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In Vivo Transduction of an Stx-Encoding Phage in Ruminants

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We assessed the ability of a kanamycin-marked Stx phage to move into a commensal, ovine *Escherichia coli* strain in the ruminant gastrointestinal tract. Transduction was detected in 19/24 sheep tested, resulting in the recovery of 47 transductants. Subtherapeutic doses of the quinolone antibiotic enrofloxacin did not increase the rate of transduction.

Ruminants are thought to be the major reservoir of Shiga toxin (Stx)-producing *Escherichia coli* (STEC), and most animals carry this organism in their gastrointestinal tract at some point in their lifetime (2). Shiga toxins are encoded on lysogenic bacteriophages that can be transferred into a variety of *E. coli* strains in vitro (11). While little is known about the factors that influence phage transduction in vivo, bacterial stress and induction of the SOS response could facilitate phage movement, as has previously been observed in vitro (10). Quinolone antibiotics trigger the SOS response in *E. coli*, and several studies have shown that these agents induce the transcription of the stx_2 genes in *E. coli* O157:H7 and increase the production of Stx both in vitro and in vivo (7, 15, 16). Furthermore, subtherapeutic doses of ciprofloxacin have been shown to increase the transduction of a marked Stx2 phage in the gastrointestinal tract of mice (16). Enrofloxacin, a fluoroquinolone antibiotic that is metabolized to ciprofloxacin in host tissues, is approved for the treatment of respiratory infections in ruminants (9). The effects of low levels of quinolone antibiotics on Stx phage dynamics in the ruminant intestine have not been explored.

The objective of this study was to determine if transduction of a marked Stx phage could be documented in a ruminant animal model and whether the administration of subtherapeutic doses of enrofloxacin would substantially induce movement of the phage.

E. coli strain C43 (recipient strain) was isolated from a healthy sheep and did not contain any known virulence genes (4). Strain 1:361, a clinical isolate of *E. coli* O157:H7, was isolated from a nationwide surveillance study (D. W. K. Acheson, K. Frankson, D. Willis, et al., Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. C-205, 1998). Strain 1:361(Δstx_2::*kan*), in which most of the stx_{2A} gene is replaced with the kanamycin resistance (Kan^r) gene from Tn*903*, was constructed using pCVD442/933-2-kan (13); this became the donor strain. The donor and recipient strains were grown to logarithmic phase and concentrated by centrifugation.

Following a 2-week period of acclimation to a commercial diet of low-energy feed concentrate (3) and alfalfa/grass hay, 24 sheep were randomly assigned to three groups. Sheep were orally inoculated with ~5×10^{11} CFU of both the donor and recipient *E. coli* strains. Group 1 did not receive antibiotics. Enrofloxacin was administered subcutaneously to the sheep in groups 2 (0.125 mg/kg/day, 5% of a therapeutic dose) and 3 (0.25 mg/kg/day, 10% of a therapeutic dose) on days 3 to 5 postinoculation (p.i.). Four additional sheep were given a therapeutic dose of enrofloxacin (2.5 mg/kg). Fecal samples were collected on days 1 to 6, 8, 10, 12, and 14 p.i. and cultured for the inoculum strains and putative transductants as previously described (3). Putative transductants were confirmed by PCR using primers directed to the stx_{2A} region containing the Kan insertion. Sheep were necropsied at 2 weeks p.i.

Transduction of the Stx-encoding phage in sheep. The mean magnitude of fecal shedding for both the donor and recipient strains in each of the three groups of sheep is shown in Fig. 1. During the time period when enrofloxacin was administered to groups 2 and 3 (samples from days 4 to 6 p.i.), the donor strain decreased 100-fold in the control group (Fig. 1A), 1,000-fold in group 2 (Fig. 1B), and ~10,000-fold in group 3 (Fig. 1C). By day 6, the 10% enrofloxacin dose decreased the quantity of the donor strain recovered by 1,000-fold compared to that recovered from the sheep that did not receive the antibiotic. In contrast, the recipient strain decreased approximately 10-fold in all three groups of sheep during the same time period. The donor *E. coli* O157:H7 strain was not recovered from three out of four sheep given the therapeutic dose of enrofloxacin but was recovered from one animal on day 12 (10^2 CFU/g) (data not shown).

Overall, transductants were recovered from seven of eight sheep in group 1, from four of eight sheep in group 2 and from eight of eight sheep in group 3. The majority of transductants (33/47) were recovered from all groups of sheep within the first 3 days (prior to the administration of enrofloxacin) (Table 1). On days 4 to 15 p.i., transductants were recovered from four of eight sheep in group 1 (five transductants), from four of eight sheep in group 2 (six transductants), and from two of eight sheep in group 3 (three transductants). In all cases, transduc-

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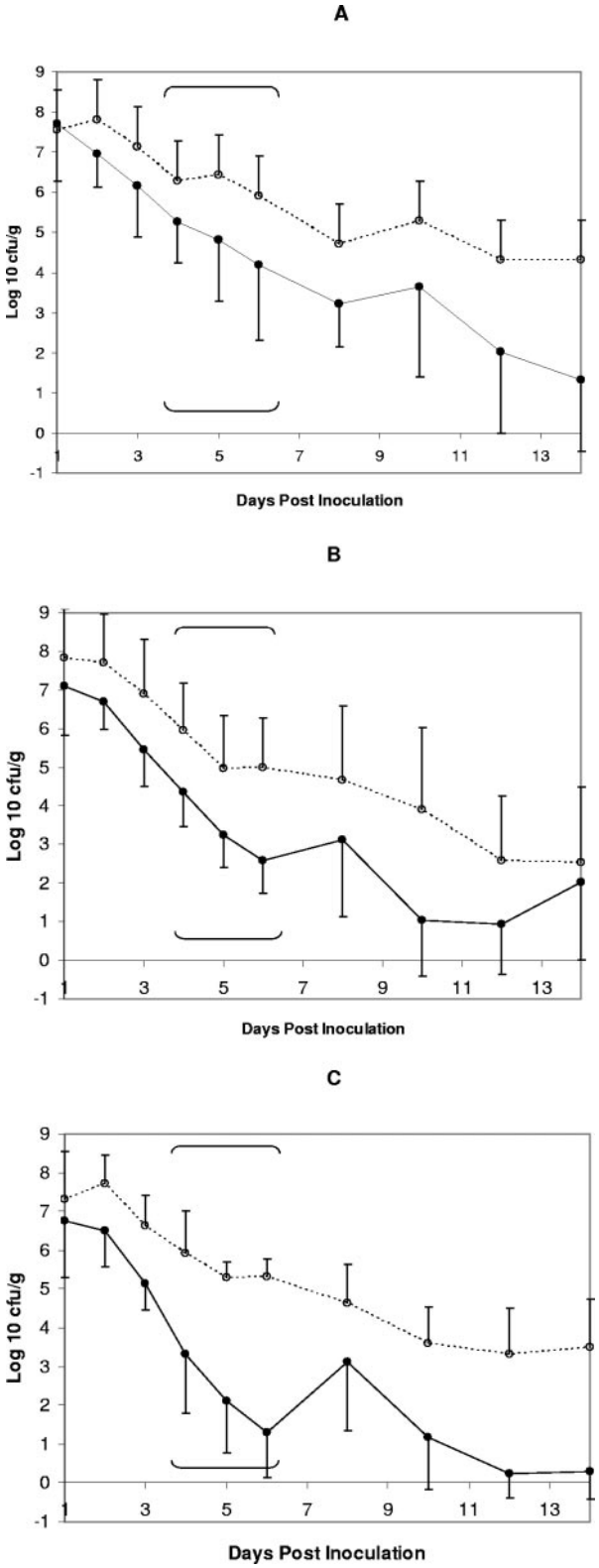

FIG. 1. Geometric mean of fecal shedding of donor *E. coli* O157:H7 (1:361Δstx_2:*kan*) (●) and recipient *E. coli* (C43) (○) strains from (A) control sheep, (B) sheep given 0.125 mg/kg/day of enrofloxacin, and (C) sheep given 0.25 mg/kg/day of enrofloxacin. Enrofloxacin was given on days 3 to 5 p.i. (samples at days 4 to 6 p.i. are shown in the bracketed areas). Bars indicate the standard errors.

TABLE 1. Recovery of transductants from sheep inoculated with donor *E. coli* O157:H7 (1:361Δstx_2:*kan*) and a recipient, commensal *E. coli* (C43)

Antibiotic dose[a]	No. of sheep from which transductants were recovered/total no. of sheep inoculated at p.i. day:										Total no. of transductants
	1	2	3	4	5	6	8	10	12	14	
None	3/8	2/8	4/8	3/8	1/8	0/8	1/8	0/8	0/8	0/8	14
0.125 mg/kg	2/8	3/8	2/8	3/8	0/8	0/8	2/8	1/8	0/8	0/8	13
0.25 mg/kg	4/8	7/8	5/8	2/8	1/8	0/8	0/8	0/8	0/8	0/8	19

[a] Enrofloxacin was given on days 3, 4, and 5 p.i.

tants were recovered only at enrichment levels (<50 CFU/g) regardless of whether the animal had received enrofloxacin or not. Transductants were not recovered from any of the tissues or samples taken at necropsy or from the four sheep given the therapeutic dose of enrofloxacin.

Our data clearly indicate that the transduction of the Stx-encoding phage can occur in vivo in the ovine gastrointestinal tract. Viable transductants were recovered from 19/24 animals tested. If our results are representative of a variety of STEC strains, it would suggest that the rate of transduction of the Stx phage is between 10^{-7} and 10^{-5} in vivo. A variety of pathogenic and commensal *E. coli* strains isolated from both humans and animals are capable of lysogenizing detoxified Stx phages (6, 11, 14). The experimental transduction of a marked Stx phage from donor *E. coli* K-12 to recipient *E. coli* K-12 strains has previously been demonstrated using an orally inoculated mouse model (1). Our data extend these findings and demonstrate that this movement also occurs in the ruminant gastrointestinal tract between a clinical *E. coli* O157:H7 isolate and a commensal, ovine *E. coli* recipient. It also suggests that naturally occurring transduction within the ruminant reservoir may be one source for the expansion of STEC strains across different serotypes of *E. coli*.

In vitro, low doses of ciprofloxacin induce both plaque formation and Stx production in *E. coli* O157:H7 (16). Furthermore, subtherapeutic doses of ciprofloxacin given to streptomycin-treated mice increased the concentration of intraintestinal Stx and mortality compared to control mice or those given fosfomycin even though the viable number of *E. coli* O157:H7 decreased by 1,000-fold. An increase in phage transduction also occurred in mice treated with subtherapeutic doses of ciprofloxacin that were inoculated with an *E. coli* K-12 strain harboring a Kan-marked Stx phage compared to the transduction rate in control mice. The increase in phage movement in mice is in contrast to what we observed in ruminants treated with an equivalent antibiotic and dosage regimen. One important point to bear in mind is that we did not have any corroboration that the doses of enrofloxacin used caused phage induction in vivo, and several factors may explain the differing responses of Stx phages to quinolone antibiotics in the mouse and sheep intestine. In our experiments, we used wild-type *E. coli* strains for both the donor and recipient strains rather than *E. coli* K-12. It is possible that *E. coli* K-12 strains are more permissive to transduction in vivo than are wild-type *E. coli* strains. Such differences have been demonstrated in vitro (8). Pretreatment of the mouse intestine with streptomycin, which removes much of the natural facultative intestinal

flora, may facilitate donor-recipient cell interaction within the intestine. In contrast, the established and competing microflora in the sheep may effectively interfere with the colocalization of the donor and recipient strains, resulting in a reduced level of interaction. Also, bile salts have been shown to attenuate the excision of Stx phages in vitro (12) and to repress the expression of cholera toxin and the toxin-coregulated pilus of *Vibrio cholerae* (5).

A better understanding of phage movement in vivo could provide clues as to why STEC strains are maintained in the gastrointestinal tract of ruminants as well as the processes behind the generation of new STEC strains.

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