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Pattern of diarrhoea in newborn beef calves in south-west France

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Abstract – A prospective study was carried out on 94 randomly selected beef herds in the Midi-Pyrénées region in France. The objective was to describe diarrhoea and mortality in beef calves from birth to 30 days of age. Calves (3 080) were followed from December 1995 to April 1996, and a total of 700 visits allowed records of herd management practices, individual data and environmental conditions to be collected. The incidence rate for diarrhoea during the neonatal period was 14.6 %, and varied markedly between herds. Eighteen herds did not suffer from diarrhoea, while five herds had an incidence of more than 50 %. Results indicate that 52 % of diarrhoea appears during the first week and only 15 % after the second week of life. The greatest risk of diarrhoea for a calf was during the first and second weeks of life (7.9 and 6.5 times, respectively). The month of birth was also significantly associated with morbidity, the highest incidence was observed in December and March (17.6 and 23.6 %, respectively). *Escherichia coli* was isolated from 20.3 % of faecal samples and appeared earlier during the first days of life. Rotavirus was frequently isolated (47.4 %) from samples. Coronavirus was positive for only 16.5 % but was significantly associated with diarrhoea. *Cryptosporidium* was less frequent (15.6 %). The global mortality rate was 3.6 % and was two-times higher in December than in other months. Forty per cent of herds did not exhibit mortality, and 10 % had mortality rates greater than 10 %. This study confirms previously reported data, and with greater precision and details on diarrhoea and mortality incidences among herds, age and month of birth in suckling beef calves. © Inra/Elsevier, Paris.

epidemiology / beef calf / diarrhoea / mortality

Résumé – **Epidémiologie de la diarrhée chez le veau nouveau-né en élevage allaitant dans le sud-ouest de la France.** Une étude prospective a été menée sur 94 élevages allaitants tirés au sort dans la région Midi-Pyrénées (France). L'objectif était de décrire la diarrhée et la mortalité de la naissance à un 1 mois d'âge chez le veau. Des veaux (3 080) ont été suivis entre décembre 1995 et avril 1996,

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environ 700 visites ont permis de recueillir des informations sur les pratiques d'élevage, d'environnement ainsi que des données individuelles. L'incidence des diarrhées chez les veaux était de 14,6 % et variait considérablement entre les élevages. Dix-huit exploitations (19 %) n'ont enregistré aucun cas de diarrhée alors que cinq autres (5,3 %) avaient une incidence supérieure à 50 %. Les résultats montrent que 52 % des cas de diarrhée sont apparus pendant la première semaine et seulement 15 % sont survenus après 15 jours. Le nouveau-né a 7,9 fois plus de risque de diarrhée au cours de la première semaine et 6,5 pendant la seconde. Le mois de naissance est également lié à l'apparition de la maladie, nous avons une incidence de diarrhée plus élevée en décembre et mars (17,6 et 23,6 % respectivement). *Escherichia coli* était retrouvée dans 20,3 % des prélèvements fécaux, notamment lors des premiers jours suivant la naissance. Le rotavirus était le plus fréquemment isolé (47,4 % des échantillons) alors que le coronavirus n'était isolé que dans 16,5 % des cas mais ce dernier était le seul significativement lié à l'apparition de la diarrhée. Les cryptosporidies n'ont été retrouvées que dans 15,6 % des échantillons. Un taux de mortalité global de 3,6 % a été observé chez les veaux d'un mois. La mortalité était deux fois plus élevée en décembre qu'au cours des autres périodes. Quarante pour-cent des élevages n'ont enregistré aucun cas de mortalité alors que 10 % notaient une incidence au-delà de 10 %. Cette étude permet de confirmer des résultats obtenus précédemment, de plus ils sont basés sur des données récentes qui ont permis une description détaillée et une estimation plus fine des incidences des diarrhées et des mortalités en élevage allaitant. © Inra/Elsevier, Paris.

épidémiologie / veau allaitant / diarrhée / mortalité

1. INTRODUCTION

Gastrointestinal disease is the most commonly reported disease of calves and the major cause of neonatal mortality [8, 11, 13, 19, 24, 26]. The incidence rates of diarrhoea in calves under 30 days of age reported by several studies varied between 15 and 20 %. The mortality rate was reported to vary between 1.5 and 8 % [6, 14, 17, 19, 20, 23, 24, 26]. The incidence of diarrhoea is reported to vary markedly among herds throughout the world (between 0 and 70 %) [9, 19, 21, 24].

Calves are at greatest risk of diarrhoea during the first week of life, and this risk subsequently decreases with age [8, 16, 20, 23, 24, 26].

This early morbidity and mortality represents an important source of economic loss to the farmer due to loss of the present value of the calf and loss of genetic potential for herd improvement. The average cost associated with prevention and mortality of gastrointestinal disease has been reported to be US\$ 33.46 per calf and per year [4, 5, 11]. One study in the USA estimated the total cost to the dairy industry at US\$ 250

million per year [14]. In Switzerland, the cost of diarrhoea was estimated to be around US\$ 30 per calf [5].

In the Midi-Pyrénées region (France), it was estimated that 80 % of herds and 20 % of calves were affected by diarrhoea [2]. Fatality from diarrhoea was reported to be 50 % [2]. Diarrhoea was estimated to cost the farmer in the region of US\$ 70 per calf and per year. Furthermore, 21 % of the veterinary surgeon's activities concerned treating calves for enteritis [2].

Many infectious agents implicated as causal agents of diarrhoea (*Escherichia coli*, rotavirus, coronavirus and *Cryptosporidium*) are commonly found in dairies. These are zoonotic and have been associated with food-borne diseases in man. To treat diarrhoea in calves, antibiotics are used, creating a potential health risk to humans in terms of drug residues and the development of resistant bacterial strains.

The majority of previous works, some of which were retrospective or cross-sectional, have been carried out on dairy heifer calves and not on beef calves. Therefore, prospective studies are needed for beef calves.

The objective of this study was to describe the pattern of diarrhoea and to evaluate the impact of age, season of birth and herd on the incidence of diarrhoea in the Midi-Pyrénées region, using a representative large sample and new statistical analysis tools.

2. MATERIALS AND METHODS

2.1. Target population

A longitudinal prospective cohort study was conducted in 94 beef herds from eight departments of the south west of France. A simple random stratified sampling design was used to recruit herds in the study. The stratification was based on the department and adjusted for the number of farms in each department using a proportional sampling method. A random number sampling algorithm was used to identify the herd to be enrolled in the study. The enumeration rule was that if a farm was selected then all the calves were enrolled in the study.

The sampling frame was the exhaustive listing of farms in that region. The inclusion criteria consisted of herds with more than 20 adult beef cows, at least three quarters of calvings during the survey and the five most important breeds of the region (Charolaise, Limousine, Gasconne, Aubrac, Blonde d'Aquitaine). A total of 7 000 herds was eligible and represented 30 % of the total herds of that region.

From 95 randomly selected beef herds in the Midi-Pyrénées, 94 were involved in this study and followed from December 1995 to April 1996.

2.2. Data collection

A team of veterinary surgeons and animal technicians were involved in data collection. All participants followed a training session before starting the project to assume uniformity in data collection.

Data on health, demography and management factors thought to be associated with the risk of diarrhoea were collected from the farm using questionnaires.

Health factors included morbidity, mortality, vaccination and preventive measures. The

demography data included number of calves, number of cows and heifers, the introduction of replacement animals and animal density. The management information concerned housing, feeding, cleaning, calving conditions and colostrum feeding.

This information was combined on three sheets (herd sheet, calf sheet and daily log sheet described later). These questionnaires were pre-tested by veterinary colleagues and a group of farmers.

All 94 selected farms were visited by members of the team. The visit included at least a veterinarian and a veterinary technician; the purpose was to describe and explain the study in more detail and to address any questions that the farmer had in relation to the study and its purpose. Subsequent visits to the farm were made by the assigned technician.

The protocol used in the study was designed so that nine visits would be made to each herd during the study period. The visits occurred three times each month at intervals of 8 to 12 days according to calving intervals. These visits allowed the collection and verification of continuous data 'daily log sheets' (e.g. dates of events, diarrhoea, death) and also to record data which varied with time (e.g. temperature, dampness, calf stocking density).

During the first visit in December 1995, the 'herd sheet' was completed during an interview with the farmer in order to determine management practices (e.g. housing, feeding, cleaning).

The 'calf sheet' was distributed to farmers to record data on each calf born during the survey (e.g. calving condition, colostrum).

To assure good quality data, at each visit different forms were verified, completed and copied by a member of the team.

In order to identify pathogens causing diarrhoea, faecal samples were taken from calves with diarrhoea and control calves which did not have diarrhoea. The veterinary laboratories of the eight departments had a standard and homogeneous analysis method to identify *Escherichia coli*, rotavirus, coronavirus and *Cryptosporidium*. An ELISA technique was used for both bacteria and virus (enterosure® Rhône Mérieux) and Ziehl coloration for *Cryptosporidium*.

2.3. Data analysis

All data collected were coded and entered on a microcomputer using a data management program.

Descriptive statistics and frequency distribution variables were performed using SAS® [18].

Density Incidence of Diarrhoea (DID) in the study population was computed as the number of cases divided by the number of Calf-Days at Risk (CDR). These estimates were stratified by age and month of birth.

The Survival analysis technique was used to compute the pattern of diarrhoea in the studied population. The pattern of diarrhoea over time was measured using the survival and Hazard function.

The survival and Hazard functions were performed using the estimators of Kaplan and Meier SAS® [18]. Let $t_1 < \dots < t_d$ be the distinct ordered failure times; noting n_i the number of calves at risk at t_i , (the calves neither failed nor were censored just before t_i) and m_i the number of animals with diarrhoea (to fail) at t_i :

$S(t) = \prod (1 - m_i/n_i)$, where $t_i \leq t \leq t_{i+1}$, ($i = 1, d$); or $S(t) = \text{Prob.}(T \geq t) = 1 - F(t)$.

$H(t) = \sum (m_i/n_i)$: the probability of a calf having diarrhoea between t and $t + dt$ with the condition that the calf was not in time t .

Because data from animals within the same herd are correlated, herd effect was considered. SUDAAN® software [22] was used to analyse the relationship between variables and diarrhoea; this specialized software for survey data analysis takes into account the stratified sample design and multilevel analysis, for example within-herd clustering when variance is estimated [25].

Due to the high mortality rate in the first 3 days of life (42.5 % of the cases in the first 24 h) and because the risk of death is constant with age, the calculation of the density incidence of mortality (DIM) (number of deaths divided by CDR) was neither more effective nor more advantageous than estimating directly the mortality rate incidence (number of dead calves divided by the number of living ones). Like morbidity, mortality was described both by age and by month of birth.

In order to assess the month effect on morbidity and mortality, we grouped calves into five cohorts according to month of birth: December, January, February, March and April. A few calves were born in the last week of November and were included in the December group while

those born in the first week of May were grouped with those of April.

3. RESULTS

3.1. Sampling description

A total of 3 157 calves were born during the study period from the 94 selected herds. Complete data were obtained from 97.9 % (3 080) of the calves in the study. The remainder was lost because of incorrect data from the calves born at the end of the study and missing follow-up data.

The average number of calves was 33.4 per herd (SD = 16.5) and 43.6 % of the herds had more than 30 calves enrolled.

The type of housing (loose versus tie stall) was equally distributed. The majority of herds (67 %) did not use a nursing location for the animal. If we consider calf density, 36.2 % of calves had an insufficient area. Newborn calves were mainly tied-up individually (73.4 %), while group pens and free calves represented 10.6 and 16 %, respectively. Vaccination practices were not frequently used either for calves or adult animals, with only about 25 % of owners vaccinating with at least one vaccine (*E. coli*, rotavirus or coronavirus).

The majority of calves (83.3 %) did not need assistance at birth while 15.2 % were born after pulling or caesarean section (1.5 %) and 20.3 % of dams were primiparous. The sex ratio (male/female) was 1.01. The proportion of premature or malformed calves was very low (0.6 %) and 88.2 % of the calves received a colostrum meal within the first 6 h of life. Navel dipping was used for 61.3 % of the calves.

3.2. Incidence of diarrhoea

A total of 496 cases of diarrhoea were recorded during the study period, which represents 16 % of the total population. The

percentage of calves that developed diarrhoea during the neonatal period was 14.6 % (450 cases).

A total of 76 814 CDR of diarrhoea was observed during the study period, which translates into an average follow-up time of 25 days per calf.

The occurrence of diarrhoea varied markedly between herds, age of occurrence and month of birth.

3.2.1. Distribution among herds

At the herd level, calves exhibit different patterns of diarrhoea. 76 herds (81 %) had at least one calf which expressed diarrhoea, five of them had more than 50 % diarrhoea. Out of the total, 49 herds (52 %) showed less than 10 % morbidity.

Similarly, the DID distribution was heterogeneous between herds. It varied from

0 to 0.05 (figure 1). The correlation between herd size and diarrhoea incidence was not significant.

Disparity between herds was the reason why the herd effect was used in the rest of the analysis.

3.2.2. Age of calves

As shown in the table 1, most of the cases of diarrhoea occurred during the first days of life. Fifty-two per cent of all cases appeared during the first week, and 84.6 % of the calves expressed diarrhoea during the first 15 days of life. Only 5.6 % of diarrhoea occurred after the third week of age.

DID was highest during the first week after birth (1.03 per 100 CDR), then declined in the second and third week. During the last week, the DID was around 0.1 per 100 CDR (table 1).

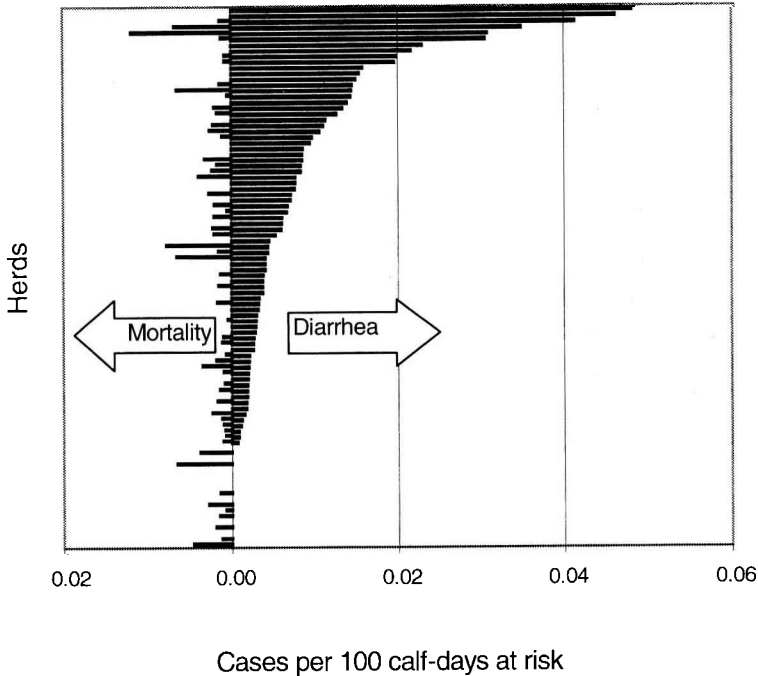


Figure 1. Diarrhoea and mortality rates for 94 beef herds in the Midi-Pyrénées region (France).

The weekly risk of diarrhoea (DID of the week divided by the DID of the fourth week as a reference) showed a higher risk during the first week (7.9 times more) compared to the fourth week of life (*table I*).

In *figure 2*, as measured by the incidence rate, the 'risk of diarrhoea' increased from birth, peaked around 1 week (two peaks at

3rd and 8th days) and then declined and remained low after the 18th day of age.

3.2.3. Month of birth

The pattern of diarrhoea according to the month of birth is shown in *table II*. The highest proportion of diarrhoea was

Table I. Distribution of diarrhoea related to age for 3 080 calves born in 94 beef herds in the Midi-Pyrénées region (France).

Age (week)	Number of		‰ ^c	CDR ^d	DID ^e	Risk ^f	P ^g
	Calves ^a	Diarrhoea ^b					
1	3 080	236	7.66	22 806	1.03	7.92	<0.001
2	2 844	145	5.09	17 254	0.84	6.46	<0.001
3	2 699	44	1.63	18 195	0.24	1.85	<0.001
4	2 655	25	0.94	18 559	0.13	1	
Total		450	14.6	76 814	0.58		

^a Calves included during the period; ^b cases of diarrhoea occurring during the week; ^c percentage of cases (rate) per week; ^d number of CDR (calf-days at risk); ^e diarrhoea incidence density (DID = number of cases/100 calf-days at risk); ^f risk of diarrhoea (DID of the week divided by the DID of the fourth week as reference); ^g statistical significance (probability) of the comparison test of risk between weeks.

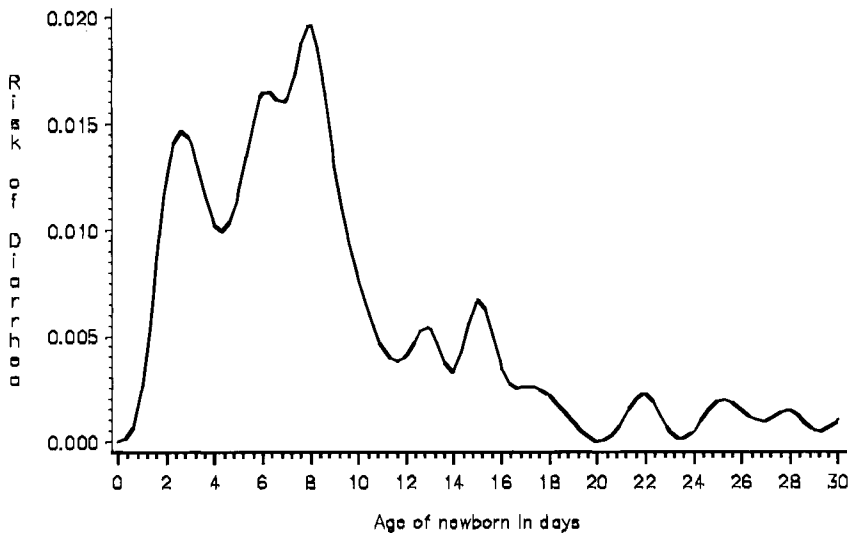


Figure 2. Daily risk of diarrhoea (per calf-day at risk) related to age of 3 080 newborn calves born between December 1995 and April 1996 in 94 beef herds in the Midi-Pyrénées region (France).

observed in March (23.6 %) and December (17.6 %) and the lowest incidence in April (4.4 %).

Likewise, the DID was highest in March and December (1.2 and 0.66 per 100 CDR, respectively) (*table II*).

Estimates of risk (RR) showed that calves born in March and December had a significantly higher risk of diarrhoea in comparison with April (RR = 4.12, $P = 0.0004$ and RR = 2.74, $P = 0.04$, respectively). These results revealed that calves born in March are four times more likely to have diarrhoea than those born in April, while those born in December were twice as likely to have diarrhoea.

On the other hand, as shown in *figures 3* and *4*, both survival and hazard functions stratified by month of birth were in accordance with the above results. The hazard function (*figure 3*) showed that the daily risk of diarrhoea was highest in March especially between the 4th and 10th days of age, whereas the risk in December was higher just after birth especially during the first 3 days of life. For the other months no significant risk was observed.

The 'survival function' (*figure 4*) stratified by month of birth also showed that no significant difference exists between birth and 3 days of age. However, after the 5th day of life, a marked and significant difference is observed in March and December rather than April (*table II*).

3.2.4. Length of diarrhoea

The length of the diarrhoea period for 354 calves was calculated by the difference between the beginning and the ending. The morbidity ranged from 1 to 8 days, with a mean of 3 days and the majority of the cases (68.1 %) had diarrhoea for 1–3 days.

3.3. Identification of infectious agents

The majority of samples were taken in the first 3 weeks of life (*figure 5a, b*). Pathogen identification appears to vary markedly with age. Globally *E. coli* was mainly isolated immediately after birth up to the 5th day of life and *Cryptosporidium* was found from day 5 to day 13 (*figure 5a*). The coronavirus was found in the first week,

Table II. Distribution of diarrhoea related to the month of birth for 3 080 beef calves from 94 herds in the Midi-Pyrénées region (France).

Month	Number of calves			CDR ^d	DID ^e	RR ^f	P ^g
	Born ^a	Diarrhoea ^b	% ^c				
December	443	78	17.60	11 816	0.66	2.74	0.04
January	768	69	8.98	22 248	0.31	1.30	0.6
February	970	134	13.81	26 330	0.50	2.00	0.12
March	673	159	23.62	13 240	1.20	4.12	0.0004
April	226	10	4.42	3 180	0.31	1	
Total	3 080	450	14.61	76 814	0.58		

^a Calves born during the month; ^b cases of diarrhoea occurring during the month; ^c percentage of cases per month of birth (rate); ^d CDR = number of calf-days at risk; ^e DID = diarrhoea incidence density per 100 calf-days at risk; ^f adjusted relative risk compared with April as the reference; ^g statistical significance (probability) of RR between months.

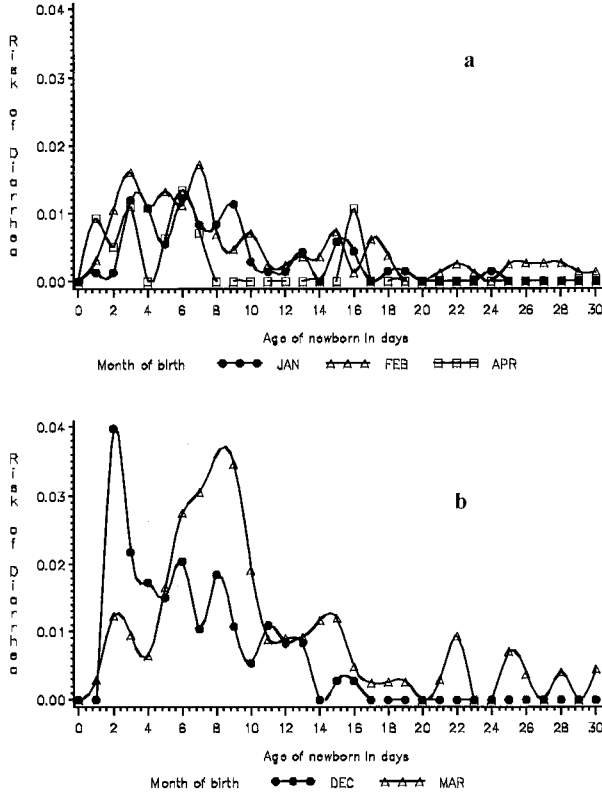


Figure 3. Daily risk of diarrhoea (per calf-day at risk) related to the month of birth of 3 080 newborn calves born between December 1995 and April 1996 in 94 beef herds in the Midi-Pyrénées region (France): 'hazard function'. a) January, February and April; b) December and March.

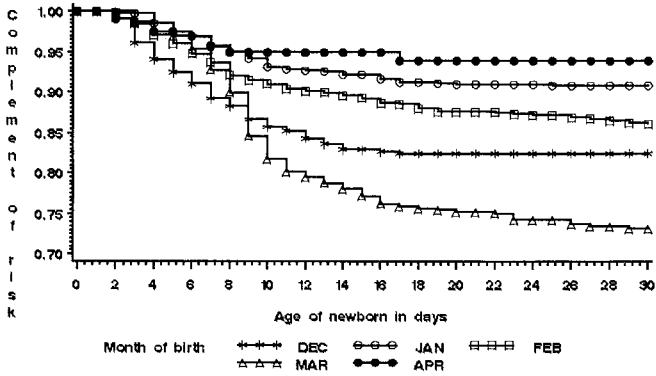


Figure 4. Daily complement of the cumulative risk of diarrhoea related to month of birth in 3 080 beef calves in the Midi-Pyrénées region (France): survival function.

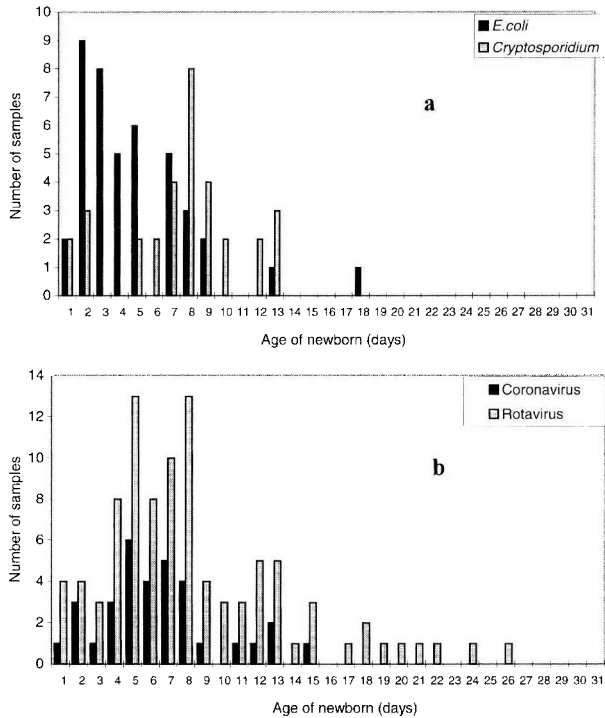


Figure 5. Identification of *E. coli* and *Cryptosporidium* (a) and coronavirus and rotavirus (b) from 94 beef herds in Midi-Pyrénées region (France).

while rotavirus was dominant from day 4 to day 15 with a peak between day 5 and day 8 (figure 5b).

The distribution between calves with and those without diarrhoea is shown in table III. From the 338 faecal samples, 211 were positive for at least one agent. The comparison of isolated pathogens in healthy and sick calves showed that *E. coli* is more frequent in healthy calves while coronavirus was more frequent in diarrhoeic calves. Rotavirus and *Cryptosporidium* appears to be distributed equally between two groups (table III). If we consider the pathogens associated with diarrhoea, no significant relationship was observed except for coronavirus. The association between combined infection and diarrhoea was not examined.

3.4. Incidence of mortality

A total of 116 deaths were observed which represents a mortality rate of 3.76 % in the study population. Ninety-seven per cent of the deaths (113) occurred during the first month, representing 3.6 % of the total population.

Because mortality appears in the first days of life and the risk of death is zero afterwards, we estimated both direct and actuarial methods for mortality incidences. The results did not show any difference between estimates, therefore we only kept the direct incidence rate (number of cases divided by number of living calves).

The mortality rate varied considerably among herds, with the age of animals and the month of birth.

Table III. Identification of pathogens in 211 positive calves with ($n = 102$) and calves without diarrhoea ($n = 109$) in 94 beef herds in the Midi-Pyrénées region (France).

	Number (%) of positive calves			
	<i>E. coli</i>	<i>Cryptosporidium</i>	Rotavirus	Coronavirus
Diarrhoea	13 (12.7)	17 (16.6)	50 (49)	22 (21.5)
No diarrhoea	30 (27.5)	16 (14.6)	50 (45.8)	13 (11.9)
Total: (211)	43 (20.3)	33 (15.6)	100 (47.4)	35 (16.5)
Risk (statistical significance)	0.5 (NS)	1.4 (NS)	1.2 (NS)	2.34 (0.01)

3.4.1. Distribution among herds

Mortality rates differed widely between herds. In fact, 38 herds (40 %) did not have mortality, while ten herds (10.6 %) had a mortality rate over 10 %.

CDR of mortality was equal to 0 for 38 herds, was very low for 47 herds (less than 0.003) and exceeded 0.007 for six herds (*figure 1*).

The mortality incidence was not correlated with herd size.

Moreover, diarrhoea and mortality were not associated. As shown in *figure 1*, among the 18 herds free of disease, nine had a high mortality rate, while herds with a high incidence of diarrhoea did not report a single case of mortality. In addition, there was no statistical association between diarrhoea and the incidence of mortality.

3.4.2. Age of calves

Stillborn calves were not included in the study. However, perinatal deaths which occurred within the first 24 h of life (dystocia, calving problems) were recorded and represented 42.4 % of dead calves and 1.57 % of total born calves.

Seventy per cent of total deaths appeared in the first week in comparison to 4.4 % in the fourth week (*table IV*).

Table IV. Distribution of mortality rates related to week of age in 3 080 beef calves from 94 herds in the Midi-Pyrénées region (France).

Week ^a	Number of		
	Calves ^b	Death ^c	% ^d
1	3 080	79	2.56
2	3 001	16	0.53
3	2 985	13	0.43
4	2 972	5	0.16
Total		113	3.6

^a Week of age of calves; ^b calves concerned during the period; ^c number of deaths; ^d percentage of cases per week of age (rate).

3.4.3. Month of birth

The mortality rate was two times higher in December than in the rest of the period: 6 % mortality rate for December compared to an approximate 3 % for other months (*table V*).

Moreover, we estimated the 'case fatality' due to diarrhoea which is why we assumed that diarrhoea was the cause of death when calves died within the 72 h following illness. The results showed that a total of 15 calves out of the 113 (13.27 %) died from diarrhoea.

Table V. Distribution of mortality rates related to the month of birth in 3 080 beef calves from 94 herds in the Midi-Pyrénées region (France).

Month ^a	Number of		% ^d
	Born ^b	Death ^c	
December	443	27	6.09
January	768	22	2.86
February	970	34	3.50
March	673	22	3.26
April	226	8	3.53
Total	3 080	113	3.66

^a Month of birth; ^b number of calves born during the month; ^c number of deaths during the month; ^d percentage of cases per month of birth.

4. DISCUSSION

This prospective study gives a good estimation of the incidence of diarrhoea in newborn calves in the Midi-Pyrénées region. The 94 farms studied are randomly and proportionally distributed among the eight departments and can be considered representative of the region and of the beef herds in France. Furthermore, the proportion of 33.4 calves per herd followed in our study is not very different from the figure observed in France [7] where 52 % of beef herds have more than 30 adult cows.

In addition to simple description incidences, we used statistical methods of survival procedures to account for intra-herd correlations and censored observations.

The majority of incidences (diarrhoea and mortality) reported in this study were similar to those found in other studies [5, 8, 13, 14, 19, 20, 24, 26]. However, some parameters must be considered in detail because a large part of the disparity between incidences could be explained by the length of the survey, the type of herds (dairy herds or beef herds), the selected samples (ran-

dom or pre-selected and particular herds), the sample size and the case definition (based on treatment rather than explicit signs). In addition, the unit of interest is important to consider, the majority of studies reported direct incidence rates rather than DID.

4.1. Diarrhoea

The proportion of diarrhoea in newborn calves under 1 month of age in this study was 14.6 % which was similar to previous results (15.2 and 17.5 %) [8, 13, 20] and lower than proportions reported by Waltner-Toews et al. [24], Wells et al. [26] and Vallet et al. [23] (20.48, 24.6 and 51 %, respectively). Those lower values were probably due to herd type (often dairy herds) and survey length (from birth to weaning). The Vallet et al. [23] study showed that the target population was herds with particularly high incidence of scours.

Diarrhoea varied markedly between herds, as previously reported [19, 24]. In our study, 19 % of the 94 herds did not report diarrhoea, as observed by Sivula et al. (23 %) in dairy heifers [20]. Schumann et al. [19], however, reported 80 % of herds without diarrhoea.

Disparity between herds is an important factor to consider, because calves within the same herd are not independent compared with calves from different herds. Estimating the incidence of morbidity or mortality could be wrong if the latter point is not taken into account.

Unfortunately, this intra-herd correlation was not considered in many previous studies. Only two authors Busato et al. [5] and Wells et al. [26] have used statistical methods for correct incidence estimation.

As found in the past, the highest risk of illness is observed in the first 2 weeks of life [6, 8, 20, 26].

Only two papers reported DID estimates. Sivula et al. [20] reported 0.9 per 100 CDR

at birth which is close to our result. Similar estimates were also found at 3 weeks as 0.1 per 100 CDR. Curtis et al. [8] reported higher DID around 6.2 and 1.8 calf-days at risk in the first and third weeks, respectively.

The hazard function (figure 2) described a logical and expected distribution with a progressive increased risk of diarrhoea from birth to 8 days then decreased rapidly and remained low and constant after the second week.

These results are clearly and easily explained by the role of virulent etiological agents (*E. coli*, rotavirus, coronavirus and *Cryptosporidium*). These agents, especially *E. coli*, appear earlier during the first days of life, followed by viruses and *Cryptosporidium*. Secondly, newborn calves may be poorly covered by maternal antibodies due to insufficient colostrum intake.

The impact of the calving season has been reported by many authors [8, 14, 16, 23]. The latter studies were conducted for longer periods of time, 2 years or more, which allows the estimation of a seasonal effect. They concluded that in pre-weaned calves morbidity is higher in winter than in spring or summer. In our survey calves were followed from December to April so that a seasonal effect could be assessed month by month. Nevertheless, the same results were observed, high morbidity from December to March rather than in April. This effect is probably due to stress produced by meteorological factors and to growth density of animals. The low incidence in April is probably due to pasture and milder weather.

Estimates of month risk factors is not frequently reported in the literature. However, Wells et al. [25] did report that calves born in the spring were better protected against diarrhoea than in the winter (OR = 0.59). Our estimates seem logical according to previous arguments. A later risk in March (between 5 and 10 days of age) than in December (between 1 and 3 days of age) could be explained biologically by the effect of infectious agents.

As reported in the literature [1, 3, 10–12, 15], *E. coli* K99 is dominant and prevalent in the herd, and may affect newborn calves in the first days of life as well as earlier in the season (December). On the other hand, rotavirus and coronavirus are reported to cause infections after the 3rd or 4th day of life, as found by previous authors [3, 11]. Infection is maintained for up to 2 weeks and contamination from sick animals or adults to newborn calves increases with the concentration of animals, which may explain the high incidence observed in March.

Because *Cryptosporidium* needs more time to cause diarrhoea, its identification occurs later and is less important than other agents [15]. The distribution described in figure 5a, b agrees with this.

Comparison between healthy calves and calves with diarrhoea showed that our results agree with those of previous studies. Coronavirus was more frequent in diarrhoeic animals [21] and increases the risk of diarrhoea. In another study [12] the same proportion was reported (42.8 % of positive samples compared with 47.4 % in our survey). On the contrary, *E. coli* was more abundant in healthy calves as previously reported [10].

Rotavirus was equally distributed between healthy and diarrhoeic animals in our study (45.8 and 49 %, respectively) whereas other authors [10] reported a higher proportion in calves with diarrhoea.

Similarly, the occurrence of *Cryptosporidium* was not different between groups (16.6 and 14.6 %) in our survey, while Gouet [15] reported higher trends in the diarrhoeic group. This could be explained by the sample population and the aims of analysis. Our study was not planned as a case-control and our objective was to describe the pattern and association of diarrhoea with several agents.

4.2. Mortality

The calculation of mortality rates excludes stillborn calves and abortion because these events were not recorded. Total mortality rate in this study was 3.6 % and similar to that reported in previous prospective studies [8, 24] but lower compared to other studies (6.3, 7.2 and 7.6 % reported by Wells et al. [26], Quigley et al. [17] and Sivula et al. [20], respectively). This difference was probably due to the target population of dairy heifers in these studies, and may also be explained by the long survey period, from birth to weaning or 4 months of age or the difference between type of production and countries. The Quigley study [17] was based upon an experimental design and not on an observational study.

Postpartum mortality was about 1.53 % (48 calves dead in the first 24 h of life); unfortunately, not many studies have reported this parameter. Fourichon et al. [13] found 6.5 % of deaths within 24 h in French dairy herds and Busato reported 3.1 % from birth to weaning in Swiss cows [5].

The remaining mortality from 24 h to 30 days represented 2.17 % and was similar to that reported by Fourichon et al. [13] for dairy calves (3.1 % between 24 h and 15 days of age).

Mortality was two times higher in December than in the other months, this may be due to climatic conditions, or virulence of pathogens early in the calving season.

Estimating mortality caused by diarrhoea 'case fatality rate' is not easy. In our study we assumed that a calf died from diarrhoea if death occurred during the 72 h following illness occurrence. We estimated 15 of 113 cases, which represents 13 % of total mortality. Two authors reported this same parameter: Busato et al. [5] and Sivula et al. [20] reported 3.1 and 43.8 %, respectively. The main difference between these studies and ours is that these authors cov-

ered the incidence from birth to weaning or 4 months while we covered only the neonatal period from birth to 30 days of age. On the other hand, the American study [20] concerning heifer calves, and the Swiss survey [5] were interrupted by the pasture period.

The results of this study were consistent with some of those previously reported in the literature. However, our survey based on a random selection of beef calves in the Midi-Pyrénées allows more details and advanced estimates with precision for diarrhoea and mortality incidences. We also found a large variation among herds. Calves were at a higher risk of diarrhoea earlier after birth. Newborn calves were at risk in the beginning of the calving season in December and later in March. This study provide baseline estimates of diarrhoea and mortality for future comparison in France.

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