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First Record of *Giardia* in Cattle in Denmark

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Iburg T., R.B. Gasser and S.A. Henriksen: First record of *Giardia* in cattle in Denmark. Acta vet. scand. 1996, 37, 337-341. – Faecal samples from asymptomatic dairy cows and calves from a farm on the Island Falster, Denmark, were examined by a sucrose gradient flotation technique. *Giardia* cysts were found in 7.6% of the 92 samples, and estimated cyst excretion rates ranged from 50-200 cysts per gram faeces. Given that *Giardia* has the potential to cause clinical disease in cattle and to be transmitted to other animal species and humans, finding the parasite in cattle may be of major epidemiological significance. Future work should focus on elucidating the pathogenicity, transmission patterns and the genetic structure of *Giardia* populations in cattle in Denmark.

parasites; protozoa; ruminants; zoonotic potential.

Introduction

Giardia is a flagellated protozoan which inhabits the small intestine of humans and a range of mammal, amphibian, reptile and bird species (see *Thompson et al.* 1993). The parasite has a world-wide distribution and is recognised as a very important causative agent of diarrhoea and other symptoms in humans, dogs, cats and other host species. *Giardia* has a direct faecal-oral transmission route, and 2 stages (the trophozoite and the cyst) are involved in the life-cycle. The motile trophozoites colonize the small intestine and divide by longitudial binary fission, and the cyst is the ('resistant') form excreted in the faeces of the host.

Based on trophozoite morphology, the genus has at least 5 different morphological groups or "types" of *Giardia,* namely *G. agilis, G. muris, G. duodenalis* (= *G. lamblia* and *G. intestinalis*) (*Filice* 1952), *G. psittaci* (*Erlandsen & Bemrick* 1987) and *G. ardeae* (*Erlandsen et al.* 1990). Traditionally, it was thought that *Giardia* was strictly host specific (e.g. *Kulda & Nohynkova* 1978), but there is a body of evidence indicating that transmission can occur between/among different host species (reviewed by *Thompson et al.* 1993). In spite of this evidence, there still remain major controversies as to the zoonotic potential of "strains" within the *Giardia duodenalis* complex. In Denmark, clinical cases of giardiasis are common in humans (*G. Gomme* and *A. Kharazmi, personal communication*). Although significant, there is a paucity of epidemiological information on *Giardia* in both humans and animals in this country. So far, there are only some observations of infection in domesticated animals. In the present study, we report for the first time the occurrence of *Giardia* infection in a cattle herd in Denmark and discuss the potential implications of this finding.

Materials and methods

A dairy farm on the Island of Falster, Denmark, was chosen for this study based on a previous

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history of scouring in calves. It was decided to visit the farm outside of the calving season and examine the entire cattle herd for evidence of *Giardia* infection. Rectal faecal samples (n=92) were collected from 59 dairy cows, 10 calves < 7 days, 3 calves 1-4 weeks, 11 calves 1-5 months and 9 calves > 5 months of age. For the detection of *Giardia* cysts in these samples, a sucrose gradient flotation technique was carried out as described previously (*Gasser et al.* 1987). In brief, 8 grams of faeces were suspended in 250 ml of water, sieved through a strainer (1 mm mesh size), and the suspension was allowed to sediment for 30 min. Thereafter, the supernatant was aspirated, the sediment centrifuged at 650 xG for 5 min and its volume adjusted to 3 ml. This suspension was overlaid on 1 M sucrose (specific gravity 1.1) and centrifuged at 650 xG for 3 min. The supernatant was aspirated to approx. 5 mm above the water-sucrose interface. The entire interface was removed, transferred to a fresh tube, diluted to 10 ml with water and centrifuged as before. The sediment was suspended in 500 µl water and vortexed. Three aliquots (each 25 µl) were pipetted on to a glass slide and cover-slipped (12 x 12 mm) and Giardia cysts counted. An estimate was made of the number of cysts per gram of faeces.

Results

Giardia cysts were found in 7 of the 92 (7.6%) faecal samples examined. Five of 59 (8.5%) cows and 2 of 33 (6.1%) calves (1 month and 5 months of age, respectively) were found to be excreting cysts in faeces. The sample from the one-month-old calf contained approx. 200 cysts per gram of faeces, the other 6 samples contained approximately 50 cysts per gram. In addition, low numbers of oocysts of *Cryptosporidium* spp. (presumably *C. parvum*) and/or *Eimeria* spp. were found in a small proportion of samples.

The morphometrical dimensions of the *Giardia* cysts detected here were in the same range as previously reported by *Gasser et al.* (1987). Using the sucrose gradient flotation technique, *Giardia* cysts are characteristic in size, shape and morphology, and there is no distorsion of the internal structure (trophozoite) of the cysts using sucrose. However, there can be a problem in differentiating *Giardia* cysts from other protozoan cysts (presumably cystic forms of the bovine forestomach flora), because of their very similar shape and size. In the present study, the main criteria for differentiation of *Giardia* cysts from these "cystic forms" was the presence of well-defined nuclei and what appears to be related to the axostyle structure. If this disctinction was not made, it would have led to significant overestimation of cyst excretion rates. This problem has been encountered previously in faceal samples from ruminants *(Gasser, unpublished observations),* but appears not to have been reported.

Discussion

To our knowledge, this is the first report of *Giardia* infection in cattle in Denmark. Although bovine infection(s) with *Giardia* had not been described before in this country, there are numerous reports from abroad (*Willson* 1982, *St Jean et al.* 1987, *Buret et al.* 1990, *Xiao et al.* 1993, *Ruest et al.* 1995). From a recent review of the literature, it is evident that the prevalence of *Giardia* in cattle (mainly calves) is high in a number of countries, including Italy (19%), the former Soviet Union (34%), former Czechoslovakia (21-100%), Romania (5%), India (52%), Cuba (11%), Canada (22%) and the USA (10-89%) (original articles reviewed by *Xiao* 1994). In Switzerland, for example, *Taminelli & Eckert* (1989) reported a prevalence of 27-45% in calves. These and other workers in Switzerland also described that the infection in calves is characterised by

intermittent excretion of cysts in faeces (*Gasser et al.* 1987, *Taminelli & Eckert* 1989, *Taminelli et al.* 1989), which is consistent with reports from other countries (e.g. *Buret et al.* 1990, *Xiao* 1994). Another characteristic of the infection in cattle appears to be high cyst excretion rates (1300 to 180000 cysts per gram of faeces) (e.g. *Gasser et al.* 1987, *Taminelli & Eckert* 1989), although the number of cysts excreted from aged cattle is lower than from calves (*Buret et al.* 1990, *Xiao* 1994). In the present study, cyst excretion rates ranged from 50-200 cysts per gram of faeces, which are significantly lower than those described in the literature (reviewed by *Xiao* 1994). The reasons for such low excretion rates (even in calves) are unclear, but a possible explanation could be that the immune status of the herd was good. However, it must be noted that faecal samples were examined on one occasion, and it is possible that some cattle were infected but were not shedding cysts and that others were shedding low numbers at the time of examination. Clearly, further work is necessary to study the dynamics of infection over a period of time. Although the numbers of cysts excreted from cattle in the present study seem low, they represent approx. 50000 to 200000 *Giardia* cysts per kilogram of faeces. Given the large amounts of faeces produced by cattle, these numbers may be quite significant from an epidemiological point of view (i.e., environmental contamination).

The clinical significance and pathogenicity of *Giardia* infection in cattle remain unclear. Several studies have indicated that the infection can cause mild to chronic diarrhoea in calves (often intermittent), apparently resulting in some cases in ill thrift and retarded growth in calves (*Willson* 1982, *St.Jean et al.* 1987, *Taminelli et al.* 1989, *Buret et al.* 1990, *Xiao et al.* 1993, *Quigley et al.* 1994, *Ruest et al.* 1995). In contrast, *Gasser et al.* (1987) found that *Giardia* infection in calves may remain asymptomatic despite high rates of cyst excretion in faeces. This information suggests that there may be (geographical) variation in clinical expression of disease in calves, which may be a consequence of differences in host susceptibility and/or virulence of the parasite. These aspects warrant detailed investigation. Another important consideration is that other pathogens may be involved in inducing clinical disease. For example, some studies have described that concurrent infections with corona and rota viruses as well as *Cryptosporidium* may play a role (*St Jean et al.* 1987, *Xiao et al.* 1993, *Quigley et al.* 1994). In the present study, oocysts of *Cryptosporidium* and *Eimeria* were detected in some faecal samples, but the "interaction" or relationship among the pathogens remains unclear. The significance of these and viral pathogens and their association with diarrhoea in calves needs to be examined in detail.

Current literature reveals that there are different "strains" (= genotypes) of *Giardia duodenalis* which may have different epidemiological, biological and physiological characteristics (e.g. *Andrews et al.* 1992a,b, *Thompson & Meloni* 1993, *Meloni et al.* 1995, *Mayrhofer et al.* 1995, *Upcroft et al.* 1995). A particularly relevant characteristic is the potential for transmission of infection among different animal species, but more importantly, the transmission from animals to humans (*Thompson et al.* 1993). Morphological parameters cannot be used to differentiate among such genotypes ("strains"), so that molecular biological and biochemical techniques have been developed to elucidate which genotypes may be transmissible to humans (e.g. *Andrews et al.* 1989, review by *Thompson et al.* 1993, *Upcroft et al.* 1995). Before such techniques can be employed to study the genetic structure of *Giardia* populations, however, it is essential to identify which potential reservoirs for human infection exist (e.g. domesticated animals, which are in close

contact with humans) and determine the prevalence of infection in these hosts. Evidence from other countries indicates that *Giardia* from cattle can be genetically quite similar to those found in humans. Several studies indicated a high degree of metabolic and genetic similarity between *Giardia* isolates from cattle and humans in Switzerland (*Strandén et al.* 1990, *Strandén & Köhler* 1991, *Meloni et al.* 1992). Based on this information, it is possible that genotypes of *Giardia* from cattle in Denmark may be transmissible to humans and other animals. Given the absence of information on *Giardia* in Denmark, future research needs to focus on identifying reservoirs of infection, determing the prevalence of infection in different host species and establishing the genetic structure of *Giardia* population(s) in order to shed light on the epidemiological transmission patterns of this fascinating parasite.

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Sammendrag

Giardia observeret for første gang hos kvæg i Danmark.

Faecesprøver (n=92) fra en malkekvægsbesætning på Falster i Danmark er undersøgt med sukkrose gradient flotation. *Giardia* cyster blev påvist i 7,6% af prøverne, primært fra voksne køer. Udskillelsen af cyster varierede fra 50-200 cyster pr. g. fæces, hvilket synes relativt lavt sammmenlignet med fund rapporteret fra udlandet. Det er så vidt vides første gang, at infektion med *Giardia* er påvist hos kvæg i Danmark. I betragtning af, at *Giardia* er i stand til at forårsage klinisk sygdom hos kvæg og at *Giardia* repræsenterer en potentiel smittekilde for andre dyr og mennesker, kan dette fund have stor epidemiologisk betydning.

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