THE INFLUENCE OF PLASMID-DETERMINED
AND OTHER CHARACTERISTICS OF
ENTEROPATHOGENIC *ESCHERICHIA COLI* ON
THEIR ABILITY TO PROLIFERATE IN THE
ALIMENTARY TRACTS OF PIGLETS,
CALVES AND LAMBS

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TRANSMISSIBLE plasmids are important in the pathogenesis of *Escherichia coli* diarrhoea. Many piglet enteropathogenic strains possess the 88 plasmid, the product of which is a proteinaceous surface antigen that promotes colonisation of the small intestine (Smith and Linggood, 1971) by facilitating adhesion of the organisms to the villi (Jones and Rutter, 1972). The diarrhoea that then ensues is principally caused by enterotoxin that is synthesised by the Ent plasmid. Many enteropathogenic strains produce plasmid-determined haemolysin (Hly) which appears to play no part in the aetiology of diarrhoea (Smith and Linggood, 1971). Practically all 88⁺ strains also possess the Raf plasmid (Smith and Parsell, 1975) which determines raffinose utilisation.

Like their piglet counterparts, calf and lamb enteropathogenic strains produce plasmid-determined enterotoxin and a plasmid-determined proteinaceous antigen designated 99 (Ørskov *et al.*, 1975) that promotes colonisation of the small intestine (Smith and Linggood, 1972). The prevalence of 99 antigen in these strains is not known, principally because its identification has presented difficulty. This has now been overcome by the introduction of a modified Minca medium (Guinée, Veldkamp and Jansen, 1977) and its use may well reveal the majority of calf and lamb enteropathogenic strains to be 99⁺. Apart from a report of the isolation of nine strains of serotype O149:K91,88:H19, a typical piglet enteropathogenic serotype, from sick calves in the USA (Ellis and Kienholz, 1977) no evidence has been presented that implicates the 88 antigen in calf and lamb diarrhoea. Attempts to produce colonisation of the small intestine and diarrhoea in calves and lambs with 88⁺ piglet enteropathogens have failed (Smith and Halls, 1967).

The demonstration of 88⁺ organisms is not difficult, and it is clear that much porcine *E. coli* diarrhoea is caused by strains that do not produce the antigen. Small intestinal colonisation by such strains in experimentally infected weaned pigs has been demonstrated by Smith and Halls (1968) and in piglets by Bertschinger, Moon and Whipp (1972*a* and *b*), Hohmann and Wilson (1975) and Nagy, Moon and Isaacson (1976). The last two groups of workers emphasised that the 88⁻ enteropathogens adhered to the villi in the posterior small intestine, whereas 88⁺ enteropathogens adhered also in the anterior part. Later, Moon *et al.*

Received 2 Feb. 1978; accepted 23 Mar. 1978.

(1977) were able to demonstrate 99 antigen in some of their 88⁻ piglet enteropathogens. These strains and four of five 99⁺ calf enteropathogens adhered to the posterior small intestine and produced diarrhoea in experimentally infected piglets. The piglets were hysterectomy-derived, colostrum-deprived and unfed. Rozemund (1977) produced adhesion and diarrhoea in a colostrum-deprived calf with a 99⁺ piglet enteropathogenic strain. In contrast to these results, Smith and Linggood (1972) had failed to produce diarrhoea in colostrum-fed, conventionally reared piglets with 99⁺ calf and lamb enteropathogenic strains. More recently, Isaacson, Nagy and Moon (1977) and Nagy, Moon and Isaacson (1977) showed that some 88⁻99⁻ piglet enteropathogenic strains possess specific pili, called 987P, which permit them to adhere to the posterior small intestine; 987P is, as yet, an untransmissible character. In these papers, and in a previous one (Nagy *et al.*, 1976), it was demonstrated that the presence of the polysaccharide K antigens was essential for some but not all strains to express their full enteropathogenicity in piglets.

Unfortunately, from the experimental viewpoint, some pigs have an hereditary resistance to infection with 88⁺ *E. coli*. At least two pig phenotypes exist, one that permits adhesion of 88⁺ organisms to the small intestinal epithelium and one that does not. The phenotypes are the product of two alleles at a single locus inherited in a simple Mendelian manner, the product of the "adhesive" allele being dominant over the product of the non-adhesive allele (Sellwood *et al.*, 1974, 1975; Rutter *et al.*, 1975). Whether 88-resistant piglets can be infected with 88⁻ enteropathogens is not known but, *in vitro*, 99⁺ organisms adhere to intestinal epithelial cells of such piglets (Burrows, Sellwood and Gibbons, 1976).

In view of the above reports we decided to obtain more information on the ability of the 88, 99 and 987P antigens to promote colonisation of the small intestine of different animal species and on the part other bacterial components play in this process. The use of laboratory-constructed forms of strains that differed from each other by the presence or absence of one or other of these characters appeared essential. Also, because of the many genetic and other variations that exist between individual animals of the same species, sets of laboratory-constructed forms of the same strain were studied in the same experimental animal; spontaneous mutants of the forms, each resistant to a different antibiotic, were used so that the concentration of each in the different parts of the alimentary tract could be estimated easily on appropriate antibiotic-containing media. Colostrum-deprived experimental animals were used to negate the unequal influence that antibody might exert on the multiplication of the different forms in the alimentary tract. The availability of 88-susceptible and 88-resistant piglets provided information on their differing susceptibility to all the colonising factors studied. Because *E. coli* multiplies profusely in the stomach of piglets in the first 16 h or so of life and floods into the small intestine (Smith and Jones, 1963), experimental animals were given an inoculum in which the *E. coli* strains under study were greatly outnumbered by organisms of a non-pathogenic *E. coli* strain and by lactobacilli. This ensured that (1) the numbers of enteropathogenic *E. coli* in the stomach were never high enough to produce sufficient enterotoxin to cause diarrhoea, and (2) the numbers of these organisms entering the small intestine were too low to give a false impression of colonisation.

MATERIALS AND METHODS

Preparation of E. coli and lactobacillus strains for animal inoculation. Implantation or removal of 88, 99 and Ent plasmids was achieved by the methods of Smith and Linggood

(1971, 1972) and of Raf plasmids by the methods of Smith and Parsell (1975) with the following additions. (1) The isolation of organisms that had acquired the 88 genes during conjugation was facilitated by using a donor strain that was 88⁺ Raf⁺ and exploiting the facts that 88 and Raf are usually co-transferred during conjugation and that a highly selective culture medium exists for identifying Raf⁺ organisms (Smith and Parsell, 1975). (2) Organisms that were 99⁺ were detected by the methods of Guinée *et al.* (1977) with modified Minca medium. (3) Auxotrophic *E. coli* K12 strains were used as donors of 88 and 99 plasmids because of the efficiency of plasmid transfer and because, if required, recipient organisms could be separated from donor organisms by culturing on minimal media. Spontaneous polysaccharide K⁻ mutants were obtained by visual examination of colonies on plates of MacConkey's agar seeded with K⁺ cultures; their K⁻ status was confirmed by demonstrating their inagglutinability by appropriate K antisera. To avoid confusion, the 88 and 99 antigens are not given the prefix "K". O⁻ strains were classified by their rough colonial appearance and failure to form a stable suspension in normal saline; they were usually obtained by the use of methyl-nitro-nitrosoguanidine, as were *lac*⁻ forms of smooth strains. *E. coli* strains possessing the 987 pilus of Isaacson *et al.* (1977) were identified by monospecific antiserum kindly provided by Dr Harley Moon.

All enteropathogenic strains were designated according to the species of animal from which they had been originally isolated under conditions that left no doubt as to their enteropathogenicity. Those that had been altered genetically still produced enterotoxin. Those that possessed, or had possessed, the 88 plasmid produced both the heat-labile and the heat-stable enterotoxin (Smith and Gyles, 1970); the others produced only heat-stable enterotoxin. Unless stated, all strains that were 88⁺ were Raf⁺ and those from which the 88 plasmid had been removed were Raf⁻.

Most *E. coli* strains used in infection experiments were spontaneous chromosomal mutants resistant to sodium nalidixate, streptomycin, spectinomycin, rifampicin or ampicillin or to combinations of these antibiotics. They were isolated by heavily inoculating plates of MacConkey's agar containing antibiotic (20–100 µg per ml) with antibiotic-sensitive cultures. The plates were incubated at 37°C for 24 h and colonies that grew were purified by plating on MacConkey's agar without antibiotics. Only colonies that were similar in appearance to those of the strain from which they were derived and that gave rise to broth cultures that yielded the same colony count on MacConkey's agar, with and without the antibiotic to which they were resistant, were retained. For use they were grown in 10 ml of Nutrient Broth (Oxoid, No. 2) for 24 h in a shaking waterbath at 37°C; they contained approximately 10⁹ viable organisms per ml.

The lactobacillus strain with which all animals were inoculated was used as a suspension washed from slopes of Rogosa's agar (Oxoid) that had been incubated anaerobically at 37°C for 2 days; it had been isolated initially from the faeces of a healthy pig.

Experimental animals. Calves were obtained mainly from one Jersey herd and lambs from the laboratory flock. Piglets classified as 88S (S = susceptible) were from matings in which the sire was known to be homozygous for 88 adhesion; they were kindly supplied by Mr R. Sellwood of the Agricultural Research Council's Institute for Research on Animal Diseases. Those classified as 88R (R = resistant) were from a herd in which we had failed to produce small-intestinal colonisation or diarrhoea in a high proportion of piglets by giving them 88⁺ enteropathogenic strains. The 88R status of this herd was subsequently shown by Gibbons and Sellwood (unpublished observations) to be due to the genetic mechanism they had previously described. All experimental animals were removed from their dams before they had obtained colostrum; colostrum-deprivation was confirmed by the zinc-sulphate turbidity test (Aschaffenburg, 1949). The animals were fed on sterilised cows' milk given by stomach tube; the calves received 1 litre three times daily, the lambs 300 ml three times daily and the piglets 40–60 ml five times daily.

Inoculation and subsequent examination of experimental animals. Calves were given, in their first feed, 10⁹ viable organisms of each of the *E. coli* strains whose abilities to proliferate in the small intestine were to be compared, together with approximately 10¹⁰ viable organisms of a non-pathogenic *E. coli* strain, P403, and the lactobacillus strain. Lambs and piglets

were inoculated similarly, lambs receiving 10^8 viable organisms of the strains to be compared and piglets, 10^7 ; both were given 10^8 viable organisms of P403 and of lactobacilli. To confirm their 88-status, all piglets from the 88-resistant herd were given in addition 10^7 viable organisms of an O141:K85,88 piglet enteropathogenic strain known to produce small-intestinal colonisation and severe diarrhoea consistently in 88S piglets. Each member of the set of strains given to any animal was resistant to a different antibiotic or antibiotics; a few possessed antibiotic-resistance plasmids, but most were spontaneous chromosomal mutants.

After inoculation, the animals were inspected at frequent intervals for evidence of diarrhoea. If death appeared imminent, they were killed by parenteral injection of sodium pentobarbitone. Otherwise, they were killed 28–32 h after inoculation; preliminary studies revealed that diarrhoea seldom commenced after this time. Immediately after death, the concentration of viable organisms of each of the inoculated *E. coli* strains in the contents of the stomach, seven equally-sized portions of small intestine, the caecum, colon and rectum, and in scrapings of the walls of the seven small intestinal portions, were estimated by the method of Smith and Halls (1968). All bacterial counts were performed on MacConkey's agar and on MacConkey's agar containing such antibiotics as were necessary to distinguish all the inoculated strains from each other. Ampicillin, chloramphenicol, neomycin, spectinomycin, streptomycin, sodium nalidixate and tetracyclines were incorporated in the MacConkey's agar at 20 μ g per ml and rifampicin at 100 μ g per ml. The spleen and, in calves, a mesenteric lymph node were also cultured to ensure that no significant bacterial invasion of the body had occurred.

RESULTS

In the experiments described below, all the animals whose posterior small-intestinal contents contained about 10^8 or more Ent⁺ *E. coli* per g when they were killed had developed diarrhoea 12–14 h after inoculation. The diarrhoea in piglets was usually most severe and the clinical signs of dehydration most pronounced in those animals given 88⁺ organisms—several had to be killed before the end of the customary 28–32-h observation period because they were near to death. Animals whose posterior small-intestinal contents contained less than 10^8 Ent⁺ *E. coli* per g either remained healthy throughout the observation period or had mild diarrhoea insufficient to produce clinical signs of dehydration. Concentrations of *E. coli* greater than 10^7 per g of contents were rarely found in the stomachs of the experimental animals. Those present in this organ usually consisted mainly of organisms of the non-pathogenic strain P403 which formed the major part of the *E. coli* component of the inocula. The median counts, with the ranges in brackets, of the numbers of P403 organisms in the contents of the stomach, parts 1, 3, 5 and 7 of the small intestine, and colon, of 34 of the piglets were ($\log_{10}$ per g) as follows: 6.0 (3.7–7.3), 5.5 (2.7–7.3), 5.4 (3.3–7.3), 5.5 (4.0–7.7), 6.2 (4.0–8.3) and 6.0 (3.3–9.7) respectively. The corresponding figures for wall scrapings of parts 1, 3, 5 and 7 of the small intestine were 4.2 (<2.0–5.7), 4.4 (<2.0–6.7), 4.7 (<2.0–6.7) and 4.8 (2.7–7.5), the concentrations of P403 organisms in the wall of a particular part of the intestine being about 10 times lower than in its contents. Organisms of strains that proliferated in the small intestine were, by contrast, often 10 times more numerous in the wall scrapings than in the contents of a particular part of the intestine.

Evaluation of the method used for counting organisms of different antibiotic-resistant mutants of a single E. coli strain in the alimentary tract of a calf

Spontaneous chromosomal mutants resistant to one or other of five different antibiotics were isolated from an 88⁻ form of an O141:K85,88 porcine enteropathogenic *E. coli* strain. The results of bacterial counts performed on different parts of the alimentary tract of a calf 28 h after it had been inoculated orally with equal numbers of each of these mutants, the parent strain and the parent strain in which a neomycin-resistance plasmid had been implanted revealed that the concentrations of all six antibiotic-resistant forms in each part of the alimentary tract examined were similar (table I). Because of extraneous *E. coli* organisms, especially in the lower alimentary tract, it was not possible to estimate the concentrations of organisms of the parent strain present. However, a comparison of the counts obtained on MacConkey's agar with and without antibiotics revealed that in the anterior intestine at least, the concentrations of these organisms were about the same as those of organisms of the antibiotic-resistant forms. Each of the six antibiotic-resistant forms with an equal number

TABLE I

*The enumeration of organisms of different antibiotic-resistant mutants of an 88⁻ form of an O141:K85,88 piglet-enteropathogenic strain of Escherichia coli in different parts of the alimentary tract of a calf to which they had been given orally**

Material examined	Number of organisms (log ₁₀ per g) that formed colonies on MacConkey's medium containing†						
	rifampicin	streptomycin	spectinomycin	ampicillin	neomycin	sodium nalidixate	no antibiotic
Stomach contents	5.5	5.7	5.7	5.7	5.7	5.8	...
Small intestine, part‡							
1 (contents)	5.7	6.2	6.0	6.3	6.0	6.4	7.0
1 (wall scrapings)	4.4	4.8	4.6	4.6	4.3	4.7	5.7
3 (contents)	5.6	6.2	6.3	5.8	5.8	6.3	7.2
3 (wall scrapings)	4.0	4.5	4.3	4.3	4.0	4.5	5.9
7 (contents)	6.5	7.2	6.7	6.9	6.9	7.2	8.9
7 (wall scrapings)	5.3	5.8	5.6	5.7	5.7	6.2	7.5
Colon contents	7.3	7.7	7.3	7.9	7.5	7.9	9.0

* The calf had been given a mixture containing 10⁹ viable organisms of each of the antibiotic-resistant mutants and of the sensitive strain from which they were derived; it was killed 28 h later. The neomycin resistance was plasmid-determined; the resistance of the other five mutants was chromosomal.

† Rifampicin at 100 µg per ml., and all other antibiotics at 20 µg per ml.

‡ The small intestine was divided into seven equal parts for counting; part 1 was nearest to the stomach and part 7 to the colon.

of organisms of the parent strain was also inoculated lightly into broth. After incubation at 37°C for 24 h, no significant difference was found between the numbers of sensitive and resistant organisms present.

The effect of the Ent, 88 and 99 plasmids on the colonisation of the alimentary tract of piglets, calves and lambs by an O9:K36:H19 non-pathogenic strain of E. coli

Ent, 88 and 99 plasmids were implanted in a non-pathogenic O9:K36:H19 strain of *E. coli* that had been isolated initially from the faeces of a healthy pig. The resulting plasmid-containing forms were given orally to an "88-susceptible" (88S) piglet, an "88-resistant" (88R) piglet, a calf and a lamb; the distributions of organisms of these forms and those of the parent Ent⁻88⁻99⁻ strain in the alimentary tracts are summarised in table II. The concentrations of all forms of the O9:K36:H19 strain in the stomachs of the four animals, were, like those of the strains used in all subsequent experiments, comparatively

TABLE II
The concentration of organisms in different parts of the alimentary tract of an 88S and an 88R* piglet, a calf and a lamb given orally a mixture of forms of a non-pathogenic O9:K36:H19 strain in which had been implanted 88, 99 and Ent plasmids†*

Animal	Forms of strain in mixture	Number of organisms of stated form (log ₁₀ per g) in					
		stomach contents	wall scrapings of small intestine, part‡				colon contents
			1	3	5	7	
88S piglet*	88-99-Ent ⁻	6.6	5.9	5.6	6.2	6.3	6.8
	88-99-Ent ⁺	5.2	4.0	4.2	4.6	5.0	5.5
	88 ⁺ 99-Ent ⁺	6.4	9.0	9.2	9.4	9.4	8.5
	88-99 ⁺ Ent ⁺	4.7	4.3	4.0	4.7	5.0	5.0
88R piglet*	88-99-Ent ⁻	5.8	3.0	5.0	7.6	6.6	8.3
	88-99-Ent ⁺	4.5	3.0	5.2	6.4	5.6	6.8
	88 ⁺ 99-Ent ⁺	3.0	<2.0	2.3	3.2	3.0	4.7
	88-99 ⁺ Ent ⁺	3.5	2.0	4.0	5.2	5.0	6.0
Calf	88-99-Ent ⁻	5.3	4.4	4.6	5.2	5.9	7.9
	88-99-Ent ⁺	4.6	4.2	4.4	4.5	5.3	6.4
	88 ⁺ 99-Ent ⁺	2.7	2.6	2.7	3.5	4.2	5.9
	88-99 ⁺ Ent ⁺	3.5	4.6	7.7	8.6	8.5	9.2
Lamb	88-99-Ent ⁺	4.2	3.0	2.5	3.0	3.6	4.2
	88 ⁺ 99-Ent ⁺	<2.0	2.5	3.0	3.0	3.2	3.2
	88-99 ⁺ Ent ⁺	3.7	3.6	8.5	8.3	8.0	8.7

* 88S = 88 Susceptible; 88R = 88 resistant.

† The piglets had been given 10⁷ viable organisms of each of the four forms; the dose of each for the calf was 10⁸ and for the lamb 10⁸ viable organisms. All animals were also given a non-pathogenic *E. coli* strain, P403, and a lactobacillus strain, 10⁸ of each for the piglets and the lamb and 10¹⁰ of each for the calf; the animals were killed 28-32 h after inoculation.

‡ The small intestine was divided into seven equal parts for counting; part 1 was nearest to the stomach and part 7 to the colon.

low. The 88⁺ form was found in high concentrations throughout the small intestine of the 88S piglet, and the 99⁺ form was found in high concentrations in the middle and posterior, but not in the anterior, small intestine of the calf and lamb. The concentrations of the 88⁺ form in the small intestine of the 88R piglet, the calf and the lamb, and of the 99⁺ form in the small intestine of the 88S and 88R piglets, were no higher than those of the 88⁻99⁻Ent⁺ form which, in most specimens examined, was present in slightly lower concentrations than the parent 88⁻99⁻Ent⁻ form itself.

The experiment was repeated in three 88S piglets. The results for two of them were similar to those for the 88S piglet referred to in table II; low concentrations of all four forms were found in the small intestine of the third. Another two 88S piglets were given 10⁹ viable organisms of each of the four forms instead of the customary 10⁷ viable organisms. Apart from the presence of high concentrations of the 88⁺ form in their small intestines, one of them had moderately high concentrations of the 99⁺ form in its lower small intestine. The viable count of 88⁺ organisms in the wall scrapings of parts 1, 3, 5 and 7 of the small intestine of this piglet were (log₁₀ per g), 9.2, 8.5, 9.0 and 9.2 respectively. The corresponding figures for 99⁺ organisms were 5.2, 6.2, 7.6 and 8.6 and for 88⁻99⁻Ent⁺ organisms 5.6, 5.4, 5.8 and 5.9.

*The effect of the Ent, 88 and 99 plasmids on the colonisation of
the alimentary tract of piglets and calves by an E. coli
K12 strain*

The results of the bacteriological examinations on an 88S and an 88R piglet and a calf given forms of an *E. coli* K12 strain that possessed different combinations of the Ent, 88 and 99 plasmids are summarised in table III; the original K12 strain was *lac*⁻ and auxotrophic and like all K12 strains it was O⁻ and K⁻. Low concentrations of the four forms were found in all parts of the alimentary tracts of the three animals; none of the plasmid-containing forms survived better in the small intestine than did the 88⁻99⁻Ent⁻ form, or as well as did the non-pathogenic P403 strain.

The above experiment was repeated in two 88R piglets and three calves. The results for the two piglets and two of the calves were similar to those in table III. The third calf developed mild diarrhoea, but otherwise appeared healthy. Although the concentrations of the 99⁺ form in its small intestine were not high, they were higher than those of the other three forms which, in all parts of the small intestine, were less than (log₁₀ per g) 2.0. The figures for the 99⁺ form in the wall scrapings of parts 1-7 were 2.9, 3.2, 3.4, 4.0, 6.2, 6.8 and 6.8; the concentrations of the P403 strain in these parts were 5-100 times greater.

The concentrations of an O8:K85,99 lamb-enteropathogenic strain in wall scrapings of parts 1-7 of the small intestine of an additional calf that had been given a mixture of this strain and the four K12 forms were (log₁₀ per g) 4.4, 5.0, 5.5, 5.8, 9.0, 9.5 and 9.3 respectively. The concentrations of all four K12 forms in these scrapings and in all the other material examined from this animal were

TABLE III

The concentration of organisms in different parts of the alimentary tract of an 88S and an 88R piglet and a calf given orally a mixture of forms of an E. coli K12 strain in which had been implanted 88, 99 and Ent plasmids

Animal	Forms of strain in mixture	Number of organisms of stated form ($\log_{10}$ per g) in					
		stomach contents	wall scrapings of small intestine, part				colon contents
			1	3	5	7	
88S piglet	88-99-Ent ⁻	<2.0	3.3	4.0	4.8	5.8	7.9
	88-99-Ent ⁺	<2.0	<2.0	<2.0	3.9	<2.0	<2.0
	88 ⁺ 99-Ent ⁺	<2.0	<2.0	<2.0	5.3	3.7	6.6
	88-99 ⁺ Ent ⁺	<2.0	<2.0	<2.0	5.5	4.4	7.0
88R piglet	88-99-Ent ⁻	5.4	<2.0	<2.0	<2.0	<2.0	6.3
	88-99-Ent ⁺	4.5	<2.0	<2.0	<2.0	<2.0	4.2
	88 ⁺ 99-Ent ⁺	5.3	<2.0	<2.0	<2.0	3.3	6.0
	88-99 ⁺ Ent ⁺	5.5	<2.0	<2.0	<2.0	4.0	5.9
Calf	88-99-Ent ⁻	4.8	4.5	5.3	5.0	6.4	5.3
	88-99-Ent ⁺	3.0	3.9	4.3	4.0	5.7	4.0
	88 ⁺ 99-Ent ⁺	4.3	4.5	4.6	4.5	4.6	5.5
	88-99 ⁺ Ent ⁺	4.3	4.3	5.3	5.5	6.9	5.0

For other details see table II.

($\log_{10}$ per g) <2.0; the non-pathogenic P403 strain was present in concentrations of ($\log_{10}$ per g) 2.0-3.0. A mixture of a pig enteropathogenic strain O141:K85,88 and the four K12 strains was also given to an 88S piglet: the concentrations of the enteropathogenic strain in wall scrapings of parts 1-7 of the small intestine of this piglet were 9.3, 9.0, 9.3, 9.5, 9.3, 9.6 and 9.6 respectively. The corresponding figures for the Ent⁺88⁺ form of the K12 strain were only 4.0, 3.7, 5.0, 5.3, 5.6, 5.3 and 5.0. They were, however, about 10-100 times higher than those for the Ent⁺99⁺ form and about 100-1000 times higher than those for the Ent⁺88⁻99⁻ form.

The effect of the 88 and 99 plasmids on the colonisation of the alimentary tract of piglets and calves by O141:K85,88 and O149:K91,88:H19 piglet-enteropathogenic strains

The results of estimating the concentration of the inoculated organisms in the alimentary tract of an 88S and an 88R piglet and a calf that had been given forms of the O141:K85,88 piglet-enteropathogenic strain that possessed either the 88 or 99 plasmid or neither are summarised in table IV. High concentrations of the 88⁺ form were found throughout the small intestine of the 88S piglet. The concentrations of this form in the small intestine of the 88R piglet and the calf, like those of the 88⁻99⁻ form, were low. High concentrations of the 99⁺ form were found in the lower, but not the upper, half of the small intestine of all three animals.

The above experiments were repeated in three 88S and three 88R piglets and a calf. Very high concentrations of the 88⁺ form were found throughout the small intestine of the three 88S piglets; high concentrations of the 99⁺ form were found in parts 2-7 of the small intestine of one, in parts 3-7 of another and in parts 5-7 of the third. In those parts of two of the piglets in which they had proliferated, the 99⁺ organisms were as numerous as 88⁺ ones. High concentrations of the 99⁺ form were found only in parts 3-7 of one of the 88R piglets and in parts 5-7 of another; in the third it did not proliferate. In the calf, high concentrations were found only in parts 3-7. Low concentrations of the 88⁺ form were found throughout the small intestine of the three 88R piglets and the calf.

Because it consistently produced intestinal colonisation and severe diarrhoea in 88S piglets, the O141:K85,88 strain had been chosen as the indicator of the 88 status of the piglets from the farm on which 88R animals were known to predominate. The median counts, with the ranges in brackets, of the numbers of these organisms in the scrapings of the walls of parts 1, 3, 5 and 7 of the small intestine of 23 piglets from this farm which were classified as 88R were ($\log_{10}$ per g) 3.5 (<2.0-6.0), 3.3 (<2.0-5.6), 4.0 (<2.0-6.4) and 3.9 (<2.0-6.9) respectively. The corresponding figures for five that were found to be 88S were 9.3 (8.0-10.2), 9.4 (8.4-10.2), 9.3 (7.0-10.3) and 9.7 (7.7-10.2).

A mixture of five wild-type strains belonging to serotype O149:K91,88:H19 (a common porcine-enteropathogenic serotype) and isolated from sick calves by Ellis and Kienholz (1977) was given to four calves. None developed diarrhoea and the inoculated organisms were found in only low concentrations in their small intestines. For a fifth calf, the dose of each of the strains was

TABLE IV

The concentration of organisms in different parts of the alimentary tract of an 88S and an 88R piglet and a calf given orally a mixture of forms of an O141:K85,88 piglet enteropathogenic strain possessing different combinations of the 88 and 99 plasmids

Animal	Forms* of strain in mixture	Number of organisms of stated form ($\log_{10}$ per g) in					
		stomach contents	wall scrapings of small intestine, part				colon contents
			1	3	5	7	
88S piglet	88-99-	6.0	5.5	4.0	5.6	5.4	6.6
	88 ⁺ 99-	6.8	9.3	9.4	9.6	9.8	9.5
	88-99 ⁺	6.0	5.0	6.7	8.0	8.0	8.5
88R piglet	88-99-	3.3	3.0	3.8	3.0	3.0	4.4
	88 ⁺ 99-	3.3	2.6	3.4	2.5	2.4	3.7
	88-99 ⁺	3.7	4.5	4.4	8.5	8.8	8.6
Calf	88-99-	5.3	3.0	3.8	3.6	4.5	6.4
	88 ⁺ 99-	6.2	4.3	4.8	4.4	5.5	6.8
	88-99 ⁺	6.9	5.9	6.5	8.9	9.2	9.3

* All forms were Ent⁺. For other details see table II.

increased to 10^{10} viable organisms and an O9:K30,99 calf-enteropathogenic strain in the customary dosage of 10^9 viable organisms was included in the inoculum. The calf developed severe diarrhoea and the viable counts of O9:K30,99 organisms in the wall scrapings of parts 1-7 of its small intestine were ($\log_{10}$ per g) 9.5, 9.8, 9.6, 9.9, 9.8, 9.8 and 9.9 respectively. The corresponding figures for the O149:K91,88:H19 organisms were 4.6, 4.3, 5.3, 5.5, 5.5, 5.8 and 6.0. To confirm that the O149:K91,88:H19 strains had not lost their virulence, the 88⁺ and 88⁻ forms of one of them were given to an 88S piglet. The piglet developed severe diarrhoea and the 88⁺ form, but not the 88⁻ form, was found proliferating in its small intestine.

The effect of the 88 and 99 plasmids on the colonisation of the alimentary tract of piglets and calves by an O8:K85,99 lamb-enteropathogenic strain

The results of estimating the concentrations of organisms in the alimentary tract of an 88S and an 88R piglet and a calf that had been given forms of the O8:K85,88 lamb-enteropathogenic strain that possessed either the 88 or 99 plasmid or neither are summarised in table V. High concentrations of the 88⁺ form were found throughout the small intestine of the 88S piglet. Apart from the presence of moderately high concentrations of the 99⁺ form in the contents and wall scrapings of part 7, the concentrations of the 99⁺ and the 88⁻99⁻ forms in the small intestine of this piglet were low. In another 88S piglet, in the 88R piglet referred to in table V and in another five 88R piglets, three of

TABLE V

The concentration of organisms in different parts of the alimentary tract of an 88S and an 88R piglet and a calf given orally a mixture of forms of an O8:K85,99 lamb enteropathogenic strain possessing different combinations of the 88 and 99 plasmids

Animal	Forms of strain in mixture	Number of organisms of stated form ($\log_{10}$ per g) in					
		stomach contents	wall scrapings of small intestine, part				colon contents
			1	3	5	7	
88S piglet	88-99-	4.8	3.3	3.9	5.6	5.8	5.4
	88 ⁺ 99-	5.7	7.7	7.6	8.3	8.9	7.7
	88-99 ⁺	3.9	2.9	3.2	6.0	8.4	7.5
88R piglet	88-99-	<2.0	3.2	3.3	4.3	7.8	9.2
	88 ⁺ 99-	<2.0	2.5	2.5	4.0	6.9	8.2
	88-99 ⁺	<2.0	2.0	2.7	4.3	5.6	8.7
Calf	88-99-	6.7	5.7	6.2	8.2	8.3	8.2
	88 ⁺ 99-	6.6	5.3	5.5	6.3	6.6	7.2
	88-99 ⁺	6.7	5.6	8.3	9.5	9.6	9.3

For other details see tables II and IV.

which had been given an inoculum increased 100 times in size, only low concentrations of all three forms were found in the small intestines. Very high concentrations of the 99⁺ form and low concentrations of the 88⁺ form were present in the small intestine of the calf; moderately high concentrations of the 88⁻99⁻ form were found in parts 4-7. In one of a further seven calves, the 99⁺ form proliferated throughout the small intestine, but in the other six it proliferated only in the middle and lower, or lower parts. The 88⁺ form did not proliferate in any of these calves, but in the small intestine of all of them unexpectedly high concentrations of the 88⁻99⁻ form were found; these concentrations were sometimes only 5-10 times lower than those of the 99⁺ form. Because of this finding, the 88⁻99⁻ form together with the 88⁻ form of the O141:K85,88 strain and of one of the O149:K91,88:H19 strains, the O9:K36:H19 strain, and *E. coli* K12 were given to a calf and an 88R piglet. The 88⁻99⁻ form proliferated in the small intestine of the calf but none of the other strains did so, their concentrations in the small intestine, especially those of the O149 and K12 strains, being lower than those of the P403 strain (table VI). By contrast, the piglet remained well and only low concentrations of the inoculated organisms were found in its small intestine.

The K85 antigen of the O8:K85,99 strain strongly promoted its colonisation of calf small intestine. In a calf that had been given mixtures of equal numbers of K85⁺ and K85⁻ organisms of this strain, the concentrations of the K85⁺ organisms in wall scrapings in parts 1-7 of its small intestine were (log₁₀ per g) 8.6, 9.3, 8.8, 8.8, 9.2, 9.4 and 9.2 respectively. The corresponding figures for the K85⁻ organisms were 5.3, 5.5, 5.0, 5.2, 5.7, 6.6 and 5.4.

TABLE VI

The concentration of organisms in different parts of the alimentary tract of a calf given orally a mixture of laboratory-prepared and wild 99⁻ or 88⁻ strains of E. coli

Material examined	Number of organisms (log ₁₀ per g) present of strain					
	O8:K85,99	O141:K85,88	O149:K91,88:H19	O9:K36:H19	K12	P403
Stomach contents	6.6	5.2	<2.0	6.3	<2.0	5.3
Wall scrapings of small intestine, part						
1	7.5	3.6	<2.0	4.2	<2.0	4.6
2	7.0	3.7	2.0	4.2	<2.0	5.0
3	6.8	3.7	2.3	4.2	<2.0	4.5
4	7.8	3.7	3.5	4.0	<2.0	4.6
5	9.0	3.7	<2.0	4.4	<2.0	4.5
6	9.3	3.7	2.3	4.4	<2.0	4.3
7	9.5	4.8	2.6	5.0	<2.0	5.4
Colon contents	8.5	6.0	4.5	7.0	<2.0	6.2

For other details see tables II and IV.

*The effect of the O, K, 88 and 99 antigens on the colonisation of
the alimentary tract of piglets and calves by 99⁺ calf- and
piglet-enteropathogenic strains*

The results of estimating the concentrations of the inoculated organisms in the alimentary tracts of an 88S and 88R piglet and a calf given forms of the O9:K30,99 calf-enteropathogenic strain possessing different combinations of its O, K and 99 antigens and the 88 antigen are summarised in table VII. Only low concentrations of the O⁻K⁻99⁻ and O⁺K⁻99⁻ forms were present in the small intestines of all three animals, the concentration of the O⁺ forms being higher than those of the O⁻ forms in the piglets but not in the calf; both survived poorly in the stomachs of the piglets. Higher concentrations of their 99⁺ forms were found in the small intestines of the piglets; this was so in the calf only for the O⁺ form. The O⁺K⁺99⁻ form proliferated in the small intestine, especially its lower half, of all three animals in concentrations usually greater than those of the O⁺K⁻99⁺ form, but less than those of the O⁺K⁺99⁺ form and, in the case of the 88S piglet, of the O⁺K⁺88⁺ form.

TABLE VII

*The concentration of organisms in different parts of the alimentary tract of an
88S and an 88R piglet and a calf given orally a mixture of forms of an
O9:K30,99 calf-enteropathogenic strain possessing different combinations
of O, K, 88 and 99 antigens*

Animal	Forms of strain in mixture	Number of organisms of stated form (log ₁₀ per g) in					
		stomach contents	wall scrapings of small intestine, part				colon contents
			1	3	5	7	
88S piglet	O-K-99 ⁻	<2.0	<2.0	2.0	5.7	3.3	3.6
	O-K-99 ⁺	4.0	5.2	5.7	7.4	7.3	7.7
	O ⁺ K-99 ⁻	<2.0	2.8	3.0	6.4	4.2	4.6
	O ⁺ K-99 ⁺	7.2	5.5	5.9	7.3	7.9	6.6
	O ⁺ K ⁺ 99 ⁻	6.3	5.2	4.6	8.9	8.3	7.9
	O ⁺ K ⁺ 99 ⁺	6.6	6.2	6.2	9.9	9.9	9.9
	O ⁺ K ⁺ 99-88 ⁺	7.5	9.4	9.6	9.8	9.9	9.9
88R piglet	O-K-99 ⁻	<2.0	<2.0	<2.0	4.4	2.0	4.8
	O-K-99 ⁺	2.7	2.5	3.3	6.8	8.3	6.9
	O ⁺ K-99 ⁻	2.0	2.0	2.3	5.7	4.0	5.9
	O ⁺ K-99 ⁺	4.5	3.6	4.3	6.9	8.5	7.3
	O ⁺ K ⁺ 99 ⁻	2.0	2.5	3.3	8.4	7.6	7.5
	O ⁺ K ⁺ 99 ⁺	4.5	4.5	4.6	9.5	9.2	8.4
Calf	O-K-99 ⁻	5.3	4.3	4.3	4.2	4.2	5.3
	O-K-99 ⁺	3.0	2.9	4.4	4.8	4.3	4.6
	O ⁺ K-99 ⁻	5.4	4.6	4.7	4.7	4.0	5.6
	O ⁺ K-99 ⁺	1.8	4.3	6.4	6.3	5.3	6.0
	O ⁺ K ⁺ 99 ⁻	5.4	6.7	8.0	8.3	8.2	8.6
	O ⁺ K ⁺ 99 ⁺	6.4	8.3	9.2	9.5	9.2	9.9

For other details see tables II and IV.

Similar results were obtained when the above experiments were repeated in two 88S piglets, four 88R piglets and one calf, except that higher concentrations of the $O^-K^-99^+$ than of the $O^-K^-99^-$ form were found in the small intestine of the calf. The $O^+K^+99^+$ form was also given, by itself, to two colostrum-fed piglets reared naturally on the same dam. Both developed diarrhoea. One was examined bacteriologically and the inoculated strain was found to be proliferating in its small intestine, the concentrations in the contents of parts 1, 3, 5 and 7 being ($\log_{10}$ per g) 5.7, 6.0, 8.0 and 9.2 respectively.

A K^+ and a K^- form of an O9:K35,99 calf-enteropathogenic strain was given to an 88R piglet and to a calf. The K antigen, as in the case of the O9:K30,99 strain, had a striking effect on the degree of small intestinal colonisation that these two forms achieved. The concentrations of the $K35^+$ form in the wall scrapings of parts 1-7 of the small intestine of the piglet were ($\log_{10}$ per g) 3.6, 4.5, 4.4, 4.5, 5.2, 8.5 and 8.6 respectively, and of the calf 8.5, 10.7, 10.7, 10.8, 10.4, 10.6 and 10.3 respectively. The corresponding figures for the $K35^-$ form were 2.0, 2.3, 2.3, 4.2, 4.2, 4.2 and 4.5 (piglet) and 5.6, 6.3, 6.9, 7.5, 8.0, 10.0 and 9.0 (calf).

Mixtures of an O101:K30,99 calf-enteropathogenic strain and an O101:K30,99 piglet-enteropathogenic strain were given to a calf and an 88R piglet. Both differed little in their ability to proliferate in the small intestine of these animals. In wall scrapings of parts 3-7 of the small intestine of the calf the concentrations of the calf strain were ($\log_{10}$ per g) 3.9, 4.2, 6.5, 7.7 and 7.7 respectively and of the piglet strain 4.6, 4.5, 7.2, 8.0 and 8.0 respectively. The corresponding figures for the piglet were 7.7, 8.8, 9.3, 9.4 and 9.5 (calf strain) and 8.0, 9.2, 9.5, 9.6 and 9.6 (piglet strain). A $K30^-$ form of the piglet strain had been included in the inoculum given to these two animals—it was present in their intestines in concentrations much lower than those of the $K30^+$ form. When a mixture of the 99^- form of the piglet strain and the 99^- form of the O9:K30,99 calf-enteropathogenic strain was given to a calf both forms proliferated in the small intestine; when the mixture was given to an 88R piglet only the piglet strain did.

The effect of the 987P and 88 antigens on the colonisation of the alimentary tract of piglets and calves by O9:K103,987P piglet-enteropathogenic strains

The results of estimating the concentrations of the inoculated organisms in the alimentary tract of an 88S and an 88R piglet and a calf that had been given forms of an O9:K103,987P piglet-enteropathogenic strain possessing different combinations of the 987P and 88 antigens are summarised in table VIII. The wild 987P⁺ strain, isolated in the USA, and its 987P⁻ mutant had been received from Dr Harley Moon. The 88 plasmid had been implanted in the mutant in this laboratory; we had failed to implant a 99 plasmid in it. The 987P⁺ form proliferated in the middle and lower parts of the small intestine of both the 88S and the 88R piglet; its concentrations in the small intestine of the calf were no higher than those of the 987P⁻ form. The 88⁺ form proliferated in parts 2-7 of

TABLE VIII

The concentration of organisms in different parts of the alimentary tract of an 88S and an 88R piglet and a calf given orally a mixture of forms of an O9:K103,987P piglet-enteropathogenic strain possessing different combinations of the 987P and 88 antigens

Animal	Forms of strain in mixture	Number of organisms of stated form ($\log_{10}$ per g) in					
		stomach contents	wall scrapings of small intestine, part				colon contents
			1	3	5	7	
88S piglet	987P-88 ⁻	3.7	5.0	5.3	5.3	4.6	5.8
	987P ⁺ 88 ⁻	3.6	4.9	5.9	9.3	9.4	9.7
	987P-88 ⁺	2.8	4.3	6.8	8.4	8.7	8.8
88R piglet	987P-88 ⁻	2.0	2.9	4.0	2.9	3.3	4.2
	987P ⁺ 88 ⁻	2.7	4.2	4.5	9.5	9.8	9.0
	987P-88 ⁺	3.3	3.5	4.2	3.0	4.4	5.5
Calf	987P-88 ⁻	<2.0	3.4	3.7	4.0	5.2	8.5
	987P ⁺ 88 ⁻	<2.0	<2.0	3.6	4.0	5.4	8.7
	987P-88 ⁺	<2.0	<2.0	3.6	4.0	5.2	7.8

For other details see tables II and IV.

the small intestine of the 88S piglet, its concentrations being considerably higher in the posterior than in the anterior parts, but it was found only in low concentrations in the intestines of the 88R piglet and the calf. The experiment was repeated in another 88S piglet, an 88R piglet and a calf, with similar results; in addition to the 987P⁺ strain previously tested, this calf was also given an O20:K101,987P strain received from Dr Harley Moon, and another O9:K103,987P strain isolated in England from a diarrhoeic piglet but they, too, failed to proliferate in its small intestine. A third calf was given 10^{10} viable organisms of each of the three 987P⁺ strains and the O9:K35,99 calf-enteropathogenic strain in the customary dosage of 10^9 viable organisms. Very high concentrations of the O9:K35,99 strain were found in its small intestine; the highest concentration of any of the 987P⁺ strains in wall scrapings of any part of this organ was only 10^6 per g.

The combined effect of the 987P and the 88 antigen on intestinal colonisation was studied in an 88S and three 88R piglets inoculated with a mixture of the wild form of the English O9:K103,987P strain and a form of it in which an 88 plasmid had been implanted (table IX). Except in part 7 of the small intestine, the 88⁺ form was present in much higher concentrations than the 88⁻ form in the small intestine of the 88S piglet; proliferation of the 88⁻ form was confined to parts 4-7. The position in the 88R piglets was quite different. As expected, proliferation was confined to the lower half of the small intestine but in most of the intestinal contents and wall scrapings examined the concentration of the 88⁻ form present was 10-100 times greater than that of the 88⁺ form.

TABLE IX

The concentration of organisms in different parts of the alimentary tract of an 88S and three 88R piglets given orally a mixture of an 88⁺ and an 88⁻ form of an O9:K103,987P piglet-enteropathogenic strain*

Animal	Forms of strain* in mixture	Number of organisms of stated form (log ₁₀ per g) in					
		stomach contents	wall scrapings of small intestine, part				colon contents
			1	3	5	7	
88S piglet	88 ⁻	4.5	4.5	5.2	7.5	9.8	8.8
	88 ⁺	4.4	7.4	7.7	9.2	9.6	8.8
88R piglet	88 ⁻	4.3	4.2	4.3	9.4	9.3	9.5
	88 ⁺	2.0	<2.0	2.0	7.3	7.0	7.2
88R piglet	88 ⁻	5.6	4.8	6.5	10.0	9.2	9.5
	88 ⁺	5.3	4.0	4.4	8.3	7.9	7.7
88R piglet	88 ⁻	6.3	3.9	5.5	9.6	9.6	9.5
	88 ⁺	6.4	3.5	5.4	8.2	8.6	7.9

* Both forms possessed the 987P antigen. For other details see tables II and IV.

The effect of plasmid-determined raffinose utilisation and chromosomally determined lactose utilisation on colonisation of the alimentary tract of piglets and calves by enteropathogenic strains

Mixtures of 88⁺Raf⁺, 88⁺Raf⁻, 88⁻Raf⁺ and 88⁻Raf⁻ forms of the O141:K85,88 strain were given to three 88S piglets. The 88⁺Raf⁺ and 88⁺Raf⁻ organisms isolated from their alimentary tracts were differentiated from each other according to their ability to grow on a synthetic medium in which raffinose was the sole energy source; so also were the 88⁻Raf⁺ and 88⁻Raf⁻ organisms. High concentrations of the 88⁺ forms and low concentrations of the 88⁻ forms were found throughout the small intestines of these piglets. Of fifty 88⁺ isolates from the inoculum given to one of them, 22 were Raf⁺. A similar proportion was found amongst isolates from the alimentary tract; the number amongst 50 from the contents of the stomach, parts 1-7 of the small intestine and the colon and from wall scrapings of parts 1-7 of the small intestine were 24, 18, 24, 17, 18, 12, 27, 21, 19, 19, 26, 20, 21, 15, 27 and 20 respectively. Six of fifty 88⁻ isolates from the inoculum were Raf⁺. Except for one isolate from the contents of part 2 of the small intestine, none of fifty 88⁺ isolates from each of the materials that had been searched for 88⁺Raf⁺ organisms were Raf⁺. The incidence of raffinose utilisation amongst fifty 88⁺ isolates from the inocula given to the other two 88S piglets was 52 and 38%. A similar incidence (52 and 44%) was found amongst eight hundred 88⁺ isolates from 16 specimens of contents or wall scrapings of different parts of the alimentary tract of each piglet.

Mixtures of Raf⁺ and Raf⁻ organisms of the 88⁺ form of the O141:K85,88 strain were also given to two 88R piglets. Of 50 isolates from the inoculum

given to both of them, 29 (58%) were *Raf*⁺. Of 294 isolates from the low concentrations of 88⁺ organisms that were found in the contents and wall scrapings of the small intestine of one of them, 127 (59%) were *Raf*⁺. The corresponding figure for 180 similarly sited isolates from the other piglet was 126 (70%).

Mixtures of *lac*⁺ and *lac*⁻ organisms of the O8:K85,99 lamb enteropathogenic strain proliferated throughout the small intestine of a calf to which it had been given. Of 50 isolates from the inoculum 21 were *lac*⁺. The numbers amongst 50 isolates from the contents of its stomach, parts 1-7 of its small intestine, and colon, and from the wall scrapings of parts 1-7 of its small intestine were 38, 46, 18, 20, 18, 24, 15, 23, 20, 45, 16, 17, 19, 16, 17 and 20 respectively. When the experiment was repeated in an 88R piglet, but with a K30⁻ form of one of the O101:K30,99 strains, the number of *lac*⁺ isolates amongst 50 from the inoculum was 20 and the numbers amongst 50 from the contents of the stomach, small intestine parts 1-7 and colon and from the wall scrapings of parts 1, 3, 5, 6 and 7 of the small intestine were 35, 22, 30, 27, 28, 36, 41, 40, 25, 24, 28, 35, 26 and 37 respectively. The inoculated strain proliferated to a slight degree only in the small intestine of this piglet.

*The effect of giving piglets E. coli strains
enteropathogenic for weaned pigs*

Mixtures of three wild strains of serotypes O141:K85, O138:K81 and O139:K82, that are enteropathogenic for weaned pigs but that are rarely isolated from piglets, were given to an 88S piglet and to two 88R piglets. None showed any sign of ill-health and only low concentrations of the inoculated strains were found in the small intestines. The experiment was repeated in three 88R piglets but they were not killed until 3 days after inoculation. They too, showed no signs of ill-health and only low concentrations of the inoculated strains were found in their small intestines.

DISCUSSION

The initial experiment, in which different antibiotic-resistant mutants of the 88⁻ form of the O141:K85,88 strain given in equal numbers to a calf were also found in approximately equal concentrations in different parts of its alimentary tract when it was killed, suggested that, in the relatively short-term infection experiments which we were to undertake, the behaviour of an antibiotic-resistant mutant would reflect the behaviour of the antibiotic-sensitive strain from which it was derived. During the subsequent experiments we had no cause to doubt this view. Some forms of a strain were used on several occasions as mutants resistant to different antibiotics and no difference in their behaviour was noted that could be attributed to their being mutant. In fact, the results of these experiments indicated that the techniques employed provided a reliable method of assessing the relative importance of plasmid-determined and other characters of *E. coli* in the colonisation of the alimentary tract.

The results of inoculating animals with the different forms of the non-pathogenic O9:K36:H19 strain have confirmed the earlier observations of

Smith and Linggood (1971) that it can be converted to enteropathogenicity for piglets by implanting 88 and Ent plasmids in it and that Ent plays no part in facilitating colonisation of the piglet small intestine. They also demonstrate that this strain can be converted to enteropathogenicity for calves and lambs by implanting 99 and Ent plasmids in it, Ent again not being involved in the process of small intestinal colonisation. Our failure, employing the same techniques, to convert the *E. coli* K12 strain to enteropathogenicity emphasises that there is more to enteropathogenicity than the possession by a bacterium of an appropriate small-intestinal colonisation factor and the ability to produce enterotoxin.

In the experiments with the O9:K36:H19 strain and with all the other strains submitted to plasmid manipulation there was no indication that the 88 antigen facilitated colonisation of calf small intestine; if anything the reverse was true. This was also the case with the O149:K91,88:H19 strains that had been isolated from sick calves by Ellis and Kienholz (1977). The possibility that these strains might have lost their enteropathogenic properties during storage was rebutted by our ability to produce small-intestinal colonisation and diarrhoea in a piglet—the common host of such strains—with one of them. In view of these findings and our previous failure to produce diarrhoea in calves with 88⁺ piglet enteropathogenic strains (Smith and Halls, 1967) it seems that further studies are needed to determine the part that O149:K91,88:H19 strains play in calf disease.

In contrast to the comparative uniformity of behaviour of the 88⁺ forms in piglets and calves and of the 99⁺ forms in calves, the 99⁺ forms of the different manipulated strains behaved differently in piglets. For example, the 99⁺ forms of the O141:K85,88 piglet-enteropathogenic strain and of the O9:K30,99 calf-enteropathogenic strain usually proliferated in piglet small intestines whereas the 99⁺ forms of the O9:K36:H19 non-pathogenic strain, except when given in greatly increased dosage, and of the O8:K85,99 lamb-enteropathogenic strain, showed very little tendency to do so. The infection experiments with the forms of the O9:K30,99 strain that possessed different combinations of the O, K and 99 antigens indicated that the K antigen in a strain with a 99 plasmid and, perhaps to a lesser extent, the O antigen were important in determining whether or not a strain would colonise the small intestine of a piglet and, for that matter, a calf. In these and other experiments the K antigens appeared to make quite as great a contribution to small intestinal colonisation as did the 99 antigen, but the presence of both was necessary to achieve maximal colonisation. It appears then that the combined contribution of the O, K and 99 antigens is not usually sufficient to promote colonisation with the O8:K85:99 and O9:K36:H19 strains in piglets. Clearly, this applied only in piglets because the 99⁺ form of both these strains consistently proliferated in the small intestine of calves. Furthermore, in the one calf to which it was given, the 99⁻ form of the O8:K85,99 strain (as did the 99⁻ form of the O9:K30,99 and O101:K30,99 strains in a calf and the 99⁻ form of the O101:K30,99⁻ form in a piglet) proliferated sufficiently to cause diarrhoea. Employing colostrum-deprived hysterectomy-derived piglets, Moon *et al.* (1977) had also failed to

achieve small-intestinal colonisation with an O8:K85,99 strain, but they did so with four other calf-enteropathogenic strains, including our O9:K30,99 strain. The strains with which Smith and Linggood (1972) failed to achieve small-intestinal colonisation in colostrum-fed conventionally reared piglets included our O8:K85,99 and O9:K30,99 strains. Their failure with the former is now not surprising and the most likely explanation of their failure with the latter would be the presence of antibody to it in the colostrum and milk of the dam of the piglets to which it had been given. The enteropathogenic nature of this strain for piglets has now been placed beyond doubt by our successful production of small-intestinal colonisation and diarrhoea with it in colostrum-fed conventionally reared piglets. It is noteworthy in this respect that the O101:K30,99 piglet-enteropathogenic strain and the O101:K30,99 calf-enteropathogenic strain proliferated in piglet intestine and in calf intestine to the same extent. It seems reasonable to look upon certain wild 99⁺ strains equally as piglet, calf and, presumably, lamb enteropathogens whereas others should be looked upon as calf and presumably lamb enteropathogens only. Whether 99⁺ strains exist that are enteropathogenic only for piglets remains to be determined.

We have confirmed the findings of Moon and his co-workers (Isaacson *et al.*, 1977; Nagy, Moon and Isaacson, 1977) that 987P is a colonising factor in piglets. The low concentrations of the American and English O9:K103,987P strains and the O20:K101,987P strain that were found in the small intestine of our calves implies, however, that the 987P antigen resembles the 88 antigen rather than the 99 antigen in that it promotes colonisation in the piglet but not in the calf. Our studies on the 987P antigen were limited by the fact that, unlike the 88 and 99 antigens, its control by a transmissible plasmid could not be demonstrated and hence exploited. However, our mixed infection experiments with the O9:K103,987P strain and the 987P⁻ mutant emphasised the potency of 987P as a colonising factor in piglets in that the small intestinal concentrations of the 987P⁺ form were very much higher than those of the 987P⁻ form. Like 99⁺ forms, the 987P⁺ forms proliferated to a much greater degree in the posterior than in the anterior small intestine, a feature of 88⁻ piglet enteropathogenic strains previously noted by Hohmann and Wilson (1975) and Nagy *et al.* (1977). Proliferation of 99⁺ forms, however, was not always restricted to the posterior small intestine. In some of the calves, for example, high concentrations were found throughout most of the small intestine. The highest concentrations, however, were always found in the lowest parts, and the concentrations decreased progressively towards the upper end until at the top they were no greater than those of the corresponding 99⁻ forms with which the animals had also been inoculated. In contrast, the 88⁺ forms proliferated throughout the small intestine of 88S piglets; the concentrations in the anterior small intestine were similar to those in the posterior small intestine and, because of the much greater susceptibility of the anterior than the posterior small intestine to enterotoxin activity (Smith and Halls, 1967), this was responsible for the severe nature of the ensuing diarrhoea. One possible explanation for this difference between the 88 antigen and the 99,987P and other small-intestinal

colonising factors, such as polysaccharide K antigens, may be that the latter are less efficient than the 88 antigen. As a consequence, organisms without the 88 antigen may be less successful in colonising the anterior small intestine where the speed of chyme flow is greatest. An alternative or additional explanation is that receptor sites for 88 antigens are evenly distributed throughout the small intestine whereas those for the other antigens are more common in the posterior than in the anterior small intestine. Support for the specific nature of 88 adhesion comes from the pig genetic studies of Sellwood *et al.* (1974, 1975) and Rutter *et al.* (1975) and our finding of a fairly clear-cut difference between piglets classed as 88-susceptible and 88-resistant in regard to colonisation by 88⁺ strains and their similarity in regard to colonisation by strains possessing other colonising factors. The argument in favour of specific receptor sites for all colonising factors becomes increasingly difficult to support as more are discovered. Also, the picture is further complicated by the implications of our failure to achieve colonisation in piglets with *E. coli* strains that possess none of the known colonising factors, yet colonise the small intestine of weaned pigs with the production of clinical disease (Smith and Halls, 1968).

The observation that *lac*⁺ and *lac*⁻ organisms of the O8:K85,99 and O101:K30,99 enteropathogenic strains were found in similar concentrations throughout the alimentary tract of the calf and piglet to which they had been given infers that, at least during the period of the experiment, the inability of the *lac*⁻ organisms to metabolise the lactose in the milk on which the animals were fed did not hinder the growth of these organisms. The failure of the plasmid-borne *Raf* genes to influence the small-intestinal colonising power of the O141:K85,88 strain is not comparable with the *lac*⁺/*lac*⁻ experiments because we were primarily investigating the reason for the high incidence of these genes in wild 88⁺ piglet-enteropathogenic strains of *E. coli* (Smith and Parsell, 1975); we were anticipating that if the *Raf* genes facilitated small-intestinal colonisation they would do so in a more subtle manner than by utilising some substrate in the milk denied to *Raf*⁻ organisms. The position of the *Raf* genes in 88⁺ piglet enteropathogenic strains resembles that of the Hly plasmid: both plasmids are commonly present in these strains and in those that infect weaned pigs, but their role has not yet been clarified in the pathogenesis of enteric disease caused by *E. coli* (Smith and Linggood, 1971).

SUMMARY

By plasmid manipulation and by other techniques, forms of non-pathogenic and of enteropathogenic *Escherichia coli* strains were obtained that possessed different combinations of the genes that control the production of enterotoxin (Ent) and of 88, 99, 987P, polysaccharide K and O antigens and the utilisation of raffinose and lactose. Mixtures of forms derived from the same strain, each resistant to a different antibiotic, together with much larger numbers of non-pathogenic *E. coli* organisms and lactobacilli were then given orally to colostrum-deprived calves and lambs and to colostrum-deprived piglets that were genetically resistant (R) or genetically susceptible (S) to infection with 88⁺ strains of *E. coli*. The concentrations that each form subsequently attained in

different parts of the alimentary tract of these animals was then determined by performing bacterial counts on culture media containing appropriate antibiotics. By this procedure it was possible to compare the part played by the different bacterial components in colonising the alimentary tract.

A non-pathogenic O9:K36:H19 strain, but not *E. coli* K12, was rendered enteropathogenic for 88S piglets by implanting an 88 and an Ent plasmid in it and for calves and lambs by implanting a 99 and an Ent plasmid in it. The 88⁺ form of this strain and of wild enteropathogenic strains proliferated throughout the small intestine of 88S piglets, but the concentrations they attained in the small intestine of 88R piglets, calves and lambs were, if anything, less than those attained by their 88⁻ counterparts; the small intestines of 88S and 88R piglets appeared equally susceptible to other colonising factors. *E. coli* O149:K91,88 strains that had been isolated from sick calves produced neither small-intestinal colonisation nor diarrhoea in calves. The 99⁺ form of the O9:K36:H19 strain and of all the wild enteropathogenic strains examined proliferated in the small intestine of calves, but only some of them proliferated in the small intestine of piglets. The enteropathogenicity for piglets of a wild 99⁺ enteropathogenic strain of calf origin was confirmed by producing small-intestinal colonisation and diarrhoea with it in two colostrum-fed conventionally reared piglets. Forms of some wild enteropathogenic strains from which the 99 plasmid had been removed proliferated in the small intestine of calves and piglets to an extent sufficient to cause diarrhoea, but they did not proliferate to the extent shown by the corresponding 99⁺ form in mixed infections.

The 987P antigen was a potent factor in the colonisation of the small intestine in piglets but not in calves. In piglets and in calves, the polysaccharide K and O antigens enhanced colonisation. The 88⁺ forms usually proliferated to a similar degree throughout the small intestine, but forms possessing the other colonising factors either proliferated only in the posterior small intestine or to a much greater degree in the posterior than in the anterior small intestine. Forms possessing both the 987P and 88 antigens proliferated throughout the small intestine of 88S piglets; they proliferated only in the posterior small intestine of 88R piglets and to a lesser degree than the corresponding 987P⁺88⁻ forms. Neither the Ent plasmid, the Raf (raffinose) plasmid nor the ability to utilise lactose influenced small intestinal colonisation. Wild 88⁻99⁻987P⁻ *E. coli* strains that were enteropathogenic for weaned pigs did not proliferate in the small intestines of piglets.

We are grateful to Mrs Frances Richards for her capable technical help. Our thanks are also due to Dr P. M. Biggs, Miss Margaret Lovell, Dr Harley Moon, Dr Ida Ørskov, Dr B. Rowe, Mr R. Sellwood and Miss Susan Shaw for assistance in a variety of ways.

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