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Prevalence of *Cryptosporidium* infection in calves in France

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Abstract

Two multicentre surveys were conducted in France to estimate the prevalence of *Cryptosporidium* infection in calves using qualitative ELISA for detection of *Cryptosporidium* coproantigens and oocysts. The first survey involved 4–12-day-old calves in six dairy-calf distribution centres, collecting calves from seven Administrative Regions (Aquitaine, Bretagne, Franche-Comté, Lorraine, Normandie, Nord, Pays de Loire). For each region, 20 calves were selected every month for 12 consecutive months (October 1995–September 1996). Prevalence of *Cryptosporidium* infection was 17.9% (Confidence Intervals (C.I.) 95%=[16.1%; 19.8%]) among the 1628 selected calves, of which only 5.3% had diarrhoea. The second survey conducted between November 1995 and May 1996 involved 4–21-day-old calves examined by veterinary practitioners who selected 189 livestock farms of dairy- or suckler-type in ten Administrative Departments (Allier, Cantal, Creuse, Doubs, Ille-et-Vilaine, Maine-et-Loire, Manche, Pas-de-Calais, Saône-et-Loire, Vendée). Cryptosporidia were detected in 105 (55.6%) of the farms. Among the 440 calves examined, of which 398 (90.5%) presented diarrhoea, cryptosporidia were found in 191 animals, i.e. a prevalence of 43.4% (C.I. 95%=[38.8%; 48.0%]). Breed of calves and type of housing had very little impact on prevalence in this survey. Some regional variations could be noticed, even if cryptosporidia infection is widespread. Monthly variations could be related to seasonal peaks in calving with a lower infection rate during summer. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cattle-Protozoa; *Cryptosporidium* spp.; Epidemiology; France

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1. Introduction

Cryptosporidia are well-recognized opportunistic protozoans mainly of the digestive or sometimes respiratory tract. *Cryptosporidium parvum* develops in a wide variety of mammals, including humans, and is responsible for diarrhoeic disorders which are particularly serious in neonates and in immunosuppressed individuals (Dubey et al., 1990; O'Donoghue, 1995). Cattle are a major source of *C. parvum* which is responsible for neonatal diarrhoea in calves and for zoonotic contamination (Angus, 1990; O'Donoghue, 1995).

Bovine cryptosporidiosis is widespread and prevalence studies show a wide range of oocyst shedding from 14 to over 80% depending on the age, clinical situation and breeding regime of the animals (Chermette and Boufassa-Ouzrout, 1988; Angus, 1990; Garber et al., 1994; Fagan et al., 1995; Olson et al., 1997; de la Fuente et al., 1999). Overall, prevalence studies in cattle ranging from 3-day-old to adult usually give results of approximately 20% of animals shedding oocysts (Quílez et al., 1996; Olson et al., 1997), while prevalence in neonates is higher, especially when diarrhoeic calves are concerned (Angus, 1990; Garber et al., 1994; Fagan et al., 1995; Quílez et al., 1996; SAC Veterinary Services, 1996; de la Fuente et al., 1999). However, the pathogenic role of cryptosporidia has remained controversial as they were often detected in combination with other enteropathogens such as rotavirus, coronavirus, *Escherichia coli* or *Salmonella*. Although some veterinary practitioners doubt the real pathogenicity of cryptosporidia, many experimental and field trials have demonstrated that cryptosporidia may act as a primary pathogen, sometimes being the predominant enteropathogens of calves (Tzipori et al., 1983; Heine et al., 1984; McDonough et al., 1994; SAC Veterinary Services, 1996, 1997; de la Fuente, 1999).

Little information has been published about bovine cryptosporidiosis in France, except the usual field cases of diarrhoeic calves (Polack et al., 1983; Contrepolis and Vallet, 1984; Nicolas et al., 1984; Navetat and Espinasse, 1985; Amedeo et al., 1995; Bourgouin, 1996), and epidemiological studies have not yet been conducted on a wide scale and in large number of animals. This paper presents the results of two epidemiological multicentre surveys which were conducted in various areas of the country to evaluate the prevalence of cryptosporidiosis in calves in France.

2. Materials and methods

2.1. Dairy calves distribution centres survey (Survey 1)

The first survey involved seven Administrative Regions of France (Table 1) through six distribution centres belonging to two industrial firms (Celtlait and V.A.L.S.) which routinely collect dairy calves from the farms of birth before sending them to specialized fattening units. Within the first week of each month for 12 consecutive months, 20 calves from each selected region were systematically tested for cryptosporidia infection on their arrival at the corresponding distribution centre, e.g. 140 calves per month, 1680 calves for the whole year. Inclusion criteria of calves were restricted to a mean age of 8 days (4–12 days) at the time of sampling and to dairy cattle of the Montbeliard, Normand or Prim'Holstein breed. The survey started on 4 October 1995 and ended on 12 September 1996.

Table 1
Prevalence of cryptosporidia infection by region (Dairy Calves Distribution Centres Survey)

Regions	Number of positive calves ^a	Total calves tested	Prevalence (%)	C.I. 95 %
Aquitaine	45	220	20.5	[15.1; 25.8]
Bretagne	32	240	13.3	[9.0; 17.6]
Franche-Comté	60	237	25.3	[19.8; 30.9]
Lorraine	43	235	18.3	[13.4; 23.2]
Nord	33	218	15.1	[10.4; 19.9]
Normandie	33	240	13.8	[9.4; 18.1]
Pays de Loire	46	238	19.3	[14.3; 24.3]
Total	292	1628	17.9	[16.1; 19.8]

^a Presence of *Cryptosporidium* antigens in faeces detected by ELISA.

Collection and shipment of faecal samples was done by technicians of the two firms concerned, taking into account the following criteria: single-use latex gloves for rectal sampling, single-use plastic sterile flask, and collection of a minimum of 5 g of faeces per sample. Each flask was identified with labels, sealed in polystyrene containers and accompanied by a case report form recording the date of sampling, the region and name of the distribution centre, the identification number of calves, breed, sex and the presence/absence of diarrhoea. All samples were mailed by express, at ambient temperature, and on the same day of sampling to the laboratory (Laboratoire Vétérinaire Départemental de Saône-et-Loire, 71000 Mâcon). *Cryptosporidium* infection was detected by a commercial ELISA kit using anti-*Cryptosporidium* monoclonal antibodies (Cryptosure[®], Rhône Mérieux Diagnostics). Faecal samples were processed according to the manufacturer's recommendations and results were expressed as 'positive' or 'negative'.

2.2. Veterinary practice survey (Survey 2)

The second survey involved ten Administrative Departments (Table 2) through their respective Laboratoire Vétérinaire Départemental. Each laboratory employed five volunteer veterinarians as investigators. A total of 1500 calves from 500 farms were expected to be tested for *Cryptosporidium* infection as each investigator had to sample 30 calves in ten different dairy or suckler livestock farms (three calves per farm). Inclusion criteria for calves were an age between 4 and 21 days at the time of sampling and presence of diarrhoea lasting for less than 72 h. Calves which had received any anticoccidial or antiprotozoal drugs, or treatment containing nitrofurans, sulfones, sulfonamides or arsenical derivatives were excluded. This survey started in November 1995 and ended in May 1996.

Collection and shipment of faecal samples were carried out by the investigators using the criteria described for the first survey. Breed, sex, age, clinical parameters, identification numbers of calves, description and name of the livestock farm were recorded on a separate form. Samples were sent as previously described to the corresponding Laboratoire Vétérinaire Départemental which used commercial ELISA kits to detect *Cryptosporidium* infection with results expressed as 'positive' or 'negative'.

Table 2
Prevalence of cryptosporidia infection by department (Veterinary Practices Survey)

Department	Number of positive calves ^a	Total calves tested ^b	Prevalence (%)	C.I. 95%
Allier	14	38	36.8	[21.5; 52.2]
Cantal	38	54	70.4	[58.2; 82.5]
Creuse	46	98	46.9	[37.1; 56.8]
Doubs	21	41	51.2	[35.9; 66.5]
Ille-et-Vilaine	10	21	47.6	[26.3; 69.0]
Maine-et-Loire	7	39	17.9	[5.9; 30.0]
Manche	7	35	20.0	[6.7; 33.3]
Pas-de-Calais	21	42	50.0	[34.9; 65.1]
Saône-et-Loire	15	22	68.2	[48.7; 87.6]
Vendée	12	49	24.5	[12.4; 36.5]
Total	191	440	43.4	[38.8; 48.0]

^a Presence of *Cryptosporidium* antigens in faeces detected by ELISA.

^b One set of data missing.

2.3. Data analyses

Confidence intervals (C.I.) have been calculated using the SAS System for Information Delivery[®].

3. Results

3.1. Dairy calves distribution centres survey (Survey 1)

A total of 1630 calves have been included through the 12 month survey period, of which 1628 were correctly tested for *Cryptosporidium* infection. The number of missing samples represents 3.1% (52 calves) of the 1680 expected samples. Missing data, regarding breed, sex and presence or absence of diarrhoea, also remained low, i.e. 9.5, 8.3 and 8.3% of the 1630 calves, respectively. With respect to available data, 66.4% of the calves were of the Prim'Holstein breed, 13.0% Normand, 13.0% Montbeliard and 7.6% of mixed breeds. The tested animal population comprised 94.6% males and only 5.4% females, which is consistent with the fact that females are kept for breeding purposes. Number of calves presenting clinical signs of diarrhoea at the time of arrival at the distribution centre was low (5.2%).

Cryptosporidia were detected in 292 out of the 1628 tested calves giving a 17.9% average prevalence nation-wide (C.I. 95%=[16.1%; 19.8%]). Monthly variations were observed (Fig. 1) with a minimum prevalence of 7% in July (C.I. 95%=[3%; 12%]) and a maximum in September when 26% of calves (C.I. 95%=[19%; 34%]) were found positive for *Cryptosporidium* infection. A clear decrease in infection was observed during July and August with a 7 and 8% prevalence, respectively. Results also indicate a geographic variation in prevalence (Table 1) with a minimum recorded in the Brittany region where 13.3% of the calves presented cryptosporidia over the survey period (C.I. 95%=[9.0%;

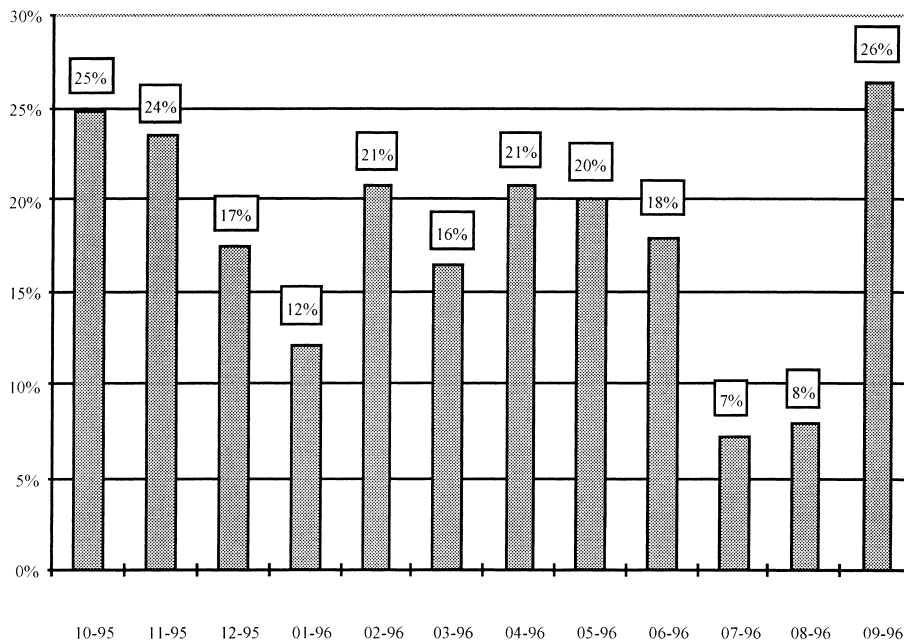


Fig. 1. Monthly prevalence of *Cryptosporidium* infection in calves (Dairy Calves Distribution Centres Survey, between October 1995 and September 1996).

17.6%) and a 25.3% maximum prevalence in the Franche-Comté region (C.I. 95%=[19.8%; 30.9%]).

3.2. Veterinary practice survey (Survey 2)

In this survey, 189 livestock farms of the 500 expected (37.8%) were included of which 57.1% were of a suckler-type, 38.1% of a dairy-type and 4.8% were mixed. Regarding housing of calves, 46.0% of the farms had loose housing allowing contacts between animals. In 52.9% of the farms, calves remained with their dam, but were separated in the remaining 47.1%. Four hundred and forty calves have been included and tested through this 7-month survey period, mainly (98.2%) between December 1995 and April 1996. They were raised as suckler (67.3%) or dairy livestock (32.7%). Sex ratio of the tested population was 53.7% male and 46.3% female and the mean age of calves was 9.8 days (C.I. 95%=[9.4%; 10.2%]). Among the 398 calves presenting diarrhoea at the time of inclusion (90.5%), 48.5% showed some degree of dehydration and 51.0% had diarrhoea symptoms for more than 24 h.

Cryptosporidium infection (at least one positive sample out of three) was detected in 105 of the 189 farms, i.e. a 55.6% prevalence (C.I. 95%=[48.5%; 62.6%]). Type of livestock farm, suckler or dairy, and type of animal housing have a marginal effect on the prevalence of farm infection.

Cryptosporidia were recovered from 191 calves out of the 440 calves tested during the survey period, showing an average prevalence of 43.4% nation-wide (C.I. 95%=[38.8%;

48.0%]). Breed and type of farming have a marginal effect on prevalence, which is 43.9 and 42.4% for suckler and dairy calves, respectively. Prevalence varies from 32.9 to 53.0% depending on the month of sampling. With respect to geographic variations, the lowest prevalence was recorded in the Maine-et-Loire and the highest in the Cantal Department with 17.9% (C.I. 95%=[5.9%; 30%]) and 70.4% (C.I. 95%=[58.2%; 82.5%]) of the calves presenting cryptosporidia, respectively (Table 2).

4. Discussion

The prevalence and the percentage of farms with oocyst-excreting calves indicate that bovine *Cryptosporidium* infection is widespread in France as many different areas of the country were involved in these two surveys. In both surveys, inclusion criteria have been generally adhered to, though the number of calves included in the second survey (veterinary practices) was much lower than expected (29.3% of the 1500 expected calves) due to the fact that only one third of the canvassed veterinarians did actually participate to the survey. With respect to the first survey (dairy calves distribution centres), only two out of the expected 84 groups of 20 calves (i.e. seven regions during 12 months) were lost and 56/82 groups (68.3%) were collected on time, i.e. in the first 7 days of the month, 24/82 groups (29.3%) late in the month, and 2/82 groups (2.4%) 1 month behind schedule.

The difference in prevalence of infection between calves in Survey 1 (17.9 %) and in Survey 2 (43.4%) must be analysed according to the respective protocols. The methodology of Survey 1 ensured a good representative sample of the entire population of dairy calves as most of the included calves came from different dairy farms. This kind of protocol also generated a lot of data for a moderate cost. However, it does not allow an estimation of infected farms and it introduces a systematic bias resulting in an underestimation of the prevalence of cryptosporidiosis infection as dairy calves presenting diarrhoea are not usually sent to distribution centres. That is demonstrated by the low rate of diarrhoea (5.2%) observed during Survey 1. This survey probably gives a good estimation of the prevalence of infection in calves populations free of clinical symptoms. Finally, prevalence of infection was pretty stable over the 1-year follow-up, except in July and August which had a lower prevalence of 7 and 8%, respectively. This result is linked to the seasonal incidence of calving which usually shows a decrease during summer. Consequently, the more susceptible calves, i.e. those younger than 1 month, represent a small population at that period of the year which might explain the lower contamination of livestock.

Survey 2 displayed opposite characteristics, with an overestimation of the prevalence of cryptosporidiosis infection, as most of the tested calves (90.5%) had diarrhoea at the time of sampling. This is reinforced by the fact that the selection of farms was the decision of the investigators who possibly selected livestock farms with a history of cryptosporidiosis. This second survey probably gives a good estimation of the prevalence of the infection in populations experiencing clinical symptoms. The 43.4% mean prevalence found in this survey confirms that the prevalence of cryptosporidiosis is higher in diarrhoeic than in non-diarrhoeic calves, as found in other studies (Angus, 1990; Quílez et al., 1996). Compared with the prevalence of oocyst excretion in diarrhoeic calves from other European countries, our results are higher than those in Denmark (17%), Hungary (27%), and UK

(23–32.9%), similar to those reported in Italy (40%), Germany (40–44%), and the Irish Republic (44.4%), but lower than those from central Spain (52.3%), the Netherlands (55%), and Finland (76%) (Angus, 1990; Fagan et al., 1995; SAC Veterinary Services, 1996; de la Fuente et al., 1999). A high prevalence was also found in France by Amedeo et al. (1995) with 56 and 45% during 1993 and 1994, respectively, and by Bourgoïn (1996) in the Corrèze Department (47.7%), but their results involved a smaller number of calves originating from limited areas and which were sampled for neonatal diarrhoea or necropsy.

In Survey 2, prevalence of cryptosporidiosis appears similar whatever the cattle breed, the type of farming, i.e. dairy or suckler calves, or the style of animal housing. It could be explained by the large number of farms where calf-to-calf contact was possible and also by a contamination from oocyst excretion by adult cattle (Chermette et al., 1985; Scott et al., 1995; Quílez et al., 1996).

Results of prevalence studies should also be discussed considering the age of animals at time of sampling and the number of samples realised per animal. Indeed, calves under 30 days of age are more susceptible than older animals. In the present studies, calves were 4–12 days of age at time of sampling in Survey 1 and between 4 and 21 days of age in Survey 2, which correspond to the time-period during which oocyst excretion of *C. parvum* by calves is highest (Fagan et al., 1995; Quílez et al., 1996; de la Fuente et al., 1999). However, our results reflect a prevalence of cryptosporidiosis at a point in time as calves were sampled only once. As the pattern of antigen and oocyst excretion in calves is intermittent and false negatives can result if animals are sampled only once (Farrington et al., 1995; McCluskey et al., 1995), a 100% prevalence for calves between 1 and 30 days of age is possible, which means that all animals will pass oocysts at least once during this period of time (Xiao and Herd, 1994; McCluskey et al., 1995).

Cryptosporidium infection was assayed by detection of coproantigens and oocysts using commercially available ELISA tests which are routinely used for diagnostic purposes by the laboratories involved in the present surveys. Various methods of detection (microscopical staining of faecal smears, directly or after concentration, formol-ether sedimentation, salt or sucrose flotation, enzyme immunoassays, immunofluorescent techniques, polymerase chain reaction) have been compared for their sensitivity (Robert et al., 1990; Xiao and Herd, 1993; McCluskey et al., 1995; Peeters and Villacorta, 1995; Webster et al., 1996). Antigen capture ELISA techniques are sufficiently sensitive to detect clinical cases of cryptosporidiosis when numerous oocysts are shed (McCluskey et al., 1995; Peeters and Villacorta, 1995), however, more sensitive techniques, such as immunofluorescence or concentration techniques, are necessary in cases of light infection with calves passing few oocysts (Robert et al., 1990; Xiao and Herd, 1993; Webster et al., 1996). Consequently, an underestimation of our results is possible especially in Survey 1 in which most of the calves were asymptomatic.

Bovine cryptosporidiosis can be due to *C. parvum* or *C. muris* and the ELISA tests used in those studies for the identification of coproantigens are not specific of *C. parvum*. However, *C. muris* which is located in the abomasum of older calves and adults, is rarely found and is not responsible for neonatal diarrhoea (Angus, 1990). Though we cannot exclude possible *C. muris* infection from our results, the probability of such an event remains low.

Diarrhoea in neonatal calves can result from various etiological factors acting alone or in combination. Cryptosporidia were detected in 43.4% (191/440) of the calves in Survey 2, while 90.5% (398/440) of the animals showed diarrhoea. The remaining diarrhoeic

animals without *Cryptosporidium* infection at the time of sampling may reflect either a lack of sensitivity of the detection method and/or the involvement of other infectious agents (e.g. rotavirus, coronavirus, *E. coli*, *Salmonella* or *Giardia*), environmental or nutritional factors, which were not investigated according to our initial objective. The present surveys demonstrate that *Cryptosporidium* infection of calves is wide-spread in France and that further studies are needed to show the relative importance of cryptosporidia, which have emerged as major enteropathogens of calves, in the neonatal diarrhoea syndrome.

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References

- Amedeo, J., Goillandeu, P., Roger, M.F., 1995. Etiologie des affections néonatales du veau: incidence de la cryptosporidiose Bull. Groupements Techniques Vétérinaires 1B, 35–41.
- Angus, K.W., 1990. Cryptosporidiosis in ruminants. In: Dubey, J.P., Speer, C.A., Fayer, R. (Eds.), *Cryptosporidiosis of Man and Animals*. CRC Press, Boston, pp. 83–103.
- Bourgouin, H., 1996. La place de la cryptosporidiose dans les maladies néo-natales du veau en Corrèze Bull. Groupements Techniques Vétérinaires 2B, 19–41.
- Chermette, R., Polack, B., Boufassa, S., Bariaud, F., 1985. Observations de cryptosporidies chez des bovins adultes. In: Navetat, H., Espinasse, J. (Eds.), *Cryptosporidiose du Jeune Ruminant*. Groupe Rech. Dév. Elev. Pathol. Veau, Lyon 16 Novembre 1984. Soc. Fr. Buiâtrie, Maisons-Alfort.
- Chermette, R., Boufassa-Ouzrout, S., 1988. Cryptosporidiosis. A Cosmopolitan Disease in Animals and in Man. (Technical Series No. 5), 2nd Edition. Office International Epizooties, Paris.
- Contrepois, M., Vallet, A., 1984. Cryptosporidiose et diarrhée néo-natale en élevage bovin Point Vétérinaire 16, 235–241.
- 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 to 30-day-old diarrheic dairy calves in central Spain *Vet. Parasitol.* 80, 179–185.
- Dubey, J.P., Speer, C.A., Fayer, R., 1990. *Cryptosporidiosis of Man and Animals*. CRC Press, Boston.
- Fagan, J.G., Dwyer, P.J., Quinlan, J.G., 1995. Factors that may affect the occurrence of enteropathogens in the faeces of diarrhoeic calves in Ireland *Irish Vet. J.* 48, 17–21.
- Farrington, M., Lloyd, S., Winters, S., Smith, J., Rubenstein, D., 1995. Patterns of *Cryptosporidium* antigen and oocyst excretion in calves studied by reverse passive haemagglutination and light microscopy *Vet. Parasitol.* 60, 7–16.
- Garber, L.P., Salman, M.D., Hurd, H.S., Keefe, T., Schlater, J.L., 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves *J. Am. Vet. Med. Assoc.* 205, 86–91.
- 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.
- McCluskey, B.J., Greiner, E.C., Donovan, G.A., 1995. Patterns of *Cryptosporidium* oocyst shedding in calves and a comparison of two diagnostic methods *Vet. Parasitol.* 60, 185–190.
- McDonough, S.P., Stull, C.L., Osburn, B.I., 1994. Enteric pathogens in intensively reared veal calves *Am. J. Vet. Res.* 55, 1516–1520.
- Navetat, H., Espinasse, J., 1985. *Cryptosporidiose du jeune ruminant*. Groupe Rech. Dév. Elev. Pathol. Veau, Lyon 16 Novembre 1984. Soc. Fr. Buiâtrie, Maisons-Alfort.

- Nicolas, J.A., Dubost, G., Gayaud, C., Noël, F., 1984. Importance des cryptosporidies dans les diarrhées néonatales du veau *Point Vétérinaire* 16, 350–351.
- O'Donoghue, P.J., 1995. *Cryptosporidium* and cryptosporidiosis in man and animals *Int. J. Parasitol.* 25, 139–195.
- Olson, M.E., Thorlakson, C.L., Deselliers, L., Morck, D.W., McAllister, T.A., 1997. *Giardia* and *Cryptosporidium* in Canadian farm animals *Vet. Parasitol.* 68, 375–381.
- Peeters, J., Villacorta, I., 1995. *Cryptosporidium*. In: Eckert, J., Braun, R., Shirley, M.W., Coudert, P. (Eds.), *Guidelines on Techniques in Coccidiosis Research*, COST 89/820, Biotechnology. Office for Official Publications of the European Communities, Luxembourg, pp. 202–240.
- Polack, B., Chermette, R., Savey, M., Bussiéras, J., 1983. Les cryptosporidioses en France. Techniques usuelles d'identification et résultats préliminaires d'enquêtes épidémiologiques *Point Vétérinaire* 15, 41–46.
- Quílez, J., Sánchez-Acedo, C., del Cacho, E., Clavel, A., Causapé, A.C., 1996. Prevalence of *Cryptosporidium* and *Giardia* infections in cattle in Aragón (northeastern Spain) *Vet. Parasitol.* 66, 139–146.
- Robert, B., Ginter, A., Antoine, H., Collard, A., Coppe, P., 1990. Diagnosis of bovine cryptosporidiosis by an enzyme-linked immunosorbent assay *Vet. Parasitol.* 37, 1–8.
- Scottish Agriculture College (SAC) Veterinary Services, 1996. Surveillance of neonatal enteritis in cattle. *Vet. Rec.* 139, 485.
- Scottish Agriculture College (SAC) Veterinary Services, 1997. *Vet. Rec.* 141, 323.
- Scott, C.A., Smith, H.V., Mtambo, M.M.A., Gibbs, H.A., 1995. An epidemiological study of *Cryptosporidium parvum* in two herds of adult beef cattle *Vet. Parasitol.* 57, 277–288.
- 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.
- Webster, K.A., Smith, H.V., Giles, M., Dawson, L., Robertson, L.J., 1996. Detection of *Cryptosporidium parvum* oocysts in faeces: comparison of conventional coproscopical methods and the polymerase chain reaction *Vet. Parasitol.* 61, 5–13.
- Xiao, L., Herd, R.P., 1993. Quantitation of *Giardia* cysts and *Cryptosporidium* oocysts in fecal samples by a direct immunofluorescence assay *J. Clin. Microbiol.* 31, 2944–2946.
- Xiao, L., Herd, R.P., 1994. Infection patterns of *Cryptosporidium* and *Giardia* in calves *Vet. Parasitol.* 55, 257–262.