of malodorous and poorly formed faeces, whereas diarrhoea was rarely observed in the flock. Laboratory investigations revealed the presence of *Giardia* in affected animals, while no other significant viral, bacterial or parasitic pathogens were identified in faeces or tissue samples. A mild to severe infiltrative enteritis with eosinophils, lymphocytes and plasma cells was detected in histological sections of the gut. *Giardia* parasites collected from duodenal aspirates were typed as *Giardia duodenalis* Assemblage B, by PCR amplification and sequencing of the TPI gene. Treatment with fenbendazole at a dose of 10 mg/kg for 3 consecutive days, successfully cleared the infection. These results show that *G. duodenalis* can cause significant economic losses in sheep farming.

Keywords: *Giardia duodenalis*; Giardiasis; Sheep; Weight loss; Italy

1. Introduction

Giardia duodenalis (sin *intestinalis*, *lamblia*) is a flagellated protozoa that causes enteric infection and clinical disease both in man and domestic animals, particularly in ruminants and dogs. Infection in sheep is relatively common, with prevalences ranging from 6.2% in Spain, to 29.8% in Switzerland and 38% in Canada (Taminelli and Eckert, 1989; Diaz et al., 1996; Olson et al., 1997). Prevalence rates of 17.6% and 3.0% were detected in lambs of Western Sicily (South Italy) and Abruzzo Region (Central Italy), respectively (Virga et al., 1998; Giangaspero et al., 2005).

However, little is known on the pathogenic role of *Giardia* in sheep. Infection has been directly related to diarrhoea and reduced growth in lambs by some authors, while no detrimental effects have been observed in other studies (reviewed by Xiao, 1994).

Olson et al. (1995) investigating the effect of giardiasis in experimentally infected growing lambs, showed a decrease in weight gain and impairment in feed efficiency compared to control animals. In infected lambs, the time needed to reach the slaughter weight increased and the

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carcass weight was lower than that of control animals. These authors concluded that giardiasis has a negative effect on lamb productivity, suggesting the need of a control of this infection in flocks (Olson et al., 1995).

The aim of the present work was to describe the clinical, pathological and molecular features of a giardiasis outbreak occurred in lambs of a milk sheep herd in Central Italy.

2. Material and methods

2.1. Case report

The farm is located in Central Italy and consists of 1400 heads of Sardinian breed, including 450 lambs whose age ranged from 30 (#150) to 90 days (#300), reared for the replacement of culled ewes. The diet was based on corn, alfa–alfa hay and weaning feed. All lambs were raised in a single pen, with a weekly replaced litter of straw.

In March 2005, in spite of a normal daily feed intake, weight loss and reduced weight gain were observed in a large percentage of lambs (approximately 50%). The owner reported an average weight of 8 and 10 kg for the 30 and 90 days-old animals, respectively; values that are lower than expected for this breed at that age (10.4 and 17.5 kg for single lambing parturition) (http://www.assonapa.com/norme_ecc/ovini_llgg/sarda-ovina.htm). Affected animals showed soft, greenish and malodorous faeces, 10% showed diarrhoea and three dead animals were reported.

The day after death, the three dead animals were submitted to the laboratory for necroscopy, routine bacteriology, virology, parasitology and histology. Briefly, two intestinal swabs (jejunum and colon) and one swab from spleen, liver and brain were collected and cultured on MacConkey agar, 5% blood sheep agar and Mannitol Salt Agar, incubated at 37 °C for 24 h; *Escherichia coli* isolates were further tested for *eae*, *stx1*, *stx2*, *sta*, *stb*, *lt* and *hle* genes by PCR (Gannon et al., 1992; Fratamico et al., 1995; Gannon et al., 1997; Osek, 1999). The presence of rotavirus and coronavirus in intestinal content was assessed by transmission electron microscopy. Faecal samples were also processed for parasites (coccidia and round worms) by flotation on saturated NaCl solution (Ambrosi, 1995) and for *Cryptosporidium* spp. using the modified Teleman–Miyagawa solution (Pasquinelli, 1971). Tissue samples from duodenum, jejunum, ileum, colon and mesenteric lymph nodes were fixed in 10% buffered formalin, routinely processed, cut at 5 μm and stained with Haematoxylin–Eosin.

These tests did not yield any result that could explain the clinical signs. Therefore, two other lambs, found in poor body condition were delivered to the laboratory, where necropsy was carried out after euthanasia (Tanax, Intervet International, i.v. injection). All laboratory tests were repeated as described above. In addition, jejunal aspirates were examined by light microscopy and *Cryptosporidium* oocysts and *Giardia* cysts were searched by immunofluorescence (IF), using a commercial kit (Merifluor, Meridian Bioscience Inc., Cincinnati, OH, USA).

2.2. DNA isolation and PCR amplification

DNA was extracted directly from concentrated faecal material according to the method of da Silva et al. (1999). Briefly, an aliquot (0.4 ml) of concentrated faecal material was homogenised using the FastPrep 120 instrument (Savant, Thermo Electro Corporation, Woburn MA, USA). The DNA released from disrupted cysts was purified using the FastDNA kit (Qbiogene, Illkirch Cedex, France), and stored at 4 °C.

The amplification of the gene fragment was performed using a nested PCR protocol. In the primary PCR reaction, a 605 bp fragment was amplified using the forward primer 5′-AAATTATGCCTGCTCGTCG-3′ and the reverse primer 5′-CAAACCTTTTCCG-CAAACC-3′, as previously described (Sulaiman et al., 2004). In the secondary, nested, PCR reaction, a 531 bp fragment was amplified using the forward primer 5′-CCCTTCATCGGTGGTAACTT-3′ and the reverse primer 5′-GTGGCCACCACTCCCGTGCC-3′. The PCR mix consisted of 1× buffer containing 1.5 mM $MgCl_2$, 200 μM of each dNTP, 10 pmol of each primer, 2.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Monza, Italy), and 1–5 μl of purified DNA in a final volume of 50 μl. PCR was performed as follows: after an initial denaturation step of 15 min at 95 °C, a set of 35 cycles was run, each consisting of 30 s at 95 °C, 30 s of annealing at 50 °C, and 60 s at 72 °C, followed by a final extension of 7 min at 72 °C. PCR products were electrophoresed on ethidium bromide-stained 1% agarose gels.

2.3. DNA sequence analysis

PCR products were purified using the Qiaquick purification kit (Qiagen S.p.a., Milan, Italy) and fully sequenced using the ABI Prism BigDye™ Terminator Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were analysed on an ABI 310 automatic DNA sequencer (Applied Biosystems). Sequences were

assembled by using the program SeqMan II (DNASTAR, Madison WI, USA). Multiple alignment of the nucleotide sequences was performed using Clustal X (Jeanmougin et al., 1998).

3. Results

Gross examination revealed poor body condition and mild dehydration; intestinal walls were thickened and contained a dense catarrhal exudate. Mesenteric lymph nodes were uniformly enlarged. Intestinal samples showed a mild to moderate diffuse chronic enteritis with blunting and fusion of villi, increased mitotic activity of enterocytes, mild dilatation of lacteals, and a severe infiltration of eosinophils in the lamina propria, with a lower number of lymphocytes and plasma cells. The number of intraepithelial lymphocytes (calculated as the mean value per 100 enterocytes on 10 randomly selected sagittally cut apical villi) was slightly increased, consisting in 38 IEL/100 enterocytes compared with 30–35 IEL/100 enterocytes in 2–4 months-old normal lambs (Reynolds and Morris, 1983). In the colon the infiltrate was lighter and mainly lymphoplasmacytic rather than eosinophilic. Mesenteric lymph nodes showed marked hyperplasia of cortical follicles with some eosinophils in the medullary sinuses.

No bacteria were isolated from liver, spleen or brain. *Escherichia coli* isolates, cultured from intestinal swabs, did not carry genes coding for the virulence factors considered in our diagnostic procedures. No viral particles were detected by electron microscopy and no parasites (coccidia, *Cryptosporidium* or round worms) were found in the samples.

Similar results were obtained from the euthanized lambs. However, direct light microscopy carried out on the small intestine content revealed the presence of a large number of flagellated organisms, which, on the basis of their morphology, were identified as *Giardia* sp. trophozoites. The IF assay was also negative, a finding that is due to the lack of the cyst wall, and therefore of the cyst wall antigens, i.e. the target of the monoclonal antibody, on the trophozoites. Molecular analysis revealed that the parasite belonged to the Assemblage B of *Giardia duodenalis*. More precisely, the highest homology (99%) was found with the subgenotype S1.

After diagnosis, all lambs were treated with fenbendazole at a dose of 10 mg/kg administered for 3 consecutive days, as suggested by other authors (Xiao et al., 1996; O'Hadley et al., 1997). After treatment, animals rapidly recovered from the symptoms and the weight gain went back to normal.

4. Discussion

Giardia infection in ruminants is considered relatively common (Taminelli and Eckert, 1989; Diaz et al., 1996; Olson et al., 1997); however, low prevalence rates have been observed in Central Italy (Giangaspero et al., 2005), and no clinical giardiasis in lambs had been previously found in this region.

Our observations show that *G. duodenalis* can cause a serious malabsorption syndrome in lambs, and consequently, relevant economic losses in sheep farming also in this geographical area. Therefore, *G. duodenalis* should be always considered in differential diagnosis in animals showing decreased weight gain or in cases of prolonged diarrhoea not responding to antibiotic therapy.

In the present case, the parasite was readily identified since necropsy was performed soon after euthanasia. Intestinal content, directly sampled from the small intestine, allowed, trophozoites to be easily visualised by light microscopy. In situations where animals are examined several hours after death, cysts detection using appropriate assays, such as staining, immunofluorescence or PCR-based methods, should be carried out to identify the parasite.

With respect to pathological features, the eosinophilic infiltration of intestinal mucosa is an uncommon finding in giardiasis (Abbas et al., 1994; Jiménez et al., 2004). However, the slight increase of IELs (Intra Epithelial Lymphocytes) observed in our case, could be consistent with the pathological pattern described by other authors in calves (Ruest et al., 1997; O'Hadley et al., 2001). Eosinophilic enteritis could be idiopathic, due to parasitism or to hypersensitivity (Jiménez et al., 2004). Since no parasites other than *G. duodenalis* were identified in the lambs and there was no record of previous treatment that could have cleared a concurrent parasitic infection, it may be supposed that parasitism did not produce the observed eosinophilic infiltration, unless *G. duodenalis* was considered the primary cause of the lesion. As an alternative hypothesis, hypersensitivity could have played a significant role in the development of the lesion; intestinal tissue eosinophilia, in fact, may depend on the synthesis of chemotactic factors for eosinophils by lymphocytes, enterocytes or mast cells (Jiménez et al., 2004). Mast cells are important for rapid control of *G. duodenalis* infections in mice (Li et al., 2004) and mast cells hyperplasia is reported in Mongolian Gerbils experimentally infected with *G. duodenalis* (Hardin et al., 1997). These cells may be involved in the host response to the parasite by modulating a hypersensitivity reaction (Hardin et al.,

1997). Consistently with this idea, soluble antigens of *G. duodenalis* have been demonstrated to elicit a Th2 response in mice, with production of IgE (Jiménez et al., 2004) and giardiasis in humans has been associated with both allergic responses and chronic urticaria (Li et al., 2004). However, further investigations are necessary to clarify the inflammatory pathways of the host response to *G. duodenalis* infection observed.

Molecular characterization revealed the etiological agent to be subgenotype S1 in the *G. duodenalis* Assemblage B, which has been previously identified in humans (GenBank accession number AY228628) and environmental (waste water) samples (GenBank accession number AY368164) in USA (Sulaiman et al., 2004). It is well known that parasites classified as *G. duodenalis*, in spite of the lack of morphological differences, show a remarkable degree of genetic variation that allows to distinguish at least seven genetic groups, or Assemblages, having different host-specificity (Thompson and Monis, 2004). Farm animals, including sheep, are predominantly infected with the so-called hoofed genotype, or Assemblage E (Ey et al., 1997), but can also be infected with the zoonotic Assemblage A (van Keulen et al., 2002; Lalle et al., 2005; Ryan et al., 2005). However, infection with the zoonotic Assemblage B was not reported so far in sheep. Whether the severity of the clinical signs and the pathogenic effects observed in this case are due to the genetic background of the parasite remains to be proven.

Regarding therapy, febendazole resulted an effective molecule, at least in terms of complete clinical recovery, confirming previous observations of other authors, who successfully treated calves infected with *Giardia* sp. using similar protocols (O'Hadley et al., 1997; Xiao et al., 1996).

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References

Abbas, Z., Qureshi, A.A., Sheikh, H., Jafri, S.M., Khan, A.H., 1994. Peculiar histopathological features of giardiasis in distal duodenal biopsies. J. Pak. Med. Assoc. 44, 206–209.

Ambrosi, M. (Ed.), 1995. Parassitologia Zootecnica. Edizioni agricole, Bologna, 389 pp.

da Silva, A.J., Bornay-Llinares, F.J., Moura, I.N.S., Slemenda, S.B., Tuttle, J.L., Pieniazek, N.J., 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Mol. Diagn. 4, 57–64.

Diaz, V., Campos, M., Lozano, J., Manas, I., Gonzalez, J., 1996. Aspects of animal giardiosis in Granada province (southern Spain). Vet. Parasitol. 64, 171–176.

Ey, P.L., Mansouri, M., Kulda, J., Nohynkova, E., Monis, P.T., Andrews, R.H., Mayrhofer, G., 1997. Genetic analysis of *Giardia* from hoofed farm animals reveals artiodactyl-specific and potentially zoonotic genotypes. J. Eukaryot. Microbiol. 44, 626–635.

Fratamico, P.M., Sackitey, S.K., Wiedmann, M., Deng, M.Y., 1995. Detection of *Escherichia coli* O157:H7 by multiplex PCR. J. Clin. Microbiol. 33, 2188–2191.

Gannon, V.P.J., King, R.K., Kim, J.Y., Thomas, E.J., 1992. Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground of beef using the polymerase chain reaction. Appl. Environ. Microbiol. 58, 3809–3815.

Gannon, V.P.J., Souza, S.D., Graham, T., King, R.K., Read, S., 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohaemorragic *E. coli* strains. J. Clin. Microbiol. 35, 656–662.

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

Hardin, J.A., Buret, A.G., Olson, M.E., Kimm, M.H., Gall, D.G., 1997. Mast cell hyperplasia and increased macromolecular uptake in an animal model of giardiasis. J. Parasitol. 83, 908–912.

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G., Gibson, T.J., 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci. 23, 403–405.

Jiménez, J.C., Fontaine, J., Grzych, J., Dei-Cas, E., Capron, M., 2004. Systemic and mucosal responses to oral administration of excretory and secretory antigens from *Giardia intestinalis*. Clin. Diagn. Lab. Immunol. 11, 152–160.

Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., Cacciò, S.M., 2005. Genetic heterogeneity at the β-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of new, potentially zoonotic, genotypes. Int. J. Parasitol. 35, 207–213.

Li, E., Zhou, P., Petrin, Z., Singer, S.M., 2004. Mast cell-dependent control of *Giardia lamblia* infections in mice. Infect. Immun. 72, 6642–6649.

O'Hadley, R.M., Buret, A.G., McAllister, T.A., Jeliski, M., Olson, M.E., 2001. Giardiasis in dairy calves: effects of febendazole treatment on intestinal structure and function. Int. J. Parasitol. 31, 73–79.

O'Hadley, R.M., Olson, M.E., McAllister, T.A., Morck, D.W., Jelinski, M., Royan, G., Cheng, K.J., 1997. Efficacy of fenbendazole for treatment of giardiasis in calves. Am. J. Vet. Res. 58, 384–388.

Olson, M.E., McAllister, T.A., Deselliers, L., Morck, D.W., Cheng, K.J., Buret, A.G., Ceri, H., 1995. Effects of giardiasis on production in a domestic ruminant (lamb) model. Am. J. Vet. Res. 56, 1470–1474.

Olson, M.E., Thorlakson, C.L., Deselliers, L., Morck, D.W., McAllister, T.A., 1997. *Giardia* and *Cryptosporidium* in Canadian farm animals. Vet. Parasitol. 68, 375–381.

Osek, J., 1999. Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. Vet. Microbiol. 68, 209–217.

Pasquinelli, F. (Ed.) 1971. Manuale per Tecnici di Laboratorio, vol. II. Rosini, Firenze, 2013 pp.

Reynolds, J.D., Morris, B., 1983. The influence of gut function on lymphoid cell populations in the intestinal mucosa of lambs. Immunology 49, 501–509.

Ruest, N., Couture, Y., Faubert, G.M., Girard, C., 1997. Morphological changes in the jejunum of calves naturally infected with *Giardia* spp and *Cryptosporidium* spp. Vet. Parasitol. 69, 177–186.

Ryan, U.M., Bath, C., Robertson, 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. Appl. Environ. Microbiol. 71, 4992–4997.

Sulaiman, I.M., Jiang, J., Singh, A., Xiao, L., 2004. Distribution of *Giardia duodenalis* genotypes and subgenotypes in raw urban wastewater in Milwaukee. Wisconsin Appl. Environ. Microbiol. 70, 3776–3780.

Taminelli, V., Eckert, J., 1989. The frequency and geographic distribution of *Giardia* infections in ruminants in Switzerland. Schweiz. Arch. Tierheilkd. 131, 251–258.

Thompson, R.C., Monis, P.T., 2004. Variation in *Giardia*: implications for taxonomy and epidemiology. Adv. Parasitol. 58, 69–137.

van Keulen, H., Macechko, P.T., Wade, S., Schaaf, S., Wallis, P.M., Erlandsen, S.L., 2002. Presence of human *Giardia* in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for giardiasis. Vet. Parasitol. 108, 97–107.

Virga, A., Baccarini, E.M., Canestri-Trotti, G., Giannetto, S., 1998. Indagine sulla fauna protozoaria intestinale in agnelli delle province di Agrigento, Palermo, Trapani (Western Sicily). Atti SIS. Vet. 52, 164–263.

Xiao, L., 1994. *Giardia* infection in farm animals. Parasitol. Today 10, 436–438.

Xiao, L., Saeed, K., Herd, R.P., 1996. Efficacy of albendazole end fenbendazole against Giardia infection in cattle. Vet. Parasitol. 61, 165–170.