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Role of *Cryptosporidium parvum* as a pathogen in neonatal diarrhoea complex in suckling and dairy calves in France

Muriel Naciri^{a,*}, Marie Paul Lefay^c, Roselyne Mancassola^a, Pierre Poirier^b, René Chermette^d

^a INRA, Unité de Pathologie Aviaire et de Parasitologie, 37380 Nouzilly, France

^b Hoechst Roussel Vet, 140 avenue Jean Lolive, 93695 Pantin Cedex, France

^c Société Protocole, Les Algorithmes, Immeuble Homère, 91194 Saint Aubin Cedex, France

^d Ecole Nationale Vétérinaire d'Alfort, UMR Biologie Moléculaire et Immunologie Parasitaires et Fongiques, INRA-CNEVA-ENVA, 7, Avenue du Général de Gaulle, 94704 Maisons-Alfort Cedex, France

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Abstract

This study was carried out to find the importance of *Cryptosporidium parvum* in diarrhoea of neonatal calves in two types of breeding – suckling and dairy calves – in France. Different agents causing neonatal diarrhoea, *E. coli*, rotavirus, coronavirus, *Salmonella* and *Cryptosporidium* were systematically researched in faeces. 1. Suckling calves: In 40 livestock farms selected for diarrhoea, 311 calves 4 to 10 days old which had diarrhoea for less than 24 h or no diarrhoea, were included in the study. A prophylaxis of neonatal diarrhoea had been carried out in 21 of the 40 livestock farms. On D0 (inclusion day), the mean age was 6 days, 82% presented a good initial general condition and 76.2% had a good appetite; 48.6% were diarrhoeic but 91.3% presented no sign of dehydration. Only 6.1% were infected by *E. coli* K99, 14.3% by rotavirus, 6.8% by coronavirus, 0.3% by *Salmonella* but 50% excreted *C. parvum* oocysts. This later percentage increases up to 84% and 86% by D3 and D7, respectively. We note that 16% of the 4-day-old calves on D0 are excreting oocysts and this percentage increases as a function of the age of the calf on D0 to reach 90% to 95% by the age of 8 days. 10 out of 12 dead calves excreted *C. parvum* oocysts. From D0 to D14 the other pathogen agents show a relative or a decreasing stability. 2. Dairy calves: 382 calves which had diarrhoea for less than 24 h or no diarrhoea, aged 8 to 15 days coming from six industrial livestock farms were included in the study. On D0, 99% of the calves presented a good initial general condition, 99.7% had a good appetite and no calf was dehydrated. At this date (D0), 16.8% of the calves excreted cryptosporidia. This percentage increases up to 23% and 51.8% on D3 and D8, respectively, then decreases to 31.9% on D14. The pressure of the other pathogenic

*Corresponding author. Tel.: +33-2-47-42-77-45; fax: +33-2-47-42-77-45
E-mail address: naciri@tours.inra.fr (M. Naciri)

agents remains relatively stable, excepted for rotavirus on D7 (from 9.9% on D0 to 27.2% on D7, then 12.6% on D14) which does not explain the concomitant peak in diarrhoea because the infection by rotavirus on D7 is more frequent in non-diarrhoeic calves than in diarrhoeic calves. Our results show that *Cryptosporidium* prevalence is higher in suckling than in dairy calves and *C. parvum* constitutes actually in both cases the major aetiological agent of neonatal diarrhoea. ©1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The neonatal diarrhoea complex (NDC) is one of the main causes of calf morbidity and mortality causing major economic losses in many dairy and beef herds. Several infectious or nutritional factors may occur either alone or in synergy and among the different infectious agents, *Escherichia coli* K99, rotavirus, coronavirus, *Cryptosporidium*, *Salmonella*, can be implicated as aetiological agents of the disease (Morin et al., 1976, Moore and Zeman, 1991). Enteric infection with these microbial agents is associated with villous atrophy, hypercellularity of the lamina propria, decreased activity of mucosal intracellular enzymes causing nutrient malabsorption, diarrhoea and debilitation. Any pathognomonic symptom does not allow to differentiate them clinically. These different aetiologies without specific symptomatology make the diagnosis and consequently the appropriate prevention or treatment difficult for veterinarians. In practice, a rapid diagnosis is *quasi* impossible and veterinarians prescribes usually an antibiotherapy and rehydration. A better knowledge of the frequency of the pathogens occurring in livestock farms would allow to the praticians a better guidance in their treatments and would avoid numerous failures in the treatment of neonatal diarrhoea, and the appearance of antibioresistance problems linked to intensive and sometimes abusive use of antibiotics. In fact, despite the cow vaccinations before calving against *E. coli*, rotavirus and coronavirus, the neonatal diarrhoea persists, and often *Cryptosporidium parvum*, a protozoan parasite is found responsible of these persistent and treatment-resistant diarrhoea. Reported worldwide, usually regarded as opportunist, it is now recognized as contributing to diarrhoea in various animal species including man (Fayer and Ungar, 1986; Moore and Zeman, 1991; O'Donoghue, 1995). This parasite seems ubiquitous because it has a broad host range, and its survival in the environment for long periods of time due to strong resistance of the oocysts. The organism was first reported in association with calf diarrhoea in 1971 (Panciera et al., 1971). In studies conducted in USA, prevalence rates in the calf population were 6–81% (Moore et al., 1988; McCluskey, 1992; Garber et al., 1994). A recent study in Spain reports that *Cryptosporidium* was detected alone enteropathogen in 52.6% of the diarrhoeic dairy calves (De la Fuente et al., 1999). All the reports from different countries (Angus, 1990), in which only one or two faecal specimens were examined per calf, likely underestimate the actual prevalence of bovine cryptosporidiosis (Fayer et al., 1998).

Informations on *C. parvum* infections in cattle in France are limited. The present paper reports results of studies about pathogenic role of *C. parvum* in the NDC in both suckling and dairy calves from different regions of France.

2. Materials and methods

2.1. Suckling calves

2.1.1. Animals, inclusion and non-inclusion criteria

The study was set up in 10 rural veterinary practices situated in three French administrative Departments Cantal, Saône-et-Loire, and Vendée. Based on the farmer's call, the calves 4 to 10 days old developing diarrhoea since less than 24 h were included as well as the calves presenting no diarrhoea of the same farm aged between 4 and 10 days (D0 = inclusion day). Afterwards, all calves which were born into the livestock farm could be included in the study when they were 4 to 10 days old, whether or not they presented a-less-than-24 h diarrhoea. In 62.5% of the livestock farms, the calves remained permanently with their dams, and in 37.5% they were with their dams during suckling time only. In all cases, they were nourished with their dam's milk.

Non-inclusion criteria concerned calves under 4 days old and more than 10 days old, those presenting a diarrhoea developing over more than 24 h or a recurrence of diarrhoea, calves infected by a previous or concomitant pathology, calves having received since birth an anticoccidial or antiprotozoal treatment or containing nitrofurans, sulfones, sulfonamides or arsenical derivatives, or animals of an individual market value over 5000 FF.

2.1.2. Procedures

Faecal samplings were collected on D0, D7 and D14. Research concerned the following NDC agents: *Cryptosporidium* oocysts, rotavirus, coronavirus, *E. coli* K99 and *Salmonella*.

Table 1

Analysis techniques used to search enteropathogens in feces specimens from suckling and dairy calves, according to laboratories in French departments

Enteropathogens	Departments				
	Cantal	Vendée	Saône et Loire	Pyrénées Atlantiques	Finistère
<i>Cryptosporidium</i> ^a	Heine staining	Modified Ziehl-Nielsen method	ELISA ^b	Sucrose	Sucrose
<i>E. coli</i> K99	ELISA	Minca ^c medium for isolation + serotyping	ELISA + bacteriology	ELISA	Bacteriology serotyping
Rotavirus	ELISA	ELISA	ELISA	ELISA	ELISA
Coronavirus	ELISA	ELISA	ELISA	ELISA	Precipitation on agar medium
<i>Salmonella</i>	Selenite isolation	Rambach's medium for isolation + SS ^d	Rambach's medium for isolation + SS	Hektoen's medium isolation	Smid's medium isolation

^a For *Cryptosporidium*, each fecal specimen was examined at INRA (Tours) by sucrose method.

^b ELISA: enzyme linked immunosorbent assay.

^c Minca: mineral casaminoacid.

^d SS: *Salmonella* and *Shigella* medium.

Table 2
Clinical parameters studied and scored

	General condition	Feeding behaviour	Dehydration	Faecal index
Score 0	Good (correct degree of fatness and good vivacity)	Normal appetite	No persistence of skinfolds No sunken eyeballs Moist mucosa	Absence of diarrhoea
Score 1	Medium (medium degree of fatness and vivacity)	Reduced appetite	Persistence of skinfolds <5 s No sunken eyeballs Moist mucosa	Semi-liquid diarrhoea
Score 2	Mediocre (thin and low vivacity)	Anorexia	Persistence of skinfolds (6 to 30 s) Sunken eyeballs Mucosa +/- moist	Liquid diarrhoea
Score 3	Poor (Cachexia and/or decubitus, coma)		Persistence of skinfolds > 30 s Very sunken eyeballs Dry mucosa	

An additional sampling was made on D3 for cryptosporidia only. Faeces were sampled directly from rectum by hand protected by a single-use latex glove. When impossible, the upper layer of the freshly deposited faecal matter was collected by hand protected by latex glove or with wooden spatula. Each faeces sample was collected in sterile polystyrene flasks with screw cap and sterile wooden spatula. All samples were rapidly sent refrigerated towards the different laboratories by express mail for researching the pathogen agents.

Cryptosporidia were researched at Institut National de la Recherche Agronomique (INRA centre de TOURS) by semi-quantitative method using Sheather's solution in which phenol is replaced by sodium azide (500 g of saccharose, 320 ml of water, 0.2 g of sodium azide per liter). Oocysts were counted by direct reading with an optical microscope at the 250 magnification and scored: score 0 = no oocyst seen per microscopic field, score 1 = less than 1 oocyst per field, score 2 = 1 to 5 oocysts per field, score 3 = 6 to 10 oocysts per field, score 4 = 11 to 20 oocysts per field, score 5 = more than 20 oocysts per field. The scores given in the table of results are the mean number of oocysts counted in 10 microscopical fields ($\times 250$).

The laboratory techniques used to research *E. coli* K99, rotavirus, coronavirus and *Salmonella* in the Departmental Veterinary Laboratories (DVL) are noted in Table 1 and the clinical parameters studied and scored are summarised in Table 2.

2.2. Dairy calves

2.2.1. Animals, inclusion and non-inclusion criteria

The study was carried out in six industrial livestock farms (fattening units) from three French administrative Departments (Finistère, Pyrénées Atlantiques and Saône-et-Loire). All dairy calves 8 to 15 days old were included in the study except those calves presenting diarrhoea developing over more than 24 h or a recurrence of diarrhoea, calves infected by a previous or concomitant pathology, and calves of an individual market value over 5000 FF. Depending on the size of the livestock farm, either all the calves of the farm or only the calves of one or two rooms were included. The animals were housed in individual, closed

or partially closed pens allowing nose-to-nose contact, on slatted flooring. They were fed reconstituted milk.

2.2.2. Procedures

2.2.2.1. Biological and clinical parameters As for the suckling calves, the faecal samples were collected on D0 (inclusion day), D7, D14 and D28 for research of the following NDC agents: cryptosporidia, rotavirus, coronavirus, *E. coli* K99 and *Salmonella*. An additional sampling was made on D3 for cryptosporidia research only. Laboratory techniques used and clinical parameters studied are summarised in Tables 1 and 2, respectively.

2.3. Statistical analysis

For each pathogenic agent, considering the distribution of sampling results on D0, this distribution was compared when the faecal index on D0 is 0 with that when the faecal index on D0 is 1 or 2. The statistical test used is the two-sided Fisher exact test at the level 5%. In order to complete analysis, an exact logistical regression model was used. The probability explained is the probability of having a faecal index equal to 1 or 2 on D0, and the explicative variables are the demonstration on D0 of the various pathogenic agents considered.

3. Results

3.1. Suckling calves

According to described criteria, 40 livestock farms and 311 suckling calves were included over a period of 3 months from February to April 1995. 92.5% of the livestock farms were suckling livestock farms and 7.5% were mixed suckling and dairy herds. The calves remained either permanently or during suckling time only with their dams in 62.5% and 37.5%, respectively.

65.3% of calves included in the study were of the Charolais breed, 18.6% other pure breeds (Salers 9%, Limousin 7.7%, Blond 1.6%, Parthenay 0.3%) and 16.1% crossed breeds. The animal population comprised 53.1% males and 46.9% females.

A prophylaxis of neonatal diarrhoea had been carried out in 21 of the 40 included livestock farms: cows were vaccinated with regard to *E. coli* K99 in three livestock farms, with regard to rotavirus and coronavirus in 4 livestock farms, and with regard to *E. coli*, rotavirus and coronavirus in 14 livestock farms.

In 50% of the livestock farms included, the farmer had the habit of taking therapeutic measures (antibiotics, anti-diarrhoeal, gastric demulcent, rehydration, vitamins and minerals) when neonatal diarrhoea occurred usually after the calving peak.

Faecal samples were collected from 311 calves on the inclusion day (D0) and from 153 calves on D7 and D14 for research of the following NDC agents: cryptosporidia, rotavirus, coronavirus, *E. coli* K99 and *Salmonella*.

On D0, the mean age of the 311 calves was 6 days (s.d. = 1.8) and weighed on an average 45 kg (s.d. = 6.1). A good initial general condition, i.e. a correct degree of fatness and a

Table 3
Distribution of calves (in percent) according to general condition scores

General condition (1) ^a	Suckling calves (%)				Dairy calves (%)			
	D0 ^b (n = 311)	D3 (n = 153)	D7 (n = 153)	D14 (n = 153)	D0 ^c (n = 382)	D3 (n = 191)	D7 (n = 191)	D14 (n = 191)
Score 0 (good)	82.0	66.0	79.1	81.7	98.9	99.5	98.4	99.5
Score 1 (average)	15.1	26.1	11.8	4.6	0.26	0.5	1.05	0
Score 2 (mediocre)	2.9	5.9	3.3	0.6	0	0	0.5	0
Score 3 (poor)	0	2.0	5.9	13.1	0	0	0	0.5

^a (1) Significance of scores – score 0: correct degree of fatness and good vivacity, score 1: average degree of fatness and vivacity, score 2: thin and vivacity weak and score 3: cachexia and/or decubitus, coma.

^b D0: Inclusion day, 311 calves 4 to 10 days old.

^c D0: Inclusion day, 382 calves 8 to 15 days old.

Table 4
Distribution of calves (in percent) according to feeding behaviour scores

Feeding behaviour	Suckling calves (%)				Dairy calves (%)			
	D0 ^a (n = 311)	D3 (n = 153)	D7 (n = 153)	D14 (n = 153)	D0 ^b (n = 382)	D3 (n = 191)	D7 (n = 191)	D14 (n = 191)
Score 0 (normal)	76.2	70.6	80.4	83.7	99.7	98.4	99.5	98.9
Score 1 (reduced)	22.2	24.2	13.7	3.3	0.26	1.05	0	0.5
Score 2 (anorexia)	1.6	5.2	5.9	13.1	0	0.5	0.5	0.5

^a D0: Inclusion day, 311 calves 4 to 10 days old.

^b D0: Inclusion day, 382 calves 8 to 15 days old.

good vivacity was noted in 82.0% (255/311) of the calves, and no calf presented a general condition initially considered as poor (Table 3) and 76.2% (237/311) of the calves presented a normal appetite (Table 4). Among the 74 calves in which feeding behaviour was affected, only five were qualified as anorexic. No sign of dehydration, i.e. neither persistence of skin folds, nor sunken eyeballs, nor dryness of the mucosa was observed in 91.3% (284/311) of the calves (Table 5). Among the 27 calves which presented signs of dehydration, only six calves were clearly affected with persistence of skin fold from 6 to 30 s, sunken eyeballs and mucosa less moist than normal. A semi-liquid or liquid diarrhoea was recorded in 48.6% of the calves (Table 6).

The general condition is affected as early as D3 (52/153 = 34%), followed by a progressive return to the initial condition on D14 (Table 3). The feeding behaviour is affected as early as D3 (Table 4). The number of calves whose appetite is affected increased on D3 then a progressive improvement is noted on D7 then D14. The number of diarrhoeic animals increased up to 63.4% and 61.4% on D3 and D7, respectively, then decreased on D14 (Table 6). Initially few animals were dehydrated (8.7% of the calves) on D0. The number of dehydrated calves increases as early as D3 (35/153 = 22.9%) then returns to the initial state from D7 onwards (11.2%) but on D0, 0% was significantly dehydrated while on D14, 11.8% presented a score 3 with a persistence of skinfold superior 30 s, with very sunken eyeballs and dry mucosa.

Table 5
Distribution of calves (in percent) according to dehydration scores

Dehydration (1) ^a	Suckling calves (%)				Dairy calves (%)			
	D0 ^b (n = 311)	D3 (n = 153)	D7 (n = 153)	D14 (n = 153)	D0 ^c (n = 382)	D3 (n = 191)	D7 (n = 191)	D14 (n = 191)
Score 0 (absent)	91.3	77.1	88.9	85.6	97.1	98.9	98.9	99.5
Score 1 (light)	6.7	16.3	3.3	2.0	2.9	1.05	0.5	0
Score 2 (distinct)	1.9	4.6	2.0	0.6	0	0	0.5	0
Score 3 (significant)	0	2.0	5.9	11.8	0	0	0	0.5

^a (1) Significance of scores – score 0: no persistence of skinfold, no sunken eyeballs, moist mucosa, score 1: persistence of skinfold <5 s, no sunken eyeballs, moist mucosa, score 2: persistence of skinfold from 6 to 30 s, sunken eyeballs, mucosa +/- moist and score 3: persistence of skinfold >30 s, very sunken eyeballs, dry mucosa.

^b D0: Inclusion day, 311 calves 4 to 10 days old.

^c D0: Inclusion day, 382 calves 8 to 15 days old.

Table 6
Distribution of calves (in percent) according to their faecal index

Faecal index	Suckling calves (%)				Dairy calves (%)			
	D0 ^a (n = 311)	D3 (n = 153)	D7 (n = 153)	D14 (n = 153)	D0 ^b n (= 382)	D3 (n = 191)	D7 (n = 191)	D14 (n = 191)
Diarrhoea Score 0 (absent)	51.4	36.6	38.6	66.0	93.7	79.6	72.8	83.8
Score 1 (semi-liquid)	25.1	31.4	39.2	17.7	4.5	14.6	13.6	13.6
Score 2 (liquid)	23.5	32.0	22.2	16.3	1.8	5.8	13.6	2.6

^a D0: Inclusion day, 311 calves 4 to 10 days old.

^b D0: Inclusion day, 382 calves 8 to 15 days old.

On D0, 50% of the calves excreted cryptosporidia (Table 7). Those were identified as *C. parvum* by their size and morphology after examination at 630 magnification with an optical microscope. This percentage increased up to 84% and 86% on D3 and D7, respectively. The Table 8 shows the percentage of animals excreting oocysts as a function of the age of the animal on D0 of its inclusion in the study. We observe that 16% of the calves 4 days old on D0 are excreting oocysts and this percentage increases progressively to reach 90% to 95% by the age of 8 days.

The results of the incidence of pathogenic agents other than cryptosporidia on D7 and D14 differ little from those observed on D0 which show a relative or a decreasing stability (Table 9).

We noted 12 deaths between D1 and D12, and 10 out of the 12 dead calves excreted cryptosporidia.

According to the results of the statistical analysis and given the fact that several pathogenic agents were isolated on the same sample, it was clearly shown that the probability of presenting diarrhoea on D0 is significantly higher for calves infected by cryptosporidia (score ≥ 1) than for calves that are not infected (score 0) ($p < 0.001$). Similarly, the probability of presenting diarrhoea on D0 is significantly higher for calves carrying rotavirus ($p = 0.03$) and coronavirus ($p < 0.001$) than for those which do not carry these viruses. It should be

Table 7
Distribution of calves (in percent) according to result of coprological analyses

Pathogenic agents	Coprological analyses	Suckling calves (%)				Dairy calves (%)			
		D0 ^a (n = 311)	D3 (n = 153)	D7 (n = 153)	D14 (n = 153)	D0 ^b (n = 382)	D3 (n = 191)	D7 (n = 191)	D14 (n = 191)
Cryptosporidia	Score 0	49.8	15.7	13.7	71.9	83.2	77.0	48.2	68.1
	Score 1	9.6	9.2	7.8	7.8	4.5	2.1	12.6	10.9
	Score 2	10.6	6.5	12.4	2.6	3.9	4.2	12.0	8.4
	Score 3	54.7	9.8	7.2	1.3	0.8	1.6	4.7	3.7
	Score 4	6.1	13.1	9.8	1.3	1.8	4.7	7.8	3.7
	Score 5	18.3	45.8	49.0	15.0	5.8	10.5	14.7	5.2
<i>E. coli</i> K99	Absence	93.9	ND	98.5	98.3	94.2	ND	98.4	95.3
	Presence	6.1	ND	1.5	1.7	5.8	ND	1.6	4.7
Rotavirus	Absence	85.7	ND	88.8	87.2	90.1	ND	72.8	87.4
	Presence	14.3	ND	11.2	12.8	9.9	ND	27.2	12.6
Coronavirus	Absence	93.2	ND	98.5	100	68.4	ND	64.4	69.5
	Presence	6.8	ND	1.5	0	31.6	ND	35.6	30.5
<i>Salmonella</i>	Absence	99.7	ND	100	100	95.3	ND	98.9	96.8
	Presence	0.3	ND	0	0	4.7	ND	1.1	31.6
Agents other than cryptosporidia	Absence	81	ND	88.9	90.2	58.6	ND	48.7	54.5
	Presence (≥ 1)	19	ND	11.1	9.8	41.4	ND	51.3	45.5

^a D0: Inclusion day, 311 calves 4 to 10 days old.

^b D0: Inclusion day, 382 calves 8 to 15 days old.

Table 8
Distribution of calves (in percent) for cryptosporidia count scores according to age on D0 (311 calves)

Age on D0 (days)	Cryptosporidia		
	Absence (score = 0)	Presence (score > 1)	% (score > 1)
4	64	12	16
5	56	22	28
6	20	36	64
7	7	25	78
8	4	29	88
9	3	14	82
10	1	18	95

noted that under the conditions of this study, we were unable to demonstrate a relationship between the presence of diarrhoea on D0 and the presence of *E. coli* and *Salmonella*.

3.2. Dairy calves

Six industrial livestock farms (fattening units) and 382 dairy calves were included from June to October 1995.

76.4% of calves were of the Friesian breed, 12% were other pure breeds (Montbeliard 10.2%, Normand 1.6%, Belgian 0.3%) and 11.5% crossed breeds. The animal population comprised 91.6% males and 8.4% females.

Table 9
Relation between diarrhoea and presence of pathogenic agents on D0

Pathogenic agents	Results of analysis	Suckling calves (%)		Dairy calves (%)	
		Diarrhoea on D0 ^a		Diarrhoea on D0 ^b	
		Absence	Presence	Absence	Presence
<i>Cryptosporidium</i>	Absence	36	13.8	79.3	3.9
	Presence	15.4	34.7	14.4	2.4
<i>E. coli</i> K99	Absence	49.5	48.8	90.3	6.3
	Presence	0.3	1.4	3.4	0
Rotavirus	Absence	45.4	40.6	84.8	5.5
	Presence	4.4	9.6	8.9	0.8
Coronavirus	Absence	49.5	43.7	65.8	3.2
	Presence	0.3	6.5	27.9	3.2
<i>Salmonella</i>	Absence	49.7	50	89	6.3
	Presence	0.3	0	4.7	0

^a D0: Inclusion day, 311 calves 4 to 10 days old.

^b D0: Inclusion day, 382 calves 8 to 15 days old.

Faecal samples were collected from 382 calves on D0 (inclusion day) and from 191 calves on D7, D14 and D28 for research of the following NDC agents: cryptosporidia, rotavirus, coronavirus, *E. coli* K99 and *Salmonella*.

On D0, the mean weight of the 382 calves was 52.4 kg (s.d. = 5.1). A good initial general condition, a correct degree of fatness and a good vivacity were noted in 99% (378/382) of the calves, no calf presented a general condition initially considered as mediocre or poor (Table 3), 99.7% (381/382) had a normal appetite (Table 4), and 97.1% (371/382) presented no sign of dehydration: neither persistence of skin folds, nor sunken eyeballs, nor dryness of the mucosa (Table 5). No calf presented signs of distinct or significant dehydration.

The clinical parameters for these dairy calves: general condition, feeding behaviour and dehydration state, were good on D0 and few were affected over the entirety of the clinical follow-up (on D7 and D14) carried out in this study.

Only 1 case of death was counted on D9.

On D0, 16.8% of the calves excreted cryptosporidia (Table 7). This percentage increases up to 23% and 51.8% on D3 and D7, respectively, then decreases to 31.9% on D14.

The pressure of the pathogenic agents researched during the study remains relatively stable (Table 7): 5.8%, 1.6% and 4.7%, respectively on D0, D7 and D14 for *E. coli*, 31.6%, 35.6% and 30.5% for coronavirus, 4.7%, 1.1% and 3.2% for *Salmonella*, with the exception of a peak of incidence, on D7, of rotavirus (from 9.9% on D0 to 27.2% on D7, then 12.6% on D14).

A semi-liquid or liquid diarrhoea was noted in 6.3% of the total population on D0 (Table 6). The presence of mucous and/or blood in faecal matter was considered as a sign of a local inflammation for which it is quite difficult nonetheless to specify the aetiology, pathological sign or difficulty in samplings.

According to the results of the statistical analysis, and given the fact that several pathogenic agents were isolated on the same sample, it was clearly shown that the probability of present-

ing diarrhoea on D0 is significantly higher for calves infected by cryptosporidia (score ≥ 1) than for calves that are not infected (score 0) ($p < 0.02$). The probability of presenting diarrhoea on D0 is higher for calves carrying coronavirus than for those which do not carry this virus, and this difference is at the limit of significance ($p = 0.08$).

Under the conditions of our study, we were unable to demonstrate a relationship between the presence of diarrhoea on D0 and the presence of rotavirus, *E. coli* and *Salmonella*.

4. Discussion

The results of this study confirm that cryptosporidia constitute one of the major aetiological agents of neonatal diarrhoea in 4- to 10-day-old suckling and dairy calves.

In suckling calves, the descriptive study of the evolution of general clinical parameters shows a deterioration of the general condition, appetite and state of dehydration of calves during the follow-up carried out from D0 to D14, principally due to cryptosporidia since the epidemiological context concerning pathogenic agents other than cryptosporidia remained relatively stable. Previously, we had reported the importance of cryptosporidia in the French Charolais or Limousin and Belgian Blue-White diarrhoeic calves (Peeters et al., 1992). Reynolds et al. (1986) reported that *C. parvum* was the second most commonly detected pathogen in diarrhoeic calves in beef suckler herds. Today, it seems that the rotavirus is not the most prevalent agent in diarrhoeic neonatal suckling calves since the enteropathogen the most often detected and statistically associated with diarrhoea is *C. parvum*. The parasite is associated with a significant high probability of diarrhoea, in suckling calves aged 4 to 10 days confirming the findings by earlier researchers that *Cryptosporidium* infection in calves is mostly a neonatal problem (Anderson, 1981; Pavlasek, 1982; Stein et al., 1983; Henriksen and Grogh, 1985; Schulz, 1986; Blewett, 1989; Naciri et al., 1993; Xiao and Herd, 1994; Quilez et al., 1996a, b). The diarrhoea and oocyst shedding may appear as soon as 4 days of age with a peak at the age of 8 to 10 days where 90% to 95% of the calves are infected in accordance with the results of previous workers (Anderson, 1981; Stein et al., 1983; Schulz, 1986; Blewett, 1989; Xiao and Herd, 1994).

Our results show that 16% of the calves 4 days old on D0 are excreting oocysts at the time of their inclusion and this percentage increases progressively as a function of the age of the calf on D0 to reach 95% by the age of 8 days. Given the duration of the parasite cycle, which is around 4 days as we had showed in experimentally infected calves (Naciri et al., 1993), we may consider as a first approximation that the percentage of 4-day-old calves on D0 contaminated by the parasite is roughly 70% to 80%; i.e. equivalent to the percentage of 7-day-old calves excreting oocysts. For the calves of 5 days of age and older, this percentage is already at a maximum (90% to 95%). Due to the age structure on D0 of the calves in the study, we may estimate that 90% of the animals were already infected at the time of their inclusion confirming their early contamination soon after birth. The calves permanently with their dams may easily get infected by sucking udders or by contact with contaminated litters. *C. parvum* has been found in the faeces of adult cattle (Chermette et al., 1984; Mann et al., 1986; Villacorta et al., 1991; Lorenzo-Lorenzo et al., 1993; Scott et al., 1994). According to Scott et al. (1994) cows apparently healthy may excrete between 750 000 and 720 million oocysts daily, thus contributing to the contamination of the newborn calf's environment. The

authors conclude that they are a significant factor in the epidemiology of cryptosporidiosis as asymptomatic carriers that intermittently shed oocysts and 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). On the other hand, Bukhari and Smith (1997) reported that half of oocysts excreted with faeces are non-viable. Nevertheless, even if the number of potentially infecting viable oocysts is smaller than previously suspected it remains sufficient to infect the calves since healthy volunteers become infected with the median infective dose of 132 oocysts (Du Pont et al., 1995).

During the study 12 deaths were counted between D1 and D12, 10 excreted *C. parvum* oocysts. Even if this observation does not allow to conclude that cryptosporidiosis is the univocal cause of mortality among these calves, it is probable that this pathology does weaken the animals' natural defences. Sandford and Josephson (1982) reported prolonged and intractable diarrhoea and high mortality in diarrhoeic calves where *C. parvum* was the sole pathogen. The presence on D0 of cryptosporidia or other pathogenic agent(s) significantly account for a higher probability of mortality.

Concerning the dairy calves, virtually each calf comes from a different breeding farm and in these conditions, it is practically impossible to know the prophylaxis and the neonatal diarrhoea treatments carried out on the calves prior to their arrival in fattening units. The oocyst excretions lead us to raise questions concerning the origin of the contamination of animals during the course of the study. Indeed, given the breeding conditions (no contact with dams, commercial milk), calves isolated in stalls, which had been previously disinfected with steam water and quaternary ammonium, one may wonder how contagion took place. The calves were only allowed nose-to-nose contact. This contamination is far from negligible as the percentage of animals excreting oocysts goes from 16.1% on D0 to 51.9% on D7. On D0, some calves are contaminated. If we consider the prepatent period is about 4 days, contamination on D0 would be revealed on D3 only for some animals (those excreting oocysts the earliest). On the other hand, by D7, all the contaminated animals have become excreting animals. If we suppose that the majority of the 16.1% of the animals excreting oocysts on D0 is no longer excreting on D7, having naturally got rid of their infection, an attentive analysis of individual results shows that 21 out of 31 calves excreting on D0 have a null score on D7; therefore, it may be estimated that around 50% to 55% of the calves not excreting oocysts on D0 were contaminated. These results are similar to those reported by Otto et al. (1995) and De la Fuente et al. (1998,1999). These dairy calves were less contaminated than suckling calves at the same age; probably the stress of transport, change of housing and feeding brings about an increase in the parasite development.

Finally only cryptosporidiosis is associated with a significantly higher probability of diarrhoea on D0, confirming the major aetiological role of this pathology in neonatal diarrhoea in calves around 8 days old. On D0, the calves were between 8 and 15 days old. At the peak of the incidence of diarrhoea; i.e. on D7, the calves are therefore 15 to 21 days old, which renders it unlikely that cryptosporidia play a role in these diarrhoea. Moreover, the increasing incidence of rotavirus does not explain the concomitant peak in diarrhoea as the analysis of individual results permits to observe that infection by rotavirus on D7 is more frequent in calves with no diarrhoea than in calves with a faecal index of 1 or 2 (Table 9). Therefore, we may conclude that the several episodes of diarrhoea encountered during the study are probably not infectious in their origin but remain principally related to stress,

change in breeding conditions, environment and feed. This does not call into question the pathogenic role of cryptosporidia in calves under 15 days old: the probability of presenting diarrhoea on D0; i.e. in calves around 8 days old, is significantly higher in animals excreting oocysts than in animals that were not excreting, and this in spite of a very low proportion of calves presenting diarrhoea on D0 (24/382).

In conclusion, our study shows that *Cryptosporidium* prevalence is higher in suckling than in dairy calves in accordance with Scott et al. (1994, 1995), Quilez et al. (1996), and the parasite actually constitutes in both cases the major aetiological agent of neonatal diarrhoea in France. Large numbers of *C. parvum* oocysts (10^6 to 10^{10} per gram of faeces) are excreted causing contamination by direct contact with other calves, other livestock species and humans. The oocysts excreted into the environment are very resistant and may remain infective for several months either in soil or water, thus contaminating the surface waters and ultimately urban water supplies causing large outbreaks of waterborne human cryptosporidiosis. *C. parvum* must not (no longer) be regarded only as an opportunistic protozoa which worsens a bacterial or viral disease. Studies must be made for research of effective specific treatments or preventive means to reduce the disease and the oocyst excretions in calves in order to reduce economic losses, the environmental contamination and the risks for animal and human health.

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