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AGENTS ASSOCIATED WITH NEONATAL DIARRHOEA IN ETHIOPIAN DAIRY CALVES

G. ABRAHAM, P. L. ROEDER[1] and ROMAN ZEWDU

Shola Disease Investigation Laboratory, Ministry of Agriculture, PO Box 7491, Addis Ababa, Ethiopia

SUMMARY

Faeces samples collected from diarrhoeic dairy calves in the first 8 weeks of life were examined for the presence of 5 enteropathogens. The majority of the 108 diarrhoea cases occurred in the first 5 weeks of life and a commercial ELISA kit detected bovine enteric coronavirus (BEC) in 38·9%, serogroup A rotavirus (RV) in 16·7% and K99 (F5) fimbrial adhesin-positive Escherichia coli *(K99 ETEC) in 11·1 per cent. Concurrent infections of these enteropathogens were detected in 14·8% of samples (30·8% of samples positive for these agents). No evidence of cryptosporidial infection was found using a differential staining method on faecal smears nor was salmonella excretion detected. On 2 of the 8 farms only BEC was present; the other 6 farms were positive for all 3 agents. It is concluded that BEC is the major infectious cause of neonatal calf diarrhoea in the Ethiopian dairy herds studied with RV and K99 ETEC also contributing to morbidity, either alone or as mixed infections.*

INTRODUCTION

In Ethiopia neonatal diarrhoea is an important cause of calf morbidity and mortality.

Until recently, lack of sophisticated laboratory facilities precluded a study of the aetiological agents involved in all but the more developed countries. However, recent advances in immunoassay technique have provided specific and sensitive tools which can be used where only basic facilities exist.

Elsewhere it has been shown that the aetiology of neonatal calf diarrhoea is complex, one component being infectious agents including rotavirus (RV), bovine enteric coronavirus (BEC), K99 fimbrial adhesin-positive enterotoxigenic *Escherichia coli* (K99 ETEC), cryptosporidia and salmonella occurring alone or in combination (Reynolds *et al.*, 1986; Snodgrass *et al.*, 1986).

The main focus of the study reported here was to determine the prevalence of these agents in diarrhoeic dairy calves in highland Ethiopia.

MATERIALS AND METHODS

Source of samples

Samples of diarrhoeic faeces were collected from dairy calves up to 8 weeks of age during periodic visits made to dairy farms over the course of 14 months. An additional 4 samples were derived from a clinic adjacent to Shola Laboratory. The source farms lie at altitudes between 1,900 and 2,600 m above sea level within 50 km of Addis Ababa.

Husbandry practices varied but calves were given access to their dams' colostrum by suckling or by being bucket fed. Once removed from their dams calves were bucket fed milk from the herd and later supplemented with concentrate feed and hay.

[1] Address for correspondence: 45, Old Portsmouth Road, Camberley, Surrey GU15 1JJ, UK.

The calves were predominantly Friesian/Holstein with a small proportion of zebu (mainly Boran) blood in some except for one farm of Jersey cattle.

Detection of disease agents

On receipt faecal smears were prepared, air dried and heat fixed (also methanol fixed in early studies) for subsequent examination for cryptosporidia by a modified Ziehl-Neelsen differential staining technique (Henriksen and Pohlenz, 1981). Smears were stained with concentrated carbol fuchsin for 25 min, rinsed in tap water, differentiated for 1·5 min in 5% sulphuric acid, rinsed in tap water and counterstained with 5% malachite green for 3 min before rinsing again in tap water and air drying. Slides were examined with a high power (× 40) dry lens and a × 100 oil immersion lens for the presence of cryptosporidia. Control positive slides were processed similarly to confirm staining efficacy. Faeces were then deep frozen pending further examination in batches, usually within one month.

Batches of faecal samples were thawed and examined in a 3 component sandwich antigen detection enzyme-linked immunosorbent assay (ELISA)[1] which employs monoclonal antibodies (MAbs) for capture and detection. The MAbs have unique specificities for the serogroup A antigen of RV, a common antigen of BEC and the K99 (F5) fimbrial adhesin of ETEC, thus detecting the major known microbial pathogens associated with calf diarrhoea (Thorns *et al.*, 1992). In brief, 10% suspensions of faeces in phosophate buffered saline (0·01 M, pH 7·6: PBS) were each applied to 3 wells of modular polystyrene ELISA plates precoated with capture MAbs. Positive and negative control antigen preparations were included in each test. After reaction for 30 min at ambient temperature (20 to 28°C) the plates were emptied and washed 5 times in PBS by filling the wells from a wash bottle and shaking out; they were then tapped dry on absorbent paper. Colour-coded preparations of the 3 horseradish peroxidase-labelled MAb conjugates were then added to appropriate wells from dropper bottles. After 15 min at ambient temperature the plates were washed as before and tapped dry. The chromogen, tetramethyl benzidine, and substrate, urea peroxide, were added to wells from dropper bottles and the plates were tapped gently to mix the reagents. After 15 minutes the controls were inspected and the test results were read by visual inspection for the development of blue colouration indicative of a positive result.

Faecal samples detected as K99-positive by ELISA were cultured on 5% sheep blood agar. After overnight growth 6 putative *E. coli* colonies were tested by a MAb based latex agglutination card test[2] (Thorns *et al.*, 1989) for the presence of K99 fimbrial adhesin.

Samples were tested for the presence of salmonella by overnight enrichment in sodium selenite broth followed by aerobic culture on MacConkey and desoxycholate citrate agar plates.

RESULTS

Detection of enteropathogens

The detection of enteropathogens is summarised in Table I together with the sources of the samples examined; 104 samples were derived from large dairy farms and 4 samples were received from the clinic, being derived from small urban dairy holdings.

[1] Pathasure Bovine Enteritis, Cambridge Veterinary Sciences, Ely, Cambridge, UK.
[2] Fimbrex K99, Central Veterinary Laboratory, Weybridge, UK.

TABLE I

Sources of faecal samples and detection of enteropathogens

Source of samples farm/clinic	Breed	Number of samples	Agent detected	BEC	RV	K99 ETEC
Adaberge	J	14	9	8	4	2
Debre Zeit	Fx	14	4	4	0	0
Holetta	Fx	23	13	10	5	3
Kurifto	Fx	9	4	2	1	1
Mulo	Fx	5	2	2	0	0
Sellale	Fx	8	6	5	3	2
Stella	Fx	31	13	11	4	4
Clinic	Fx	4	1	0	1	0
Total		108	52	42	18	12
Percentage			48·2	38·9	16·7	11·1

J = Jersey, Fx = Friesian/Holstein cross zebu.

Although control slides were clearly identified as containing cryptosporidia, none were present in the 108 faeces samples examined. Excretion of salmonella was not detected. Enteropathogens were detected in 52 of the 108 samples (48·2%) examined with 36 (33·3%) being single infections.

BEC was the dominant enteropathogen detected being present in 42 of 108 samples (38·9%), representing 80·8% of positive samples. In 26 of these calves (24·1% of samples; 61·9% of positive samples) BEC was the only agent detected; in the remaining 16 calves BEC was present in combined infections with RV (7 calves), K99 ETEC (5 calves) or both RV and K99 ETEC (4 calves) (Table II).

RV infections were detected in 18 calves (16·7%) representing 34·6% of positive samples. RV was the only agent detected in 7 calves, the remainder of RV infections being combined with BEC or both BEC and K99 ETEC (see above). The combination of RV and K99 ETEC was not encountered.

K99 ETEC was present in 12 calves (11·1%) representing 23·1% of positive samples. Only in 3 calves was this a single infection uncomplicated by intercurrent infection; 5 were in combination with BEC and 4 with both BEC and RV. Attempts were made to confirm 11 of these identifications by culture; 10 were successful. One

TABLE II

Occurrence of multiple infections

Source of samples farm/clinic	Number of samples	BEC alone	RV alone	K99 ETEC alone	BCV + RV	BCV + K99 ETEC	BCV + RV + K99 ETEC
Adaberge	14	4	0	1	3	0	1
Debre Zeit	14	4	0	0	0	0	0
Holetta	23	6	3	0	1	2	1
Kurifto	9	2	1	1	0	0	0
Mulo	5	2	0	0	0	0	0
Sellale	8	2	1	0	1	1	1
Stella	31	6	1	1	2	2	1
Clinic	4	0	1	0	0	0	0
Total	108	26	7	3	7	5	4
Percentage		24·1	6·5	2·8	6·4	4·6	3·7

TABLE III

Prevalence of enteropathogens at different ages

	Number (%) of samples positive at each week of life							
	1	2	3	4	5	6	7	8
Sample number	33	14	23	13	9	3	5	8
Samples +ve	21(63·6)	8(57·1)	14(60·9)	4(30·8)	2(22·2)	0	0	3(37·5)
BEC	17(51·5)	6(42·9)	11(47·8)	4(30·8)	2(22·2)	0	0	2(25·0)
RV	10(30·3)	6(42·9)	1 (4·3)	0	0	0	0	1(12·5)
ETEC	7(21·2)	0	4(17·4)	0	0	0	0	1(12·5)

sample from a 6 day old calf was not examined and one isolate from a 3 day old calf could not be confirmed because of autoagglutination. The last was from a farm where K99 ETEC was confirmed in other calves on the same occasion and previously.

Concurrent infections of 2 or more of BEC, RV and K99 ETEC were present in 14·8% of samples (30·8% of samples positive for these agents).

Occurrence of enteropathogens on individual farms

BEC was present alone on 2 farms; BEC, RV and K99 ETEC were present on all of the other 6 farms.

RV was the only enteropathogen detected in the clinic samples and only one of the 4 samples was positive.

Age distribution of infections

Table III summarises the proportions of samples positive for each enteropathogen during each week of life.

Cases of diarrhoea were concentrated in the first 5 weeks of life with 85·2% of samples being derived from this period. The overall detection rate declined during this period until, in weeks 6 and 7, enteropathogens were not detected. However, all agents were again present in 3 of the 8 samples collected from calves in the eighth week of life; these were one single BEC infection, one single RV infection and one combined BEC and K99 ETEC infection.

Considering only the first 5 weeks of life, BEC was detected in 17 of 33 calves (51·5%) in the first week of life being present in 81·0% of positive samples. The prevalence of BEC remained at a similarly high level for the next 2 weeks, at week 2 being 42·9% (75·0% of positive samples) and at week 3 being 47·8% (78·6% of positive samples). It was the only agent detected in samples taken during the fourth and fifth week of life, being present in 30·8% and 22·2% of samples, respectively. In the first 3 weeks of life BEC infections were present alone in 24·2%, 14·3% and 39·1% of samples, representing 38·1%, 25·0% and 64·3%, respectively, of positive samples (47·1%, 33·3% and 81·8%, respectively, of BEC infections). Rotavirus infections were mainly restricted to the first 2 weeks of life being detected in 10 of 33 samples (30·3%) and 6 of 14 samples (42·9%), respectively, accounting for 47·6% and 75·0% of positive samples, respectively. Approximately a third of these were uncomplicated by intercurrent infections (30·0% and 33·3%, respectively, of RV infections). By week 3 RV infection was detected in only one of the 23 samples examined (4·3%). K99 ETEC was present in 7 of 33 samples (21·2%) at week one and 4 of 23 samples (17·4%) at week 3, being present in 33·3% and 28·6% of positive samples, respectively. Single K99 ETEC infections were present in 3·0% and 8·7% of all samples, 4·8% and

14·3% of positive samples and 14·3% and 50·0% of K99 ETEC-positive samples at weeks one and 3, respectively.

DISCUSSION

BEC was the predominant enteropathogen associated with diarrhoea, occurring either alone or in combination with other enteropathogens. The prevalence rate of BEC (38·9% of all samples and present on all farms visited) was considerably higher than the 8·5% and 14% recorded in the UK by Snodgrass *et al.* (1986) and Reynolds *et al.* (1986) respectively, but was of the same order as that reported from continental Europe (Wellemans *et al.*, 1977; Woode *et al.*, 1978) and North America (Marsolais *et al.*, 1978; Langpap *et al.*, 1979; Morin *et al.*, 1980). This enteropathogen is considered to be an important cause of diarrhoea in calves, rarely being found in healthy animals (Moon *et al.*, 1978; Reynolds *et al.*, 1986). BEC appears to have been the major enteropathogen causing diarrhoea in this study.

In contrast, RV infections were detected less frequently than in previous studies, at a prevalence rate of 16·7% compared to the 42% and 50% reported by Reynolds *et al.* (1986) and Snodgrass *et al.* (1986), respectively. RV is also considered to play an important role in the pathogenesis of calf enteritis although the presence of virus shedding in symptom-free calves (Snodgrass *et al.*, 1986) and avirulent strains of RV (Bridger, 1988) complicates diagnostic interpretation. Being present in only a relatively small proportion of samples and in over 60% of these being associated with BEC and/or K99 ETEC, RV did not appear to be a dominant prime pathogen in the present study although it might be expected to have exacerbated the effects of concurrent infections. This effect has been demonstrated experimentally with RV and K99 ETEC (Snodgrass *et al.*, 1982) but this combination was not encountered in the present study nor in that reported by Snodgrass *et al.*, (1986).

In other studies (Snodgrass *et al.*, 1986; Reynolds *et al.*, 1986) K99 ETEC was recognised to be an important cause of diarrhoea in the first 6 days of life. In the present study, 7 of 12 cases were also in calves less than 6 days of age in keeping with the classical syndrome recognised, although 6 were associated with BEC and 5 of these with RV. However, 4 isolates were from calves in the third week of life (all confirmed by culture) and one was in a calf aged 8 weeks (also confirmed by culture). The significance of these isolations from older calves is unknown. However, the sensitivity of the K99 ELISA is such that it gives positive results only when K99 ETEC are present at clinically significant levels of more than 10^7 bacteria per gram of faeces (C. J. Thorn, pers. comm.) and thus these results should also have been clinically significant.

The absence of cryptosporidia in this study was surprising since it was regularly encountered in other studies (Reynolds *et al.*, 1986; Snodgrass *et al.*, 1986) and is an important enteropathogen (Tzipori, 1985); its absence requires verification.

It was expected that salmonella infections would be found, since in earlier studies in Addis Ababa both *Salmonella dublin* and *S. typhimurium* were reported as causes of disease in calves (Pegram *et al.*, 1981). The potential for salmonella infections to cause morbidity therefore, should not be discounted.

It was not possible to obtain comprehensive data from all farms to estimate the contribution of calf enteritis to calf mortality. However, on 4 farms (Adaberge, Mulo, Kurifto and Holetta) it was possible to arrive at a reasonable estimate; overall mortality during the period studied amounted to 245 of 640 calves (38·3%) of which 93 cases (14·5%; 38·0% of mortality) were attributed to enteritis on clinical grounds. At Adaberge farm 135 of 203 Jersey calves died (66·5%) and of these 71 cases (35·0%

of mortality) were attributed to enteritis on clinical grounds. BEC, RV and K99 ETEC were demonstrated on this farm and evidence for the important role played by these enteropathogens derives from the response to vaccination. When late gestation vaccination of cows with BEC, RV and ETEC vaccines[3] was introduced following this study, overall mortality fell to approximately 12% (Paulos Wolde-Semait, pers. comm.).

Thus, there appears to be an association between the agents detected and diarrhoea in the first 5, and particularly the first 3, weeks of life. However, the poor detection rate thereafter suggests that the cause in this age group must be sought elsewhere, possibly in nutritional and husbandry factors (the majority of samples were also examined for coccidial oocysts without evidence of a causal role).

Care should be exercised in drawing too many conclusions from this relatively small study, but it does appear that in central Ethiopian dairy farms cryptosporidial infection is not the problem that it is in temperate climates, and that BEC plays a dominant role in causing enteritis supported by RV and K99 ETEC.

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AGENTS ASSOCIES A UNE DIARRHEE NEONATALE CHEZ DES VEAUX DE LAIT EN ETHIOPIE

Résumé—Des échantillons de fèces, collectés sur des veaux de lait dans les huit premières semaines de leur vie, ont été examinés afin de rechercher la présence de 5 germes entéropathogènes. La majorité des 108 cas de diarrhée a été constatée au cours des cing premières semaines de vie. Un nécessaire du commerce pour le test ELISA a permis la détection d'un coronavirus entérique bovin (BEC) dans 38,9 p.100 des cas, un rotavirus du groupe A (16,7 p.100) et une réaction positive à l'adhésine fimbriée K99 (FS) d'*Escherichia coli* (K99 ETEC) dans 11,1 p.100 des cas. Des infections simultanées impliquant ces différents entéropathogènes ont été trouvées dans 14,8 p.100 des prélèvements dont 30,8 p.100 provenaient des prélèvements positifs vis-à-vis de ces agents. Aucune preuve d'une infection cryptosporidienne n'a pu être décelée à l'aide d'une méthode de coloration différentielle sur des étalements fécaux, non plus qu'une excrétion de salmonelles. Pour deux des huit fermes, seul le BEC était présent. Les six autres exploitations étaient positives aux trois autres agents. Les auteurs en concluent que le coronavirus entérique ou intestinal bovin (BEC) est l'agent infectieux majeur de la diarrhée néonatale des veaux dans les troupeaux laitiers éthiopiens étudiés; un rotavirus et *Escherichia coli* K99 ETEC ont contribué, par ailleurs à la morbidité, soit seuls, soit dans des affections associées.

AGENTES ASOCIADOS CON DIARREA NEONATAL EN TERNEROS ETIOPES DE LECHERIAS

Resumen—Se examinaron muestras fecales provenientes de terneros diarreicos, en las primeras ocho semanas de vida, para detectar cinco enteropatógenos. La mayoría de los 108 casos de diarrea, ocurrieron en las primeras cinco semanas de vida y una prueba ELISA commercial, detectó coronavirus entérico bovino (CEB) en 38·9%m rotavirus serogropo A (RV) en 16·7% y K99 (F5) *Escherichia coli* fimbrial-adhesiva positiva (K99 ETEC) en 11·1 por ciento. Infecciones concurrentes con éstos enteropatógenos, se detectaron en el 14·8% de muestras (30·8% de las muestras positivas para éstos agentes). No se detectó criptosporidia o salmonela. En dos de las ocho fincas se detectó CEB solamente; las otras seis fincas fueron positivas para todos los tres agentes. Se concluye, que el CEB es la causa principal de diarreas en animales jóvenes en las lecherías estudiadas, con el RV y K99 ETEC, contribuyendo a la tasa de mortalidad, solos o en infecciones mixtas.