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## PREVALENCE OF VARIOUS ENTEROPATHOGENS IN THE FECES OF DIARRHEIC AND HEALTHY CALVES

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### Résumé

FRÉQUENCE DE PLUSIEURS AGENTS ENTÉROPATHOGENES DANS LES FÉCES DE VEAUX DIARRHÉIQUES ET SAINS.— Différents agents entéropathogènes ont été recherchés dans les matières fécales de veaux de lait élevés à la ferme, dans une aire géographique limitée (Nord-Ouest du département d'Indre-et-Loire, France) pendant l'hiver 1983-1984. Deux enquêtes différentes ont été effectuées: une étude cas-contrôle incluant 32 veaux diarrhéiques et 21 veaux sains élevés dans 53 fermes différentes et une étude séparée sur neuf cas de diarrhée apparus dans l'élevage de la station. Les agents infectieux suivants ont été recherchés à l'aide de méthodes spécifiques de détection: *Escherichia coli* entérotoxigènes et létaux pour la souris, *Salmonella* sp., *Yersinia enterocolitica*, *Campylobacter jejuni*, *Clostridium perfringens* entérotoxigènes, *Chlamydia psittaci*, rotavirus, coronavirus, *Cryptosporidium*. Dans l'étude cas-contrôle, aucune souche de *E. coli* entérotoxigène (K99+) n'a été détectée dans les deux groupes de veaux. Quatre agents ont été plus fréquemment diagnostiqués chez les veaux malades que chez les veaux sains: rotavirus (12/32 vs 1/21), *E. coli* létaux pour la souris (6/32 vs 1/21), *Cryptosporidium* (2/32 vs 0/21) et *Salmonella typhimurium* (1/32 vs 0/21). L'un au moins de ces quatre agents était présent chez 16 veaux diarrhéiques (50%) contre seulement deux veaux sains (10%) ( $P < 0,01$ ). En revanche, les fréquences de *C. jejuni* d'une part et de l'entérotoxine de *C. perfringens* d'autre part, étaient similaires dans les deux groupes d'animaux, s'élevant respectivement à 20% et à 10%. Les particules coronavirus-like étaient même significativement plus fréquentes chez les veaux sains (11/21) que chez les veaux malades (7/32) ( $P < 0,05$ ). Dans la deuxième enquête, limitée à une seule exploitation, tous les agents entéropathogènes étudiés ont été détectés pendant la période d'observation, à l'exception de *E. coli* entérotoxigène. Cependant, chaque veau pris individuellement excréta rarement plus de deux agents à la fois. Par ailleurs, tous les veaux des deux enquêtes possédaient dans leur sérum des anticorps contre l'entérotoxine de *C. perfringens*, tels que mis en évidence par un test ELISA spécifique. Cette étude confirme le rôle primordial des rotavirus et de *Cryptosporidium* comme agents de la diarrhée des veaux de moins de trois semaines. Elle suggère également la participation de souches de *E. coli* létales pour la souris et souligne le danger potentiel pour la santé humaine des réservoirs bovins de *C. jejuni* et *C. perfringens* entérotoxigènes.

Numerous infectious agents including bacteria, viruses and protozoa have been related to calf diarrhea (Tzipori, 1981). Some of these agents are

able to cause diarrhea in experimentally-infected calves and can therefore be considered as primary enteropathogens. These are enterotoxigenic *E. coli*

(Dubourguier *et al.*, 1978), *Salmonella* species (Lintermans and Pohl, 1983), rotaviruses (Mc Nulty, 1983), coronaviruses (Scherrer and Laporte, 1983), and *Cryptosporidium* (Naciri and Yvoré, 1983). For other agents, the experimental evidence suggests a milder enteropathogenicity, i.e. *Campylobacter jejuni* (Al-Mashat and Taylor, 1983), enterotoxigenic *C. perfringens* (Niilo, 1980), and chlamydial agents (Storz *et al.*, 1971). A third group contains agents, known as potential enteropathogens for humans, that have been isolated from calves' intestinal tract without being directly related to diarrhea, i.e. *Yersinia enterocolitica* (Swaminathan *et al.*, 1982) and *Aeromonas hydrophila* (Al-Mashat and Taylor, 1983).

Besides their possible contribution to enteritis of calves, some of these organisms may also be agents of food-borne diseases in humans. The importance of bovine reservoirs has been outlined for *Salmonella* (Who, 1980) *Campylobacter jejuni* (Blaser and Reller, 1981) and is suspected for enterotoxigenic *C. perfringens* (Shandera *et al.*, 1983) and *Yersinia enterocolitica* (Swaminathan *et al.*, 1982).

Therefore, the detection of these enteropathogens in calves' feces can serve a double purpose: etiological by relating enteritis with the significant presence of an organism in the digestive tract, and epidemiological by assessing the potential hazard for human health of calf sources of food borne diseases agents. These were the objectives of the present investigation, which was carried out on homebred dairy calves less than three weeks old and within a restricted geographical area of France (North-West of County of Indre-et-Loire). This investigation includes:

- a case-control study performed on two independent samples of diarrheic and healthy calves, each calf being raised in a different farm (study n° 1).
- a study in the herd of the research station where several cases of diarrhea occurred during the period of observation (study n° 2).

The reliability of diagnostic methods has been insured by the collaboration of specialists of each group of agents examined. One of the original findings of this study was the association of *E. coli* highly lethal for mice with diarrheic calves. The complete analysis of this observation will be published in a separate article (in redaction). A special emphasis has been given to enterotoxigenic *C. perfringens* because of the growing interest for this agent, a cause of food poisoning in humans (Shandera *et al.*, 1983). We carried out the detection of *C. perfringens* enterotoxin in the calves' feces, and looked for a possible relationship between the presence of this enterotoxin and such factors as level of seric antitoxic antibodies,

number of fecal *C. perfringens* organisms, and age of the calf.

## Materials and Methods

### Sampling of calves

This investigation, carried on from December 1983 to April 1984 in the North West area of the County of Indre-et-Loire, comprises two studies:

- a case control study, including 32 diarrheic calves and 21 healthy calves reared in 53 different farms located in the area of three veterinary practices (study n° 1).
- a study in the herd of the research station, including nine cases of diarrhea which occurred during the period of observation (study n° 2).

In both studies, the eligible calves, either diarrheic or healthy, were homebred dairy calves, less than three weeks old. Diarrhea was defined as the emission of soft or fluid feces, with or without general symptoms. The feces of any eligible calf were sampled only if no antimicrobial agents had been administered recently. To check the eligibility of the calves selected and to obtain information about the composition of the samples of calves, a questionnaire was filled up for each animal, including name of the owner and location of the farm; identification number, age, breed and sex of the animal; and, for diarrheic calves, the symptomatology, including general symptoms, dehydration signs and characteristics of feces. The feces and the blood of each animal were sampled once, and before the treatment for diarrheic calves.

In the case-control study, the practitioners were asked to collect the feces of all diarrheic eligible calves whom they had to care for during the period of investigation. The control calves were selected in separate farms of the same area, where, according to the owners, no cases of diarrhea had been recorded for at least one year. One calf was selected per eligible farm.

In the other study, all the calves less than three weeks old that became diarrheic during the period of observation were considered.

Feces samples were transported to the laboratory on the day of collection, and immediately submitted to the bacteriological analysis and processed for the detection of viruses and the enterotoxin of *Clostridium perfringens*. Blood sera were stored at -70 °C.

### Detection of enteropathogenic organisms and *C. perfringens* enterotoxin in the feces

#### Bacteria

The various methods of bacterial isolation and characterization are reported in table 1. In addition to the bacterial agents mentioned in this table, some of the methods allowed isolation of other species of *Enterobacteriaceae* and *Vibrionaceae*.

*Chlamydia psittaci* was detected by a plaque assay on Mc Coy cells (Rodolakis and Chancerelle, 1977). Prior to inoculation on cells, feces were diluted to 1:10 in PBS-DEAE that contained gentamicin (200 µg/ml), vancomycin (200 µg/ml) and amphotericin B (2.5 µg/ml). Dilutions 1:10, 1:50, and 1:500 were tested. Nearly half of the cell cultures seeded with dilutions 1:10 developed a non-specific cytopathogenic effect and were therefore

Table 1. -- Methods of isolation and characterization of bacterial agents

Bacteria	Media		Enumeration	Characterization
	Enrichment	Plating		
<i>Escherichia coli</i>	...	Drigalski agar	yes	enterotoxigenic : K99 seroagglutination  <i>E. coli</i> lethal for mice by IP route (lethal dose 50 % less than $1 \times 10^7$ organisms) <sup>a</sup>
<i>Salmonella species</i>	Selenite Broth	Salmonella- Shigella agar	no	serotyping Centre National des Salmonella Inst. Pasteur, Paris (Pr L. Le Minor)
<i>Yersinia enterocolitica</i>	PBS (4° C, 4 weeks)	Mac Conkey agar	no	...
<i>Campylobacter jejuni</i>	...	Shirrow agar + Butzler agar	no	Antigenic typing <sup>b</sup> Laboratoire Central Bactériologie-Virologie, Hopital Necker, Enfants Malades (Pr M. Véron)
<i>Pseudomonas aeruginosa</i>	...	Citrimid agar	no	...
<i>Clostridium perfringens</i>	...	VL agar + Sheep blood (5 %) + Neomycin (50µg/ml) + Cycloserine (25µg/ml)	yes	...

a: the complete procedure of selection and virulence testing of *E. coli* isolates is described in a separate paper (in redaction).

b: according to Penner (1980).

not readable. This effect was observed with about 20 % of 1:50 dilutions and with none of the 1:500 dilutions.

#### Viruses

Rotavirus was detected by the ELISA test (Scherrer and Bernard, 1977). Five degrees of reaction were recorded according to the level of spectrophotometric absorbance: -, ±, +, ++ and +++.

Coronavirus-like particles were examined by direct electron-microscopy (EM) after coloration with phosphotungstate (Mc Nulty *et al.*, 1980).

#### Cryptosporidium

Cryptosporidium oocysts were demonstrated by direct microscopic observation of smears of feces concentrated by the technique of fecal flotation (Anderson, 1981).

#### Enterotoxin of *C. perfringens*.

The enterotoxin was detected by the technique of counterimmuno-electrophoresis first described by Naik and Duncan (1977). The specific antiserum was produced in rabbits against the enterotoxin that had been purified

by the polyacrylamide gel electrophoresis technique (Popoff, 1984). This method allows quantitation of enterotoxin in the supernatants of feces. Titer was expressed as the reciprocal of the highest dilution of feces giving a clear precipitin line. The preparation of samples before testing included dilution 1:10 in PBS, centrifugation 1500 rpm during 10 min, and congelation of supernatants at -70 °C.

#### Detection of seric antibodies against *C. perfringens* enterotoxin

A specific ELISA technique was developed using the purified enterotoxin as an immobilized antigen (Popoff, 1984) and a peroxidase-conjugated antiovine IgG (Miles, Paris). Titer was expressed as absorbance × 500 (dilution of serum).

#### Statistical analysis

The data were analyzed by the chi-square test (Snedecor and Cochran, 1980)

Table 2 -- Case control study: prevalence of enteropathogenic organisms and of *C. perfringens* enterotoxin in the feces of calves<sup>a</sup>

	Number of calves		Statistical analysis (Chi-square)
	diarrheic (total: 32)	healthy (total: 21)	
Rotavirus <sup>b</sup>	12	1	7.3**
<i>E. coli</i> lethal for mice <sup>c</sup>	6	1	2.2
<i>Cryptosporidium</i>	2	0	1.4
<i>Salmonella</i> <i>typhimurium</i>	1	0	0.3
At least one of the preceding agents	16	2	9.3**
<i>Campylobacter jejuni</i>	6	4	0.1
<i>Clostridium</i> <i>perfringens</i> enterotoxin	3	2	0.1
Coronavirus	7	11	5.3*
Combinations:			
no agent	9	8	...
one	12	8	...
two	9	4	...
three	1	1	...
four	1	0	...

\*: P<0.05; \*\*: P<0.01

a: No *E. coli* K99<sup>+</sup>, *Yersinia enterocolitica* and *Chlamydia psittaci* was detected.

b: Levels of infection ++ and +++ in the ELISA test.

c: Lethal dose 50% in mice by IP route less than  $1 \times 10^7$  organisms.

## Results

### 1. Case-control study

#### Characteristics of the calves

The mean age in days ( $\pm$  standard error) of the 32 diarrheic calves and the 21 healthy calves was respectively 9.7 ( $\pm$  0.8) and 7.9 ( $\pm$  1.0). Fifteen diarrheic calves (47%) were of the French Frison breed, as compared to 16 healthy calves (76%). The remaining calves were exclusively of the Normand breed. Approximately 50% of the calves were male/female in both groups.

The clinical examination of the diarrheic calves, based on a single examination, indicated that 8 calves (26%) were severely ill, i.e. with signs of intense dehydration and/or systemic signs of shock (rectal temperature over 40 °C; anorexia; collapse).

Detection of enteropathogenic organisms and *Clostridium perfringens* enterotoxin in the feces of calves (table 2)

No *E. coli* K99<sup>+</sup>, *Yersinia enterocolitica* and *Chlamydia psittaci* was detected. At least one of the other agents was present in 72% of diarrheic calves and 62% of healthy calves. Rotavirus, *E. coli* highly lethal for mice, *Cryptosporidium*, and *Salmonella typhimurium* were more frequently excreted in diarrheic calves than in healthy calves, but a significant difference was observed for rotavirus only (P < 0.01). At least one of the last four agents was detected in 50% of the diarrheic calves versus 10% of the healthy calves (P < 0.01).

The prevalence of *Campylobacter jejuni* was approximately 20% in both groups of calves. Ten isolates (one per infected calf) were serotyped. Of these ten isolates, two only were serotypable. They belong respectively to the serotypes 0:2 and 0:3:1.

The enterotoxin of *Clostridium perfringens* was demonstrated in approximately 10% of the calves, with no significant difference between the two groups. The titers of enterotoxin were respectively

80, 40 and 20 for the three positive diarrheic calves, and 160 and 20 for the two positive healthy calves.

Coronavirus-like particles were significantly more frequent in healthy calves (52 %) than in diarrheic calves (33 %) ( $P < 0.05$ ).

Combinations of more than two agents were rarely observed, accounting for two cases only. The most frequent association was rotavirus and coronavirus in diarrheic calves (three cases) and *Campylobacter jejuni* and coronavirus in healthy calves (four cases).

*C. perfringens* enterotoxin in relation to titer of enterotoxigenic antibodies in the serum, number of *C. perfringens* organisms in the feces, and age on the calf (fig.1).

All the calves, either diarrheic or healthy, displayed antibodies against *C. perfringens* enterotoxin in their serum. The titers ranged from 100 to 1000 and the mean titers were not significantly different in diarrheic and healthy calves (respectively  $490 \pm 25$ , and  $566 \pm 43$ ;  $P > 0.05$ ).

Of the five calves with *C. perfringens* enterotoxin in the feces, all but one displayed a titer of antibodies greater than 600, the median value for the total population. Three of these five calves contained more than  $10^5$  *C. perfringens*

organisms in their feces. These five calves were 7 or 8 days old (mean of the sample of 53 calves:  $8.8 \pm 0.7$  d).

*Subsidiary bacteriological results* (table 3)

Several bacteria that belonged to the families of *Enterobacteriaceae*, *Vibrionaceae* and *Pseudomonaceae*, and not considered so far as major enteropathogens in calves, were isolated during this investigation. In one calf, which was diarrheic, the feces contained approximately  $10^8$  *Citrobacter freundii* organisms/g as numbered after primary isolation on Drigalski agar plates. The other *Enterobacteriaceae* were isolated either after direct isolation on Drigalski agar or after culture in enrichment media designed primarily for the detection of *Salmonella* sp. and *Yersinia enterocolitica*. In particular, *Enterobacter* sp. and *Aeromonas* sp. were selected principally after enrichment in PBS maintained at 4 °C during four weeks and isolation on Mac Conkey Agar.

*Pseudomonas aeruginosa* was detected in 28 % of calves.

2. Study n°2 (table 4)

Nine of 40 calves that were born during period of observation exhibited diarrhea. Most calves were more than four day-old. None of them died

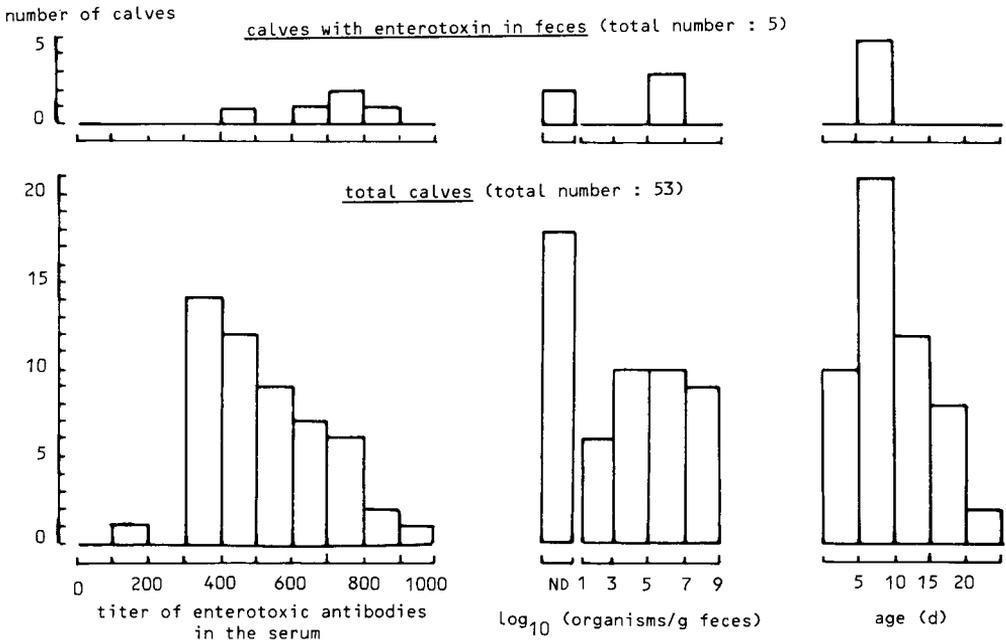


Fig. 1. — *Clostridium perfringens* enterotoxin in feces of calves in relation to titer of specific antibody in serum, number of organisms in feces and age. ND: Not determined.

Table 3. — Case control study: subsidiary bacteriological results

Organisms	Diarrheic calves (32)	Healthy calves (21)
<i>Enterobacteriaceae</i>		
<i>Proteus mirabilis</i>	15	9
<i>Proteus vulgaris</i>	7	3
<i>Klebsiella pneumoniae</i>	3	5
<i>Enterobacter hafniae</i>	4	3
<i>Citrobacter</i> species	2 <sup>a</sup>	4
<i>Enterobacter cloacae</i>	0	2
<i>Enterobacter agglomerans</i>	2	0
<i>Serratia liquefaciens</i>	2	0
<i>Proteus morgani</i>	1	0
<i>Providencia</i> species	0	1
<i>Levinea amalonitica</i>	1	0
<i>Vibrionaceae</i> <sup>b</sup>		
<i>Aeromonas hydrophila</i>	1	0
<i>Aeromonas caviae</i>	1	0
<i>Pseudomonaceae</i>		
<i>Pseudomonas aeruginosa</i>	10	5

a: one calf had about 10<sup>8</sup> organisms/g of feces.

b: diagnosis confirmed by C. Richard, service des Entérobactéries. Institut Pasteur, Paris.

(the treatment consisted in parenteral rehydration and oral administration of colistin). None of the fecal *E. coli* isolates was K99<sup>+</sup> antigen (ten isolates per calf). The lethality for mice of these isolates was not tested. Rotavirus (level + + +) and coronavirus were the most prevalent agents, accounting for respectively two and three calves. *Cryptosporidium*, *C. jejuni* and *S. infantis* were also present in the herd, each of these agents having been isolated once, in different calves. Although no *C. perfringens* enterotoxin was detected, all but one calf were shedding high numbers of *C. perfringens* organisms in their feces and displayed specific antibodies against the enterotoxin in their serum.

## Discussion

The main purpose of the case-control study was to examine the possible involvement of various enteropathogens in diarrhea by comparing the prevalence of these agents in the feces of diarrheic and healthy calves. The control calves were chosen in farms where no cases of diarrhea had been recorded for at least one year, in order to

avoid the selection of healthy carriers of enteropathogens, a situation likely to arise when control calves are in contact with diarrheic calves. As compared to a design where controls are in contact with diarrheic calves, the present design was expected to reveal more accurately a difference in shedding of enteropathogens between the two groups of calves. A possible confounding factor associated with this design was the sanitary conditions of the farms.

If each agent is considered individually, rotavirus only was significantly associated with diarrheic calves ( $P < 0.01$ ). Nevertheless, when we consider together rotavirus, *E. coli* highly lethal for mice, and *Cryptosporidium*, it appears that 50 % of diarrheic calves were shedding at least one of these agents versus only 10 % of healthy calves ( $P < 0.01$ ). The association of these agents with diarrheic calves suggests that they may have caused the disease. The enteropathogenicity of rotavirus and *Cryptosporidium* has conclusively been demonstrated in experimental oral infections of calves (reviewed respectively by Schwerts *et al.*, 1983, and Pivont and Antoine, 1982). The role of *E. coli* highly lethal for mice in enteritis of calves

Table 4. -- Prevalence of enteropathogens in the feces of nine diarrhetic calves raised at the research station (Study n° 2)

Identification Age (d)	Calves									
	43 2	44 7	52 18	54 11	55 8	56 6	57 10	61 6	62 5	
Rotavirus <sup>a</sup>	—	—	—	—	—	—	—	+	+	
Coronavirus	—	—	+	+	—	—	—	—	+	
<i>Cryptosporidium</i>	—	+	—	—	—	—	—	—	—	
<i>Campylobacter jejuni</i>	—	—	—	+	—	—	—	—	—	
<i>Salmonella sp.</i>	—	—	—	—	+ <sup>b</sup>	—	—	—	—	
<i>Clostridium perfringens</i> log (number/g feces)	8.0	6.7	8.7	7.9	7.9	8.0	7.0	ND	8.2	
enterotoxin in feces	—	—	—	—	—	—	—	—	—	
titer of seric antitoxic antibodies	196	431	231	342	NT	212	480	230	255	

a: +: levels of infection ++ and +++ in the ELISA test.

b: *Salmonella infantis*.

ND: not detected.

NT: not tested.

over three days of age was suspected in a previous field investigation (De Rycke *et al.*, 1982). The present investigation strengthens this hypothesis. The detailed analysis of *E. coli* isolates, including careful selection based on biotyping and testing of virulence by intraperitoneal inoculation of adult mice will be published separately (in redaction). The lethal strains were defined by a lethal dose 50% on mice less than  $1 \times 10^7$  organisms, a level of virulence which characterizes classical septicemic strains. Their possible contribution to enteritis of older calves remained to be elucidated.

The absence of *E. coli* K99<sup>+</sup> is probably due to the low proportion of calves under four days of age, the period of maximum receptivity to this agent.

Coronavirus particles, as detected by electron microscopy, were significantly associated with healthy calves ( $P < 0.05$ ). The reliability of electron microscopy for the detection of coronavirus has been questioned because of the possible confusion of typical particles with pleomorphic particles (Woode *et al.*, 1978). In the present study, the validity of our conclusions is reinforced by the agreement between results in electron microscopy and in a specific ELISA test that was performed subsequently (Laporte, personal

communication). There are very few reports on the detection of coronavirus in healthy calves, but Morin *et al.* (1978), during a field survey in Canada including 51 diarrhetic calves and 21 healthy calves, found that respectively 47% and 52% of them had coronavirus particles in the intestine, a result which is somewhat similar to ours. According to our results coronaviruses appear to be widespread in the calves of the area concerned by the study. Furthermore, these results suggest that the presence of coronaviruses in the intestine is not a reliable indicator of their participation to enteritis, or that only some strains of coronaviruses are actually pathogenic.

From our results, the actual virulence of *Campylobacter jejuni* for calves is also questionable since approximately 20% of both diarrhetic and healthy calves were shedding the organisms in their feces. An equal occurrence of *Campylobacter sp.* in diarrhetic and normal calves was also observed by Snodgrass *et al.* (1982). The same authors failed to produce any clinical disease by experimental infection of gnotobiotic calves (Snodgrass, 1983), whereas Al-Mashat and Taylor (1980) provoked only a mild diarrhea by oral inoculation of massive doses of the organism. These data suggest that *Campylobacter jejuni* is only moderately pathogenic for calf intestine.

The same conclusion probably holds true for enterotoxigenic *C. perfringens*. In the present study, approximately 10 % of healthy and diarrheic calves had detectable amount of enterotoxin in their feces. To our knowledge, there is no other report in the literature of systematic detection of enterotoxin in the feces of diarrheic or healthy calves. The available data concern the detection of enterotoxin in strains isolated from the digestive tract of cattle. According to these data, 12 % of the strains isolated in Canada from *post-mortem* cases of diarrhea were enterotoxigenic (Niilo, 1978), whereas the prevalence rate was 60 % in normal cattle in California (Tsai *et al.*, 1974). The titers of enterotoxin in the present study ranged up to 1:160. In humans involved in *Clostridium perfringens* food poisoning outbreaks, Naik and Duncan (1978), using the same method of counterimmunoelectrophoresis, found titers up to 1:128.

Seric antibodies against *C. perfringens* enterotoxin were found in all the calves, with titers ranging from 100 to 1000. This observation could indicate that enterotoxigenic strains of in the *C. perfringens* were highly prevalent in the environment of the calves. But this must be interpreted cautiously in the absence of data concerning the sensitivity and the specificity of the test. Niilo and Cho (1984) tested a similar ELISA technique for the detection of *C. perfringens* enterotoxin antibodies. Their technique was more sensitive than the concurrent techniques used so far. These authors considered the titer 100 as the inferior level of positivity. Using the reverse passive hemagglutination technique, Niilo and Bainborough (1980) observed a low incidence of specific antibodies against the enterotoxin in cattle in Western Canada. Conversely, in another study conducted on Zebu cattle in Brazil, using a fluorescent antibody test (P.C. Brant, 1974, PhD thesis, University of California, Davis), all the sera were found to contain enterotoxin antibodies, as in the present survey. Whether these differences are attributable to the actual geographical distribution of enterotoxigenic *C. perfringens* or to the methods of detection remains debatable in the absence of comparative analysis of the different techniques and definition of levels of positivity. However, our whole set of data suggests that enterotoxic *C. perfringens* are widespread in the area studied, even though their enteropathogenicity for calves is not established.

The main conclusion of the second study, restricted to a single herd, is that all the major enteropathogens were detected at least once, with the exception of *E. coli* K99 (which had been isolated in the previous years). This observation may suggest that all the enteropathogens associa-

ted so far with diarrhea of calves are present endemically in every herd.

Besides the association of various enteropathogens with calf diarrhea, this study provides information about the bovine reservoirs of potential agents of food borne diseases in humans, particularly for *C. jejuni* and enterotoxigenic *C. perfringens* (Prescott and Bruin-Mosch, 1980; Prescott and Munroe, 1982; Shandera *et al.*, 1983). In the area concerned with the present study, bovine reservoirs of *Campylobacter jejuni* seem important (20 % of carriers). Elharrif *et al.* (1982) detected this organism in 18 out of 50 adult healthy cows examined in the area of Bordeaux (South-West of France). In other countries, the isolation rates varied greatly according to the geographical location and to the health status of animals. As for enterotoxigenic *C. perfringens*, the importance of the bovine origin in the human contamination is suggested by the frequent implication of beef products in food poisoning outbreaks (33 % of the outbreaks in the USA, according to Shandera *et al.*, 1983). But data about the carriage of this organism by animals are lacking. Such an information is dependent upon a preliminary standardization of the methods of detection of the enterotoxin and of its specific antibodies. In any case, our study suggests that this organism is widespread in cattle in the area studied.

In the same way, the importance for human health of animal reservoirs of *Salmonella* species has long been recognized, and their distribution is followed on a world scale (Who, 1980). Of the two serotypes that were detected in this study, *S. typhimurium* is considered as the most prevalent worldwide, whereas *S. infantis* is much less commonly found, at least in France (Corbion and Gledel, 1981).

In spite of thorough searching, no *Yersinia enterocolitica* was found in this study. This result is consistent with previous reports, indicating a very low frequency of isolation in bovine feces (Inoue and Kurose, 1975; Wooley *et al.*, 1980).

The other enteropathogens examined in the present study have not been considered to date as agents of zoonosis. Yet, relationships have been demonstrated between human and bovine rotaviruses (Mebus *et al.*, 1976), *Cryptosporidium* (Current and Long, 1983) and coronaviruses (P. Bobulesco, 1984, Thèse de 3ème cycle, Université de Paris VII).

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### Summary

The presence of various enteropathogens was examined in the feces of homebred dairy calves reared in a restricted geographical area of France (North West of County of Indre-et-Loire) during winter 1983-1984. Two distinct surveys were carried on: a case-control study including 32 diarrheic calves and 21 healthy calves bred in 53 different farms; and a separate study on nine diarrheic calves in another farm. The following infectious agents were looked for, by specific methods of detection: *Escherichia coli* K99 and *E. coli* lethal for mice, *Salmonella* species, *Yersinia enterocolitica*, *Campylobacter jejuni*, enterotoxigenic *Clostridium perfringens*, *Chlamydia psittaci*, rotaviruses, coronaviruses, *Cryptosporidium*. In the case-control survey, no enterotoxigenic *E. coli* (K99<sup>+</sup>) was detected in either group of calves. Four agents were more often detected in diarrheic calves than in healthy calves, i.e. rotavirus (12/32 vs 1/21), lethal *E. coli* (6/32 vs 1/21), *Cryptosporidium* (2/32 vs 0/21) and *Salmonella typhimurium* (1/32 vs 0/21). One at least of these four agents was present in 16 diarrheic calves (50%) vs only 2 healthy calves (10%) ( $P < 0.01$ ). On the other hand, the occurrence of *Campylobacter jejuni* and of *C. perfringens*, enterotoxin was similar in both groups of calves, accounting respectively for about 20% and 10% of total calves. Moreover, coronavirus-like particles were significantly associated with healthy calves (7/32 vs 11/21;  $P < 0.05$ ). In the other study, all the main categories of enteropathogens were detected throughout the period of observation in the same farm with the exception of enterotoxigenic *E. coli*. But each calf taken individually was rarely shedding more than two agents at a time. In addition, specific antibodies against *C. perfringens* enterotoxin, as tested in an ELISA test, were present in the serum of all the calves examined in both surveys. This study confirms the primary role of rotavirus and *Cryptosporidium* as agents of diarrhea in calves under three weeks. It also suggests the possible participation of *E. coli* strains that are lethal for mice and underlines the potential hazard for human health of bovine reservoirs of *Campylobacter jejuni* and enterotoxigenic *C. perfringens*.

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