Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

ELSEVIER

Veterinary Parasitology 69 (1997) 177–186

veterinary
parasitology

Morphological changes in the jejunum of calves naturally infected with *Giardia* spp. and *Cryptosporidium* spp.

N. Ruest [a,*], Y. Couture [a], G.M. Faubert [b], C. Girard [a]

[a] *Faculté de Médecine Vétérinaire, Université de Montréal, 3200 rue Sicotte, St-Hyacinthe, Québec J2S 7C6, Canada*

[b] *Institute of Parasitology, McGill University, 21 111 LakeShore Road, Macdonald Campus, Ste-Anne-de-Bellevue, Québec H9X 3V9, Canada*

Received 27 March 1996; accepted 22 October 1996

Abstract

Giardiosis and cryptosporidiosis are frequently diagnosed in calves at the large animal clinic of the veterinary school. Few studies have been reported in the literature regarding pathogenesis of these two intestinal protozoa. The aims of this study were to follow the histological changes in the villi and crypts and the changes in the number of intraepithelial lymphocytes in the jejunum of naturally infected calves during the acute phase of infection. For this purpose, 29 calves aged between 7 and 10 days were bought at a local auction. The animals were housed in individual pens to avoid cross-contamination. Fecal samples were examined microscopically for the presence of *Giardia* cysts and *Cryptosporidium* oocysts, three times per week for a period of 45 days. Six calves did not pass any cysts or oocysts and were used as controls. Fifteen calves passed *Giardia* cysts only, five passed both cysts and oocysts, and three passed oocysts only. The villus to crypt ratio index was 1.76 in the control group and 1.08 in the *Giardia*-infected group. In the *Cryptosporidium*-infected calves, the ratio was 1.18 and calves infected with both parasites had an index of 1.37. The number of intraepithelial lymphocytes per millimeter of jejunum tissue was 21 in the control group. This number was doubled in the calves infected with *Giardia*, but was

* Corresponding author.

0304-4017/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved.
PII S0304-4017(96)01121-1

slightly lower in the other infected groups. All of the infected calves had intermittent diarrhea and mucus was seen in many fecal samples. © 1997 Elsevier Science B.V.

Keywords: *Giardia* spp.; *Cryptosporidium* spp.; Cattle; Calf; Protozoa; Pathogenesis

1. Introduction

Giardiosis is the most widespread intestinal protozoal disease of humans in the world. Not only is the infection diagnosed in developing countries, but it also occurs among people living in developed countries where public hygiene is good and water supplies are purified and piped. Today, epidemiological studies indicate that infections are spreading not only in humans, but also in farm animals (Xiao, 1994). The first case of giardiosis in cattle was reported by Fantham (1921). Since this first report, many studies on the incidence of giardiosis in cattle have been reported (Deshpande and Shastri, 1981; Taminelli and Eckert, 1989; Buret et al., 1990). However, the pathogenesis of intestinal mucosal damage in cattle giardiosis has received little attention. After infecting calves in the laboratory with bovine *Giardia* isolates, Taminelli et al. (1989) studied cellular infiltration and villus atrophy in the jejunum of infected calves. After a pre-patent period of 8 days, all calves excreted cysts during a maximum period of 112 days. Intestinal blunting, flattening of the villi and cellular infiltration of the mucosa of the small intestine were present. They also observed that these changes also occured when the intestine was densely populated by bacteria and viruses (Taminelli et al., 1989). In humans, the distribution of trophozoites is practically unknown since the small intestine is accessible only by invasive techniques involving intubation. However, trophozoites have been detected in the duodenum and proximal jejunum of infected patients (Ferguson et al., 1990). Using the gerbil–*Giardia lamblia* animal model, Campbell and Faubert (1994) found that trophozoites are distributed evenly in the small intestine. In humans and laboratory animal models, it has been reported that *Giardia* trophozoites are capable of causing a decrease in the villus to crypt ratio (VCR) at the site of infection. For example, it has been reported that gerbils infected with 2×10^5 *G. lamblia* trophozoites have a significant decrease in the VCR during the acute phase of the infection (Belosevic et al., 1989). Meanwhile, the presence of acute inflammatory cells in the epithelial layer was reported in clinical human giardiosis (Hoskins et al., 1967). Intraepithelial lymphocyte (IEL) counts, performed both in adults (Gillon, 1985) and children (Ferguson et al., 1976), showed high numbers at the time the patients passed cysts in the feces.

The aims of this study were to follow the histological changes occuring in the villi and crypts in the jejunum of calves naturally infected with *Giardia*, and to count the number of IEL during the various phases of the infection. We also wished to determine whether a concomitant infection with *Cryptosporidium* spp. increased the pathogenesis observed in the jejunum of calves infected with *Giardia* trophozoites. For this purpose, we measured the VCR and counted the number of IEL in various sections of the jejunum using standard histological procedures. The bacterial and viral flora of the small intestine were also determined. The morphometric measurements were compared and

analysed by the Spearman rank correlation test in order to determine if there was a relationship between the VCR and the number of IEL.

2. Materials and methods

2.1. Animals

For this study, 29 Holstein calves aged between 7 and 10 days were bought at a local auction. On arrival, they were placed in individual pens to avoid cross-contamination. The pens were elevated from the ground and had slatted floors. A commercial milk substitute (Lacvor® Nutrinor CAA, Chambord, Qc) was the primary diet until weaning at 8 weeks of age. No antibiotics were added to the milk substitute. Concentrates were provided to the calves at 2 weeks of age and remained as the primary diet after weaning. Water was available to each calf ad libitum.

Six of the calves were housed in a different barn and were used as controls. They were kept in individual pens and received the same diet as the other calves.

2.2. Fecal collection and examination

The day after arrival, fecal samples from all calves were collected and examined by microscopy. Samples were collected from the floor when freshly passed or from the rectum using disposable plastic gloves. They were stored in individual plastic fecal pots at 4°C until examination. The zinc sulfate concentration technique was used to detect *Giardia* cysts. Approximately 10 g of feces was mixed thoroughly with 10 ml of tap water and stirred through a gauze into 15-ml centrifuge tubes. The filtrate was centrifuged for 2 min at 1500 rpm. The supernatant was discarded and the sediment resuspended in a zinc sulfate solution with a specific gravity of 1.18. The tubes were centrifuged again for 2 min at 1500 rpm. After centrifugation, the tubes were filled to the rim with zinc sulfate solution and covered with a coverslip touching the meniscus. After 2 min, the coverslip was then placed on a glass slide and examined unstained with a microscope at 200 × or 400 × magnification for a period of 5 min. A direct examination technique was used to detect *Cryptosporidium* oocysts (Casemore et al., 1985). Fecal samples were taken three times per week during a period of 6 weeks, and were examined within the first 36 h after collection. All the fecal samples were graded according to the level of moisture content: 0 = normal; 1 = partly-formed feces; 2 = mostly watery content; and 3 = watery diarrhea.

2.3. Necropsy

The calves were necropsied at 6 or 8 weeks of age while the animals were still being fed with milk substitute to avoid any variables at the intestinal level following weaning. At 6 weeks of age, 21 calves were necropsied: three controls, three infected with *Cryptosporidium*, five infected with both parasites and 10 infected with *Giardia*. In order to determine the progression of the lesions in the intestine, eight calves were

necropsied at 8 weeks of age (three controls and five infected with *Giardia*). Calves were passing *Cryptosporidium* oocysts or *Giardia* cysts in their feces immediately before slaughter.

2.4. Intestine tissue collection and examination

Sections of abomasum, duodenum, proximal, mid and distal jejunum, ileum and spiraled colon were fixed in 10% buffered-formalin. Tissue specimens varied in length from 2.5 to 10 cm. Tissues were set in paraffin, cut into 6 μm sections and stained with haematoxylin, phloxin and saffron (HPS). Inflammation was graded as + (light), + + (moderate) or + + + (severe). Presence or absence of *Giardia*, *Cryptosporidium* and *Eimeria* was also noted.

2.5. Observations on BVD, Rota and Corona viruses and Salmonella spp.

At necropsy, spleen and ileal tissues and rectal content of each calf were taken. The presence of *BVD* in the spleen and ileal histological sections (6 μm) was determined with a direct immunofluorescence assay which used a polyclonal antiserum of bovine origin (VMRD, Pullman, WA) which detects non-cytopathogenous strains of *BVD* in tissues. The presence of *Rota* and *Corona* viruses in the ileal sections was determined with an indirect immunofluorescence assay using a rabbit antiserum against the antigens of these viruses (Armand-Frappier Inst., Laval, Qc.). Detection of *Salmonella* spp. in fecal samples was carried out by using tetrathionate broth (Difco, Detroit, MI) as enrichment medium. Tubes were incubated at 37°C and, after 24 h, the broths were poured on SS agar (Difco) plates. The SS agar plates were incubated at 37°C for 18 h. Lactose-negative colonies were submitted to a biochemical identification according to the procedures used in the clinical bacteriology laboratory at the veterinary school and the Manual of Clinical Microbiology (Murray et al., 1995).

2.6. Measurement of villus to crypt ratio and IEL numbers

The method of Roberts-Thomson et al. (1976) for the determination of villus to crypt ratio in murine giardiosis was used, except for the following modification. The jejunum was removed, fixed in formalin and processed in paraffin for light microscopy. All histological specimens (HPS 6 μm) were examined at 100 × with a morphometric analysis computer program called: 'R and M biometric analysis volume morphometry' (R and M Biometrics Inc., Nashville, TN).

The method of Rosekrans et al. (1981) was used for the determination of the number of IEL per mm surface epithelium, except for the following modification. The graphic tablet (Tektronix) used by Rosekrans et al. (1981) was replaced by the morphometric analysis computer program described above to determine the IEL in the jejunum in each calf. For both measurements, 10 representative specimens of infected and uninfected calves were used.

2.7. Statistical analysis

The Kruskal–Wallis one-way analysis of variance by ranks and a multiple comparison test were used to determine differences between groups for the given parameters. The Kruskal–Wallis test was also used to evaluate the effect of the duration of infection with *Giardia* on the VCR and the IEL. The Spearman rank correlation was used to determine if there was a relation between the VCR and the IEL. The alpha chosen was 0.05.

3. Results

3.1. Fecal examination and clinical symptoms

The first faecal examinations revealed that, upon arrival, three calves were passing *Giardia* cysts and two were passing *Cryptosporidium* oocysts. None of the infected calves had a mixed infection. One week later, eight calves were passing *Giardia* cysts, 11 calves were passing *Cryptosporidium* oocysts and four had a mixed infection. Therefore, in less than a month, all the 23 calves in one barn had become infected with one or both parasites. The six calves raised separately did not become infected. One month after arrival, the infection rates for *Giardia* spp. and *Cryptosporidium* spp. were 87% and 34.8%, respectively. Five calves were infected with both parasites. All infected calves had intermittent diarrhea of 2–3 days average duration. Mucus was seen in the feces of calves infected with *Giardia* spp. and to a lesser extent in the feces of calves infected with *Cryptosporidium* spp. No differences were observed in feed intake between the infected and non-infected calves. In spite of the presence of diarrhea, dehydration was not a clinical feature. No differences were noted in the duration and severity of the diarrhea between the three infected groups. Some calves had fever (40°C and over) for 1–6 days during their stay. The fever was attributed to respiratory bacterial infection and responded well to penicillin (22000 UI Kg^{-1}, BID).

3.2. Histological examination

3.2.1. Parasite observations

Giardia trophozoites were observed at the base of the villi in 50% of the infected calves. Seven calves were infected in the proximal jejunum, five in the mid jejunum and four in the distal jejunum; one calf had trophozoites in all jejunal segments. Trophozoites were found in the duodenum of only one animal; none was found in the abomasum, the ileum or the colon. *Cryptosporidium* spp. were found in the abomasum or the jejunum of the calves. *Eimeria* spp. were found in the jejunum of three calves infected with *Giardia*. In one animal the parasite was located in the ileum and in the colon. No parasites were observed in the control group.

3.2.2. Histological observations

A moderate to severe diffuse inflammation was seen in the jejunal chorion of all calves infected with *Giardia*. The cellular infiltration consists mainly of mononuclear

Fig. 1. Cross-section of the proximal jejunum of a calf infected with *Giardia* (100×). The villi and the space between crypts are filled with inflammatory cells. Villi are atrophied and crypt distortion is noted.

cells; plasmocytes were predominant. Villus atrophy and distortion of the crypts were common findings in calves infected with *Giardia* (Fig. 1). Purulent cryptitis, varying from rare to multifocal, was observed in the proximal jejunum of 10 calves. The inflammation was less severe in the duodenum, ileum and colon.

The inflammation was more severe in the proximal jejunum than in other intestinal segments in calves infected with both parasites or with *Cryptosporidium* alone. In the control group, the inflammation was light to moderate in all intestinal segments. However, this inflammation could not be attributed to the presence of the two parasites since they were not observed in histological examination and no cysts or oocysts were detected in feces. There was no distortion in the crypts, and the villi were not atrophied (Fig. 2). As reported by Barker et al. (1993), no pathological lesions compatible to a *BVD* virus infection were observed in the intestinal tract of these calves. Histological changes compatible with *Cryptosporidium* spp. or *Eimeria* spp. infections were not observed in the abomasum or in the large intestine of these animals, as had been reported previously by Angus (1990) and Barker et al. (1993).

3.3. Viral and bacterial flora

The absence of *Rota*, *Corona* and *BVD* viruses in the ileum section and *BVD* in the spleen sections was confirmed by using the immunofluorescence assay. On the other hand, the absence of *Salmonella* in the fecal samples was determined by the routine bacterial procedures used in our clinical bacteriology laboratory.

Fig. 2. Cross-section of the proximal jejunum of a calf used as a control (100×). Cellular infiltration by mononuclear cells is light. The villi are long and no distortion of the crypts are seen.

3.4. IEL results

The mean number of IEL per mm of tissue in the control group was 20.93 (Fig. 3). In the *Giardia*-infected group, the number of IEL per mm doubled (42.32) and reached a peak in the group infected with *Cryptosporidium*. It was interesting to note that the number of IEL per mm for the group of animals with the mixed infection was lower when compared with the group of calves infected with *Giardia* only. There was no correlation between the IEL and the duration of infection with *Giardia* ($0.1 < P < 0.95$). However, a direct correlation existed between the number of IEL per mm and the intensity of the inflammation observed at histology ($0.005 < P < 0.01$).

3.5. Villus and crypt measurements

The VCR for *Giardia*-infected calves was significantly lower when compared to the control group (Fig. 4). However, no significant differences were observed between the

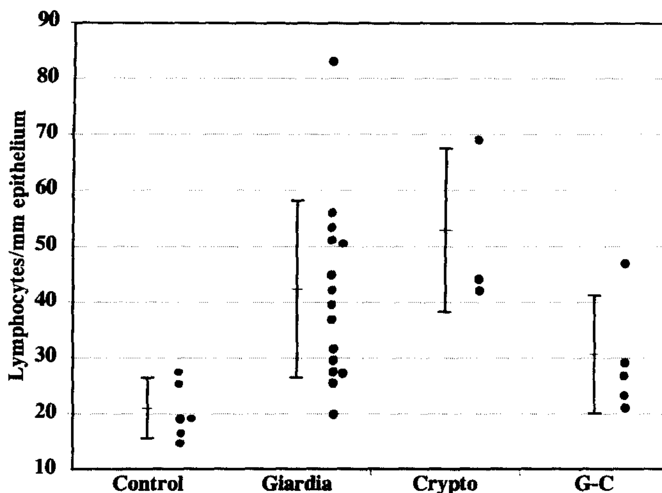

Fig. 3. Jejuna were removed from calves and the number of IEL was determined by computer using a program designed for morphometric analysis. Each dot represents one calf. The mean number of IEL for each group, together with S.D., is presented.

control group and the *Cryptosporidium*-infected calves or the calves with the mixed infection. It is worth mentioning that the variation in the VCR for all the groups was rather large. There was no correlation between the VCR and the duration of infection with *Giardia* ($0.1 < P < 0.95$).

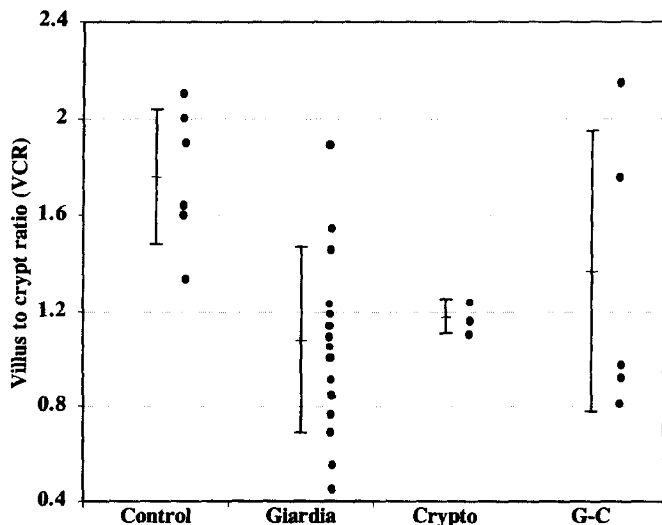

Fig. 4. Jejuna were removed from calves and the VCR was measured at 100× (magnification) with a morphometric analysis computer program. The height of the villi and crypts was determined on 10 specimens. Each dot represents one calf. The mean of the villus to crypt ratio for each group, together with S.D., is presented.

4. Discussion

The data obtained in this study show that only the group of calves infected with *Giardia* spp. had a reduced VCR. However, the number of IEL per mm of tissue increased significantly in the three groups of infected calves, when compared to the non-infected group. It appears that the morphological changes in the jejunum, together with the increase in the number of IEL, can be attributed to the presence of these two protozoans since the results of tests for detecting the presence of bacteria and viruses were negative. We have observed an important range in the VCR value and the number of IEL per mm of tissue. The low number of calves per group and the infective dose are likely to be important factors in this variation. Furthermore, the animals were naturally infected and we were not able to determine the intensity of the infection. On the other hand, the state of the immune system at the time of infection, together with the variation in virulence of different strains, are also important factors to consider in the variability of pathogenesis. We have ruled out these two parameters as explanations for the variation in our data. The calves were all of the same age, were in excellent health and all originated from the same region. Therefore, it is unlikely that the immune system might have played an important role or that the animals were infected with different strains. From a clinical point of view, the results emphasize the difficulties in diagnosing these infectious diseases. Animals may be infected with a low number of parasites that are not detectable by routine fecal examination or they may show few clinical signs or evidence of pathology. However, they remain as an important source of contamination for the herd since they may be carriers of the parasites. All the infected calves in this experiment had episodes of diarrhea lasting 2–3 days. Recently, it has been reported that lambs infected in the laboratory with *Giardia* also became diarrheic (Olson et al., 1995). The calves infected with *Cryptosporidium* spp. in the present work did not have a reduced VCR and this was attributed to the fact that the site of *Cryptosporidium* is the ileum, not the jejunum (Barker et al., 1993). On the other hand, villus atrophy and crypt hyperplasia, in response to infection with *Giardia*, are well known phenomena. The infection is more detrimental to the mucosal architecture of the upper portion of the small intestine. Buret et al. (1992) observed an increase in the numbers of immature enterocytes, which appears to be associated with crypt hyperplasia. In human giardiosis, it has been reported that malabsorption is associated with a low VCR and high numbers of IEL present in the jejunum. In our study, an elevated VCR was associated with a low number of IEL in the jejunum. It is worth mentioning that the mixed infection caused less morphological damage in the jejunum than was the case when the calves were infected with a single parasite. These results are contrary to our hypothesis, since we reasoned that a mixed infection would exacerbate the pathogenesis and the clinical signs caused by infection with only one of the protozoans. It is possible that the low number of calves in the group with the two parasites (five) was not enough to observe differences. Another possibility would be that the parasites may have an antagonistic effect on each other rather than a synergistic effect. The housing of calves in individual pens did not prevent cross-contamination since, at the time of housing in the barn, there were only three calves infected with *Giardia* spp. and two calves infected with *Cryptosporidium* spp. In less than a month, all the animals became infected. We believe

that the common procedures of husbandry were responsible for the rapid spread of the infection amongst the herd.

Acknowledgements

The authors thank 'Le fond du Centenaire' of the Faculté de Médecine Vétérinaire of l'Université de Montréal for their financial support, Dr Alain Villeneuve and Dr Béatrice Doizé for welcoming us and allowing us to work in their respective laboratories, and Dr Roger Prichard and Mr Shawn Mohammed for critique of the manuscript. Dr Faubert would like to acknowledge NSERC grant number OGP9374.

References

Angus, K.W., 1990. Cryptosporidiosis in ruminants. In: J.P. Dubey, C.A. Speer and R. Fayer (Editors), Cryptosporidiosis of man and animals. CRC Press, Boca Raton, FL, pp. 83–103.

Barker, I.D., van Dreumer, A.A. and Palmer, N., 1993. The alimentary system. In: K.V.F. Jubb, P.C. Kennedy and N. Palmer (Editors), Pathology of domestic animals, Fourth edition, vol. 2. Academic Press, San Diego, CA, pp. 149–315.

Belosevic, M., Faubert, G.M. and Maclean, J.D., 1989. Dissacharidase activity in the small intestine of gerbils (*Meriones unguiculatus*) during primary and challenge infections with *Giardia lamblia*. Gut, 30: 1213–1219.

Buret, A., den Hollander, N., Wallis, P.M., Befus, D. and Olson, M.E., 1990. Zoonotic potential of giardiasis in domestic ruminants. J. Inf. Dis., 162: 231–237.

Buret, A., Hardin, J.A., Olson, M.E. and Gall, D.G., 1992. Pathophysiology of small intestinal malabsorption in gerbils infected with *Giardia lamblia*. Gastroenterology, 103: 506–513.

Campbell, D. and Faubert, G.M., 1994. Comparative studies on *Giardia lamblia* encystation in vitro and in vivo. J. Parasitol., 80: 36–44.

Casemore, D.P., Armstrong, M. and Sands, R.L., 1985. Laboratory diagnosis of cryptosporidiosis. J. Clin. Pathol., 38: 1337–1341.

Deshpande, P.D. and Shastri, U.V., 1981. Incidence of *Giardia* infection in calves in Maharashtra state, India. Trop. Anim. Hlth. Prod., 13: 34.

Fantham, H.B., 1921. Some parasitic protozoa found in South Africa. S. Afr. J. Sc., 18: 164–170.

Ferguson, A., McLure, J.P. and Townley, R.R.W., 1976. Intraepithelial lymphocyte counts in small intestinal biopsies from children with diarrhea. Acta Paedia. Scand., 65: 541–546.

Ferguson, A., Gillon, J. and Munro, G., 1990. Pathology and pathogenesis of intestinal mucosa damage in giardiasis. In: E.A. Meyer (Editor), Giardiasis. Elsevier, Amsterdam, pp. 153–174.

Gillon, J., 1985. Clinical studies in adults presenting with giardiasis to a gastro-intestinal unit. Scott. Med., 30: 89–95.

Hoskins, L.C., Winawer, S.J., Britman, S.A., Gottheb, L.S. and Zemcheck, N., 1967. Clinical giardiasis and intestinal malabsorption. Gastroenterology, 53: 265–279.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. (Editors), 1995. Manual of Clinical Microbiology, Sixth Edition. ASM Press, Washington, D.C., 1482 pp.

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

Roberts-Thomson, I.C., Stevens, D.P., Mahmoud, A.A.F. and Warren, K., 1976. Giardiasis in the mouse; an animal model. Gastroenterology, 71: 57–61.

Rosekrans, P.C.M., Lindeman, J. and Meyer, C.J.L., 1981. Quantitative histological and immunohistochemical findings in jejunal biopsy specimens in giardiasis. Virchows Archiv Pathol. Anat., 393: 145–151.

Taminelli, V. and Eckert, J., 1989. Haufigkeit und geographische Verbreitung des *Giardia* Befalls bei Wiederkauern in der Schweiz. Schweiz. Arch. Tierheilk., 131: 251–258.

Taminelli, V., Eckert, J., Sydler, T., Gottstein, B., Corboz, L. and Hofmann, M., 1989. Experimentelle infektion von Kälbern und schafen mit bovinen *Giardia*-isolaten. Schweiz. Arch. Tierheilk., 131: 551–564.

Xiao, L., 1994. *Giardia* infections in farm animals. Parasitology Today, 10: 436–438.