Cryptosporidium parvum and *Giardia intestinalis* in Calf Diarrhoea in Sweden

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Björkman C, Svensson C, Christensson B, de Verdier K: *Cryptosporidium parvum and Giardia intestinalis* **in calf diarrhoea in Sweden. Acta vet. scand. 2003, 44, 145-152.** – The objective of this study conducted in 75 herds was to investigate the presence and significance of *Cryptosporidium parvum* and *Giardia intestinalis* in Swedish dairy calves in comparison with rotavirus, coronavirus and *Escherichia coli* K99+. The farmers were asked to collect faecal samples from each heifer calf that had diarrhoea between birth and 90 days of age, and also from a healthy calf of the same age. In total, 270 samples were collected and analysed. *C. parvum,* either alone or together with *G. intestinalis* and/or rotavirus, was detected in 16 (11%) and 6 (5%) of the samples from diarrhoeic and healthy calves, respectively. Even though a higher proportion of diarrhoeic calves shed *C. parvum,* the difference between the groups was not statistically significant (p=0.067), possibly due to the low number of positive samples. *G. intestinalis* was found in 42 (29%) of the diarrhoea samples and in 29 (23%) of the samples from healthy calves. Rotavirus and coronavirus were demonstrated in 24% and 3% of the diarrhoea samples, respectively, whereas *E. coli* K99+ was only found in samples from 2 healthy calves. *C. parvum* and *G. intestinalis* were found in samples from calves 7 to 84 days of age and during all seasons. The results confirm that *C. parvum* is present in Swedish dairy herds and might have clinical significance. *G. intestinalis* was the most common agent found but the importance of this parasite remains unclear. Both parasites have suggested zoonotic potential and thus warrant further attention. In addition, rotavirus is a major pathogen in neonatal enteritis in Sweden, whereas coronavirus and *E. coli* K99+ seem to be of less importance.

Cryptosporidium parvum; Giardia intestinalis; rotavirus; coronavirus; Escherichia coli; calf diarrhoea.

Introduction

Cryptosporidium parvum is an intracellular protozoan parasite causing gastrointestinal disease and diarrhoea in a variety of animal species including cattle, sheep and man. The first report on bovine cryptosporidiosis was published in 1971, when *C. parvum* parasites were identified in the faeces from an 8-month-old heifer with chronic diarrhoea (*Panciera et al.* 1971). Today *C. parvum* is recognised as an important infection in young calves. The most prominent finding in an infected calf is diarrhoea, sometimes accompanied by depression, inappetence, fever, dehydration and/or poor condition. The calf most often recovers spontaneously within 1-2 weeks even though there is a large variation between individuals in how they respond to and recover from infection (*Tzipori et al.* 1983, *O'Donoghue* 1995). Concomi-

tant infection with other enteric pathogens can aggravate the clinical signs and prolong the duration of disease (*O'Donoghue* 1995). *C. parvum* is transmitted as microscopic oocysts that are excreted in the faeces from infected animals. Finding oocysts in diarrhoeic faeces is indicative of *C. parvum* being the cause of the disease. During the first two weeks of infection a calf can shed millions of oocysts (*Fayer et al.* 1998, *Uga et al.* 2000) which ensures efficient dissemination of the parasite.

The intestinal flagellate *Giardia intestinalis* (synonyms *G. duodenalis* and *G. lamblia*) has a global distribution and is a common protozoan parasite in many animal species and in man (*Xiao* 1994, *Kulda & Nohýnková* 1995). In calves it can cause diarrhoea, malabsorption and growth retardation. However, the severity of disease is highly variable and many *G. intestinalis* infections are asymptomatic (*Xiao* 1994, *Quílez et al.* 1996). An infected individual sheds microscopic cysts with the faeces and in calves intermittent cyst shedding can continue for several weeks (*Xiao & Herd* 1994).

Infections with rotavirus and coronavirus are well-known causes of acute diarrhoea in young calves, usually occurring in calves at 1-3 weeks of age (*Mebus et al.* 1973, *Saif et al.* 1994). The clinical picture is similar to that of cryptosporidiosis and comprises diarrhoea, depression, anorexia and mild fever. In several surveys, rotavirus has been the most commonly detected infectious agent in bovine neonatal enteritis (*Snodgrass et al.* 1986, *Saif et al.* 1994). Other infectious agents – *Eimeria* spp, bredavirus, calicivirus, astrovirus, parvovirus, *Salmonella* spp, *Campylobacter* spp., *Clostridium perfringens* – are also reported as enteric pathogens in neonatal calf diarrhoea (*Tzipori* 1981). Bovine viral diarrhoea virus has the ability to cause immunosuppression and enhance the severity of calf diseases, including neonatal calf diarrhoea. Enterotoxigenic *Escherichia*

coli (*E. coli*) K99+ causes diarrhoea in very young calves and the clinical outcome may be fatal. A rapid and extensive loss of body water and electrolytes can cause severe weakness, recumbence and hypothermia (*Butler & Clarke* 1994).

From a zoonotic point of view the most interesting of the calf enteric pathogens are *C. parvum, G. intestinalis* and *Salmonella* spp. However, *Salmonella* spp. are uncommon in Swedish dairy herds as a result of many years of intensive control measures (*Wahlström* 2001). Information about the presence of *C. parvum* infection in Swedish cattle is limited. In a study comprising 279 dairy calves aged 0-14 days, 5% of the calves harboured *C. parvum* (*Viring et al.* 1993). In a more recent investigation, *C. parvum* oocysts were detected in 3 out of 14 herds with calf diarrhoea problems (*de Verdier Klingenberg & Svensson* 1998). Bovine *G. intestinalis* infection has been reported in Sweden (*Anonymous* 2001) but the prevalence and importance of giardiosis in the Swedish cattle population is not known.

The objective of the present study was to investigate the presence and significance of *C. parvum* and *G. intestinalis* in Swedish dairy calves in comparison with rotavirus, coronavirus and *E. coli* K99+.

Materials and methods
Animals and samples
The study was conducted in dairy herds in the former Skaraborg county in the southwest of Sweden between 1 January 1998 and 31 March 1999. The herds participated in an a long-term research project about health and growth rate in dairy heifer calves. Details about the herds, which had 29-94 cows, considered a reasonably representative sample of Swedish dairy farms, have been reported elsewhere (*Svensson et al.* 2003). The farmers were requested to take faecal samples from each up to 90 day-old heifer

calf born in 1998 that had diarrhoea, and from a healthy calf of the same age. Diarrhoea was defined as faeces with a consistency that was looser than normally observed in calves, continuing for ≥2 days. Healthy was defined as absence of diarrhoea. The samples were to be collected the second day after the onset of diarrhoea and before any possible antibiotic treatment was given. The samples together with information about date of birth of the calves were sent by mail to the laboratory. The age of a calf was calculated as days between date of birth and date of arrival of sample to the laboratory. For healthy calves kept in group pens, the birth date was calculated as the mean between the birth dates of the youngest and oldest calf in the pen. At arrival, the samples were immediately analysed for presence of *C. parvum,* rotavirus and coronavirus. An aliquot of each sample was stored at -70°C and later analysed for *G. intestinalis* and *E. coli* K99+.

Laboratory methods

Presence of *C. parvum* oocysts was demonstrated by microscopic examination of faecal smears stained with the modified Ziehl-Neelsen technique (*Henriksen & Pohlenz* 1981). Rotavirus was analysed by a group A rotavirus ELISA (*Svensson et al.* 1986) using an optical density value of 0.1 to discriminate between positive and negative samples. Coronavirus was analysed by ELISA with an in-house method developed and used for routine diagnosis at the Swedish National Veterinary Institute. Commercially available ELISAs were used for demonstration of *G. intestinalis* (Prospect Giardia microplate assay, Alexon-Trend, Ramsey, MN, USA) and *E. coli* K99+ (BioX, Bruxelles, Belgium).

Statistical analysis

Associations between presence of enteric pathogens and diarrhoea were investigated by

Table 1. Detection of enteropathogens in faecal samples from diarrhoeic and healthy calves younger than 90 days in a study comprising 75 dairy herds from south-east Sweden.

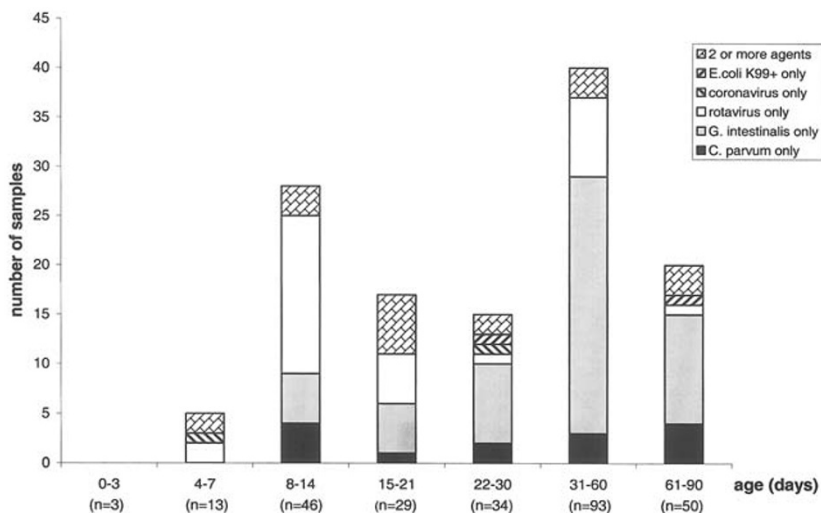|	*C. parvum* only	*G. intestinalis* only	Rotavirus only	Coronavirus only	*E. coli* K99+ only	*C. parvum* + *G. intestinalis*	*C. parvum* + rotavirus	*G. intestinalis* + rotavirus	Rotavirus + coronavirus	*C. parvum* + *G.intestinalis* + rotavirus
Diarrhoea (n=146)	10 (7%)	31 (21%)	25 (17%)	3 (2%)	0	4 (3%)	1 (0.5%)	6 (4%)	2 (1%)	1 (0.5%)
Healthy (n=124)	4 (3%)	26 (21%)	10 (8%)	0	2 (2%)	2 (2%)	0	1 (1%)	0	0
Total (n=270)	14 (5%)	57 (21%)	35 (13%)	3 (1%)	2 (1%)	6 (2%)	1 (0.5%)	7 (3%)	2 (1%)	1 (0.5%)

Figure 1. Distribution of infectious agents in diarrhoeic and healthy calves (n=268) younger than 3 months of age in 75 Swedish dairy herds.

Chi square test (Stata Statistical Software Release 7.0, Stata Corp., College Station, TX, USA).

Results

Of 122 herds invited to participate in the study, 75 (61%) submitted samples. In total, samples from 146 diarrhoeic and 124 healthy calves were sent to the laboratory and analysed. The farmers sometimes submitted samples from several diseased calves but only a single sample from a healthy calf, and this is the reason for the discrepancy between the number of diarrhoeic and healthy calves. Of the 3081 heifer calves born in the study herds during 1998, 302 developed diarrhoea before 90 days of age (*Svensson et al.* 2003). Thus, faecal samples were submitted from 48% of the diarrhoeic calves in the herds.

C. parvum, either alone or together with *G. intestinalis* and/or rotavirus, was detected in 16 (11%) of the 146 samples from diarrhoea calves, whereas *G. intestinalis,* rotavirus, coro-

navirus and *E. coli* K99+ were demonstrated in 42 (29%), 35 (24%), 5 (3%) and 0 of the diarrhoea samples, respectively (Table 1). The presence of rotavirus was significantly associated with diarrhoea (P=0.001) and a tendency to an association was found between diarrhoea and the presence of *C. parvum* oocysts (P=0.067). *C. parvum, G. intestinalis* and rotavirus were found in 17/75 (23%), 38/75 (51%) and 28/75 (37%) or the herds, respectively.

C. parvum, G. intestinalis and rotavirus were found in calves that were between 7-84, 9-84 and 5-78 days old, respectively (Figure 1). These agents were found in the faecal samples throughout the year.

Discussion

This is the first large scale investigation of *C. parvum* and *G. intestinalis* infections in Swedish dairy calves. *Giardia* antigen was found in almost a quarter of the investigated samples, and 50% of the 75 herds that sent samples to the laboratory had at least one positive

calf. This agrees with the relatively high prevalences of *Giardia* infection in calves reported from point prevalence studies performed in other parts of the world (*Quílez et al.* 1996, *O'Handley et al.* 2000, *Appelbee et al.* 2003). The only previous investigation of *G. intestinalis* in Swedish farm animals was done in sheep and revealed that 22% and 68% of investigated lambs and sheep flocks, respectively, harboured the infection (*Ljungström et al.* 2001). *G. intestinalis* is assumed to be a potential pathogen in cattle (*Xiao & Herd* 1994) but in the present study the parasite was equally common in diarrhoeic as in healthy calves, giving an ubiquitous impression. Similar findings have also been reported by others (*Quílez et al.* 1996, *Huetink et al.* 2001).

The zoonotic potential for *Giardia* is under debate. However, the high prevalence of infection in Swedish cattle and sheep together with recent reports that cattle and sheep can shed cysts of a genotype that infect humans (*O'Handley et al.* 2000, *van Keulen et al.* 2002) warrant further attention. *C. parvum* in livestock is by current knowledge indicated to be an important reservoir for cryptosporidiosis in humans, and contaminated water may be a key vector for the parasite. It is therefore noteworthy that 8% of the calves in the present study showed oocyst shedding, which was not restricted to scouring calves.

These overall results regarding cryptosporidiosis are in accordance with international studies showing that rotavirus and *C. parvum* are the infectious agents most often found in young calves with enteritis (*Snodgrass et al.* 1986, *McDonough et al.* 1994). They are also consistent with a previous finding that rotavirus and *C. parvum* are the predominant agents causing neonatal diarrhoea in Swedish cattle herds (*de Verdier Klingenberg & Svensson* 1998). That *E. coli* K99+ was not found in any of the diarrhoea samples and in only 2 samples collected from

clinically healthy calves, partly contradict results from a Swedish investigation performed in 1987-88. In that study, *E. coli* K99+ was demonstrated in 11.5% of faecal samples from dairy calves younger than 14 days and a similar percentage of diarrhoeic and healthy calves were infected (*Viring et al.* 1993). However, our results support a previous suggestion that there was a substantial over-treatment with antimicrobial drugs in the farms included in the present study; 30% of the calves ≤90 days with diarrhoea were treated with antibiotics (*Ortman & Svensson* 2003).

In the present study *C. parvum* and rotavirus were found throughout the 90 days sampling period, except for the first neonatal days. A similar observation was reported by e.g. *Quílez et al.* (1996) who in an epidemiological investigation found that even though *C. parvum* infection was more common among neonatal calves, 30% of the 1.5-4 month-old calves were infected. Also asymptomatic adult cattle have been found to excrete *C. parvum* oocysts (*Scott et al.* 1995) and have been implied to transmit the infection in cattle herds.

Although *C. parvum* is recognised as an enteric pathogen in calves, an association between shedding of oocysts and presence of diarrhoea is not always seen (*Snodgrass et al.* 1986, *Xiao & Herd* 1994). A lack of relationship between oocyst shedding and diarrhoea has been reported from an investigation of a Swedish herd with cryptosporidiosis (*Tråvén et al.* 1989). In the present study the higher proportion of *C. parvum* infected calves in the diarrhoea group compared with the healthy calves was not statistically significant. However, further studies to investigate any association between diarrhoea and *C. parvum* are desirable since the number of infected calves in this study was low (n=22). There is a great variability in the clinical outcome of infection depending on e.g. the infection dose and the immune status of the calf

(*de Graaf et al.* 1999). It is therefore not surprising that subclinical *C. parvum* infection does occur. Further, it has been shown that there is a difference in the oocyst shedding pattern between individuals; the shedding often continues for several days and can start before the onset of scouring and continue after the diarrhoea has ceased (*Fayer et al.* 1998, *Enemark* 2002).

In the present study *C. parvum* was found in faeces from calves sampled during all seasons. An absence of association between season of the year and prevalence of the parasite was reported by *Wade et al.* (2000). However, others have found seasonal variation in the prevalence of bovine *C. parvum* infection, maybe owing to climate factors and various managemental factors such as seasonal production of livestock (*de Graaf et al.* 1999).

Concomitant infections with *C. parvum* and other enteric pathogens increase the risk for diarrhoea and might aggravate the clinical signs (*de Graaf et al.* 1999, *Enemark* 2002). In the present study it is noteworthy that the calves with both *C. parvum* and rotavirus had diarrhoea and were very young, 7 and 9 days old.

To conclude, our results confirm that *C. parvum* is present in Swedish dairy herds and might have clinical significance. *G. intestinalis* was the most common agent found but the importance of this parasite remains unclear. Both parasites have suggested zoonotic potential and thus warrant further attention. In addition, rotavirus is a major pathogen in neonatal enteritis in Sweden whereas coronavirus and *E. coli* K99+ seem to be of less importance.

Acknowledgements

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Sammandrag

Cryptosporidium parvum och Giardia intestinalis vid kalvdiarré i svenska mjölkkobesättningar.

Avsikten med denna studie var att undersöka förekomst och betydelse av *Cryptosporidium parvum* och *Giardia intestinalis* hos svenska kalvar, jämfört med rotavirus, coronavirus och *Escherichia coli* K99+. Djurägarna i 75 mjölkkobesättningar tog träckprover från upp till 90 dagar gamla kalvar som hade diarré, samt från friska kalvar i samma ålder. Totalt insamlades och analyserades 270 prover. *C. parvum,* enbart eller tillsammans med *G. intestinalis* och/eller rotavirus, påvisades i 16 (11%) av proverna från kalvar med diarré och 6 (5%) av de friska kalvarna. Skillnaden mellan grupperna var inte statistiskt signifikant (p=0,067), möjligen beroende på det låga antalet positiva prover. *G. intestinalis* påvisades hos 42 (29%) prover från kalvar med diarré och hos 29 (23%) friska kalvar. Rotavirus påvisades i 24% och coronavirus i 3% av proverna från diarrékalvarna medan *E. coli* K99+ bara återfanns i prover från 2 friska kalvar. *C. parvum* och *G. intestinalis* påvisades

i prover från kalvar mellan 7 och 84 dagars ålder och under hela året. Resultaten bekräftar att *C. parvum* förekommer i svenska mjölkkobesättningar och kan ha klinisk betydelse. *G. intestinalis* var det agens som påvisades hos flest kalvar, men parasitens betydelse för kalvhälsan är oklar. Fynden är intressanta då båda parasiterna anses ha zoonotisk potential. Resultaten styrker även tidigare iakttagelser att rotavirus är den vanligaste infektiösa orsaken till kalvdiarré i Sverige.