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DYSENTERY IN CALVES CAUSED BY AN ATYPICAL STRAIN OF *ESCHERICHIA COLI* (S102-9)

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ABSTRACT

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Dysentery lasting 4—8 days was produced in five 4-day-old colostrum-fed calves, after inoculation with an atypical strain of *Escherichia coli* S102-9; peak excretion of S102-9 occurred during the period of dysentery. Two calves were killed when clinical signs were most severe and bacteria were seen attached to the surfaces of enterocytes in the large intestine; microscopic lesions were seen in these areas. The lesions were identical to those previously reported in a natural outbreak of dysentery in calves, from which *E. coli* S102-9 was isolated, and to those seen in gnotobiotic calves experimentally infected with S102-9. Reinfection of the three surviving calves 16—20 days later with S102-9 and primary infection of two calves aged 24 and 51 days did not cause dysentery. Four of 659 coliforms isolated from field outbreaks of calf diarrhoea resembled the atypical strain S102-9. These four isolates and S102-9 did not produce heat-stable enterotoxin, but all produced a toxin cytopathic for Vero and HeLa cells. Two of the four isolates were inoculated alone into 4-day-old gnotobiotic calves deprived of colostrum; neither calf developed dysentery but microscopic lesions identical to those produced by S102-9 were detected in the large intestines of both animals.

INTRODUCTION

An atypical strain of *Escherichia coli* (designated S102-9) was isolated from the faeces and intestinal contents of farm calves aged 8—21 days old during an outbreak of dysentery (Hall et al., 1985) and reproduced the disease after experimental infection of gnotobiotic calves (Chanter et al., 1984). The distinctive characteristics of S102-9 included an atypical colony morphology on MacConkey agar, production of urease and anaerogenicity, but it was identified as *E. coli* in particular by its abilities to produce acid in MacConkey broth at 44°C and indole at 44°C. S102-9 was shown by an immunoperoxidase method to adhere to the mucosae of the large bowel in

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gnotobiotic and farm calves with dysentery, and microscopic lesions were seen in the colonic and rectal mucosae (Hall et al., 1985). These were identical to those seen in piglets (Moon et al., 1983), man (Rothbaum et al., 1982) and rabbits (Takeuchi et al., 1978) infected with *E. coli* which do not produce the classical enterotoxins and which are not invasive.

The objectives of the experiments reported here were firstly to establish the pathogenicity of *E. coli* S102-9 for conventional calves, secondly, to determine whether *E. coli* with atypical characteristics were an important cause of calf diarrhoea by examining a culture collection of 659 isolates from field outbreaks of diarrhoea, and thirdly, to investigate by experimental infection of gnotobiotic calves, the association between the atypical characteristics of these isolates and pathogenicity. Electron microscopy was used to examine more precisely lesions produced by atypical *E. coli* and to compare them with those described previously.

MATERIALS AND METHODS

Experimental infection of conventional calves with E. coli *S102-9*

Experimental design

Five Friesian cross calves were infected with *E. coli* S102-9 and compared with two uninfected calves for signs of dysentery. Two of the five infected calves were killed when clinical signs were maximum to investigate the presence of lesions. Immunity and age resistance to infection were investigated by allowing the other three calves to recover and then reinfecting them and comparing the results with two primarily infected calves, one age matched and one 27 days older.

Calves

The nine calves were left with their dams for 12 h, then moved to a loose box, separate from other calves, and fed on a milk replacer diet. On the fourth day, the calves were moved to rooms in an isolation unit; they were fed sterilised canned milk. Infected and control animals were housed in separate rooms. Calves were inoculated orally after the morning feed; 5 ml of sterilised canned milk (Carnation Ltd.) containing $5.0 \times 10^9 - 2.3 \times 10^{10}$ colony forming units (cfu) of *E. coli* grown on bovine blood agar at 37°C for 18 h, was given by syringe. Calves 1—5 were infected when 4 days old; Calves 4 and 5 were killed on days when clinical signs were judged to be most severe. Calves 1—3 were allowed to recover and then reinfected when 20, 22 and 24 days old, respectively. Calves 6 and 7 were infected for the first time at 24 and 51 days old respectively and were controls for the reinfection of Calves 1—3. Calves 8 and 9 were housed together until 42 and 41 days of age, respectively. Faeces were sampled daily. Blood samples were taken at 4 days, at reinfection and daily from calves with dysentery. Rectal temperatures were measured twice daily.

Microbiology

The strain of atypical *E. coli* S102-9 (O5:K⁻:H⁻) was used (Chanter et al., 1984).

Rotavirus and coronavirus were detected by enzyme linked immunosorbent assays of faecal samples taken daily (Reynolds et al., 1984) and calicivirus by electron microscopic examinations of faeces taken on the first day of illness (Bridger and Hall, 1979).

Smears of faeces stained with Giemsa were examined for cryptosporidial oocysts on the first and last day of illness.

E. coli were enumerated in scrapings of mucosa from the ileum, caecum, colon and rectum, and in faeces and intestinal contents as described previously (Chanter et al., 1984). The mucosae were washed, scraped off with a glass slide and ground to make 10^{-1} suspensions in saline. One gram of the mucosal suspensions, intestinal contents or faeces were serially diluted 10-fold in saline and 0.1 ml of appropriate dilutions spread on to MacConkey agar plates (Mackie and MacCartney, 1953) in triplicate before incubation at 37°C for 18 h. *E. coli* g^{-1} were enumerated according to the dilution of sample and the number of lactose fermenting colonies per dilution. Atypical *E. coli* were initially identified by their characteristic colonies on MacConkey agar; these had a red centre, a clear outer zone and the surrounding medium was clear. Isolates from experimental infections were occasionally tested for anaerogenicity and production of urease. Faeces taken from calves aged 2 and 4 days and at onset of diarrhoea or dysentery were examined for salmonellae (Jones et al., 1983).

Pathology

Tissues were fixed, processed and examined for the presence of lesions as described previously (Hall et al., 1985).

Immunoperoxidase staining methods

Paraffin sections of mercuric formol fixed tissue were stained by an indirect immunoperoxidase method with primary antisera to bovine rotavirus prepared in calves (Parsons et al., 1984) or by a peroxidase-antiperoxidase method (Sternberger et al., 1970) with primary rabbit antisera to live *E. coli* S102-9 produced by the method of Sojka (1965).

Detection of serum immunoglobulins

Immunoglobulins were detected in calf serum taken at 4 days of age by the zinc sulphate turbidity test (McEwan et al., 1970).

Detection of agglutinins of E. coli *S102-9*

Serum samples were serially diluted in 0.1-ml volumes of isotonic saline in a U-bottomed microtitre tray. A suspension of *E. coli* S102-9 (0.1 ml equivalent to Browns tube No. 4) was added to each well and to wells con-

taining saline alone. Tests were incubated at 37°C for 1 h followed by 18 h at 4°C. Rabbit antisera to live *E. coli* S102-9 was used as a positive control.

Detection and characterisation of atypical E. coli *in a culture collection*

Bacteria

During a survey of field cases of calf diarrhoea in southern England during the winters of 1981/82 and 1982/3 isolates that grew on MacConkey agar (Mackie and MacCartney, 1953) at 37°C and fermented lactose, were subinoculated into vials of ferrous metabisulphate pyruvate (FBP) semi-solid medium (Oxoid) and were frozen at −20°C. Stored cultures (total 659) were revived by scraping frozen medium from vials onto trypticase glucose extract (TGX) agar (Orskov et al., 1975), subsequently incubated at 37°C for 24 h. *E. coli* strains K12 and B41 (0101:K99) were used as controls in characterisation tests.

Biochemical characterisation of bacteria

Tests described by Cowan and Steel (1974) were used. *E. coli* B41 was used as a typical control.

Serotyping of E. coli

Rabbit antisera to live *E. coli* S102-9, produced by the method of Sojka (1965), was used in a slide agglutination test. Further typing of somatic antigens was kindly undertaken by Dr. B. Rowe at the Central Public Health Laboratory, Colindale, London.

Haemagglutination test

The microtitre method of Burrows et al. (1976) was used. Tests were incubated at 4°C or 37°C in the presence or absence of 1% D-mannose.

Cytotoxin test

E. coli isolates which were to be tested for production of cytotoxin were inoculated into 10 ml of Evans medium (Evans et al., 1973) which was incubated at 37°C for 24 h and shaken at 150 r.p.m. A 0.1-ml aliquot of this culture was subinoculated into a second Evans medium and treated in the same way as the first. Cultures were then centrifuged at 1000 × *g* for 20 min and the supernatant fractions filtered through an 0.45-μm Millipore membrane and the filtrate tested for the presence of toxin. Aliquots of 180 μl of 10^5 Chinese hamster ovary (CHO) or HeLa cells ml^{-1} or 2×10^5 Vero cells ml^{-1} were placed into all wells of a flat-bottomed microtitre tray (Intermed, Nunc); 20 μl of filtrate was placed in a duplicate set of wells and serially diluted. Microtitre trays were sealed and incubated in a CO_2 cabinet at 37°C for 3 days. Cells were stained with a mixture of equal volumes of 0.2% (w/v) crystal violet and 12% (v/v) neutral buffered formalin for 1 h, washed, dried at 37°C and examined.

Detection of heat-stable enterotoxin

Toxin was prepared in Evans medium and tested in the suckling mouse test (Guinee et al., 1981).

Detection of fimbriae

Bacteria were washed from an overnight culture on either 5% sheep blood agar or TGX medium with phosphate buffered saline (pH 7.2) and dropped onto Formvar/carbon-coated grids. After 30 s, excess fluid was removed with filter paper. The grids were rinsed in distilled water and then stained with 2% (w/v) phosphotungstate (pH 7.0), excess stain removed, air-dried and examined in a Philips 300 electron microscope.

Experimental infection of gnotobiotic calves with atypical E. coli *isolates 37/1 or 6/193*

Bacteria

Two out of four atypical isolates from the culture collection were selected for experimental infections. *E. coli* 37/1 had been isolated from a calf with diarrhoea from which no other enteropathogen was detected and *E. coli* 6/193 had been isolated from a calf which did not have diarrhoea.

Gnotobiotic calves

Gnotobiotic calves were derived by the methods of Dennis et al. (1976). One was infected with *E. coli* 37/1 and another with *E. coli* 6/193.

Infection of calves, pathology and immunoperoxidase staining methods

These methods were as described for the experimental infection of conventional calves with *E. coli* S102-9.

RESULTS

Experimental infection of conventional calves with E. coli *S102-9*

The occurrence of dysentery in calves and the excretion of enteropathogens are summarised in Table I. All five calves (1—5) infected with *E. coli* S102-9, at 4 days of age, developed dysentery, whereas older calves (6—7) infected for the first time or reinfected (1—3), and control calves (8—9) did not. The dysentery was characterised by the presence in faeces of bright, fresh blood (sometimes copious) and large amounts of clear or faeces-stained mucus. The faecal consistency was soft or semi-liquid.

Peak excretion of S102-9 ($>10^8$ cfu g^{-1} of faeces) occurred during signs of dysentery in all 5 calves (1—5) infected at 4 days of age. In contrast only 2 (1 and 2) of the 5 older calves (1—3, 6 and 7) excreted these numbers; this occurred on the first day after reinfection.

Rotavirus or coronavirus were excreted by Calves 1—3, but not at times

TABLE I

Infection of conventional calves with *E. coli* S102-9

Calf no.	Age (days)				
	Infection	Occurrence of dysentery	Excretion of S102-9	Excretion $>10^6$ cfu g^{-1}	Excretion of other agents
1	4 and 20	5—9	5—12, and 21	5, 9 and 21	24—26[a]
2	4 and 22	6—9	5—11 14—16 23—25	5—9 and 23	13, 15 and 17[a]
3	4 and 24	5—6 8 13—20	5—23 25—29	6—9 13—18	1 and 2[a] 10[b]
4	4	6—8[c]	5—8	6—7	7—8[a]
5	4	7—8[c]	5—8	8	7—8[a]
6	24		25—30		
7	51		52—57		
8					13—18[a]
9					12—16[a]

[a]Rotavirus.
[b]Coronavirus.
[c]Killed at 8 days.

when dysentery was seen. Calves 4 and 5, infected at 4 days of age and necropsied at the peak of dysentery (8 days), were excreting rotavirus whereas control calves (8—9) excreted rotavirus at times when mild diarrhoea was seen. Salmonellae, cryptosporidia and calicivirus were not detected.

Pyrexia or bacteraemia did not occur consistently during the period of dysentery. Prior to infection, the serum of calves contained widely different amounts of immunoglobulin (2—33 zinc sulphate turbidity units). Prior to infection the amount of serum immunoglobulins of Calf 3 was the lowest recorded. Calf 3 alone responded to infection by the production of agglutinins to *E. coli* S102-9 (titre of 10), and this calf had the longest period of dysentery and the longest period of excretion of S102-9.

In Calves 4 and 5, necropsied at the peak of dysentery, *E. coli* S102-9 colonised the colonic and rectal mucosae in approximately equal or greater numbers than in the contents. In contrast *E. coli* of other types colonised the mucosae in fewer numbers than in the contents (Table II). The mean number of S102-9 in these mucosae was $\log_{10}$ 7.9 g^{-1} whereas for other *E. coli* it was $\log_{10}$ 6.6 g^{-1}. The same distribution of *E. coli* was seen in the caecum of Calf 4. *E. coli* of all types colonised the mucosae of the small intestine in lower numbers than in the contents.

Petechial haemorrhages were seen in the rectal mucosae of Calves 4 and 5; all of the rectal mucosa of Calf 4 and the longitudinal folds of Calf 5 were markedly reddened. The same changes were seen in the colon of Calf 4 and

TABLE II

Colony forming units g^{-1} (log_{10}) in necropsy samples of calves infected with *E. coli* S102-9

	Calf 4		Calf 5	
	Typical *E. coli*	Atypical *E. coli*	Typical *E. coli*	Atypical *E. coli*
Caecum contents	9.0	7.9	7.3	6.9
Caecum mucosa	7.4	8.0	7.3	6.5
Colon 1 contents	9.6	7.7	<6.5	<6
Colon 1 mucosa	7.9	8.5	<6.5	<6
Colon 2 contents	8.3	8.7	6.5	7.2
Colon 2 mucosa	7.9	8.5	<6	7.4
Colon 3 contents	8.0	8.7	8.0	7.7
Colon 3 mucosa	6.7	8.6	<6	7.6
Rectum contents	8.6	8.1	7.2	7.2
Rectum mucosa	<6	8.9	<6	8.4

were most noticeable over the gut-associated lymphoid tissue of the anterior colon and along the tops of longitudinal folds. In the caecal mucosa of Calf 4 there were areas of reddening 1—2 cm in diameter.

In the caecum, colon and rectum of Calves 4 and 5 foci of bacteria were seen adherent to clumps of irregularly arranged and exfoliated enterocytes and there were neutrophils in the lamina propria. In the caecum, neutrophils were in foci in the lamina propria; in the colon and rectum, neutrophils were more numerous and were also present in foci in an exudate on the mucosal surface with mucus and exfoliated enterocytes. Lesions were most severe in the colon.

Immunoperoxidase staining of sections of tissue with antiserum to *E. coli* S102-9 revealed microcolonies of bacteria adherent to the colonic and rectal rugae of Calves 4 and 5. All adherent bacteria that were seen were immunostained. Rotavirus was detected in the ileal enterocytes by immunoperoxidase staining and in the small intestine there were lesions of a viral enteropathy (stunted and fused villi covered by cuboidal enterocytes).

Detection and characterisation of atypical E. coli *amongst isolates collected during a survey of outbreaks of calf diarrhoea*

Of 659 isolates, including 373 from calves with diarrhoea, four had an atypical colony morphology on MacConkey agar indistinguishable from that of *E. coli* S102-9, were anaerogenic and produced urease. On the basis of other tests these isolates were identified as *E. coli* (Table III). The four isolates originated from different farms and three were isolated from calves with diarrhoea. Coronavirus was isolated from one of these calves and *Salmonella typhimurium* from another; the third isolate from a normal calf was

TABLE III

Identification of the atypical *E. coli*

	E. coli B41	*E. coli* S102-9	Four isolates from outbreaks of diarrhoea
Gram-negative rod	+	+	+
Facultative anaerobe	+	+	+
Oxidase reaction	–	–	–
Catalase reaction	+	+	+
Indole produced at 44°C	+	+	+
Methyl red	+	+	+
Citrate utilised	–	–	–
Acid (A) or Gas (G) from glucose	A + G	A	A
Acid produced in MacConkey broth at 44°C	+	+	+
Urease	–	+	+
Colony morphology on MacConkey agar	Typical	Atypical	Atypical

designated 6/193 and the fourth from a calf with diarrhoea in which other enteropathogens were not detected was designated 37/1.

The results of further characterisation of the four atypical isolates and S102-9 are in Table IV. Preliminary serotyping of *E. coli* 37/1 and 6/193 revealed that they produced the same somatic antigen (05) as S102-9. In addition to the four atypical isolates, another isolate, which was aerogenic but otherwise indistinguishable from *E. coli* S102-9, produced a toxin active on HeLa and Vero cells but not CHO cells. Fifty-five isolates were detected with either 1 or 2 of the atypical characteristics of *E. coli* S102-9; 16 of these were tested for the production of cytotoxins. They comprised four isolates which produced urease, five anaerogenic isolates, five which agglutinated in antiserum to *E. coli* S102-9, one with an atypical colony morphology and one with an atypical colony morphology which agglutinated in antiserum to *E. coli* S102-9. One isolate produced a cytotoxin active on Vero cells; it also produced urease.

Experimental infection of gnotobiotic calves with atypical E. coli *isolates 37/1 or 6/193*

In a preliminary investigation, two gnotobiotic calves were infected with either *E. coli* 37/1 or 6/193; they did not develop dysentery, although the calf infected with *E. coli* 37/1 produced mucoid liquid faeces from 5 to 9 days after infection, when it was killed. *E. coli* 6/193 was isolated from a normal calf and the faeces of the gnotobiotic calf infected with 6/193 were unchanged until 10 days after infection, when the animal was killed. This

TABLE IV

Characteristics of atypical *E. coli*

Isolates	Motility	Absorption of antibody to S102-9[a]	Fimbriae (F) or haemagglutinins (H) produced on			Toxins			
			TGX agar + O_2	Blood agar + O_2	Blood agar – O_2	Vero	HeLa	CHO	ST
E. coli S102-9	–	+	F^+H^-	$F(NT)H^-$	F^-H^+[b]	+[c]	+[d]	–	–
Four isolates from outbreaks of diarrhoea	–	+	F^+H^-	$F(NT)H^-$	F^-H^+[b]	+[c]	+[d]	–	–
E. coli K12	+	–	F^+H^+	NT	F^-H^-	–	–	–	–
E. coli B41	–	–	F^+H^+	NT	F^-H^-	–	–	–	+

[a] Slide agglutination test and immunoperoxidase method.
[b] Agglutinated sheep erythrocytes at 37°C with mannose at titres of 16—64.
[c] Titres of 10^3—10^5.
[d] Titres of 10.
NT, not tested.

suggested that isolate 6/193 was avirulent. Calves excreted between 1.2 $\times 10^9$ and 2.2 $\times 10^{10}$ *E. coli* g^{-1} of faeces until they were killed. Three days after infection, faeces of the gnotobiotic calf infected with *E. coli* 37/1 inoculated onto MacConkey agar revealed *E. coli* with two different colony morphologies in approximately equal numbers. One was atypical and of the kind produced by *E. coli* S102-9, and the other was typical of most isolates of *E. coli*. *E. coli* of both types, however, were anaerogenic, produced urease and agglutinated in antiserum to *E. coli* S102-9.

At necropsy of the two gnotobiotic calves there were more *E. coli* in the caecal, colonic and rectal contents ($\geqslant 3.9 \times 10^9$ cfu g^{-1}) than in the respective mucosae ($8.3 \times 10^6 - 4.8 \times 10^8$ cfu g^{-1}) which indicated that colonisation of the large intestinal mucosae was less than that seen with *E. coli* S102-9 infections of gnotobiotic calves (Chanter et al., 1984). In the mucosae of the colon and rectum of both calves there was marked congestion and occasional small haemorrhages. Neutrophils were numerous in the lamina and they were present as foci on the mucosal surface forming an exudate, together with mucous and exfoliated enterocytes. These lesions were apparently as severe as those seen with *E. coli* S102-9 infections of gnotobiotic calves (Hall et al., 1985).

Scanning electron microscopy revealed many short, rod-shaped bacteria attached to the mucosal surface of the large intestines of both calves. Enterocytes to which bacteria were attached, and adjacent enterocytes, lacked microvilli or microvilli were abnormally orientated or were shortened or lengthened. Changes to microvilli were confirmed by transmission electron microscopy which also showed that the bacteria were closely associated with the enterocyte cell membrane. At the point of attachment, enterocyte cytoplasm was often cup-shaped or arranged as a pedestal.

Immunoperoxidase staining revealed microcolonies of *E. coli* adherent to the colonic and rectal rugae of both calves.

DISCUSSION

E. coli S102-9 was pathogenic for conventional calves; all animals infected at 4 days of age developed dysentery similar to that previously seen in gnotobiotic and farm calves (Chanter et al., 1984; Hall et al., 1985). The calves excreted other enteropathogens, but in two these were not associated with dysentery, and in a third they were not excreted during the major episode of dysentery. In two calves, slaughtered at the estimated peak of clinical signs, rotavirus was detected in the ileum and was not associated with lesions that could have been a source of fresh blood in faeces. Lesions in the large bowel, which appeared to be the source of blood in faeces, were consistently associated with adherent *E. coli* and were identical in nature to those produced in gnotobiotic calves and those seen previously in naturally-affected farm calves (Hall et al., 1985).

Primary infection of two older calves did not reproduce dysentery, which

suggested that the calves had acquired resistance to the effects of infection. In this respect, resistance might be similar to that acquired with age by calves to infection with K99$^+$ *E. coli* (Smith et al., 1967).

Detection of four atypical *E. coli* out of 659 isolates collected from natural outbreaks of calf diarrhoea suggested that they were not an important cause of enteric disease in calves in that survey. One isolate with these properties has previously been associated with diarrhoea in a calf in France (De Ryke et al., 1982).

Two isolates of atypical *E. coli* (37/1 and 6/193) caused microscopic lesions indistinguishable from those produced by *E. coli* S102-9 (Hall et al., 1985). However, one (6/193) did not cause disease and the other (37/1) caused mucoid diarrhoea. Failure of these strains to cause dysentery may have been related to the extent of bacterial colonisation and the lesions caused. There were approximately 10-fold fewer *E. coli* 37/1 or 6/193 in the mucosae of the large intestine than were previously reported for *E. coli* S102-9 in gnotobiotic calves (Chanter et al., 1984). The extent of microscopic lesions in the gnotobiotic calves was difficult to assess, particularly because of their focal nature, which was apparently similar to that seen in calves infected with *E. coli* S102-9. Consequently, the virulence of these *E. coli* may vary not only with the ability to colonise but also the extent of the lesions they cause or, alternatively, it may differ with the production of an unknown pathogenic determinant. In man infected with different strains of verotoxic *E. coli*, which cause the same type of lesion, signs of disease vary from mild diarrhoea to dysentery (Anon, 1983).

An alternative explanation for the failure of *E. coli* 37/1 and 6/193 to cause dysentery may have been a variation of animals in their susceptibility. Moon et al. (1983) reported considerable animal to animal variation in the response of pigs to infection with *E. coli* enteropathogenic for humans; identical lesions were seen in the colons of pigs with or without diarrhoea.

Historically, the term enteropathogenic *E. coli* or EPEC was first applied to strains of *E. coli* implicated in epidemic infantile diarrhoea of humans. Some of these strains and others virulent for a number of species have been shown to cause highly characteristic microscopic lesions of the gut mucosa and to produce a cytotoxin detectable in vitro and have been called EPEC (Anon, 1983). The microscopic lesions and production of cytotoxin may be unifying features of an emerging sub-group of enteropathogenic *E. coli* for which the term EPEC is not sufficiently descriptive. Takeuchi et al. (1978) suggested that these lesions in rabbits were induced by a cytotoxin which showed similarities to the shiga toxin produced by *Shigella dysenteriae* (O'Brien et al., 1982). *E. coli* enteropathogenic for man have been shown to produce a toxin (Konowulchuk et al., 1978) which is biologically and serologically related to the shiga toxin (O'Brien et al., 1982). The *E. coli* described here may form a distinctive sub-group in view of their isolation from cattle, their atypical characteristics and their distinctive properties in tests for fimbriae and haemagglutinins. These *E. coli* produced a mannose-resis-

tant haemagglutinin in anaerobic but not aerobic conditions which was of particular interest; apparently the haemagglutinin was not associated with fimbriae. In aerobic conditions these bacteria produced fimbriae without detectable haemagglutinating activity which, although previously described (Isaacson, 1981), are nonetheless unusual.

The unusual colony morphology produced on MacConkey agar might usefully serve as a primary selective characteristic for potentially pathogenic *E. coli* of the calf. However, strain 37/1 produced in vivo a variant with a colony morphology indistinguishable from that produced by most *E. coli*. Furthermore, if in vitro the common characteristic of the enteropathogenic *E. coli* is cytotoxigenicity then it is probable that *E. coli* which can cause enteric disease in the calf may be found with otherwise typical properties.

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