F41 Antigen as a Virulence Factor in the Infant Mouse Model of *Escherichia coli* Diarrhoea

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(Received 22 February 1985; revised 4 June 1985)

The properties responsible for the virulence in infant mice of the bovine enterotoxigenic *Escherichia coli* strain B41 were investigated. A B41K99⁻ variant previously found to be nearly as virulent as the original strain B41 (B41K99⁺) possessed F41 antigen and haemagglutinating properties. Two variants that did not haemagglutinate sheep and human erythrocytes were isolated from strain B41K99⁻. These variants simultaneously lost their ability to agglutinate with F41 antiserum and their haemagglutinating properties. They still produced heat-stable enterotoxin. The first B41K99⁻F41⁻ variant was much less virulent than strains B41K99⁺ and B41K99⁻, the second was not virulent at all. F41 properties were not acquired by other *E. coli* strains by plasmid transfers. Non-haemagglutinating variants could not be obtained from the original strain B41K99⁺. However, a B41K99⁺F41⁻ strain was obtained by a four-step procedure: (i) spontaneous loss of the K99 plasmid, (ii) obtaining a nalidixic acid-resistant mutant, (iii) obtaining a non-haemagglutinating F41⁻ variant, (iv) reacquisition of the K99 plasmid. This B41Nal^rK99⁺F41⁻ strain, although producing heat-stable toxin, was not at all virulent, whereas reacquisition of the K99 plasmid by the strain B41Nal^rF41⁺ restored virulence. These results show that F41 antigen is an important virulence factor of strain B41 in the infant mouse model.

INTRODUCTION

Virulence factors have been identified in enterotoxigenic *Escherichia coli* (ETEC), including components responsible for adherence of bacteria to the intestinal epithelium, and toxins (Gaastra & De Graaf, 1982; Gyles *et al.*, 1974; Smith & Halls, 1967; Smith & Linggood, 1971, 1972). The ETEC adherence factors include plasmid-determined pili, antigenic structures, such as K88 found in ETEC isolated from piglets (Jones & Rutter, 1972; Stirm *et al.*, 1967), K99 antigen found in ETEC strains isolated from calves, lambs and piglets (Ørskov *et al.*, 1975) and CFA/I and CFA/II antigens found in *E. coli* isolated from humans (Evans *et al.*, 1975; Evans & Evans, 1978). Recently, however, other surface structures, both pilus-like and non pilus-like, and assumed to have the same role, have been at least partly characterized (Awad-Masalmeh *et al.*, 1982; Gaastra & De Graaf, 1982). Furthermore, some adhesins are more complex than at first realized. For example, ETEC strains possessing CFA/II antigen have been found to possess two antigens of three antigenic types (CS₁ + CS₃) or (CS₂ + CS₃) with different haemagglutinating properties (Cravioto *et al.*, 1982; Mullany *et al.*, 1983; Smyth, 1982). Also strain B41, which produces K99 antigen, produces another antigenic and haemagglutinating surface structure, not expressed at 18 °C, which causes adherence to intestinal cells (De Graaf & Roorda, 1982; Morris *et al.*, 1978, 1980, 1982). This antigen, called F41 (De Graaf & Roorda, 1982; Morris *et al.*, 1982), is believed to be a pilus-like structure (De Graaf & Roorda, 1982) and a variant strain, B41K99⁻F41⁺, was still able to produce diarrhoea in gnotobiotic piglets (Morris *et al.*, 1982).

Abbreviations: ETEC, enterotoxigenic *E. coli*; ST toxin, heat-stable enterotoxin.

An infant mouse model of *E. coli* diarrhoea was established by Duchet-Suchaux (1980). In this model the oral inoculation of infant mice with strain B41 provokes rapid death (Duchet-Suchaux, 1980). A B41 variant which does not produce K99 antigen is still fully virulent, while *E. coli* strains having acquired the ability to produce K99 antigen and heat-stable enterotoxin (ST toxin) by plasmid transfer are much less virulent than strains B41 and B41K99⁻, or not virulent at all (Bertin, 1983). These results suggested that another property (or properties) of the B41K99⁻ strain, absent from the transconjugant clones, was (were) responsible for the virulence of strain B41 for the infant mouse and that F41 antigen was a good candidate for this role (Bertin, 1983).

This report describes further study of the virulence of strain B41 for the infant mouse with particular consideration of the role of F41 antigen.

METHODS

Bacterial strains. *Escherichia coli* strain B41 is a bovine reference strain (Smith, 1971) provided by Ph. Gouet, INRA, Theix, France. Strain B41* is supposedly the same strain but was provided by P. Pohl, Institut National de Recherches Veterinaires, Groeselenberg, Bruxelles, Belgium. This strain originated from I. Ørskov. Strain B41A is a B41K99⁻ variant obtained in this laboratory that still has the ability to produce the ST toxin (Bertin, 1983). Strains C600(1), H510(1), 3404(1) and K14(1) are *E. coli* K12 and non-ETEC *E. coli* reference strains of serotypes O101, O8 and O9 which have acquired by plasmid transfer from strain B41 (selection for tetracycline resistance) a plasmid coding for tetracycline (Tc), streptomycin (Sm) and sulphonamide (Su) resistances (Bertin, 1983). Strains C600(2), H510(2), 3404(2) and K14(2) have additionally acquired from strain B41 a plasmid coding for K99 antigen and ST toxin (Bertin, 1983). The main characteristics of all these strains are listed in Table 2.

The strain used for plasmid mobilization was 28R315 (J53-R1-19*drd*) originating from Dr M. M. McConnell, Central Public Health Laboratory, London, UK. Reference plasmids were R702 (46 MDal), RI (62 MDal), RA1-1 (85 MDal), Rts1 (120 MDal) provided by C. Waalwijk, Vrije University, Amsterdam, Holland, V517 (Macrina *et al.*, 1978) and C600RP4 (Datta *et al.*, 1971).

Production of antiserum. Antisera were produced in rabbits by multiple intravenous injections of bacteria (live or formalin-treated) that had been grown at 37 °C on minca agar (Guinée *et al.*, 1977). Specific anti-K99 serum was prepared by absorbing B41 antiserum with the B41K99⁻ variant grown at 37 °C on minca agar. F41 antiserum was prepared against the B41K99⁻ variant and was absorbed by the same strain cultivated at 18 °C on minca agar.

Haemagglutination tests. These were done by slide agglutination at room temperature. One drop of a dense suspension of bacteria grown for 18–24 h at 37 °C on minca medium was added to one drop of washed erythrocytes diluted 1/5 in Mayer veronal buffer (Mayer *et al.*, 1948) containing 0.5% (w/v) mannose. Immediate and strong agglutination was recorded as ++, and slower agglutination appearing in less than 5 min was recorded as +.

Determination of other characteristics of the strains. Biochemical metabolic profiles were determined by the API system, and resistance to antibiotics by using disks (Bio-Mérieux) on Mueller–Hinton agar. ST toxin production was determined by the suckling mouse assay (Dean *et al.*, 1972) by oral inoculation (Stavric & Jeffrey, 1977).

Selection of nonhaemagglutinating (MRHA⁻) variants. A haemadsorption method derived from that of Sedlock *et al.* (1982) was used. Broth cultures of strains B41 or B41A were diluted and plated on minca agar so as to obtain about 100 isolated colonies. The plates were incubated for 48 h at 37 °C and cooled to 4 °C just before testing. A volume (5 ml) of a 10% (v/v) suspension of washed sheep erythrocytes in Mayer veronal buffer containing 0.5% (w/v) mannose was poured slowly onto each plate and rocked gently for about 2 min. The erythrocyte suspension was then aspirated. Colonies that appeared not to adsorb erythrocytes were sampled for cloning and further characterization.

Plasmid analysis. The plasmid content of the strains was analysed by agarose gel electrophoresis after alkaline extraction by the method of Kado & Liu (1981). Electrophoresis was done in a vertical apparatus (gel dimensions 16 × 14 × 3 mm) with ten sample slots (15 × 8 × 3 mm). Gels (0.8%, w/v, agarose; Sigma type II, medium EEO) were electrophoresed for 150 min at 120 V, stained for 20 min with ethidium bromide (0.4 µg ml⁻¹) and photographed under UV light (short-wave transillumination type C61) onto Polaroid type 665 film with a yellow filter. The molecular weights of the plasmids were estimated by calculation from a linear regression curve obtained from mobility of standard plasmids.

Selection of nalidixic acid resistant (Nal^r) mutants. Spontaneous Nal^r mutants (resistant to 50 µg ml⁻¹) were isolated on trypticase soy agar (TSA) (Bio-Mérieux) plates containing increasing concentrations of nalidixic acid. Nal^r clones were chosen from those having the same size and appearance as the original strain on TSA.

Plasmid transfers. Donor, recipient and mobilizing strains were each cultured in 10 ml trypticase soy broth for 18–24 h at 37 °C. Then 0.02 ml of each culture was added to 10 ml trypticase soy broth and cultivated for a further 18–24 h at 37 °C. Selection of transconjugants was made on TSA with tetracycline (16 µg ml⁻¹) and nalidixic acid (40 µg ml⁻¹).

Table 1. Mannose-resistant haemagglutinating properties of strains of *E. coli* and their reaction with the anti-F41 serum

Strain†	Haemagglutination of erythrocyte types:					Agglutination with anti-F41
	Human A	Bovine	Horse	Sheep	Guinea-pig	
B41, B41* and B41A	++	+	++	++	++	+
C600(1), H510(1), 3404(1) and K14(1)	—	—	—	—	—	—
C600(2), H510(2), 3404(2) and K14(2)	—	+	++	—	—	—
B41B ₁ and B41B ₂	—	+	+	—	+	—
B41*C	++	+	+	++	++	+
B41*CB	—	+	—	—	+	—

† See text and Table 2 for details.

Pathogenicity for infant mice. Infant OF1 mice (IFFA-Credo, L'Arbresle, France) born from at least nine animals for each experiment were randomly redistributed (eight per mother) 18 h before inoculation. At inoculation they were 18–48 h old and weighed about 2 g. Each experiment was done using three mothers (= 24 infant mice). For inoculation, bacteria were grown on TSA slants for 18–24 h at 37 °C, and suspended in saline (9 g NaCl l⁻¹). Infant mice were inoculated orally with 10³ or 10⁵ bacteria by means of a calibrated platinum loop. Mortality was recorded each day for 6 d.

RESULTS

Strain B41K99⁻ (B41A) did not agglutinate with a K12K99 antiserum, and when cultivated at 37 °C did not absorb K99 antibodies (Bertin, 1983). A serum (referred to as anti-F41 serum) against this B41K99⁻ variant absorbed with the same strain cultivated at 18 °C was obtained. This serum agglutinated the B41K99⁻ variant cultivated at 37 °C, giving evidence in this strain of at least one antigenic structure not expressed at 18 °C (Table 1). This is a characteristic reported for F41 antigen (De Graaf & Roorda, 1982; Morris *et al.*, 1982). Like the original B41 strain, the B41K99⁻ variant, when grown at 37 °C on minca medium, induced immediate mannose-resistant haemagglutination of human group A, horse, sheep and guinea-pig erythrocytes at room temperature while bovine erythrocytes reacted more slowly (Table 1). Mannose-resistant haemagglutinating properties have also been reported associated with the presence of F41 antigen (De Graaf & Roorda, 1982; Morris *et al.*, 1982).

Non-ETEC *E. coli* strains having acquired only antibiotic resistances by plasmid transfer from strain B41 did not acquire haemagglutinating properties (Table 1). The same strains which had in addition acquired the ability to produce K99 antigen and ST toxin also acquired the ability to haemagglutinate horse erythrocytes immediately and bovine erythrocytes more slowly (Table 1). None of these strains agglutinated with the anti-F41 serum (Table 1).

Isolation of non-haemagglutinating (MRHA⁻) F41⁻ variants from strain B41A

Two colonies which did not appear to adsorb sheep erythrocytes on agar plates were cloned twice and characterized. They had the same metabolic profile, antibiotic resistance (Sm^r Tc^r Su^r) and apparent plasmid profile as strains B41 and B41A (Fig. 1). These clones, called B41B₁ and B41B₂, had totally lost the ability to haemagglutinate human and sheep erythrocytes, and haemagglutination of guinea pig and horse erythrocytes appeared more slowly than with the original strain (Table 1). Clones B41B₁ and B41B₂ had also lost the ability to be agglutinated by the anti-F41 serum (Table 1).

Obtaining B41Na^r K99⁺ MRHA⁺ (F41⁺) and B41Na^r K99⁺ MRHA⁻ (F41⁻) strains

It proved impossible to isolate non-haemagglutinating variants from strain B41, perhaps because K99 antigen might have masked, at least in part, the F41 structure. Surprisingly, strain B41 from another source (called B41*; see Methods) was found to contain only one large plasmid of about 90 MDal (Fig. 1, lane 7), which was transferable after mobilization to *E. coli* C600 (Fig. 1, lane 6). Presence of this plasmid was associated with the Sm^r Tc^r Su^r, K99 and ST

Fig. 1. Plasmid content of various *E. coli* strains. Agarose gel electrophoresis (0.8%, w/v) was done after alkaline extraction by the method of Kado & Liu (1981) as described in Methods. Lanes 1 and 5, strain B41; lane 2, first MRHA⁻ variant B41B₁; lane 3, second MRHA⁻ variant B41B₂; lane 4, B41K99⁻ variant (strain B41A); lane 6, transconjugant C600 clone (B41* × C600) Sm⁺Tc⁺Su⁺K99⁺ST⁺; lane 7, strain B41*; lane 8, strain B41*C (Sm⁺Tc⁺Su⁺K99⁻ST⁻ derivative of B41*). The sizes (MDal) of the plasmids are indicated. Chr, chromosomal DNA.

characteristics. The sizes of the two large plasmids of strain B41 were estimated as about 67 MDal and 55 MDal under the same conditions (Fig. 1). In addition to these plasmids, two plasmid DNA bands migrating ahead of the chromosome were seen after alkaline extraction (Fig. 1). One or the other was sometimes cotransferred without the appearance of detectable phenotypic characteristics.

Forty-one clones of strain B41* were taken from the medium of Bochner *et al.* (1980) for positive selection for loss of tetracycline resistance, and characterized. Of these, 35 had lost tetracycline resistance only, two had lost tetracycline resistance and ST toxin, and one had lost the resistances to tetracycline, streptomycin and sulphonamides as well as K99 antigen and ST toxin production. This last clone, B41*C, had lost the 90 MDal plasmid (Fig. 1, lane 8). It was still able to produce F41 antigen and haemagglutinated horse erythrocytes more slowly than the original strain B41* (Table 1). From clone B41*C, a NaI^r mutant was obtained, and from this mutant, a sheep erythrocyte non-haemagglutinating variant, B41*CB. This last clone had the same haemagglutinating properties with sheep and human erythrocytes as the other sheep erythrocyte non-haemagglutinating strains and did not agglutinate with anti-F41 serum (Table 1). The 90 MDal B41* plasmid was transferred by mobilization into these two NaI^r strains, restoring the B41* phenotype in the former (strain B41*CD) and giving a B41*NaI^rK99⁺MRHA⁻(F41⁻)ST⁺ variant in the latter (strain B41*DB).

The main characteristics of all these strains are listed in Table 2.

Virulence of strains for infant mice

It has been established that infant mice less than 48 h old are very sensitive to K99⁺ST⁺ ETEC strains such as strain B41 when orally inoculated; death of the animals is associated with intestinal fluid accumulation, diarrhoea and abnormal proliferation of the inoculated strain in the intestine (Duchet-Suchaux, 1980). Doses as small as 10 bacteria kill 40–60% of the animals in less than 6 d, and inoculation with 10³ bacteria regularly kills 80–100% of the animals. The

Table 2. *Main characteristics of the strains*

Strain†	Characteristics associated or supposedly associated with pathogenicity				Presence of plasmid(s) coding for:		
	K99 antigen	F41 antigen	ST toxin	MRHA‡	K99-ST (67 MDal)	Sm ^r Tc ^r Su ^r § (55 MDal)	K99-ST Sm ^r Tc ^r Su ^r § (90 MDal)
B41	+	+	+	+	+	+	—
B41A ^a	—	+	+	+	+	+	—
C600(1), H510(1), H510(1), 3404(1) and K14(1) ^b	—	—	—	—	—	+	—
C600(2), H510(2), 3404(2) and K14(2) ^c	+	—	+	—	+	+	—
B41B ₁ and B41B ₂ ^d	—	—	+	—	+	+	—
B41* ^e	+	+	+	+	—	—	+
B41*C ^f	—	+	—	+	—	—	—
B41*CD ^g	+	+	+	+	—	—	+
B41*CB ^h	—	—	—	—	—	—	—
B41*DB ⁱ	+	—	+	—	—	—	+

† *a*, Variant of strain B41 which does not produce K99 antigen but still has the other characteristics of strain B41; *b*, non-ETEC transconjugants which have acquired the Sm^rTc^rSu^r (55 MDal) plasmid from strain B41; *c*, non-ETEC transconjugants which have acquired the Sm^rTc^rSu^r (55 MDal) and K99-ST (67 MDal) plasmids from strain B41; *d*, variants isolated from strain B41A as non-haemagglutinating clones and not agglutinating with F41 antiserum; *e*, this reference strain B41 (different source from B41: see Methods) possesses only a single large plasmid (90 MDal) coding for K99-ST and Sm^rTc^rSu^r; *f*, strain B41* having lost the 90 MDal plasmid; *g*, Nal^r mutant of strain B41*C which has reacquired the 90 MDal plasmid; *h*, variant of a Nal^r mutant of strain B41*C, isolated as a non-haemagglutinating clone and does not agglutinate with F41 antiserum; *i*, strain B41*CB which has reacquired the 90 MDal plasmid.

‡ Mannose-resistant haemagglutination with sheep erythrocytes.

§ Each strain possessing this plasmid is resistant to streptomycin, tetracycline and sulphonamide.

Table 3. *Virulence of E. coli strains assessed in the infant mouse model*

Strain	Mortality† 5 d after inoculation with:	
	10 ³ bacteria	10 ⁵ bacteria
B41	23/24	24/24
B41*	22/24	24/24
B41A	23/24	24/24
B41B ₁	Expt 1 0/24	0/24
	Expt 2 0/24	0/24
B41B ₂	Expt 1 6/24	9/24
	Expt 2 10/24	10/24
B41*CD	Clone 1 15/24	24/24
	Clone 2 23/24	24/24
B41*DB	Clone 1 0/24	0/24
	Clone 2 1/24	0/24

† No. of infant mice dead/no. of infant mice inoculated.

susceptibility of the mice decreases with age, and after 10 d they are no longer susceptible (Duchet-Suchaux, 1980). In the present study standard inocula of 10³ (or, for less pathogenic strains, 10⁵) bacteria were used.

Non-ETEC *E. coli* strains C600, H510, K14 and 3404 which had acquired the K99-ST plasmid by matings from strain B41 killed, respectively, 0/24, 1/23, 10/48 and 0/24 of the animals after inoculation with 10⁵ bacteria. These strains were thus much less virulent than strain B41 (Bertin, 1983). The B41K99⁻ variant strain B41A was still very virulent: the same final rate of death as the original strain B41K99⁺ was obtained (Table 3), but death was delayed by about 24 h (Bertin, 1983).

Strain B41* had similar virulence to strain B41 (Table 3). Strain B41B₁, which does not produce F41 antigen, was not virulent at all, whereas the second F41⁻ variant (B41B₂) did show some virulence (Table 3).

Strains that did not produce ST toxin were not virulent at all. Virulence was restored when the Nal^r mutant of strain B41*C reacquired the K99-ST Sm^rTc^rSu^r 90 MDal plasmid (strain B41*CD) (two transconjugant clones tested, Table 3). The F41⁻ variant of the same strain which had acquired the same plasmid and phenotypic characteristics (strain B41*DB) was not virulent at all (two transconjugant clones tested, Table 3).

DISCUSSION

F41 antigen has been found in the bovine reference ETEC strain B41 (De Graaf & Roorda, 1982; Morris *et al.*, 1983). It mediates bacterial adherence to intestinal epithelial cells (Morris *et al.*, 1983), but its exact role in pathogenicity is not known. In this report, a genetic approach to the problem, especially by obtaining F41⁻ variants, has shown that the F41 antigen is an important virulence factor in an infant mouse model.

In this model of *E. coli* diarrhoea a B41K99⁻ variant (strain B41A) was nearly as pathogenic as the original strain B41, and transconjugant K99⁺ST⁺ *E. coli* clones were much less virulent or not virulent at all (Bertin, 1983). The B41K99⁻ variant strain B41A, but not the *E. coli* strains having acquired by plasmid transfer K99 and ST characteristics or other plasmids from strain B41, had properties reported to be F41-associated, namely agglutination with a serum against antigens of strain B41 different from K99 antigen and also not expressed at 18 °C on minca medium, and mannose-resistant haemagglutinating properties (De Graaf & Roorda, 1982; Morris *et al.*, 1982).

F41 antigen was found by titration assay at 4 °C in the presence of mannose to haemagglutinate strongly human group A erythrocytes and guinea-pig erythrocytes and, to a lesser extent, sheep and horse erythrocytes (De Graaf & Roorda, 1982). Also, the haemagglutinating properties of the F41 antigen were not readily reproducible at low temperatures but at room temperature this structure haemagglutinated sheep, guinea-pig and horse erythrocytes (Ollier & Girardeau, 1983). The B41K99⁻ variant strain B41A also induced mannose-resistant haemagglutination of human A type, horse, sheep and guinea-pig erythrocytes.

Each MRHA⁻ variant isolated had entirely lost the ability to induce rapid haemagglutination at room temperature of human group A and sheep erythrocytes, and haemagglutination of horse and guinea-pig erythrocytes was markedly slowed. At the same time they lost the ability to be agglutinated by a serum against a structure from B41K99⁻ not expressed at 18 °C on minca medium. It seems likely that the MRHA⁻ variants have lost entirely, or have very greatly reduced amounts of the F41 antigen. This structure is incompletely characterized and has been provisionally called F41 by De Graaf & Roorda (1982). The purified material has a filamentous structure and their B41K99⁻ variant (strain B41M) has very thin pili that are not produced at 18 °C (De Graaf & Roorda, 1982). However, two mannose-resistant haemagglutinins have also been found in a B41K99⁻ variant and it is not certain that the B41 anionic adhesin is a fimbrial structure (Chanter, 1983). The full characterization of this structure and its role in pathogenesis has been complicated by the failure to obtain F41⁻ variants (Morris *et al.*, 1982).

I found that strain B41 from one origin had two large plasmids, the first coding for K99 and ST characteristics and the second for resistances to streptomycin, tetracycline and sulphonamide (Bertin, 1983). Surprisingly, strain B41 from a different origin (strain B41*) had only a single, larger plasmid coding for all these characteristics. This suggests that plasmid recombination can occur, perhaps facilitated by the presence of transposons, since ST toxin is coded by a transposon in this strain (So *et al.*, 1979). Before my first report (Bertin, 1983), production of K99 antigen and ST toxin had not been reported as being coded by the same plasmid in strain B41 (Meyers *et al.*, 1976; So *et al.*, 1976; Van Embden *et al.*, 1980). Strains B41 and B41* had identical phenotypic characteristics and both were virulent for infant mice.

In agreement with earlier studies (De Graaf & Roorda, 1982) I did not detect F41 antigen or F41-specific haemagglutinating properties in any of the transconjugant clones. Also the B41* clone which had lost the 90 MDal plasmid possessed F41-associated properties. All these results suggest that F41 is not a plasmid-determined characteristic.

Loss of K99 antigen by the strain B41 does not modify its haemagglutinating properties, least of all its horse and bovine haemagglutinating properties. However, these properties were transferred with the K99 plasmid since K99⁺ transconjugants of *E. coli* agglutinated horse and bovine erythrocytes. This poses the question of the structural relationships between K99 antigen and haemagglutinins and the possible existence of several haemagglutinins with the same specificity in *E. coli* B41.

ST toxin is essential for virulence, The B41 MRHA⁻ (F41⁻) variants, still producing ST toxin, were much less virulent for infant mice than strains B41 and B41A or not virulent at all. So far, no difference in phenotypic characteristics has been detected between these variants. The second variant may be able to produce a very small amount of F41 structure, as suggested by electron microscopy (unpublished observations). Mortality was not appreciably increased by increasing the inoculum from 10³ to 10⁵ bacteria. This could reflect a characteristic relevant to the model. The degree of virulence could not be exactly represented as a simple toxic effect. It is probable that for the inoculated bacteria to implant successfully in the intestine they must implant before, or successfully compete with, other micro-organisms. The second step is abnormal proliferation of the bacteria, probably relevant to the death of the animals, and would depend on the characteristics of the bacteria, especially their adherence ability. Perhaps for this second step, inoculated doses are of less importance. I found that the non-pathogenic or poorly pathogenic strains H510(2), K14(2) and 3404 (2) comprised the dominant enterobacterial population of the intestines of survivors 5 d after inoculation (Bertin, 1983).

Strain B41*DB, which is F41⁻ but possesses K99 antigen and produces ST toxin is not virulent at all, showing that F41 antigen is indispensable for virulence.

A B41K99⁻F41⁺ variant produces diarrhoea in gnotobiotic piglets (Morris *et al.*, 1982). F41 antigen has now been found in numerous ETEC strains, in K99⁺ strains of serotypes O9 and O101 (Morris *et al.*, 1980), and also in porcine strains that do not produce K88, K99 and 987P antigens (Morris *et al.*, 1983; To, 1984).

Because ETEC diarrhoea is not easily reproduced in natural hosts, and experiments with a great number of animals are costly, studies have been made in an infant mouse model (Bertin, 1983; Duchet-Suchaux, 1980). Analysis of the B41 virulence factors in this model shows that ST toxin is necessary for virulence but not sufficient alone to render any ST⁺ *E. coli* virulent. The most important surface structure involved in the virulence of strain B41 appears to be an F41-like structure. The validity of the model for other ST-producing *E. coli* strains is currently being assessed.

I thank the people mentioned in this paper for providing me with *E. coli* strains, P. Menanteau for her technical assistance in some of the experiments, and E. Rabouan and H. Le Roux for care and maintenance of the experimental animals. I also thank M. Plommet for constant interest.

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