Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain)

José A. Castro-Hermida, Yolanda A. González-Losada,
Elvira Ares-Mazás*

*Departamento de Microbiología y Parasitología, Laboratorio de Parasitología, Facultad de Farmacia,
Universidad de Santiago de Compostela, Avda. de Vigo s/n, 15782 Santiago de Compostela, La Coruña, Spain*

Abstract

An epidemiological study was carried out on farms in Galicia (NW Spain) to investigate the prevalence of and the risk factors associated with the spread of infection by *Cryptosporidium parvum* in calves of less than 3 weeks of age. A total of 22 cattle farms (10 beef herds and 12 dairy herds) were visited once every 21 days between January and December 2000. A faecal sample was collected directly from the rectum of each of the 844 calves born during the study period. Each sample was studied macroscopically to establish its consistency as liquid, soft or solid, and the presence of mucus or blood noted. *C. parvum* oocysts were identified by direct microscopic examination and the intensity of infection established semiquantitatively as slight, moderate or severe. Of the 844 calves, 404 were found to have the parasite in their faeces, i.e. the prevalence was 47.9%. Statistical analysis of the risk factors such as general characteristics of the farm and the housing conditions of the calves, revealed three variables that significantly effect the risk of cryptosporidial infection in suckling calves: the method of cleaning, the type of flooring and the frequency of cleaning. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cryptosporidium parvum; Cattle-protozoa; Prevalence; Risk factors; Odds ratio

1. Introduction

Cryptosporidiosis is a widespread parasitic disease caused by obligate and opportunistic parasites of the genus *Cryptosporidium* (Tyzzer, 1907), which develop and multiply in

---

the epithelial cells of the intestines and respiratory tracts of vertebrates, and for which 152 mammalian hosts have been described (Fayer et al., 2000). Two species of *Cryptosporidium* can be distinguished in cattle: *C. parvum*, which infects the distal small intestine (spherical oocysts, $5.0 \times 4.5 \,\mu m$) and *C. andersoni* (syn. *C. muris*), which infects the abomasum (elipsoidal oocysts, $7.4 \times 5.5 \,\mu m$) (Lindsay et al., 2000).

Infection of farmed cattle by *Cryptosporidium* sp. was first described by Panciera et al. (1971). However, due to its association with other bacterial and viral enteropathogens, its role as a primary pathogen was not established until the 1980s, when Tzipori et al. (1980) attributed an outbreak of neonatal diarrhoea in calves to parasitization by *Cryptosporidium*. Some years later, Tzipori et al. (1983) and Heine et al. (1984) demonstrated experimentally, the capacity of the parasite to cause diarrhoea in calves. Only *C. parvum* is known to be associated with diarrhoea in neonatal ruminants (de Graaf et al., 1999).

At present, *C. parvum* is one of the most frequently found pathogenic agents in calves of less than 3 weeks of age (Moore and Zeman, 1991; de la Fuente et al., 1999). However, cryptosporidiosis should not only be considered from the perspective of animal health and production; its zoonotic character and the possibility that animals may act as a source of infection to humans, via foodstuff and water, should also be considered. Although the infection leads to few deaths, serious economic losses can occur because of problems associated with the resulting diarrhoea—dehydration, weight loss and slow growth (Sanford and Josephson, 1982)—and the costs involved in the treatment—oral administration of electrolytic solutions and drugs—and the application of suitable hygiene practices (de Graaf et al., 1999). *C. parvum* is usually present without other agents, however, the costs involved increase in the case of multiple infections (Vanopdenbosch et al., 1979).

Taking into account the importance of both dairy and beef cattle farming in Galicia, we decided to find the prevalence of cryptosporidiosis in suckling calves as well as to determine those factors associated with housing and hygiene which influence the spread of infection in calves, so that possible control measures can be established.

2. Material and methods

2.1. Choice of farms

The study was carried out in Galicia (NW Spain), a region where predominant the livestock cattle. The only condition placed on the choice of farm was that it had a large number of adult cattle, thus guaranteeing the birth of calves throughout the period of the study. A total of 22 farms were selected, and a questionnaire about the general characteristics of each farm and the calves' housing conditions was completed. The information thus compiled included, the size of the farm, the number of adult cattle and how this varied throughout the year, the type of flooring, the treatment and use of excretia, and the presence of other animals on the farm. Furthermore, there was a series of questions on the birth and raising of the calves, as well as the type of housing, the drinking water and hygiene practices used. Each farm was visited once every 21 days between January and December 2000, so that each farm was visited 16 times.

2.2. Collection of samples

On each visit, a single faecal sample was collected from each of all the calves born since the previous visit (calves of less than 21 days). The samples were taken directly from the rectum using plastic gloves or containers. An index card was filled out for each animal, with the following data, sampling date, origin and number of animal identification. The plastic flasks containing the samples were transported to the laboratory in a cool box then stored for a maximum of 24 h before analysis. The samples were then examined macroscopically to establish their consistency as liquid, soft or solid and to check for the presence of blood or mucus.

2.3. Detection of oocysts

Faecal smears were examined in the microscope and Heine's (1982) negative stain was used to enable identification of *C. parvum* oocysts. The intensity of infection was estimated semiquantitatively according to the average number of oocysts in 20 randomly selected fields at $1000\times$ magnification. The categories established were: negative (absence of oocysts); slight (1–5 oocysts); moderate (6–10 oocysts); severe (>10 oocysts).

2.4. Statistical analyses

Data was analysed by multivariate logistic regression in which the dependent variable was the status of the calves (infected or non-infected) and the independent variables were the general characteristics of the farm such as the calves' housing and hygiene conditions. The results were each expressed as an odds ratio (OR) with a 95% confidence interval (CI 95%).

To study the possible association between those variables in which there is no temporal relationship and which do not allow causal relationships to be established, a χ^2-test of association, which gives a measure of the relationship among variables, was carried out. For those variables with more than two categories, the linear trend was examined by the Maentel–Haenszel test. Results were considered to be significant at $P < 0.05$.

Analyses were carried out using the computer software SPSS (Statistical Package for Social Sciences) for Windows, Version 10.0, 1999.

3. Results

Analysis of a single sample of each of the 844 calves born during the study period revealed that 404 calves were shedding oocysts, i.e. the prevalence of infection was 47.9%.

The contingency tables containing the variables considered (consistency, mucosity and presence of blood) and on which the statistical analysis was carried out show that in all the calves examined (844) there was a significant association between parasitization with *C. parvum* and the consistency of the faeces (χ^2, $P < 0.001$; linear trend test, $P < 0.001$). Thus, 52.5% of the parasitized calves had liquid faeces compared with 2.2% with solid faeces. Most of the animals that were not infected had soft faeces (65.5%).

There was a significant association (χ^2, $P < 0.001$) between parasitization by *C. parvum* and the presence of mucus in the faeces: 22.0% of the parasitized calves had mucus in their

faeces, compared with 10.9% of the non-infected calves. Likewise, there was a relationship between the presence of *C. parvum* and the existence of blood in the faeces (χ^2, $P = 0.017$), 3.2% of the infected calves had blood in their faeces, the incidence being three times higher than in non-infected animals.

Furthermore, the relationship between the intensity of infection in the 404 infected calves and the macroscopic characteristics of their faeces was also studied. Statistical analysis showed a significant association between the intensity of infection and the consistency of the faeces (χ^2, $P < 0.001$; linear trend test, $P = 0.007$). Thus, none of the calves with solid faeces had severe infection. However, it was found that there was no association between the intensity of infection and the presence of either mucus or blood in the faeces. In contrast, analysis revealed a significant association between the presence of both blood and mucus, and infection, independently of the intensity (χ^2, $P = 0.002$).

3.1. General characteristics of the farm

On the 12 dairy farms, a total of 535 calves were born and 254 (47.5%) became infected, whereas on the 10 beef cattle farms, there were 309 calves born, 150 (48.5%) of which shed oocysts in their faeces. Analysis showed that the risk of infection by *C. parvum* was not influenced by the type of farm (dairy or beef) (OR: 0.9; CI 95%: 0.7–1.2). The prevalence of infection on the 22 farms is shown in Fig. 1.
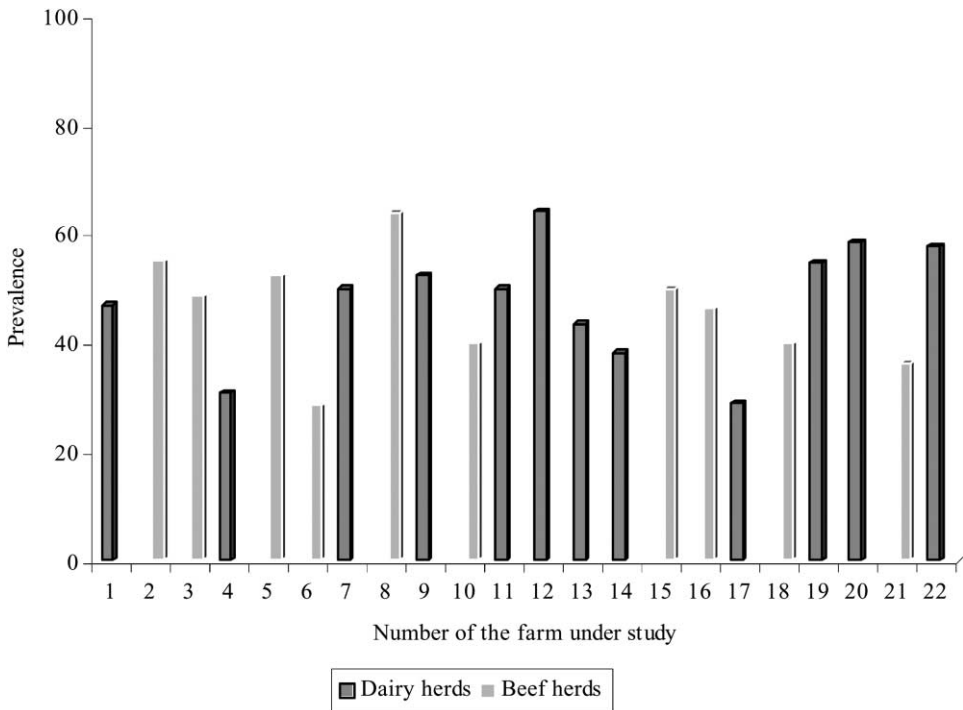

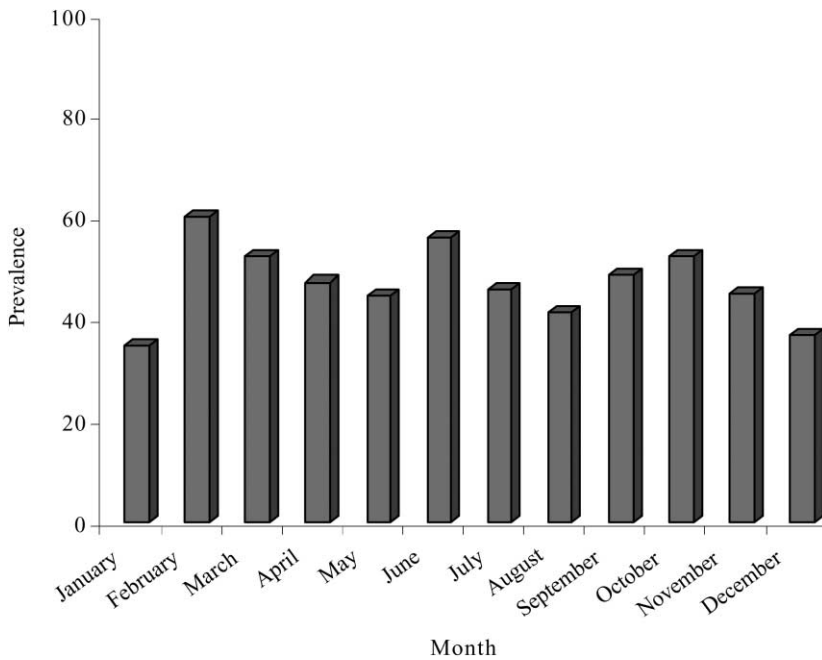Fig. 1. Prevalence of cryptosporidiosis on the farms under study.

Fig. 2. Monthly prevalence of infection by *C. parvum* in calves.

The percentage of parasitization in calves born under intensive and semiextensive rearing conditions was 45.9 and 50.4%, respectively; the farm regime did not have any significant effect on the risk of infection by *C. parvum* (OR: 0.8; CI 95%: 0.6–1.1). The source of water, whether from a municipal supply or from a well, did not have any significant effect on the risk of infection (OR: 0.9; CI 95%: 0.6–1.4); the prevalence in calves on the farms being 48.2 and 47.8% for each type of water source, respectively. Furthermore, there was no significant association between the time of year and the prevalence of infection (Fig. 2).

However, the number of adult cattle on the farm may have influenced the prevalence of parasitization of new-born calves, the influence being greater the smaller the number of adult cattle. Thus, on farms with 100–150 head of cattle the risk of infection was 52.0% higher than on farms with more than 200 head of cattle (OR: 1.5; CI 95%: 1.0–2.3). The risk increased to 74.0% on farms with between 50 and 100 head of cattle (OR: 1.7; CI 95%: 1.1–2.7).

The remaining characteristics of the farms—treatment and use of excretia, presence of other animals, etc.—were not subject to statistical analysis because they were common to all of the farms.

3.2. Calf housing and hygiene conditions

Type of flooring was one of the risk factors considered and results showed significant differences in the prevalence of cryptosporidiosis on farms with straw/earth flooring and cement flooring (OR: 1.6; CI 95%: 1.2–2.3). Thus, the risk of infection was 66.0% higher in calves housed in pens with straw/earth floors than in those in pens with cement flooring.

The type of floor was found to be directly related to the type of cleaning, i.e. cement floors were washed with water, using a pressure hose whereas the other types of floor were swept. Identical results were therefore obtained when comparing the effect of the type of cleaning or the type of flooring on the risk of infection (Fig. 3a and b).
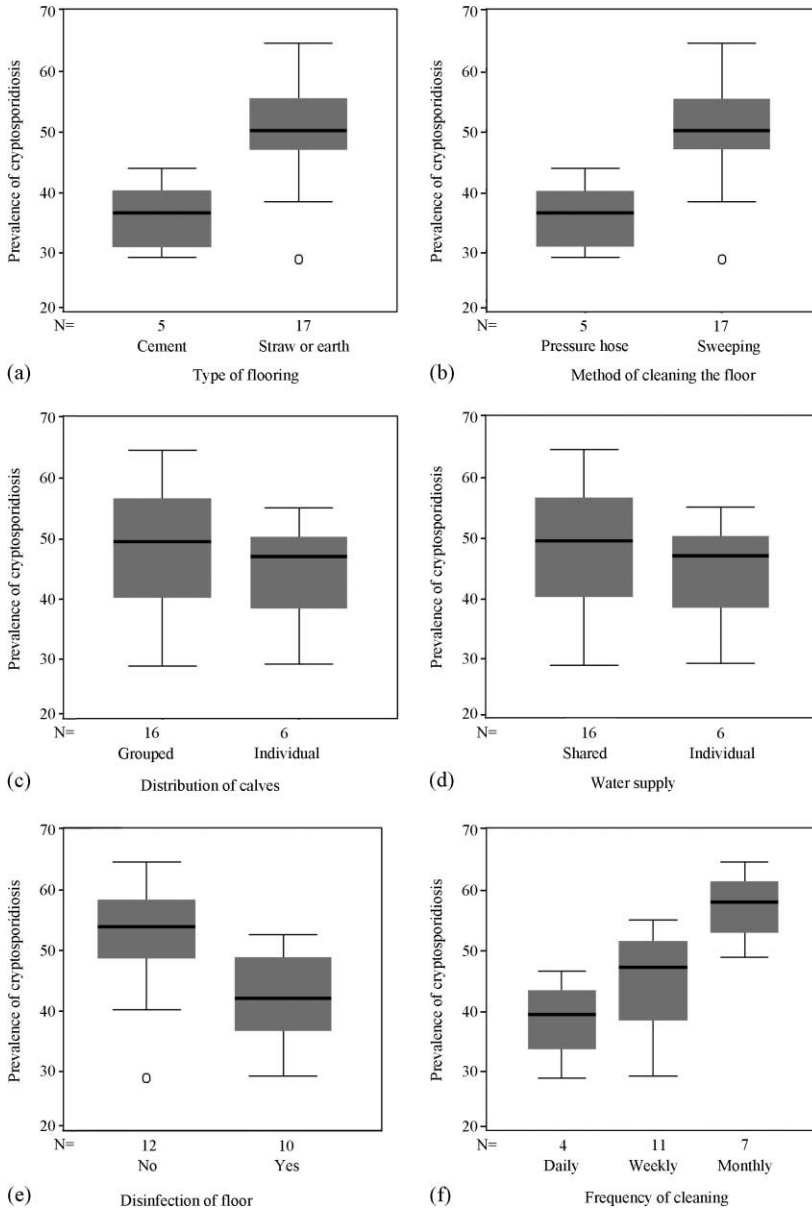

Fig. 3. Effect of housing and hygiene conditions on the prevalence of cryptosporidiosis in calves.

Whether calves were housed individually or in groups did not have a significant effect on the risk of infection, however, there was a trend of increasing risk of infection in calves housed in groups (OR: 0.1; CI 95%: 0.9–1.7). The grouping of calves was directly associated with the method of supplying water to the animals; individually housed calves had an individual water supply, whereas grouped calves had a shared supply; identical results were therefore obtained for the two factors (Fig. 3c and d).

The type of housing—whether open or closed—did not have any effect on the risk of cryptosporidial infection (OR: 0.9; CI 95%: 0.7–1.2). Disinfection of floors was found to be important with there being 62.0% higher risk of infection in calves housed in pens where the floor was not disinfected (OR: 1.6; CI 95%: 1.2–2.1) (Fig. 3e). The frequency of cleaning (daily, weekly or monthly) also had a significant effect on the risk of infection. On farms where pens were cleaned monthly, calves were almost twice as likely to contract the infection than those on farms where pens were cleaned daily (OR: 1.9; CI 95%: 1.3–2.9) (Fig. 3f).

When the three variables which had a significant influence on the risk of infection by *C. parvum* (type of flooring, frequency of cleaning and disinfection of floors) were analysed it was found that although the type of floor did not have a significant impact, there was an increasing trend in the statistical significance and the risk of parasitization was greater in calves housed in pens with straw/earth flooring than in those housed in pens with cement flooring (OR: 1.6; CI 95%: 0.9–2.6). Furthermore, the significant difference between the effect of monthly and daily cleaning was maintained, although with a slight loss of significance (OR: 1.8; CI 95%: 1.1–3.1). However, calves kept in pens that were cleaned monthly were 87.0% more at risk of infection than those calves whose pens were cleaned daily. Weekly cleaning also led to a certain degree of risk of infection, although this was not significant (OR: 1.5; IC 95%: 0.9–2.5). There was a loss of statistical significance of the effect of disinfection of the floor by this analysis. Therefore, the type of flooring and the frequency of cleaning remained those variables which had a clear effect on infection by *C. parvum* in new-born calves.

4. Discussion

It is difficult to compare our data with prevalence of infections reported in other studies because they involve animals of different ages or the farms were chosen because of a history of diarrhoea, or because animals displayed symptoms of enteric illness. However, we believe that the prevalence found, 47.9%, reflects a serious situation because, although the study was carried out on animals in a high risk category, the only conditions for selecting the farms were the collaboration of the farmers and the presence of a sufficient number of adult animals to guarantee that calves would be born throughout the study period.

In a study carried out in central Spain, of calves of less than 30 days of age with diarrhoea, the prevalence of infection varied considerably with the age of the animals. The lowest prevalence was found in calves at either extreme of the age range (43.8% in calves <7 days old and 6.9% in calves between 22 and 30 days old); in contrast, the prevalence in calves of 8–14 and 15–21 days of age rose to 71.9 and 63.2%, respectively (de la Fuente et al., 1999).

Furthermore, we believe that the prevalence was underestimated because the value was the result of direct (therefore limited) examination of a single sample from each calf. Other authors (Fayer et al., 1998) have reported that the prevalence of bovine cryptosporidiosis is underestimated because of the low number of samples taken during the pre-weaning period. Thus, when the faeces of 3–6-day-old calves chosen at random in a market were examined twice weekly for 1 month, the prevalence of infection was found to be 93.0% (Villacorta et al., 1991). In contrast, when only one or two faecal samples per calf were examined during the pre-weaning period, less than 30.0% of the calves were found to have oocysts in their faeces (Garber et al., 1994; Maldonado et al., 1998).

The prevalence of infection in each type of herd considered—dairy and beef—was very similar, 47.5 and 48.5%, respectively. However, Atwill et al. (1999) found that the maximum values of prevalence were reached at different ages in the two types of calves—in calves from dairy herds, at 15 days of age and in calves from beef herds, at 2 months of age. These authors suggest that the delay may be due to passive immunity acquired by the calves, which remain with their mothers for longer periods than in dairy herds. However, various studies have shown that passive immunity acquired via colostrum and maternal milk does not provide protection in mice and calves experimentally infected by *C. parvum* (Moon et al., 1988; Harp et al., 1989). Furthermore, Quigley et al. (1994) and Mohammed et al. (1999) observed a decrease in the risk of infection in calves which were separated from their mothers after only a few hours and fed manually. All of the calves in the present study were separated from their mothers immediately after birth, although they were fed with colostrum pooled from different cows, during the first few hours of their lives.

Several authors have described an association between the size of the farm and the risk of infection, where the higher the density of animals, the greater the number of calves which become infected and which in turn, contaminate their surroundings (Garber et al., 1994; Quigley et al., 1994; Mohammed et al., 1999). In the present study, we found the opposite to be true, i.e. the risk of infection was lower on those farms with a large number of cattle. This finding may be due to the particular circumstances found in rural Galicia, as housing and hygiene conditions on large farms tend to be better.

In accordance with the recommendations for housing and hygiene previously described by Fayer and Ungar (1986) and Heath (1992) and consistent with the findings of Garber et al. (1994), Quigley et al. (1994) and Mohammed et al. (1999), we found a tendency for the risk of infection to decrease when animals were housed individually in pens previously disinfected with bleach or lime, and that there was also less risk of infection associated with the use of cement flooring, which was washed daily with water, using a pressure hose. Thus the risk of severe infection was seven times greater when cleaning was carried out monthly.

Although some authors have observed an increase in the prevalence of cryptosporidiosis during certain seasons, related with high rainfall or the number of births, seasonal effects can only be correctly evaluated when the study is repeated over several consecutive years. However, Atwill et al. (1999), who carried out a study over a period of 6 months, used statistical analysis to show that in California, calves are at most risk of contracting the infection in the month of May, possibly because this is when the animals have most contact with the source of infection, or their resistance to the infection is low, or environmental conditions most favour transmission of the infection. In contrast, in a year long study on the prevalence of neonatal bovine cryptosporidiosis in France, Lefay et al. (2000) found

that the lowest levels of parasitization were found in July and August. Taking into account these contradictory reports, we did not find any significant association between the time of year and the prevalence of infection. The values obtained fluctuated between a minimum of 34.5% in January and a maximum of 60.0% in February. The prevalence found in May, July and August was 44.5, 45.9 and 41.4%, respectively. We assume that the absence of notable climatic changes in Galicia throughout the year as well as the lack of a particular calving season explain our results. The absence of seasonality in the presence of infection has been described by Wade et al. (2000) in a study of 109 dairy farms in five regions in the southeast of New York state.

As already mentioned the main symptom of cryptosporidiosis is diarrhoea. Of the 844 calves examined, 330 had liquid faeces (39.1%), and *C. parvum* oocysts were detected in 212 (52.5%). A large number of animals (471) had soft faeces and infection was diagnosed in 45.3% of cases. However, in the study carried out by Atwill et al. (1999), the low percentage of infected calves with liquid faeces (8–9%) suggests the involvement of other enteropathogens in the process. Thus, de la Fuente et al. (1998) found coexistence of *C. parvum*, rotavirus, coronavirus and *Salmonella*, among others, in calves of less than 1 month of age, with diarrhoea. *Cryptosporidium* infection usually does not cause a bloody diarrhoea due to the very superficial location of the parasite. The fact that infection by *C. parvum* is associated with the presence of blood in the faeces may suggest coexistence with other enteropathogens.

Acknowledgements

References

Atwill, E.R., Johnson, E., Klingborg, D.J., Veserat, G.M., Markegard, G., Jensen, W.A., Pratt, D.W., Delmas, R.E., George, H.A., Forero, L.C., Phillips, R.L., Barry, S.J., McDougald, N.K., Gildersleeve, R.R., Frost, W.E., 1999. Age geographic and temporal distribution of fecal shedding of *Cryptosporidium parvum* oocysts in cow–calf herds. Am. J. Vet. Res. 60, 420–425.

de Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H., Peeters, J.E., 1999. A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 29, 1269–1287.

de la Fuente, R., García, A., Ruíz-Santa-Quiteria, J.A., 1998. Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. Prev. Vet. Med. 36, 145–152.

de la Fuente, R., Luzón, M., Ruiz-Santa-Quiteria, J.A., García, A., Cid, D., Orden, J.A., García, S., Sanz, R., Gómez-Bautista, M., 1999. *Cryptosporidium* and concurrent infections with other major enteropathogens in 1–30-day-old diarrheic dairy calves in central Spain. Vet. Parasitol. 80, 179–185.

Fayer, R., Ungar, B.L.P., 1986. *Cryptosporidium* spp. and Cryptosporidiosis. Microbiol. Rev. 50, 458–483.

Fayer, R., Gasbarre, L., Pasquali, P., Canals, A., Almeria, S., Zarlenga, D., 1998. *Cryptosporidium parvum* infection in bovine neonates: dynamic clinical, parasitic and immunologic patterns. Int. J. Parasitol. 28, 49–56.

Fayer, R., Morgan, U., Upton, S.J., 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int. J. Parasitol. 30, 1305–1322.

Garber, L.P., Salman, M.D., Hurd, H.S., Keefe, T., Schater, J.L., 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc. 205, 86–91.

Harp, J.A., Woodmansee, D.B., Moon, H.W., 1989. Effects of colostral antibody on susceptibility of calves to *Cryptosporidium* infection. Am. J. Vet. Res. 50, 2117–2119.

Heath, H.E., 1992. Neonatal diarrhea in calves: investigation of herd management practices. Comp. Cont. Educ. Pract. Vet. 14, 385–395.

Heine, J., 1982. An easy technique for the demonstration of Cryptosporidia in faeces. Z. Vet. B 29, 324–327.

Heine, J., Pohlenz, J.F.L., Moon, H.W., Woode, G.N., 1984. Enteric lesions and diarrhea in gnotobiotic calves monoinfected with *Cryptosporidium* species. J. Infect. Dis. 150, 768–775.

Lefay, D., Naciri, M., Poirier, P., Chermette, R., 2000. Prevalence of *Cryptosporidium* infection in calves in France. Vet. Parasitol. 89, 1–9.

Lindsay, D.S., Upton, S.J., Owens, D.S., Morgan, U.M., Mead, J.R., Blagburn, B.L., 2000. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporidiidae) from cattle, *Bos taurus*. J. Eukaryot. Microbiol. 47, 91–95.

Maldonado, C.S., Atwill, E.R., Saltijeral-Oaxaca, J.A., Herrera, A.L.C., 1998. Prevalence of and risk factors for shedding of *Cryptosporidium parvum* in Holstein Freisian dairy calves in central Mexico. Prev. Vet. Med. 36, 95–107.

Mohammed, H.O., Wade, S.E., Schaaf, S., 1999. Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. Vet. Parasitol. 83, 1–13.

Moon, H.W., Woodmansee, D.B., Harp, J.A., Abel, S., Ungar, B.L.P., 1988. Lacteal immunity to enteric cryptosporidiosis in mice: immune dams do not protect their suckling pups. Infect. Immun. 56, 649–653.

Moore, D.A., Zeman, D.H., 1991. Cryptosporidiosis in neonatal calves: 277 cases (1986–1987). J. Am. Vet. Med. Assoc. 198, 1969–1971.

Panciera, R.J., Thomassen, R.W., Garner, F.M., 1971. Cryptosporidial infection in a calf. Vet. Pathol. 8, 479–484.

Quigley, J.D., Martin, K.R., Bemis, D.A., Potgiete, L.N.D., Reinemeyer, C.R., Rohrbach, B.W., Dowlen, H.H., Lamar, K.C., 1994. Effects of housing and colostrum feeding on the prevalence of selected infectious organisms in feces of Jersey calves. J. Dairy Sci. 77, 3124–3131.

Sanford, S.A., Josephson, G.K.A., 1982. Bovine cryptosporidiosis: clinical and pathological findings in forty-two scouring neonatal calves. J. Can. Vet. 23, 340–343.

Tyzzer, E.E., 1907. A sporozoa found in the peptic glands of the common mouse. Proc. Soc. Exp. Biol. Med. 5, 12–13.

Tzipori, S., Campbell, I., Sherwood, D., Snodgrass, D.R., Whitelaw, A., 1980. An outbreak of calf diarrhoea attributed to cryptosporidial infection. Vet. Rec. 107, 579–580.

Tzipori, S., Smith, M., Halpin, C., Angus, K.W., Sherwood, D., Campbell, I., 1983. Experimental cryptosporidiosis in calves: clinical manifestations and pathological findings. Vet. Rec. 112, 116–120.

Vanopdenbosch, E., Wellemans, G., Dekegel, X., Strobbe, R., 1979. Neonatal calf diarrhoea: a complex etiology. VI Dierg Tijdschr. 48, 512–526.

Villacorta, I., Ares-Mazás, E., Lorenzo, M.J., 1991. *Cryptosporidium parvum* in cattle, sheep and pigs in Galicia (NW Spain). Vet. Parasitol. 38, 249–252.

Wade, S.E., Mohammed, H.O., Schaaf, S.L., 2000. Prevalence of *Giardia* sp., *Cryptosporidium parvum* and *Cryptosporidium muris* (*C. andersoni*) in 109 dairy herds in five counties of southeastern New York. Vet. Parasitol. 93, 1–11.