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Pathology of calves with diarrhoea in southern Britain

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**Twenty-one moribund calves with diarrhoea were
purchased from 11 farms, their faeces examined for
enteropathogens and samples of intestinal tissue
removed under anaesthesia. Lesions and presence of
enteropathogens on the mucosal surface were scored
by histological examination of immunostained
paraffin sections. Two or more enteropathogens were
detected in 19 calves. Cryptosporidium appeared to
be the principal cause of diarrhoea in six calves, rota-
virus in four, *Salmonella typhimurium* in two,
bacteria adherent to the surface of the large intestine
in two, coronavirus in one and K99 + *Escherichia coli*
in one calf. Diarrhoea in four calves was the con-
sequence of mixed infections in which no one entero-
pathogen appeared to predominate. In one calf no
enteropathogen was detected. Diarrhoea was asso-
ciated with infections and lesions throughout the
small and large intestines. The enteropathogens most
frequently associated with lesions in the small
intestines were rotavirus, coronavirus and crypto-
sporidium; in the large intestines they were corona-
virus and bacteria apparently adherent to the mucosal
surface.**

THE aetiology of neonatal calf diarrhoea is complex;
many infectious agents, singly or in combination,
have been associated with field outbreaks (Acres et al
1975, Morin et al 1976, Reynolds et al 1986,
Snodgrass et al 1986) and non-infectious factors con-
tribute to the disease process (Roy 1980). The
infectious agents thought to contribute significantly
are rotavirus, coronavirus, cryptosporidium, K99 +
Escherichia coli and *Salmonella* species, and the
importance of these agents has been investigated
recently by means of a survey of the microbiology of
calf diarrhoea in southern Britain (Reynolds et al
1986). During the survey, 21 moribund calves with
diarrhoea were purchased and examined post mortem
with the following objectives: to describe the
pathology of the natural disease and to investigate
diagnostic problems by studying the relationship
between the lesions and infectious agents.

The lesions detected in eight of the 21 calves, which
were infected with rotavirus, have been described in a
preliminary report (Hall et al 1986).

Materials and methods

Animals

Twenty-one calves with diarrhoea were purchased
from 11 of the 34 outbreaks surveyed (Reynolds et al
1986) (Table 1). The mean age of the calves was 11·4
days (range one to 23) and the mean duration of
diarrhoea before necropsy was 2·9 days (range 0·5 to
14). Several breeds and crossbreeds were represented
within the group which contained males and females
in almost equal numbers. Some calves had been
treated with antibiotics. Faeces were collected from
all calves on the day of necropsy and from seven
calves on one or two occasions during the week before
necropsy. They were examined for rotavirus and
coronavirus by enzyme-linked immunosorbent assay,
for cryptosporidium by microscopical examination of
faecal smears stained by the Giemsa method, for
K99 + *E coli* by slide agglutination tests of 10 colonies
per faecal sample and for *Salmonella* species by
enrichment of faeces in Rappaport and selenite-
brilliant green broths and subculture to brilliant green
agar (Jones et al 1983). Five grams of faeces, prepared
for electron microscopy by differential centrifugation
followed by negative staining, were examined for
Newbury agent (Bridger et al 1978).

Necropsy

Intestinal tissues for microscopy were removed
under pentobarbitone sodium anaesthesia. Short
lengths (10 cm) of small intestine (SI), referred to as
duodenum and SII to SI9, and four lengths (5 cm) of
spiral colon were ligated and filled with fixative; fixa-
tives used were mercuric formol, a fixative comprising
nine parts saturated mercuric chloride and one part
glacial acetic acid, 12 per cent neutral buffered
formalin and 0·1 M phosphate buffered 3 per cent
glutaraldehyde, pH 7·3. Duodenum was sampled
adjacent to the pylorus, SII close to the ligament of
Treitz and SI9 adjacent to the ileocaecal junction.
Sites SI2 to SI8 were spaced as equally as possible
between sites 1 and 9.

The calves were killed by intravascular injection of
an overdose of anaesthetic and the sacs excised and,

TABLE 1: Enteropathogens detected in faeces ante mortem and in histological sections post mortem; examinations not made ante mortem for the enteropathogens in brackets. Faeces were collected on the day of necropsy

Calf	Age (days)	Farm of origin	Duration of diarrhoea (days)	Enteropathogens detected in Faeces ante mortem	Mucosa post mortem
1	19	I	4	R, S	R, (A)
2	4	II	2	R	R, (A)
3	10	III	<1 ·	R	R
4	10	IV	5	Cr	Cr, (A), R
5	4	V	1	R	R, (A)
6	20	VI	1	—	R, Cv, (A)
7	9	VII	3	R	R, Cr (N)
8	5	III	1	R	R, (A)
9	15	VI	4	*	Cv, (A), Cr
10	11	VI	2	R	R, Cv, (A), Cr
11	15	VI	4	Cr	Cr, (A), Cv
12	17	VI	3	—	Cv, (A), Cr
13	9	VIII	4	†	Cv
14	10	IX	2	Cr	Cr, R, Cv, (A)
15	11	VI	1	Cv, Cr	Cv, (A), Cr
16	7	VII	1	Cr, S	Cr, S, (A)
17	23	VI	1	Cv, S	Cv, S
18	10	VI	5	R	Cr, (N)
19	1	X	<0·5	E	E, (N)
20	9	III	2	—	Cr, Cv
21	21	XI	14	—	—

R Rotavirus
Cv Coronavirus
N Newbury agent
S *Salmonella typhimurium*
E K99 + *E coli*
A Adherent bacteria
Cr Cryptosporidium
— No enteropathogen detected
* Rotavirus detected in faeces four days before necropsy
† Rotavirus and coronavirus detected in faeces three days before necropsy

together with other samples, immersed in fixative (Table 2). After fixation for 24 hours the mercuric formol and the mercuric chloride and acetic acid mixture were replaced with 80 per cent alcohol.

Enteropathogen scores

The immunostaining methods used were an indirect immunoperoxidase method with primary antisera to bovine rotavirus, bovine coronavirus, Newbury agent SRV-1 and purified K99 adhesin prepared in calves and rabbits (Parsons et al 1984) and the peroxidase-antiperoxidase method (Sternberger et al 1970) with primary antisera to atypical *E coli* (S102-9) (Hall et al 1985). To obtain the enteropathogen scores the extent of infection of the cytoplasm or surface of enterocytes in the small and large intestines by enteropathogenic viruses, bacteria (salmonellae excluded) or protozoa, seen in a histological cross-section of intestine was assessed subjectively and scored as follows:

0 — no enteropathogen-infected enterocytes detected;
1 — at least one enteropathogen-infected enterocyte detected;

2 — scattered enteropathogen-infected enterocytes detected;
3 — many enteropathogen-infected enterocytes detected;
4 — majority of enterocytes infected with enteropathogens; and
5 — all enterocytes infected with enteropathogens.

The sum of the scores of enteropathogens detected at a site was divided by five (the number of enteropathogens scored) to give a mean enteropathogen score for each site. Calves infected with *Salmonella typhimurium* were excluded from the calculations because in salmonellosis, surface infection comprises only part of the disease process and severe lesions may result from infection in the lamina propria.

Lesion scores

To obtain lesion scores, one section from each of the 10 small intestinal sites was scored on a scale from 0 to 5 for the following 11 lesions: villus stunting, villus fusion, presence of degenerate cells in the lamina propria, presence of leucocytes in lacteals, cuboidal enterocytes, exfoliated enterocytes, vacuo-

TABLE 2: Details of tissues collected and their fixation, staining and examination

Tissue	Fixation			
	Mercuric formol	Mercuric/acetic	Formalin	Glutaraldehyde
Duodenum, S12, S14, S16,				
SI7, SI8	+	−	−	−
SI3	+	+	+	−
SI1, SI5, SI9;				
caecum, colon	+	+	+	+
Abomasum+, rectum, mesenteric lymph nodes*,				
lung, liver, gall bladder	+	−	+	−
Kidney, spleen	−	−	+	−
	HE. Immunostain: Rotavirus Coronavirus K99 *E coli* *E coli* S102-9	Immunostain: Newbury agent	HE. Giemsa: for bacteria and *Cryptosporidium* species	Scanning and transmission electron microscopy

+   Three samples
*   Three samples corresponding to sites SI1, SI5 and SI9
SI   Small intestine
HE   Haematoxylin and eosin

lated enterocytes, abnormally arranged enterocytes and presence of neutrophilic polymorphonuclear leucocytes in the lamina propria, crypts and intestinal lumen. One section of caecum, colon and rectum was scored similarly for the following five lesions: exfoliated enterocytes, abnormally arranged enterocytes and presence of neutrophils in the lamina propria, crypts and intestinal lumen. A maximum score of 55 could be allocated to each small intestinal site and 25 to each site in the large intestine. Scores at 10 sites in the small intestines of 15 calves, nine sites from three calves, eight sites from one calf and six sites from two calves were pooled to derive the contribution of each lesion type to the overall pathology. Similarly, in the large intestines, scores from three sites from 17 calves, two sites from three calves and one site from one calf were pooled. Intestinal tissues which were not scored and non-intestinal tissues were examined and abnormalities which were detected were recorded.

To gain an overall impression of which parts of the intestinal tract were most severely damaged, the sum of the lesion scores at each site was divided by the number of calves in which that site was examined, to give a mean lesion score; the mean lesion score at each site was also calculated, excluding the three salmonella-infected calves.

Statistical method

For statistical analysis mean lesion scores and the mean of all enteropathogen scores were calculated for upper (SI1 to SI3), middle (SI4 to SI6) and lower (SI7 to SI9) small intestine and large intestine (caecum, colon and rectum); salmonella-infected calves were excluded from the analysis. Correlations were calculated between the two scores at each region.

Electron microscopy

When histological examination revealed the presence of a lesion or infection which required further investigation, intestinal tissues fixed in glutaraldehyde were examined by scanning electron microscopy and transmission electron microscopy. Pieces of intestine were stored in the original glutaraldehyde at 4°C until required and processed for examination in transmission and scanning electron microscopes (Hall et al 1976, 1985).

Results

Detection of enteropathogens

Faeces were not examined ante mortem for Newbury agent or for bacteria apparently adherent to the mucosal surface of the large intestine, and detections of these agents have been excluded from comparisons of results of faecal and mucosal examinations. Detection of enteropathogens by examination of faeces or the intestinal mucosa gave identical results in nine of the calves (2, 3, 5, 8, 15, 16, 17, 19 and 21) (Table 1). More enteropathogens were detected by mucosal examination than by faecal examination in nine calves (4, 6, 7, 9, 10, 11, 12, 14 and 20); the opposite was true for two calves (1 and 13) and in another (18) rotavirus was detected in the faeces and coronavirus in the mucosa. Combined faecal and mucosal examinations detected four enteropathogens in two calves, three in nine calves, two in seven, a single enteropathogen (rotavirus) in one calf and no enteropathogens in another.

Rotavirus

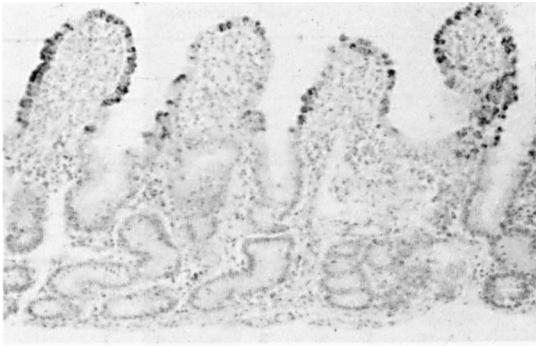In one calf (3) of 13 rotavirus-infected calves, rota-

FIG 1: Small intestine of calf 7, in which infection by rotavirus and Newbury agent was detected. The majority of the villous enterocytes are rotavirus-infected and villi are stunted and fused. Immunoperoxidase

virus was the only enteropathogen detected by mucosal examination (Table 1). High rotavirus scores were recorded throughout the small intestine and infection was present in the large intestine (Table 3); in this calf diarrhoea appeared to have been caused by rotavirus infection alone. In five of the remaining 12 rotavirus-infected calves, one additional enteropathogen was detected by mucosal examination, in four calves two additional enteropathogens and in three calves three additional enteropathogens were detected. Bacteria, apparently adherent to the surface of the large intestine, were seen in nine rotavirus-infected calves, coronavirus in five, cryptosporidium in six and Newbury agent in two. In three rotavirus-infected calves (2, 5 and 7), despite the presence of other enteropathogens, the severity (Fig 1) and extent (Table 3) of rotavirus infection in the small intestine suggested that rotavirus was the important aetiological agent. Lesions detected in the small intestines of calves in which rotavirus appeared the sole or principal cause of diarrhoea (2, 3, 5 and 7) were identical to those described in natural (Pearson et al 1978, Reynolds et al 1985) and experimental (Mebus and Newman 1977) infections. Severe infection and lesions were distributed widely in the small intestines of these calves (Fig 1, Table 3).

Coronavirus

Coronavirus was detected in 10 calves by either faecal or mucosal examination; by both methods in three calves, and by mucosal examination alone in seven calves. In one calf (13) high coronavirus scores were present throughout the small and large intestines and coronavirus was the only enteropathogen detected by mucosal examination; in this calf, diarrhoea appeared to have been caused by coronavirus infection. The lesions in the small and large intestines of this calf were identical to those described

in natural (Langpap et al 1979) and experimental (Mebus et al 1973) infections. In the nine calves remaining, coronavirus was either absent from the small intestines (five calves), or coronavirus scores in the small intestines were low (four calves). In these nine calves other enteropathogens were detected which probably contributed to the disease process; one additional enteropathogen was detected by mucosal examination in two of the nine calves, two additional enteropathogens in five calves, and three additional enteropathogens in two of the nine calves. Bacteria were detected apparently adherent to the surface of the large intestine in seven calves. Cryptosporidium was detected by mucosal examination in seven of the nine, rotavirus in three and *S typhimurium* in one.

Cryptosporidium

Cryptosporidium was detected in 11 calves by either faecal or mucosal examination, by both methods in four calves, and by mucosal examination alone in seven calves. In six calves (4, 12, 14, 16, 18 and 20) mucosal examination showed infection with cryptosporidium to be severe and extensively distributed in mid and lower small intestines (Table 3), suggesting that it was the principal cause of diarrhoea. Infection with cryptosporidium was restricted largely to the small intestines of these six calves and the lesions were similar to those described previously (Pohlenz et al 1978, Pearson et al 1982, Sanford and Josephson 1982). In all 11 calves other enteropathogens, which could have contributed to the disease process, were detected by mucosal examination. One additional enteropathogen was detected by mucosal examination in two of the 11 calves, two additional enteropathogens in seven calves and three additional enteropathogens in two calves. Bacteria were detected apparently adherent to the surface of the large intestines of eight of the 11 cryptosporidium-infected calves. Coronavirus was detected by mucosal examination in seven of the 11, rotavirus in four Newbury agent in two and *S typhimurium* in one.

Adherent bacteria

Bacteria, apparently adherent to the surface of the large intestines, were detected by mucosal examination in 13 calves, always in association with one or more additional enteropathogens which could have contributed to the disease process. Faeces were not examined ante mortem for these bacteria because their presence was not suspected until mucosae were examined post mortem. One additional enteropathogen was detected by mucosal examination in four of the 13 calves, two additional enteropathogens in seven of the 13 calves and three additional entero-

TABLE 3: Summary of lesion and enteropathogen scores* in six intestinal sites from 21 calves with diarrhoea

Le and P	Calf	D	U	M	Lo	Ca	Co	Calf	D	U	M	Lo	Ca	Co
Le	1†	3	10	17	17	3	3	12	0	1	10	8	8	9
R		0	2	1	0	0	0		0	0	0	0	0	0
Cv		0	0	0	0	0	0		0	0	0	0	1	0
A		0	0	1	1	1	0		0	0	0	0	3	3
Cr		0	0	0	0	0	0		0	1	3	2	0	0
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	2	0	0	3	3	3	2	13	0	9	13	13	5	8
R		0	1	3	2	1	1		0	0	0	0	0	0
Cv		0	0	0	0	0	0		2	4	5	5	3	5
A		0	0	0	1	1	1		0	0	0	0	0	0
Cr		0	0	0	0	0	0		0	0	0	0	0	0
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	3	0	12	11	2	0	0	14	2	10	8	10	0	2
R		0	4	3	2	0	1		0	1	0	1	1	1
Cv		0	0	0	0	0	0		0	0	0	0	1	1
A		0	0	0	0	0	0		0	0	0	0	2	3
Cr		0	0	0	0	0	0		0	0	3	3	0	2
N		0	.	0	0	0	0	0		0	0	0	0	0	0
Le	4	0	4	10	9	3	5	15	3	6	6	8	8	12
R		0	0	0	1	0	1		0	0	0	0	0	0
Cv		0	0	0	0	0	0		1	0	0	1	4	4
A		0	0	0	0	2	2		0	1	0	0	3	4
Cr		0	2	3	4	0	0		0	0	2	3	0	0
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	5	0	13	17	17	8	6	16	0	0	6	7	3	8
R		0	3	3	3	0	1		0	0	0	0	0	0
Cv		0	0	0	0	0	0		0	0	0	0	0	0
A		0	0	0	1	3	3		0	0	0	0	1	2
Cr		0	0	0	0	0	0		0	0	3	3	0	0
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	6	0	1	0	1	5	6	17†	0	9	15	30	14	10
R		0	0	0	0	0	1		0	0	0	0	0	0
Cv		0	0	0	0	0	2		0	0	0	0	3	3
A		0	0	0	0	2	4		0	0	0	0	0	0
Cr		0	0	0	0	0	0		0	0	0	0	0	0
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	7	0	11	10	9	0	0	18	0	0	8	9	3	2
R		0	3	3	1	0	0		0	0	0	0	0	0
Cv		0	0	0	0	0	0		0	0	0	0	0	0
A		0	0	0	0	0	0		0	0	0	0	0	0
Cr		0	0	0	1	0	0		0	0	3	3	0	0
N		0	1	1	1	1	1		0	2	0	0	0	0
Le	8	0	6	15	7	8	7	19‡	1	1	13	7	2	0
R		0	1	3	1	0	0		0	0	0	0	0	0
Cv		0	0	0	0	0	0		0	0	0	0	0	0
A		0	0	0	1	1	1		0	1	1	4	3	0
Cr		0	0	0	0	0	0		0	0	0	0	0	0
N		0	0	0	0	0	0		0	1	0	0	0	0
Le	9	0	7	3	1	7	9	20	0	9	7	9	3	5
R		0	0	0	0	0	0		0	0	0	0	0	0
Cv		0	0	0	0	0	2		0	0	1	1	1	1
A		0	0	0	0	3	3		0	0	0	0	0	0
Cr		0	0	0	2	0	0		0	0	2	4	0	1
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	10	2	4	1	3	2	2	21	0	1	2	4	5	4
R		0	0	1	1	2	0		0	0	0	0	0	0
Cv		2	0	0	0	1	0		0	0	0	0	0	0
A		0	0	0	0	3	2		0	0	0	0	0	0
Cr		0	0	0	3	0	0		0	0	0	0	0	0
N		0	0	0	0	0	0		0	0	0	0	0	0
Le	11	0	5	12	13	5	5							
R		0	0	0	0	0	0							
Cv		0	0	0	0	1	3							
A		0	0	0	1	2	3							
Cr		0	0	0	1	0	0							
N		0	0	0	0	0	0							

Le Lesions, P Pathogens, R Rotavirus, Cv Coronavirus, A Bacteria adherent to the mucosal surface, Cr Cryptosporidium, N Newbury agent, D Duodenum, U Upper small intestine, M Mid small intestine, Lo Lower small intestine, Ca Caecum, Co Colon

* At each site, the maximum score possible was 55 for lesions in the small intestine, 25 for lesions in the large intestine and five for each pathogen. Values in upper, mid and lower small intestine are means of values from small intestine sites 1 to 3, 4 to 6 and 7 to 9, respectively

† High lesion scores attributed to infection with *S typhimurium*, which was not scored

‡ Adherent bacteria were K99⁺ *E coli*

FIG 2: Scanning electron micrograph of colonic mucosa of calf 12, in which adherent bacteria were detected in the large intestine. Bacteria are attached to the surface of enterocytes and there are exfoliated cells and mucus; the microvilli are absent or reduced in number and disorganised
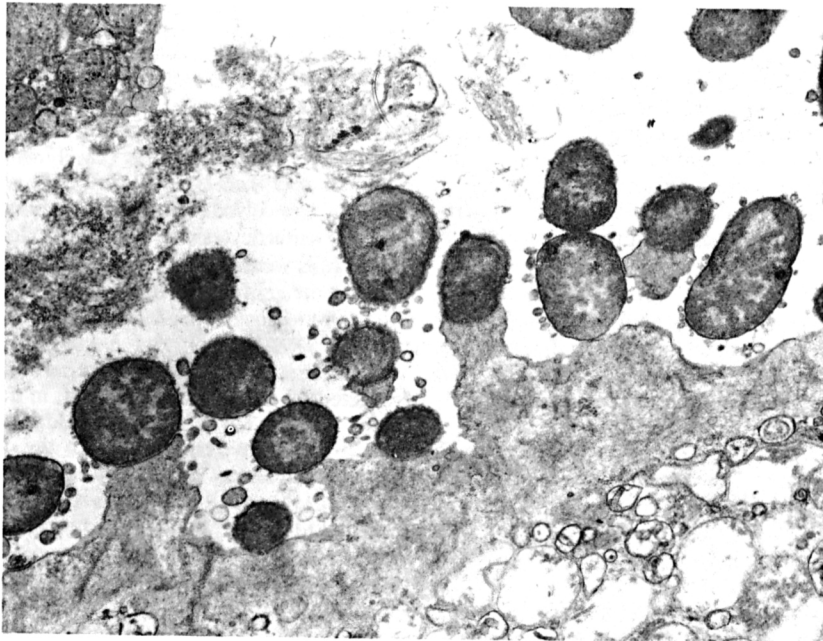

FIG 3: Transmission electron micrograph of the caecal mucosa of calf 6, in which adherent bacteria were detected in the large intestine. The bacteria, which did not immunostain with antiserum to *E coli* (S102-9) are associated closely with the enterocyte surface membrane, the microvilli are effaced and the terminal web is absent. Bacteriophages are associated with the bacteria

pathogens in two calves. Rotavirus was detected in eight calves, coronavirus in seven, cryptosporidium in eight and *S typhimurium* in one calf. In two calves (6 and 9), the surface of the large intestine was infected severely and the severity of infection of the small and large intestines by other enteropathogens was slight; in these calves the adherent bacteria appeared to be the principal cause of diarrhoea. Villi in the small intestines of these calves (6 and 9) were slightly stunted and some were fused, but these lesions could have been caused by enteropathogenic viruses which were detected in these calves. Lesions seen in the large intestines were disarrangement and exfoliation of surface enterocytes and the lamina propria was infiltrated by neutrophils. Light and electron microscopic examination of the mucosal surfaces of the large intestines of the 13 calves, in which bacteria were visible adherent to the mucosal surface, revealed variation in the nature of the bacterial colonisation and in the severity of the lesions. In seven calves (1, 6, 9, 10, 11, 12 and 15), electron microscopic examination revealed that the bacteria were closely attached to the surface of enterocytes (Fig 2), often by means of 'cups' or 'pedestals', and microvilli were effaced (Fig 3). In the remaining six calves (2, 4, 5, 8, 14 and 16) the bacteria appeared, by light microscopy, to be adherent to the mucosal surface but examination by transmission electron microscopy revealed that, although closely associated with the musocal surface, they were not attached to the surface of enterocytes; they were detected most frequently in folds and crevices in the mucosa (Fig 4). In some calves, bacteria formed part of an exudate together with mucus and exfoliated cells. The lesions detected in both groups were similar and comprised disarranged enterocytes, exfoliated enterocytes (Fig 5) and infiltration of the lamina propria by neutrophils; lesions varied in severity. Scanning electron microscopy revealed that in some animals bacteria were associated closely with enterocytes which were disarranged and exfoliated, but in other animals bacteria were not adherent to the abnormal enterocytes although bacteria were associated with adjacent cells.

Histological sections from the large intestines of the 13 calves in which bacteria were visible apparently adherent to the mucosal surface, were stained by the immunoperoxidase method (Parsons et al 1984), with a primary antiserum raised against *E coli* (S102-9) which had been identified as a cause of dysentery in neonatal calves (Hall et al 1985). In seven calves in which there were lesions identical to those caused by S102-9 the bacteria were stained in five calves (1, 9, 10, 11 and 15) but not in two calves (6 and 12).

Lesions were not detected in the large intestines of two of the eight calves in which bacteria were not associated with the mucosal surface of the large intestines (3 and 7); the lesions detected in four of the
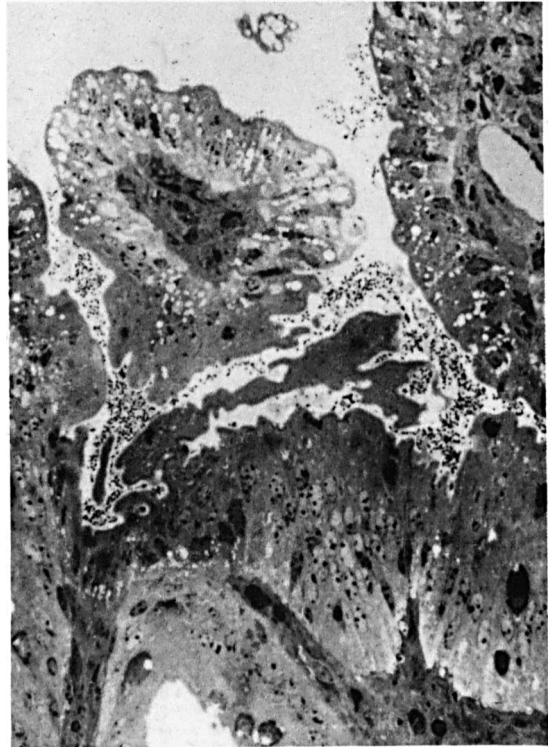

FIG 4: Caecum of calf 5, in which adherent bacteria were detected in the large intestine. The mucosal surface is irregular and enterocytes are disarranged. Many bacteria are present, not adherent to the enterocyte surfaces but associated closely with the mucosal surface in folds and crevices. Toluidine blue

remaining six calves appeared to be the result of infection with coronavirus (13, 17 and 20), cryptosporidium (20), *S typhimurium* (17) or K99+ *E coli* (19). In two calves (18 and 21), there was disarrangement of enterocytes and some had exfoliated; enteropathogens were not detected.

Newbury agent

Enterocytes infected with Newbury agent were detected by mucosal examination, in the intestines of three calves (7, 18 and 19); additional pathogens which could have contributed to the disease process were also detected. *Cryptosporidium* was detected in one calf (18) and K99+ *E coli* in another (19). In the third calf (7), infection with Newbury agent extended throughout the small and large intestine but it was not possible to conclude that Newbury agent was a causal agent because the calf was infected extensively with rotavirus and cryptosporidium. Nevertheless, lesions in the small intestines of the three calves were identical to those described in calves infected experimentally with Newbury agent (Hall et al 1984).

FIG 5: Scanning electron micrograph of the surface of the caecal mucosa of calf 4, in which adherent bacteria were detected in the large intestine. The surface of the mucosa is ridged and many degenerate enterocytes appear to be exfoliating from the ridges. Bacteria are associated with the enterocyte surfaces in the grooves between the ridges

Salmonella typhimurium

S typhimurium was detected in three calves by either faecal or mucosal examination, by both methods in two calves and by faecal examination alone in one. Mucosal examination detected other enteropathogens in three calves; coronavirus in one (17), cryptosporidium and bacteria apparently adherent to the surface of the colon in another (16) and rotavirus and *E coli* (S102-9) adherent to the surface of the small and large intestine in a third (1). The extent of infection and the nature of the lesions in two calves (1 and 17) suggested that *S typhimurium* was the principal cause of diarrhoea. Villi in the small intestine of these calves were stunted and fused, enterocytes had exfoliated and remaining enterocytes were cuboidal and disarranged. Neutrophils were numerous in the lamina propria, crypts (numerous crypt abscesses were seen) and intestinal lumen.

Escherichia coli

K99$^+$ *E coli* were detected, by faecal and mucosal examination, in one calf (19). Newbury agent was detected also in the small intestinal mucosa of this calf, but the severity and extent of infection of the small intestinal surface by *E coli* suggested that this agent was the principal cause of diarrhoea. The lesions in the small intestine of this calf comprised mild enterocyte exfoliation and slight stunting and fusion of villi, which could have been caused by Newbury agent (Hall et al 1984), and infiltration of

the lamina propria by neutrophils. Lesions were not detected in the large intestines.

Mixed infections

Lesions were detected in the small intestines of four calves (8, 10, 11 and 15) in which no one enteropathogen appeared to play a major role; several enteropathogens were detected and bacteria, apparently adherent to the surface of the large intestines, were present. The lesions in these calves comprised stunted and fused villi covered by dis-
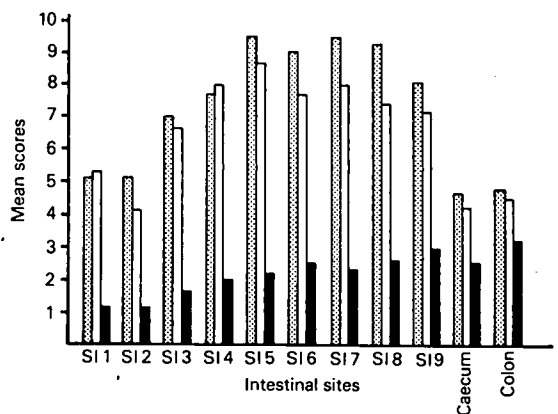

FIG 6: Mean lesion scores ▩ at nine small intestinal sites, caecum and colon, in 21 diarrhoeic calves and mean lesion □ and mean pathogen scores ■ in 18 diarrhoeic calves (Salmonella-infected calves excluded)

arranged and cuboidal enterocytes; exfoliated enterocytes were present. In the large intestines of these calves there were disarranged and exfoliated enterocytes on the mucosal surface and mild infiltration of the lamina propria and crypts by neutrophils.

Enteropathogen scores

Mean enteropathogen scores (Fig 6) increased along the small intestine and were highest in the colon. The reason for high scores in the lower small intestine and the large intestine was the presence of multiple infections; coronavirus, rotavirus and cryptosporidium in the small intestine, and coronavirus and bacteria apparently adherent to the mucosal surface in the large intestine. The duodenum appeared resistant to infection by all enteropathogens except coronavirus, which was detected in the duodenum of three calves (10, 13 and 15).

Lesion scores

Lesions were detected in the small intestines of all calves (Table 3) and scores at sites 5 to 9 were higher than those at sites 1 to 4 (Fig 6); lesions were detected rarely in the duodenum. Lesions were detected in the large intestines of 19 of the 21 calves (Table 3) and in nine of these calves (5, 6, 8, 9, 12, 13, 15, 16 and 17) severe lesions were seen (Table 3). The contribution of the 11 lesions scored in the nine small intestinal sites, as a percentage of the overall pathology was: stunted villi 23 per cent, fused villi 18 per cent, cuboidal enterocytes 15 per cent, disarranged enterocytes 12 per cent, exfoliated enterocytes 12 per cent, neutrophils in the lamina propria 6 per cent, vacuolated enterocytes 5 per cent, neutrophils in crypts 4 per cent, neutrophils in the gut lumen 2 per cent, degenerate cells in the lamina propria 2 per cent and cells in lacteals 1 per cent. The contribution of the five lesions scored in the large intestine as a percentage of the overall pathology was: exfoliated enterocytes 35 per cent, disarranged enterocytes 30 per cent, neutrophils in the lamina propria 20 per cent, neutrophils in crypts 9 per cent and neutrophils in the lumen 6 per cent.

Lesion scores and pathogen scores were correlated significantly. Examination of each region separately indicated correlations between 0·53 and 0·59, which were significant statistically; P<0·05 in upper and lower small intestine and large intestine and P<0·01 in the mid small intestine.

Discussion

Detection of infectious agents in faeces before death, and in the intestinal mucosa after death, was more helpful in understanding the aetiology of diarrhoea than faecal examination alone. With the combined approach, cases where rotavirus, coronavirus, enterotoxigenic E coli, Salmonella species and cryptosporidium appeared to be the sole or principal causative agent were identified, although mixed infections were more common than single infections. Lesions caused by these infections were recognised as identical to those described in natural and experimental infections (Jubb et al 1985) and in outbreaks of undifferentiated neonatal diarrhoea in North America (Morin et al 1976, 1980, Moon et al 1978). Previous authors recommended that outbreaks should be investigated by the combined use of bacteriological and virological methods, together with histological and immunofluorescent studies of fresh intestinal tissue from diarrhoeic calves. This study has confirmed the value of histological examination and shown the value of immunocytochemical examination of intestinal tissue removed under anaesthesia from diarrhoeic calves. The particular advantage of the immunoperoxidase method was that it allowed evaluation of mucosal infection and lesions in one histological section of paraffin-embedded tissue. The finding that enteropathogens were detected more frequently by immunocytochemical examination of tissues than by microbiological examination of faeces does not lessen the value of faecal examination. Examination of faeces from several diarrhoeic calves from an outbreak, and faeces from contemporary normal calves on the same farm, is a valuable investigative procedure (Reynolds et al 1986). Tissue examination may be helpful in instances where faecal studies have not resulted in a diagnosis.

Pathogen and lesion scores were correlated significantly overall, although in one calf (2) high pathogen scores were detected in association with low lesion scores and in some calves lesions were present where enteropathogens were absent or present in small amounts. It is probable that in these latter calves the lesions were the result of a preceding infection by an enteropathogen; also the possibility exists in all calves that lesions could have been caused by unrecognised enteropathogens. Evaluation of infection and lesions in numerous intestinal sites revealed the severity and extent of infection and lesions in the small and large intestines. It is assumed that the cumulative effect of different infectious agents, present in the calf at the same time, infecting and damaging different parts of the intestinal tract caused loss of digestive and absorptive function sufficient to result in diarrhoea. The concept of an additive effect on intestinal structure and function by enteropathogens infecting different parts of the alimentary tract, is supported by the results of an earlier study of the lesions in the intestines of clinically normal rotavirus-infected calves (Reynolds et al 1985), in which the methods

used to score infection and lesions were similar to those used in this study. In rotavirus-infected calves which did not have diarrhoea, lesions and infection were severe in the upper small intestine and mild in the lower small intestine; and the large intestines were virtually undamaged. In these calves it is probable that normal absorptive function in the lower small intestine and large intestine compensated for rotavirus-induced malabsorption in the upper small intestine; this hypothesis was proposed previously to explain why experimental infection of colostrum-deprived calves with rotavirus caused only a mild diarrhoea (Logan et al 1979). In the diarrhoeic calves in this study, where lesions caused by mixed infections occurred throughout the intestines, it is proposed that absorptive capacity was overwhelmed.

Analysis of the contribution of each lesion type to the overall pathology in the small intestine revealed lesions common to several infectious agents. Regardless of infectious agent, disarrangement and exfoliation of enterocytes, with a change to stunted and fused villi covered by cuboidal enterocytes, were the principal lesions. In calves infected with cryptosporidium, cuboidal enterocytes were especially prevalent, while in calves infected by *S typhimurium*, neutrophils were numerous in the lamina propria, in crypt abscesses and in the intestinal lumen. It was possible, however, even in the absence of immunocytochemistry, to make a presumptive diagnosis of the cause of diarrhoea by histological examination of lesions in the small intestine. Cryptosporidium and enterotoxigenic *E coli* were recognised on the mucosal surface following staining by the Giemsa method and *S typhimurium* infection was suspected by the presence of crypt abscesses and neutrophils in the lamina propria and intestinal lumen. The possibility of confusion between the lesions produced by *Salmonella* species and enteropathogenic *E coli* remains because both cause infiltration of the mucosa with neutrophils. Lesions induced by enteropathogenic *E coli* have been described as occurring primarily in the large intestine with little involvement of the small intestine (Hall et al 1985). However, a strain which causes severe lesions in the small intestine has been recognised (G. R. Pearson, personal communication) and in this study a mixed infection of the small intestine with *S typhimurium* and an *E coli* serologically identical to the enteropathogenic strain *E coli* (S102-9) was detected in one calf (1). Remaining cases were assumed to be virus-induced but the association of lesions with a particular virus could only be made by immunostaining for viral antigen.

Three types of infection which could have caused lesions were detected frequently in the mucosa of the large intestine; bacteria apparently adherent to the surface, coronavirus and rotavirus. In rotavirus-infected calves only a few enterocytes were infected in the large intestine and infection was not thought to have caused lesions. Previous studies of rotavirus infections in calves, which have examined the distribution of rotavirus-infected cells using the immunofluorescence technique on cryostat sections, have failed to detect rotavirus-infected enterocytes in the large intestine (Mebus and Newman 1977, Pearson et al 1978, Jubb et al 1985). The detection of rotavirus-infected enterocytes in the large intestines of calves in this study is probably the result of a greater sensitivity shown by the immunoperoxidase method. Six calves were affected by bacteria adherent to the mucosal surface, in the absence of coronavirus infection, and bacterial infection resulted in disarranged and exfoliated enterocytes, together with neutrophils in the lamina propria. In two calves, coronavirus had caused characteristic lesions (Mebus et al 1973). Bacteria apparently adherent to the surface of the large intestine of diarrhoeic calves have not been recognised in neonatal calf diarrhoea except in a dysentery of calves caused by *E coli* (S102-9) (Hall et al 1985).

In the present study five of the 13 calves in which bacteria were detected adherent to the large intestinal mucosa were found, by immunoperoxidase staining, to be infected by *E coli* (S102-9). Four of the calves originated from one farm and this was the farm on which the outbreak of calf dysentery had occurred (Hall et al 1985); the fifth calf originated from a separate outbreak. Two other calves were examined from the farm on which the former outbreak of calf dysentery occurred. Lesions were detected in the large intestines of these calves which were indistinguishable from those described in association with enteropathogenic *E coli* (Hall et al 1985), but the bacteria were not *E coli* (S102-9). The nature of the adherent bacteria in the other seven calves is unknown, and their role in the pathogenesis of diarrhoea is unclear. It is possible that, because they always occurred in association with other enteropathogens, their presence was the result of an altered bacterial flora, following diarrhoea caused by another enteropathogen, or by the use of antibiotics. Alternatively, they may have contributed to the pathogenesis of diarrhoea by colonising the mucosal surface of the large intestine, causing lesions and inhibiting absorptive function. The lesions with which they were associated were identical in nature to those seen in the four calves infected by the confirmed enteropathogen *E coli* (S102-9) (Hall et al 1985), although the severity was variable; this suggests a pathogenic role for these unidentified bacteria which may be confirmed by isolation and identification of the bacteria involved and studies of experimental infections.

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