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Prevalence of *Cryptosporidium* and *Giardia* infections in cattle in Aragón (northeastern Spain)

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Abstract

Faecal samples from 554 bovines randomly selected at 30 farms in Aragón were examined to investigate the prevalence of *Cryptosporidium* and *Giardia* infections. *C. parvum* oocysts were identified by using the Ziehl–Neelsen modified technique in 109 (19.7%) bovines ranging from 3 days old to adults. Positive animals were found in 19 (63.3%) farms. As much as 44.4% of calves aged 3–4 days were infected, but infection rates peaked at 6–15 days of age (76.7%). Nevertheless, prevalence was also high in weanling calves aged 1.5–4 months (14%), fattening calves and heifers 4–24 months old (7.7%) and adults (17.8%). Diarrhoea was recorded in 78.6% of suckling and 29.4% of weanling calves infected by *C. parvum*, but it was only found to be statistically associated with infection in suckling calves ($P < 0.01$). All calves shedding moderate or many oocysts had diarrhoea, whereas asymptomatic infection was always correlated with few oocysts in faeces. Cryptosporidial infections were always asymptomatic in bovines older than 4 months. *Giardia* cysts were identified in 65 bovines (11.7%) from 16 (53.3%) of the farms surveyed. Infection rates were significantly higher in suckling (14.1%) and weanling calves (38%) than in bovines older than 4 months (2.2%) ($P < 0.001$). Diarrhoea was recorded in 45.5% of suckling and 10.9% of weanling calves infected by *Giardia*, but it was not found to be statistically associated with infection. In fact, infection rates were higher in non-diarrhoeic than in diarrhoeic calves.

Keywords: *Cryptosporidium parvum*; Epidemiology; *Giardia* sp.; Cattle–Protozoa

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1. Introduction

Enteric cryptosporidial infection is a well-recognized cause of diarrhoea in man and animals. Both severity of infection and prevalence increase in immunodeficient humans, such as those with AIDS, as well as in neonates of some mammal species, such as ruminants. At present, the control of cryptosporidiosis is difficult, bearing in mind the lack of a clearly effective drug therapy and the resistance of oocysts to environmental factors and chemical agents (O'Donoghue, 1995; Martins and Guerrant, 1995).

In cattle, *C. parvum* is recognized worldwide as one of the most common enteropathogens causing neonatal diarrhoea, in addition to rotavirus, coronavirus, enterotoxigenic *Escherichia coli* and *Salmonella* spp. (Angus, 1990). Since neonatal enteric infections have a negative effect on the performance of the calves during the first month of life, the economic significance of cryptosporidiosis is remarkable. Furthermore, *C. parvum* is a potential zoonotic pathogen, as it is considered to be the species infecting humans. In fact, numerous cases of zoonotic transmission have been documented from epidemiological studies, involving direct transmission from pets and farm animals as well as water-borne outbreaks (O'Donoghue, 1995).

Giardia infection has also long been considered as a cause of diarrhoea in humans and companion animals (Zajac, 1992; Thompson et al., 1993). However, recent works confirm that this protozoan is more common than currently believed in large animals, especially in cattle (Xiao, 1994). Additionally, high levels of infection in young calves are considered to be clinically significant. Continuous or intermittent diarrhoea, weight loss and growth retardation because of maldigestion and malabsorption have been associated with giardiasis in cattle and subclinical infections are thought to increase requirements for some nutrients (Kirkpatrick, 1989). On the other hand, like cryptosporidiosis, *Giardia* infection in large animals may have epidemiological implications for human health. The zoonotic potential of *Giardia* has been controversial for long and there is some epidemiological evidence of giardiasis of zoonotic origin mainly based on studies of water-borne outbreaks in humans (Kasprzak and Pawlowski, 1989). Although no irrefutable evidence for such transmission outside the laboratory has been recorded, at present, most authors recognize that at least some isolates of *Giardia* are not host-specific and that humans and a variety of others animals share the parasite, regarding giardiasis as a zoonosis (Thompson et al., 1993).

Epidemiological studies on *C. parvum* infections in cattle in Spain are limited, although infection has been reported in some regions. Similarly, no information is available about the importance of *Giardia* infections in cattle. The objective of the current study was to determine the prevalence and age distribution of *Cryptosporidium* and *Giardia* infections in cattle in Aragón, as well as the occurrence of both protozoa in cases of neonatal calf diarrhoea.

2. Materials and methods

Between December 1990 and August 1993, stool specimens were collected from 554 bovines randomly selected at 30 dairy and beef cattle farms in Aragón (northeastern

Spain). A minimum of five animals per farm were tested. On average, 10% of the total number of animals on each farm were sampled. For each bovine, a single faecal sample was taken from the rectum by using a disposable plastic bag. In calves with diarrhoea, disposable plastic containers were used and the faeces classified according to the consistency: soft, semiliquid or liquid. Animals were classified based on the age range: suckling calves less than 1.5 months old (78), weanling calves aged 1.5–4 months (121), fattening calves and heifers aged 4–24 months (130) and adults (225).

Fresh stool specimens were processed by the formalin-ethyl acetate sedimentation technique (Young et al., 1979) and smears of the concentrate (20 μl) were stained by the modified Ziehl–Neelsen technique (Henriksen and Pohlenz, 1981). The complete surface of the smear was examined for the presence of *Cryptosporidium* oocysts at ×200 and ×400 magnifications. Infection intensities were evaluated semiquantitatively according to the average number of oocysts in 20 randomly selected fields at ×200 magnifications: few (0–1 oocysts per field), moderate (1–5 oocysts per field) and many (more than 5 oocysts per field). This method has been shown to be as accurate as haemacytometer counting (Kao and Ungar, 1994). Additionally, wet mounts and iodine-stained preparations of the sediment were examined by light microscopy for gastrointestinal parasites.

Infection rates between different age groups and between diarrhoeic and non-diarrhoeic bovines were compared by using chi-square and Fisher's exact tests, according to the number of expected values. Significance was determined at $P < 0.05$.

3. Results

The overall prevalence of cryptosporidial infection in cattle was 19.7%. Infected bovines were found in 19 out of 30 (63.3%) farms surveyed. Oocyst shedding was recorded in bovines ranging from 3 days old to adults, although infection rates were significantly higher in suckling calves (53.8%) than in the remaining age groups ($P < 0.0001$) (Table 1). The size and structure of the oocysts identified in positive samples was consistent with that of *C. parvum* (Upton and Current, 1985). Analysis of selected infection rates in suckling calves showed that 44.4% of calves 3–4 days old were infected, although the percentage of calves shedding oocysts peaked at 6–15 days (76.7%), decreasing to 40% in calves aged 1 month.

Table 1
Prevalence of *Cryptosporidium* and *Giardia* infection according to the age range

Age range	No. studied	*Cryptosporidium*		*Giardia*	
		No. infected	%	No. infected	%
< 1.5 months	78	42	53.8	11	14.1
1.5–4 months	121	17	14	46	38
4–24 months	130	10	7.7	3	2.3
Adults	225	40	17.8	5	2.2
Total	554	109	19.7	65	11.7

Semiquantitative counting of cryptosporidia positive smears showed that infection intensities were usually low, since 88.1% of infected animals excreted few oocysts. Moderate or many oocysts were detected in some of the suckling calves less than 1.5 months old (4.8% and 23.8%, respectively) and weanling calves aged 1.5–4 months (0% and 5.9%, respectively), whereas all the infected bovines older than 4 months excreted few oocysts.

At sampling, diarrhoea was reported in 51 (65.4%) suckling calves and 18 (14.9%) weanling calves. As much as 78.6% of suckling calves and 29.4% of weanling calves infected by *C. parvum* had diarrhoea, whereas infection was always asymptomatic in bovines older than 4 months. Cryptosporidial infection rates were higher in diarrhoeic than in non-diarrhoeic calves, in both suckling (64.7% and 33.3%, respectively) and weanling calves (27.8% and 11.6%, respectively), but the differences were only statistically significant in suckling calves ($P < 0.01$).

It is worth noting that all calves shedding moderate or many oocysts had diarrhoea, whereas asymptomatic infection was always correlated with few oocysts in faeces. Nevertheless, diarrhoea was also associated with low intensity of infection, since 70% of suckling and 25% of weanling calves shedding few oocysts had diarrhoea. Similarly, an association between infection intensities and faecal consistency was found in diarrhoeic suckling calves, as the number of those excreting liquid stools significantly increased with the number of oocysts found in the faeces ($P < 0.05$) (70% of suckling calves shedding many oocysts had liquid stools, in contrast with 50% of those shedding moderate and 23.8% of those shedding few oocysts).

On the other hand, *Giardia* cysts were identified in 65 bovines (11.7%) from 16 (53.3%) of the farms surveyed. Infection rates were significantly higher in suckling (14.1%) and weanling calves (38%) than in the other age groups ($P < 0.001$) (Table 1). Cysts were classified as belonging to the *Giardia duodenalis* group based on their structure (Thompson et al., 1993). Diarrhoea was recorded in 45.5% of suckling and 10.9% of weanling calves infected by *Giardia*, but it was not found to be statistically associated with infection. In fact, rates were higher in non-diarrhoeic than in diarrhoeic calves, in both suckling (22.2% and 9.8%, respectively) and weanling calves (39.8% and 27.8%, respectively).

Table 2
Prevalence of enteric parasites in suckling and weanling calves with diarrhoea

	Calves < 1.5 months old	Calves 1.5–4 months old
C. parvum	29 (56.9%)	2 (11.1%)
Giardia sp.	2 (3.9%)	–
Eimeria spp.	1 (1.9%)	5 (27.8%)
C. parvum + *Giardia* sp.	2 (3.9%)	–
C. parvum + *Eimeria* sp.	1 (1.9%)	2 (11.1%)
Giardia sp. + *Eimeria* sp.	–	4 (22.2%)
C. parvum + *Giardia* sp. + *Eimeria* spp.	1 (1.9%)	1 (5.5%)
No parasites	15 (29.4%)	4 (22.2%)
Total	51	18

Prevalence of enteric parasites in calves with diarrhoea, including monoinfections and mixed infections, is presented in Table 2.

4. Discussion

Prevalence rates and percentage of farms testing positive show that cryptosporidial infection is widespread in cattle in our geographical area. *Cryptosporidium* oocysts were detected in bovines ranging from 3 days old to adults, although infection rates were significantly highest in suckling calves. The high percentage of suckling calves shedding oocysts at the age of 3–4 days (44.4%) indicates that most of them are infected immediately after birth. The short prepatent period of *Cryptosporidium* infection found here is similar to that previously reported (Xiao and Herd, 1994a) and consistent with the life cycle of the parasite (Fayer et al., 1990). It may also indicate heavy environmental contamination in the calving area (Angus, 1990). Nevertheless, the percentage of calves shedding oocysts peaked at 6–15 days of age (76.7%) whereas the rate decreased to 40% in calves aged 1 month. These results are similar to those of previous workers concluding that cryptosporidiosis in calves is generally established during the initial 2 weeks of life (Anderson, 1981; Henriksen and Krogh, 1985; Hiepe et al., 1988; Ongerth and Stibbs, 1989; Garber et al., 1994).

Shedding of oocysts from bovines older than 2 months has long been considered as rare (Henriksen, 1989) suggesting that dams are not a potential infection source for their calves. However, results of the current study show that infection is also common in calves after weaning and even in adults. Although, in agreement with results by Henriksen and Krogh (1985), infection intensities were always light and asymptomatic in animals older than 4 months. Recent surveys have recorded infection rates ranging from 62.4% to 71.7% in asymptomatic adult cattle, even higher than that detected in our study (Lorenzo-Lorenzo et al., 1993; Scott et al., 1995) and some authors have considered that asymptomatic adult carriers are responsible for the diarrhoea in calves born during early calvings. Contamination is then amplified by the infection of first new-born calves (Aurich et al., 1990). This opinion is consistent with the recent report, although not confirmed in cattle, of a periparturient rise in the excretion of *Cryptosporidium* oocysts in sheep (Xiao et al., 1994).

Although few oocysts are detected in faecal smears, the significance of asymptomatic carriers may be greater than that usually presumed, taking into account the large amount of faeces daily excreted by cows. In this way, Scott et al. (1994) showed that a cow may excrete between 750000 and 720 million oocysts daily, concluding that it contributed to the contamination of the new-born calf's environment and was a significant factor in the epidemiology of cryptosporidiosis. On the other hand, acid-fast stains have been shown to have a low sensitivity, since no less than 500000 oocysts g^{-1} faeces may be detected when processing formed human stool specimens (Weber et al., 1991).

We found that as much as 78% of suckling calves positive for *Cryptosporidium* had diarrhoea. However, this clinical symptom is not necessarily attributed only to cryptosporidiosis, since neither bacterial nor viral infections were checked. In fact, although *C. parvum* may be the only pathogenic agent isolated in many calves with cryptosporidiosis (Krogh and Henriksen, 1985; Moore and Zeman, 1991), concurrent infections with other microorganisms are common, especially between rotavirus and cryptosporidia (Snodgrass et al., 1986; Aurich et al., 1990; Moore and Zeman, 1991). Nevertheless, epidemiological surveys of enteric infections in calves conclude that *C. parvum* is the most frequent enteropathogen detected in diarrhoeic veal calves in some areas of the United States (McDonough et al., 1994), or only second to rotavirus in other areas of the same country (Moore and Zeman, 1991), Britain (Reynolds et al., 1986) and The Netherlands (de Visser et al., 1987).

In the current study, infection rates were higher in diarrhoeic than in non-diarrhoeic animals, in both suckling and weanling calves. However, the difference was only statistically significant in suckling calves. Other workers have also reported a statistically significant association between cryptosporidial infection and diarrhoea (Reynolds et al., 1986; Sobieh et al., 1987). Reynolds et al. (1986) stated that distribution of enteropathogens in healthy animals should be considered in interpreting results, but rates of cryptosporidial infection above 25%, as reported in our study, may be considered significant with regards to the involvement of *C. parvum* in the aetiology of an outbreak. Snodgrass et al. (1986) and Kaminjolo et al. (1993) found no statistically significant association between infection and diarrhoea, although cryptosporidia were detected more frequently in diarrhoeic than in healthy calves.

Results on the prevalence of *Giardia* in cattle revealed that infection is frequent in both suckling and weanling calves, in accordance with previous reports regarding giardiasis as a common infection in immature farm animals (Xiao, 1994; Ruest et al., 1995). Nevertheless, we found that cyst shedding occurred in all age groups although, unlike *Cryptosporidium*, the low rates of infection detected in bovines older than 4 months (2.2%) suggest that adult carriers do not play an important role as a source of infection to calves. By contrast, studies on *Giardia* infection in horses found that infection initiates later than in ruminants and rates are high in mares, concluding that they may be a major source of infection for foals (Xiao and Herd, 1994b).

On the other hand, it is worth mentioning that infection rates detected in the current study may be an underestimation of the true infection, since *Giardia* cysts are intermittently excreted in faeces. Intermittent excretion has been detected in humans (Thompson et al., 1993) and different mammals, such as companion animals, horses and calves (Kirkpatrick, 1989; Xiao, 1994; Xiao and Herd, 1994b), advising that at least three faecal specimens taken on non-consecutive days should be examined for reliable diagnosis of infection.

Giardia infection has been implicated as a cause of diarrhoea in calves (St Jean et al., 1987; Xiao et al., 1993). Ruest et al. (1995) reported that as much as 47% of the calves positive for *Giardia* cysts had diarrhoea, but it could not be attributed only to giardiasis since neither bacterial nor viral infections were checked. We found that 45.5% of suckling and 10.9% of weanling calves infected by *Giardia* had diarrhoea, although no statistically significant association between infection and occurrence of diarrhoea was detected, as the parasite was more frequent in non-diarrhoeic than in diarrhoeic calves. Some of the infected calves had additional parasites that could be involved in the aetiology of the clinical symptoms, such as *Cryptosporidium parvum* and *Eimeria* spp. Only two diarrhoeic suckling calves had a monoinfection by *Giardia*, but the role of the parasite in the aetiology of the diarrhoea is unknown since microbial infections were not excluded.

In conclusion, results of the current study demonstrate that *C. parvum* is frequently involved in the aetiology of calf neonatal diarrhoea in our region and adult carriers seem to play an important role as a source of infection. *Giardia* infections have also been found to be unexpectedly frequent in cattle, although the role of this protozoan in the aetiology of diarrhoea in calves remains unclear.

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