ELSEVIER

Preventive Veterinary Medicine 33 (1998) 195–205

PREVENTIVE VETERINARY MEDICINE

Infectious agents associated with diarrhoea of calves in the canton of Tilarán, Costa Rica

E. Pérez [a,*], A. Kummeling [b], M.M.H. Janssen [b], C. Jiménez [c], R. Alvarado [d], M. Caballero [c], P. Donado [a], R.H. Dwinger [a]

[a] *Proyecto Salud de Hato, Universidad Nacional, P.O. Box 304–3000, Costa Rica*
[b] *University of Utrecht, Utrecht, Netherlands*
[c] *PIET, Universdad Nacional, Costa Rica*
[d] *Dept. of Parasitology, Universdad Nacional, Costa Rica*

Accepted 21 April 1997

Abstract

A case-control study of calves under 3 months of age was carried out by weekly visits to 15 farms in the canton of Tilarán, Costa Rica. Most farms were dedicated to beef or dual-purpose (DP) production. Faecal samples were collected over a 6-month period from a total of 194 calves with clinical signs and from 186 animals without clinical signs of diarrhoea as assessed by a scoring system. The samples were investigated for the presence of viruses, bacteria and parasites. Torovirus was detected for the first time in Costa Rica and was present in 14% of calves with diarrhoea and in 6% of the controls. Coronavirus and Rotavirus were less frequently encountered in either one of the groups (in 9 and 7% of scouring calves and in 1 and 2% of controls, respectively). *Escherichia coli* was detected in 94% of all the faecal samples, but isolates from only three samples from calves with diarrhoea contained the K99 antigen. Similarly, *Salmonella* was found only in scouring calves. *Cryptosporidium* oocysts were detected in animals with signs of diarrhoea, while other coccidia oocysts, *Strongylida* and *Strongyloides* eggs were frequently found in animals both with and without diarrhoea. A conditional logistic regression (CLR) analysis to compare healthy and scouring calves showed a significant difference with regard to the presence of Torovirus, Rotavirus and Coronavirus. © 1998 Elsevier Science B.V.

Keywords: Diarrhoea; Torovirus; Cattle-microbiological diseases; Mortality and morbidity; Case-control studies

* Corresponding author. E-mail: eperez@ns.medvet.una.ac.cr.

PII S0167-5877(97)00038-X

1. Introduction

Diarrhoea in young pre-weaned calves is one of the most important causes of calf morbidity and mortality (Oxender et al., 1973; Tzipori, 1981; Olsson et al., 1993). Disease incidence in young calves has an adverse effect on their immediate health status, longevity in the herd and productivity performance and thus causes great economic loss (Britney et al., 1984; Vanopdenbosch and Pohl, 1993). In order to increase productivity per livestock unit without increasing livestock numbers, it is important to identify the etiological and predisposing factors involved in calf diarrhoea in order to devise preventive measures and reduce losses during the initial months of life. Previously, such studies have been conducted on various dairy farms in Costa Rica (Oviedo et al., 1987; Cordero and Osorio, 1988; Hird et al., 1990). However, most cattle in Costa Rica (60%) are kept in extensive beef production systems and 18% are kept in dual-purpose (DP; beef and dairy) systems. Moreover, beef production has increased recently in Costa Rica from 37.1 in 1974–1976 to 51.7 kg in 1986–1988 per head of cattle and calves (Simpson and Conrad, 1993). Furthermore, the dual-purpose system is expected to increase in relevance to the foreseeable future (Perez, 1994). Consequently, we devised a case-control study to compare the prevalence of selected pathogens in calves with and without diarrhoea in 15 beef or dual-purpose farms in a tropical dry-forest ecosystem.

2. Materials and methods

2.1. Study area

A convenient sample of 15 farms was selected in the canton of Tilarán. The farms were selected on the basis of the owner's willingness to participate in the study and to form a representative selection of the various management systems in the region (Table 1). The selected farms belonged to a pilot project initiated by the staff of the Veterinary School of the Universidad Nacional as part of a livestock-information system (Dwinger et al., 1994). The calf-rearing units at these farms were visited weekly over a period of 6 months during the dry season (November 1993 to April 1994). The canton of Tilarán is situated at 10°29′58″ northern latitude and 84°54′26′ western longitude and contains areas with tropical dry forest and tropical moist forest. The average annual rainfall ranges between 2000 and 4000 mm and the ambient temperature between 24 and 30°C. Four farms were extensive enterprises (average area: 300 ha) and dedicated to beef production. The calves on these farms remained with the dams for several months. Ten farms were dedicated to dual-purpose production. In these farms, the calves remained with the dams for several days postpartum before milking started. After the daily milking routine, the calves were allowed to suckle the dams once or twice a day. At nine farms, milking was done mechanically and manually in the remaining one. In three of the dual-purpose farms, the calves were housed together with the dams and were only separated before milking, when they were temporarily housed in small pens. On the remaining seven dual-purpose farms, the calves were housed separately from the dams, either inside large communal pens or during the day when they grazed outside as a

Table 1
Characteristics of 15 farms[a] visited weekly in a case-control study on calf diarrhoea in the canton of Tilarán

Farm number	Pasture area (ha)	Altitude (m)	Production type	Number of animals		Breed
				All cattle	Calves	
1	140	450	Beef	198	20	Zebu
2	300	260	Beef	370	50	Zebu
3	112	300	Beef	100	12	Zebu
4	770	500	Beef	850	60	Zebu
5	260	600	DP	300	15	H, O, C
6	225	760	DP	186	30	H, O, C, Zebu
7	175	740	DP	300	20	H, O, C
8	56	707	DP	70	8	H, J
9	175	700	DP	260	21	H, O, C, Zebu
10	280	850	DP	434	23	H
11	105	900	DP	200	15	H, O, C, Zebu
12	14	900	DP	45	4	H
13	56	770	DP	170	10	H
14	200	640	Dairy	223	15	H, J
15	1190	50	DP	1400	90	H, O, C, Zebu

DP = dual purpose; H = Holstein; O = other breeds (e.g., Swiss Brown); C = crosses; J = Jersey.
[a]As of December 15, 1993.

group. The time of weaning varied from 3 months to 1 year of age. One farm was a specialised dairy and raised the calves in individual pens with whole milk.

2.2. Sampling techniques

All calves under 3 months of age with signs of diarrhoea were sampled, provided they had not received prior treatment with antibiotics. Diarrhoea was defined according to a scoring system which took into account colour (1 = normal; 2 = abnormally light or dark), smell (1 = normal; 2 = ambiguous; 3 = foul) and consistency (1 = normal; 2 = soft; 3 = watery). Other aspects, such as the presence of blood or particles (of undigested food, blood clots or pieces of intestinal tissue) in the faeces, mucosity and the presence of a dirty tail, hocks or ischiadic processes were also recorded. An animal with an additive score of more than 4 using colour, smell and consistency, was considered a case of diarrhoea. If an animal had a score of 4 and dirty hind parts, it was also considered a case. For each case calf, a corresponding calf with normal faeces, closest in age (with a maximum age difference of 2 weeks), of the same breed and on the same farm was sampled as control. Whenever an animal was sampled, a form was completed with relevant information. Sample size was calculated as the minimum number of calves necessary to detect a difference between 15% (prevalence rate of Rotavirus in calves with diarrhoea; Hird et al., 1990) and 6% (prevalence rate of Rotavirus in calves without diarrhoea; Hird et al., 1990) at a confidence level of 95% and a power of 80%; 179 calves with diarrhoea and 179 controls were needed. Controls were discarded whenever they turned into cases within 1 week of sampling and, consequently, the match was not used in the analysis.

Faecal samples were collected directly from the rectum in disposable plastic bags and stored at 4°C. The marked plastic bags containing the samples were transported in coolboxes on ice to the central laboratory of the Universidad Nacional in Heredia (a 5-h drive) within 2 days of the sampling. The faecal samples were investigated for the presence of agents considered to play an important role in neonatal calf diarrhoea, such as *Escherichia coli* K99, *Salmonella*, Rotavirus, Coronavirus, Torovirus, *Strongyloides* sp., *Strongylida*, *Cryptosporidium* sp. and *Coccidia* spp. (Vermunt, 1994).

2.3. Bacteriological examination

A subsample of faeces using a metal loop with a diameter of 4 mm was placed in a tube with 5 ml of tetrathionate–iodide broth and incubated at 37°C for 24 h. The broth from the tetrathionate–iodide tube was streaked on to a MacConkey, an Eosin Methylene Blue Levin and a *Salmonella shigella* agar and incubated at 37°C. After 24 h, the colonies were identified. All colonies were inoculated on Triple Sugar Iron, Christensen Urea agar, Sulfur Indol Mobility and Simmon's Citrate and identified after incubation for 24 h at 37°C. Thus, *Salmonella* was identified and colonies were further checked by means of a serum agglutination test (Difco).

Colonies identified as *E. coli* were transferred to a Tripto Soya agar tube and after an incubation period of 24 h at 37°C stored at 4°C. To identify the K99-strain, a tube with Minca agar was inoculated and incubated 24 h at 37°C. After that, a serum agglutination test (CDI-DLO, Lelystad, The Netherlands) was done.

2.4. Virological examination

Samples of 4 g of faeces were diluted with 10 ml of PBS, thoroughly mixed using a Vortex-Genie 2® (VWR-Scientific) and centrifuged for 10 min at 2830 g. Two Eppendorf bottles (1.5 ml each) were filled with the supernatant and frozen at −70°C until further investigation. Bovine Coronavirus, Rotavirus and Torovirus were detected by means of a DOT–ELISA as described by Jiménez (1990). Briefly, filtration paper was used to support the nitrocellulose membrane (Transblot® Transfer Medium, Bio-Rad). Before preparing the Bio-dot® microtiter plate (Bio-Rad), the membrane and filter paper were washed in distiled water and all bubbles were removed. The microtiter plate was closed as tightly as possible, washed with carbonate–bicarbonate buffer (pH 9.6) and all the fluid was sucked away with vacuum. Thereafter, each cup was filled with a 100 μl sample, which was thawed at 7°C and centrifuged for 10 min at 8160 g using an Eppendorf centrifuge. The samples were filtered through the nitrocellulose membrane and the membrane was removed from the microtiter plate. Subsequently, the nitrocellulose membranes were blocked with 1% casein in PBS, prepared according to Vogt et al. (1987) and the antisera were added. For Rotavirus, a monoclonal antibody against the P-6 polypeptide (Liprandi et al., 1990) and for Toro- and Coronavirus, a polyclonal monospecific antiserum conjugated to *N*-hydroxysuccinimidobiotin was used (Jiménez, 1990). The membranes were incubated with agitation at room temperature for 90 min and washed three times with PBS 0.05% Tween solution. Thereafter, in the case of Rotavirus, Fab' specific anti-mouse peroxidase conjugate (Sigma) was added and in the

case of Corona- and Torovirus, a commercial avidin-peroxidase conjugate (Sigma) was used. The membranes were incubated for 90 min as described above, washed two times with PBS 0.05% Tween solution and once with PBS. A solution of 4-chlor-1-naphtol (Sigma), prepared according to Voller and Bidwell (1986), was used as substrate. The chromogen was added to the nitrocellulose membranes and read after 5 or 10 min. A clear blue reaction was considered positive. Intensities were not measured.

2.5. Parasitological examination

To investigate the presence of *Cryptosporidium* sp., a qualitative test was done. A smear was prepared, fixed in methanol and coloured for 1 min with Giemsa. The smears were examined by 10×100 magnification (oil immersion).

The qualitative Shearer test was performed to detect *Strongylida* and *Strongyloides* sp. eggs. If this test was positive, the quantitative McMaster method originally described by Gordon and Whitlock (1939) and modified for use in cattle by Roberts and O'Sullivan (1950) was used. In the Shearer test, 4 g of faeces were mixed and homogenised with a hypersaturated solution of sugar. Then hypersaturated sugar solution (gravid density 1.235) was added to approximately 50 ml, the mix was sifted and a Borrel cylinder was filled with the solution until a head of fluid was formed. A microscope slide (5×7 cm) was placed on top of the cylinder and after at least 30 min, the slide was examined under the microscope at an amplification of $100 \times$. This method permitted the detection of oocysts of *Coccidia* as well. A quantitative test for *Coccidia* was not done.

2.6. Statistical analysis

A conditional logistic regression (CLR) for matched sets (Breslow and Day, 1980) was performed. Each matched set consisted of all observations with the same value for the matching variables (farm, breed). The analysis consisted of a 1-to-1 matching with two covariates (parity of the dam and gender of the calf).

3. Results

3.1. Descriptive statistics

Faecal samples were collected from 380 calves, of which 186 showed signs of diarrhoea and 194 were considered healthy. Two-thirds of the investigated animals were female in both (case and control) categories. Thirty-four calves in each group were born from heifers and the remainder were calves from cows with a history of more than one parturition. Most animals sampled were either Holstein ($n = 158$), Zebu ($n = 50$) or crosses ($n = 134$) with some Zebu blood. Sixty-two percent of the calves were females. Farms dedicated to beef production generally had fewer problems with diarrhoea than other farms.

Table 2
Number (and percentage) of samples tested positive for various enteropathogens in animals affected by diarrhoea and healthy calves at farms in the canton of Tilarán

Enteropathogen	Calves with diarrhoea		Healthy calves	
	n	%	*n*	%
Torovirus	194	14	186	6
Coronavirus	194	9	186	1
Rotavirus	194	7	186	2
Salmonella	188	2	179	0.00
E. Coli K99	188	2	179	0.00
Coccidia spp.	190	53	182	66
Strongylida	190	5	182	11
Strongyloides	190	14	182	32
Cryptosporidium	187	11	177	0.00

Salmonella species were detected in only three of the calves with diarrhoea; two of the isolates were identified as *S. derby* and the third could not be identified with the sera available (but was not *S. typhimurium* or *S. dublin*). The K99 antigen was demonstrated in 2% of the calves with diarrhoea and in none of the animals without diarrhoea (Table 2).

Coccidia oocysts were detected frequently in both groups. A total of 23% of the faecal samples tested positive for *Strongyloides* sp. eggs and 8% for *Strongylida* eggs. *Cryptosporidium* oocysts were detected only in animals with symptoms of diarrhoea (Table 2).

No infectious agents were detected in 25% (46 of 181) of samples collected from calves with diarrhoea and in 21% (37 of 175) of the samples from healthy calves. In 32% (58 of 181) of the calves with diarrhoea, two or more pathogens were detected; the same was true in 35% (61 of 175) of the healthy calves. The *Coccidia* eggs and either *Strongylida* and/or *Strongyloides* eggs were more frequent in healthy calves than in calves with diarrhoea (Table 2).

The average age of a scouring calf was 31 days (median 25 days with a range of 1 to 97 days) and for the control animals, 32 days (median 26 with a range of 2 to 99 days). In scouring calves younger than 1 month of age, viruses were detected most frequently, while in older calves parasite eggs were predominantly present. In calves younger than 1 month of age, one agent was detected in 42% of the scouring calves and in 46% of the healthy calves. In relatively few cases of scouring calves older than one month (5 of 42; 12%), no etiological agent could be detected.

3.2. Conditional logistic regression (CLR)

Comparisons between healthy and scouring calves using CLR showed significant differences in odds ratios for the presence or absence of specific enteropathogens (Table 3). The excretion of virus in the faeces of scouring calves was significantly higher than in healthy calves for Torovirus (OR 2.11), Coronavirus (OR 19) and for Rotavirus (OR 4.79, Table 3). In one sick calf, all three viruses were detected. Differences between

Table 3
Results of the conditional logistic regression model to match pairs consisting of the diarrhoeic case and non-diarrhoeic matched control: Tilarán study of infectious agents associated with diarrhoea

Enteropathogen	Odds ratio (OR)	OR 95% confidence interval
Torovirus	2.11	1.02–4.33
Parity[a]	1.74	0.97–3.23
Gender[b]	0.85	0.52–1.39
Coronavirus	19	2.51–147
Parity	2.11	1.13–3.95
Gender	0.83	0.50–1.38
Rotavirus	4.79	1.06–22
Parity	1.79	0.98–3.27
Gender	0.91	0.55–1.48
Salmonella	na	na
Parity		
Gender		
E. coli K-99	na	na
Parity		
Gender		
Coccidia spp.	0.49	0.30–0.80
Parity	1.81	0.98–3.35
Gender	0.84	0.50–1.39
Strongylida	0.28	0.09–0.85
Parity	1.66	0.91–3.08
Gender	0.92	0.55–1.52
Strongyloides	0.18	0.08–0.42
Parity	1.72	0.90–3.29
Gender	0.87	0.51–1.47
Cryptosporidium	na	na
Parity		
Gender		

[a]First parity heifer used as base category.
[b]Male used as base category.
na: not applicable. Odds ratio is an infinite value. None of the control animals tested positive.

animals with and without diarrhoea were statistically significant for each group of helminths and intestinal protozoa investigated (Table 3).

4. Discussion

A case-control study was performed to examine the possible involvement of various enteropathogens in diarrhoea by comparing the presence of these agents in the faeces of scouring and healthy calves younger than 3 months of age.

We have detected Torovirus for the first time in Costa Rica and found it to be the most frequently detected virus in the faeces of scouring calves (Table 2). The virus was discovered in 1979 (Woode et al., 1982) and is widespread among farms in the USA and Europe, while more than 88% of dairy cattle in The Netherlands have circulating antibodies (Koopmans et al., 1989).

Other studies reported the presence of Coronavirus in scouring calves, for example 14% in southern Britain (Reynolds et al., 1986), 4% in Scotland (Snodgrass et al., 1986) and 9% in The Netherlands (Moerman et al., 1982). The presence of Coronavirus in the faecal matter of clinically healthy calves is in agreement with previous reports (Crouch et al., 1985).

In contrast to the higher numbers of samples containing Rotavirus as reported in other countries (Moon et al., 1978; Moerman et al., 1982; Snodgrass et al., 1986; Pohjola et al., 1986), only 7% of the scouring and 2% of the healthy calves investigated in the region of Tilarán excreted Rotavirus.

Not more than 2% of calves showing signs of diarrhoea were infected with *E. coli* K99 in the region of Tilarán. A similar percentage has been reported in previous studies (Reynolds et al., 1986; Snodgrass et al., 1986). *E. coli* enteritis occurs mainly in calves younger than 10 days old (Haggard, 1985). In our study, a total of 16 animals with symptoms of diarrhoea were younger than 10 days of age, while 17 of the controls were less than 10 days old.

Salmonella spp. were detected in 2% of the calves with diarrhoea, which is in agreement with the results of previous studies (Pohjola et al., 1986; Snodgrass et al., 1986; Hird et al., 1990). However, in contrast to previous findings in animals from Poás, Costa Rica (Hird et al., 1990), the species isolated from the calves in Tilarán was *S. derby*.

Infection levels up to 1250 epg were detected for *Strongylida*, which is considered a severe infection (Troncy, 1989). Similarly, high epg levels were detected for *Strongyloides*. These elevated levels could have been caused by management and housing conditions in the region of Tilarán, such as frequency of deworming, hygienic conditions and helminth infestation of calf pastures. In a previous investigation in the regions of Poás and Irazú, which are specialised dairy areas, the infestations detected were few and light (Oviedo et al., 1987).

Cryptosporidium oocysts were detected in 11% of the calves showing signs of diarrhoea and not in any of the healthy calves. This result is comparable to the findings reported in a previous study of Costa Rican calves (Oviedo et al., 1987).

The percentage of scouring calves in which no enteropathogen could be detected was comparable to results of other surveys (Reynolds et al., 1986; Hird et al., 1990). This relatively high percentage might indicate that many cases of diarrhoea were not associated with infectious disease agents, but due to nutritional or other management factors. Alternatively, the failure to detect infectious agents could be due to insufficient sensitivity of the methods of detection, intermittent shedding of pathogens or the existence of other agents that cause diarrhoea but were not investigated.

In the CLR, significant differences were detected between healthy and scouring calves with regard to the presence or absence of enteropathogens. The presence of any of the three investigated viruses did increase the odds for diarrhoea in calves. On the other hand, *Coccidia* spp. and *Strongyloides* had a tendency to be detected more frequently in healthy calves.

Many calves in our investigation were infected with more than one species of potential enteropathogen. All calves infected with Rotavirus in our study were simultaneously infected with *E. coli*, none of which possessed the K99 pilus antigen. However,

somatic antigens or adherence antigens different from K99 are present in *E. coli* strains and can be similarly associated with secretory forms of diarrhoea (Heath, 1992). A combination of Rotavirus and enteropathogenic *E. coli* has been reported to cause a more severe infection than either one of the agents alone (Mebus et al., 1969).

In other studies, the lactation number of the cow was a source of variation in immunoglobulin concentration of the colostrum (Besser and Gay, 1985) and calves born alive of primiparous cows had a higher mortality and morbidity due to diarrhoea than calves born by older cows (Olsson et al., 1993; Perez et al., 1990). However, we found no difference in morbidity between calves of primiparous heifers and cows with a higher number of parturitions (Table 3). The same is true for the difference in morbidity rates in male and female calves. In some reports, morbidity and mortality rates did not differ between gender (Umoh, 1982; Debnath et al., 1990). Others studies showed differences between gender (Olson et al., 1989; Vaccaro, 1990).

Finally, the investigation demonstrated calf morbidity due to enteropathogens on beef and dual-purpose farms in Costa Rica. It is suggested that improvements in hygiene, housing and management should be implemented to alleviate calf morbidity and mortality in the region of Tilarán.

Acknowledgements

We are grateful to Dr. E. Rodríguez and Mr. E. Gómez for their support with the field work, to the laboratory staff in general and to Mr. J. Hernández and Dra. G. Dolz in particular. The initial support from Dr. J. van Amerongen is gratefully acknowledged. We thank Dr. Ferdinando Liprandi from Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas (IVIC) for providing the monoclonal antibody against Rotavirus and Prof. Dr. M.C. Horzinek for providing antisera against Torovirus. Financial support was obtained from the Dutch Ministry of Foreign Affairs through the interuniversity collaborative project between the Universidad Nacional in Costa Rica and the University of Utrecht in The Netherlands.

References

Besser, T.E., Gay, C.C., 1985. Septicemic colibacillosis and failure of passive transfer of colostral immunoglobulin in calves. Vet. Clin. North Am. (Food Anim. Pract.) 1, 445–459.

Breslow, N.E., Day, N.E., 1980. Statistical methods in cancer research. Vol. 1—The Case-control Studies, Lyon IARC, p. 162.

Britney, J.B., Martin, S.W., Stone, J.B., Curtis, R.A., 1984. Analysis of early calfhood health status and subsequent dairy herd survivorship and productivity. Prev. Vet. Med. 3, 45–52.

Cordero, R.R., Osorio, S.M.L., 1988. Factores de riesgo sobre morbilidad y mortalidad en terneras menores de tres meses en tres zonas lecheras de Costa Rica. Thesis, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica, 78 pp.

Crouch, C.F., Ohmann, H.B., Watts, T.C., Babiuk, L.A., 1985. Chronic enteric shedding of bovine enteric coronavirus antigen–antibody complexes by clinically normal cows. Gen. Virol. 66, 1489–1500.

Debnath, N.C., Sil, B.K., Prodhan, M.A.M., Howlader, M.M.R., 1990. A retrospective study on calf mortality and morbidity on smallholder traditional farms in Bangladesh. Prev. Vet. Med. 9, 1–7.

Dwinger, R.H., Cappella, E., Pérez, E., Baaijen, M., Müller, E., 1994. Application of a computerised herd management and production control program in Costa Rica. Trop. Agric. (Trinidad) 71, 74–76.

Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. Council Sci. Industrial Res. (Australia) 12, 50–52.

Haggard, D.L., 1985. Bovine enteric colibacillosis. Vet. Clin. North Am. (Food Anim. Pract.) 1, 495–507.

Heath, S.E., 1992. Neonatal diarrhoea in calves: diagnosis and intervention in problem herds. Comp. Cont. Educ. Pract. Vet. 14, 995–1002.

Hird, D., Pérez, E., Caballero, M., Rodríguez, L., Velázquez, J., 1990. Identification of selected disease agents from calves on Costa Rican tropical cloud-forest dairy farms. Prev. Vet. Med. 9, 221–231.

Jiménez, C., 1990. Vergleichende labordiagnostische Untersuchungen von Kotproben (EM, Virusisolierung, immunologische Verfahren) zum Nachweis einer bovinen Coronavirus-Infektion bei durchfallkranken Kälbern. Vet. Med. Diss. Justus- Liebig-Universität Giessen, Germany, 170 pp.

Koopmans, M., van den Boom, U., Woode, G., Horzinek, M.C., 1989. Seroepidemiology of Breda virus in cattle using ELISA. Vet. Microbiol. 19, 233–243.

Liprandi, F., López, G., Rodríguez, I., Hidalgo, M., Ludert, J.E., Mattion, N., 1990. Monoclonal antibodies to the VP6 of porcine subgroup I rotaviruses reactive with subgroup I and non-subgroup I non-subgroup II strains. Gen. Virol. 71, 1395–1398.

Mebus, C.A., Underdahl, N.R., Rhodes, M.B., Twiehaus, M.J., 1969. Calf diarrhoea (scours) reproduced with a virus from a field outbreak. Univ. Nebr. Agric. Exp. Sta. Res. Bull. 233, 1–16.

Moerman, A., de Leeuw, P.W., van Zijderveld, F.G., Baanvinger, T., Tiessink, J.W.A., 1982. Prevalence and significance of viral enteritis in Dutch dairy calves. XII World Congress on diseases of cattle. The Netherlands. Proceedings, 1: 228–234.

Moon, H.W., McClurkin, A.W., Isaacson, R.E., Pohlenz, J., Skartvedt, S.M., Gillette, K.G., Baetz, A.L., 1978. Pathogenic relationships of Rotavirus, *Escherichia coli*, and other agents in mixed infections in calves. J. Am. Vet. Med. Assoc. 173, 577–583.

Olson, T.A., Elzo, M.A., Koger, M., Butts, W.T., Adams, E.L., 1989. Direct and material genetic effects due to the introduction of Bos taurus alleles into Brahman cattle in Florida: 1. Reproduction and calf survival. J. Anim. Sci. 68, 317–323.

Olsson, S.-O., Viring, S., Emanuelsson, U., Jacobsson, S.-O., 1993. Calf diseases and mortality in Swedish dairy herds. Acta Vet. Scand. 34, 263–269.

Oviedo, M.T., Araya, L.N., Hernández, F., 1987. Agentes bacterianos, parasitarios y virales involucrados en la etiologia de la diarrea de terneros en Costa Rica. Cienc. Vet. (Costa Rica) 9, 29–35.

Oxender, W.D., Newman, L.E., Morrow, D.A., 1973. Factors influencing dairy calf mortality in Michigan. J. Am. Vet. Med. Assoc. 162, 458–460.

Perez, E., 1994. Epidemiological aspects of morbidity, mortality and growth of calves in Costa Rica. PhD thesis. University of Utrecht, The Netherlands, pp. 87–105.

Perez, E., Noordhuizen, J.P.T.M., Van Wuijkhuise, L.A., Stassen, E.N., 1990. Management factors related to calf morbidity and mortality rates. Livest. Prod. Sci. 25, 79–93.

Pohjola, S., Oksanen, H., Neuvonen, E., Veijalainen, P., Henriksson, K., 1986. Certain enteropathogens in calves of Finnish dairy herds with recurrent outbreaks of diarrhoea. Prev. Vet. Med. 3, 547–558.

Reynolds, D.J., Morgan, J.H., Chanter, N., Jones, P.W., Bridger, J.C., Debney, T.G., Bunch, K.J., 1986. Microbiology of calf diarrhoea in southern Britain. Vet. Rec. 119, 34–39.

Roberts, F.H.S., O'Sullivan, P.J., 1950. Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. Aust. J. Agric. Res. 1, 99–102.

Simpson, J.R., Conrad, J.H., 1993. Intensification of cattle production systems in Central America: why and when. J. Dairy Sci. 76, 1744–1752.

Snodgrass, D.R., Terzolo, H.R., Sherwood, D., Campbell, I., Menzies, J.D., Synge, B.A., 1986. Aetiology of diarrhoea in young calves. Vet. Rec. 119, 31–34.

Troncy, P.M., 1989. Helminths of livestock and poultry in tropical Africa. In: Manual of Tropical Veterinary Parasitology. CAB International, Wallingford, pp. 33–61.

Tzipori, S., 1981. The aetiology and diagnosis of calf diarrhoea. Vet. Rec. 108, 510–514.

Umoh, J.U., 1982. Relative survival in calves in an University herd in Zaria, Nigeria. Br. Vet. J. 138, 507–514.

Vaccaro, L.P., 1990. Survival of European dairy breeds and their crossess with zebus in the tropics. Anim. Bre. Abst. 58, 475–494.

Vanopdenbosch, E., Pohl, P., 1993. Infectieuze kalverdiarree: therapie en preventiemogelijkheden op basis van etiologische, pathofysiologische en immunologische gegevens. Tijdschr. Diergeneesk. 118, 731–734.

Vermunt, J.J., 1994. Rearing and management of diarrhoea in calves to weaning. Aust. Vet. J. 71, 33–41.

Vogt, R.F., Phillips, D.L., Henderson, L.O., Whitfield, W., Spierto, F.W., 1987. Quantitative differences among various proteins as blocking agents for ELISA microtiter plates. J. Immunol. Meth. 101, 43–50.

Voller, A., Bidwell, D., 1986. Enzyme-linked immunosorbent assay. In: Rose, N.R., Friedman, H., Fahey, J.L. (Eds.), Manual of Clinical Laboratory Immunology. American Society for Microbiology, Washington DC, pp. 99–109.

Woode, G.N., Reed, D.E., Runnels, P.L., Herrig, M.A., Hill, H.T., 1982. Studies with an unclassified virus isolated from diarrhoeal calves. Vet. Microbiol. 7, 221–240.