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Increased Survival in Calves of *Escherichia coli* K-12 Carrying an Ent Plasmid

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Escherichia coli K-12 strains with and without an Ent plasmid were fed to calves, and the survival of each was monitored by viable bacterial counts of the feces. The *E. coli* K-12 strain carrying the Ent plasmid survived in the calves at significantly higher levels and for a longer period of time than the *E. coli* F^- strain.

In the past 10 years there has been compelling evidence to show that certain *Escherichia coli* strains are causally related to a significant proportion of diarrheal disease seen in man and his domestic animals (1, 8). Data obtained by Smith and Linggood (6) with piglets strongly suggest that, in order for *E. coli* to produce diarrhea in a particular animal, it must possess at least two properties: it must be able to produce an enterotoxin active on the epithelium of the small bowel, and it must also be able to multiply in the small intestine of the animal. Enterotoxin production has been shown to be mediated in many instances by a plasmid called Ent (2, 5). The ability to proliferate in the small bowel has been associated with a plasmid-mediated K antigen that permits organisms to adhere to the small bowel epithelium. In *E. coli* isolated from piglets the plasmid encoding for the K antigen has been termed K88 (3, 6), whereas the plasmid encoding for the K antigen of bovine *E. coli* strains has been termed K99 (7, 8). Ent^+ K^+ organisms often give rise to a severe or even lethal diarrheal disease in both piglets and newborn calves. Ent^- K^+ cells may give rise to a mild diarrhea which probably reflects a host response to the large number of organisms proliferating in the small bowel, a region that ordinarily contains relatively few enterobacteriaceae. Ent^+ K^- organisms fed to animals appears to be totally innocuous.

Although the possession by *E. coli* of the Ent plasmid alone does not have any noticeable selective advantage with respect to colonizing the small bowel and the toxin is ineffective against large bowel epithelium, it has not been clear whether Ent might still provide a host bacterium with some selective advantage over other *E. coli* strains. We wish to report that, in fact, *E. coli* K-12 harboring a single Ent plasmid received from a bovine *E. coli* isolate shows a significantly increased period of survival in calves as compared to a homogenic F^- strain.

E. coli strain B41 (O101:K99), previously isolated by Smith (4) from a calf, was used in genetic crosses, and its plasmids were characterized by using methods described in earlier work from this laboratory (2). A nalidixic acid-resistant mutant of strain B41 was isolated for oral feeding experiments. B41 was found to possess three distinct plasmids: a 65-Mdal plasmid which encoded for a heat-stable enterotoxin, a 65-Mdal plasmid encoding for the K99 antigen, as well as a 60-Mdal R plasmid encoding for streptomycin and tetracycline resistance (J. A. Meyers and S. Falkow, unpublished observations). All three plasmids were transmitted independently, although the K99 plasmid did not appear to be self-transmissable but required mobilization by Ent or the R plasmid. The *E. coli* K-12 recipient 711 (*phe his pro trp lac nal*) was used as a recipient of plasmid species.

Calves were purchased from selected farms in northern Colorado at 1 to 2 days of age and transported to the Contagious Disease Laboratory at Colorado State University. They were fed colostrum for 3 to 4 days and then placed on an appropriate milk replacer. The calves were observed for clinical signs, and fecal samples were taken daily. Serial dilutions of the fecal samples were plated on MacConkey agar and MacConkey agar containing 25 μg of nalidixic acid per ml. Before oral challenge none of the calves exhibited >10 colony-forming units/g (dry weight) of feces of nal^r organisms which could grow on MacConkey agar plus nalidixic acid, whereas the total counts of organisms appearing on MacConkey agar ranged from 10^8 to 10^{10}/g (dry weight) of feces. Calves 5 to 10 days old were chosen at random, placed in

groups in separate rooms, and dosed orally with the appropriate *E. coli* strain. The organisms for oral challenge were grown in brain heart infusion broth to the early logarithmic phase of growth and centrifuged, and the cells were resuspended in 50 ml of fresh brain heart infusion broth and mixed with 150 ml of pasteurized milk. After dosing, the animals were observed for clinical signs, and fecal samples were obtained daily for enumeration on MacConkey agar and MacConkey agar plus 20 μg of nalidixic acid per ml. The counts on the latter medium were assumed to reflect the challenge strain, and at least five colonies from the plates were picked to confirm this assumption.

Whereas strain B41 was capable of causing a severe diarrhea or even death in calves less than 24 h old, the 5- to 10-day-old animals infected with this strain did not show any clear-cut clinical symptoms. Nevertheless, animals receiving this strain shed this organism at high levels for 7 days after dosing and, indeed, all three animals were excreting detectable organism 2 weeks after dosing (Table 1). A B41 K99$^-$ derivative fed calves did not colonize the animals as well as the K99$^+$ form and the counts were, on average, considerably lower by day 4 after dosing. Nevertheless, the K99$^-$ derivative continued to be excreted for 2 weeks in one of three animals.

As might be expected, *E. coli* K-12 711 did not survive well after being fed to calves. In two of the four calves fed this strain the organism was detected for only 1 day, and none of the animals excreted this strain after day 3 after challenge. In rather marked contrast, the *E. coli* K-12 711 strain harboring the 65-Mdal Ent plasmid was found in significant numbers for 7 days after dosing in three of four animals and could be detected in two of four animals for 9 days. The *E. coli* 711 K-12 Ent$^+$ K99$^+$ isolate-fed animals showed slightly higher levels of excretion compared to the Ent$^+$ strain for the first 4 days after dosing and was excreted in two of four animals for 11 days.

The results reported here indicate that *E. coli* K-12 carrying an Ent plasmid can survive in calves at significantly higher levels and for a longer period of time than a homogenic F$^-$ strain. The K99 plasmid that is of critical im-

TABLE 1. *Effect of Ent on the survival of Escherichia coli K-12 in calves*

Organism given	No. of viable organisms (log_{10}) per gram of feces voided on the following days after administration						
	1	2	3	4	5	6	7
E. coli K-12 711 F$^-$							
Calf 35	5.3	0[a]	0	0	0	0	0
Calf 36	6.2	4.7	2.9	0	0	0	0
Calf 37	5.5	3.5	2.7	0	0	0	0
Calf 38	6.3	0	0	0	0	0	0
E. coli K-12 711 Ent$^+$ K99$^-$							
Calf 31	8.2	6.5	5.6	4.4	4.4	5.8	4.1
Calf 32	7.6	6.4	3.4	3.1	2.2	0	0
Calf 33	8.1	7.8	4.5	4.7	4.1	5.1	4.0
Calf 34	6.7	7.2	3.4	3.3	3.8	2.9	3.5
E. coli K-12 711 Ent$^+$ K99$^+$							
Calf 39	8.0	6.7	7.2	6.8	3.6	0	0
Calf 40	8.5	7.0	6.3	5.3	2.0	5.0	2.7
Calf 41	8.0	8.4	5.1	3.2	3.0	2.0	3.6
Calf 42	6.0	7.5	5.5	4.7	4.4	3.3	3.3
E. coli B41 *nal* Ent$^+$ K99$^+$							
Calf 50	5.9	8.4	7.9	7.9	7.0	7.5	6.5
Calf 51	6.3	9.1	7.8	7.9	6.9	6.4	5.7
Calf 52	6.1	8.7	7.9	7.9	7.0	6.9	6.1
E. coli B41 *nal* Ent$^+$ K99$^-$							
Calf 53	9.5	7.1	6.4	4.0	5.1	5.0	3.1
Calf 54	7.5	7.1	5.8	5.4	3.6	5.2	3.0
Calf 55	9.0	6.7	7.6	6.9	5.8	5.9	5.0

[a] 0 = $log_{10} < 1.0$. The total numbers of organisms administered per calf were: *E. coli* K-12 711 F$^-$, 11.9; *E. coli* K-12 711 Ent$^+$, 12.0; *E. coli* K-12 711 Ent$^+$ K99$^+$, 12.2; *E. coli* B41 *nal* Ent$^+$ K99$^+$, 11.4; *E. coli* B41 *nal* Ent$^+$ K99$^-$, 11.4.

portance in the pathogenesis of diarrhea in calves did not have a remarkable additive effect upon the ability of *E. coli* K-12 to survive in calves. As noted by Smith (4), the physiological state of the epithelium of the small bowel might well be important in this respect since the apparent advantage of K99 for proliferation in the small bowel of calves is largely lost within 36 h after birth. It is not clear whether the apparent survival value of the Ent plasmid is directly related to enterotoxin production or to the product of some other plasmid gene. It should be noted, however, that the Ent plasmid of strain B41 belongs to the F incompatibility group and exhibits polynucleotide sequence homology with both FI and FII plasmids for a contiguous stretch of deoxyribonucleic acid from 15×10^6 to 24×10^6 daltons in size, encompassing the Tra and Rep genes (So and Falkow, unpublished observations). *E. coli* K-12 strains carrying such related FI and FII R plasmids do not show increased survival when fed to calves.

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