Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6609–6613, September 1990
Microbiology

Inter- and intraspecies spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage

(ecology/colonization/antibiotic resistance)

BONNIE MARSHALL*†, DIANE PETROWSKI*, AND STUART B. LEVY*‡

Departments of *Molecular Biology and Microbiology and of ‡Medicine, Tufts University School of Medicine and New England Medical Center, Boston, MA 02111

Communicated by Robert K. Selander, May 17, 1990

ABSTRACT **The spread of wild-type *Escherichia coli* bearing a transferable plasmid was studied in a farm environment. *E. coli* of bovine and porcine origin were marked by resistance to nalidixic acid (Na^r) or rifampicin (Rf^r), and a transferable, multiple resistance plasmid (pSL222-1 derivative of plasmid R222) was introduced by conjugation. In separate experiments, the two mutant derivatives were fed back to the respective host animals, which were housed adjacent to, but separate from, one or more "recipient" animals. No antibiotic was given. Although the Rf^r derivatives declined rapidly to undetectable levels within 1 week, the Na^r mutants of bovine and porcine origin persisted in the original hosts and in their bedding throughout most of each 4-month test period. Test bacteria were isolated from mice residing in the same pen as the donor animals and from multiple secondary hosts having direct or indirect contact with the inoculated donors, but not from neighboring animals maintained in isolation. The bovine mutant was excreted by two caretakers for >4 weeks and was recovered for 4–6 weeks from pigs, fowl, and flies. Although the porcine mutant appeared to colonize less effectively, it spread rapidly to flies and mice and was recovered transiently from humans and fowl. Despite high transfer rates of plasmid pSL222-1 from *E. coli* K-12 *in vitro*, transfer of the plasmid from the animal *E. coli* host was very low and transfer *in vivo* was not detected among indigenous gut or environmental bacteria. *E. coli* of animal origin can spread rapidly and can colonize the intestinal tract of humans and of other animals in the absence of antibiotic selection.**

The frequency, direction, and conditions of the natural spread of bacteria and their plasmids within the environment are of obvious importance in understanding the epidemiology of pathogenic and antibiotic-resistant bacteria and the means by which natural and genetically engineered strains and plasmids may be maintained in the environment. Antibiotic resistance genes commonly exist in human and animal flora (1, 2), but it has been difficult to track the flow of these genes and their bacterial hosts between animal species (3–5), and minimal or no colonization has been reported in the absence of antibiotic use (6–9). The degree to which animal host specificity contributes to biologic containment of *Escherichia coli* is also not well understood. To address these questions, we designed studies in a controlled, yet natural, farm environment in which ingress and egress of participants were known and different elements that could affect spread of *E. coli* and its plasmids could be examined. The effect of direct and indirect contact with an animal hosting a marked *E. coli* was evaluated and the spread and persistence of the organism in new hosts were documented. The results demonstrated a rapid spread of *E. coli* among different humans and animals and environmental sources in the absence of antibiotic selection.

MATERIALS AND METHODS

Bacterial Strains. For the bovine studies, two morphologically identical, antibiotic-susceptible *E. coli* were selected from among the predominant lactose-fermenting enteric flora [titer = 6×10^6 colony-forming units (cfu)/g of feces] of the heifer. They were isolates of the same clone as shown by identical chromosomal DNA fragment profiles after endonuclease digestion with three or more enzymes (*Eco*RI, *Bam*HI, *Bgl* II, *Hin*dIII, and *Sal* I) and multilocus enzyme electrophoresis (performed by T. Whittam, University Park, PA). One isolate was mutated to nalidixic acid resistance (Na^r) (30 μg/ml) (SLC1) and the other was mutated to rifampicin resistance (Rf^r) (100 μg/ml) (SLC2) by plating on the antibiotic-containing medium. We introduced plasmid pSL222-1 (a transfer-derepressed mutant derived from R222 by ethylmethane sulfonate mutagenesis) conferring resistance to tetracycline (Tc^r), chloramphenicol (Cm^r), sulfonamide, and streptomycin/spectinomycin into each strain, creating SLC1-R and SLC2-R. Both hosts maintained the plasmid through ≈100 generations of growth in L broth (per liter: 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, 1 g of glucose, pH 7.0). Although transfer out of the *E. coli* K-12 host was efficient (10^{-1} per donor cell), transfer from the new host was low (≈10^{-8} per donor cell). All four strains showed identical growth rates in L broth and minimal medium (10).

For the porcine studies, two antibiotic-susceptible *E. coli* were selected from among the lactose-fermenting porcine fecal flora. Both showed identical chromosomal digestion patterns and multilocus enzyme electrophoresis (see above) but were distinctly different from the bovine strains. Spontaneous Na- (SLP1) or Rf-(SLP2) resistant derivatives were made as above. Kanamycin resistance (Kn^r) was added to plasmid pSL222-1 by incorporating the Tn*5* transposon. Plasmid pSL222-1::Tn*5* was then mated into the *E. coli*, creating SLP1-R and SLP2-R. No plasmid loss was observed through 40 generations of growth; some spontaneous loss (≈10%) was noted following the subsequent 10 generations. This plasmid also exhibited repressed transfer (≤10^{-8} per donor cell) from the wild-type *E. coli*. The parental strains and their resistant derivatives had identical growth rates in L broth and minimal medium.

Inocula were grown to midlogarithmic phase in L broth, collected by centrifugation, resuspended in buffered saline, and transported on ice to the experimental site. Doses were administered orally in gelatin capsules.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: cfu, colony-forming unit(s); Na, nalidixic acid; Rf, rifampicin; Tc, tetracycline; Cm, chloramphenicol; Kn, kanamycin; r, resistance.

†To whom reprint requests should be addressed.

Selective Media. Primary isolations were performed on L agar or MacConkey (MAC) agar containing Tc (10 μg/ml) and Cm (25 μg/ml) for the selection of strains bearing pSL222-1 and on Cm (25 μg/ml) and Kn (10 μg/ml) for strains carrying pSL222-1::Tn*5*. L plates also contained amphotericin B at 2 μg/ml (Sigma) to inhibit molds. To ensure detection of test bacteria that might have undergone plasmid loss, representative "negative" samples were confirmed by plating directly onto MAC agar with Rf or Na only. Gram-negative organisms were enumerated on plain MAC agar. Primary isolates were confirmed by replica-plating onto MAC agar having either Na at 30 μg/ml (to identify Na^r test strains) or Rf at 50 μg/ml (to identify the Rf^r mutant).

Experimental Design. Experiments were conducted in a privately owned barn (Sherborn, MA), housing domestic animals that received no antibiotics. The experimental pens (A and B) consisted of two wooden stalls (3.5 m × 2.8 m) with concrete floors, divided by a 1.5-m-high solid wooden barrier. Attached to each side (1.0 m from the floor) was a long, narrow wooden cage (1.5 m × 0.15 m × 0.15 m) with a screened front, each housing five commercial feeder mice. This arrangement prevented physical contact between the test animals but permitted access to air, insects, and wild birds (mostly sparrows).

One yearling heifer (which received the test strains) and one yearling bull (potential recipient) were introduced into the adjacent pens. Each received a normal diet of hay, Coarse 14 (Purina), and water ad lib. The mice received water and commercial rodent feed consisting of seeds and grains. Antibiotic-free feed was purchased; no antibiotics were given except where noted. The animal bedding of straw and wood shavings was rotated periodically by replacing half of the soiled portion with fresh material. Contaminated bedding from the donor side was placed into autoclave bags and sealed. Following inoculation of the donor animal with the test strains, sampling was performed daily for the first week and one to three times weekly thereafter by selecting the most recently deposited feces from the test pens. Mice were sampled by removal from cages to a decontaminated plastic container where newly excreted fecal pellets were pooled into sterile test tubes. Where containment procedures were observed, the caretaker entered the recipient cage (pen A) initially, performed all required procedures, and then, wearing disposable plastic boots, entered the donor side (pen B). Upon exit, the boots were removed to autoclave bags.

Following the bovine studies, the cages were thoroughly cleaned using a mild disinfectant. Two 7-month-old female Yorkshire pigs (raised without antibiotics) were introduced into each of the two isolation pens. Donor pigs received the test bacteria and containment and sampling procedures were performed as before. All pigs were fed nonmedicated Hog Grower Chow (Purina) and water ad lib. The bedding (wood shavings) was rotated and disposed of as described above.

Sampling Methodology. Recovery assays employed 10-fold serial dilution into sterile buffered saline (SBS) of 50-g fecal samples from humans and the larger test animals and 0.5- to 10-g samples from mice and chickens. Fifty grams of randomly collected and mixed bedding samples from several areas was diluted 10-fold (wt/vol) in SBS, homogenized for 60 sec in a Waring blender, and assayed by plating. Water samples were diluted 10-fold in SBS or concentrated by filtering 100 ml through a 0.45-μm (Nalgene) filter. The filter was "vortexed" thoroughly in 5 ml of SBS and plated (1 ml and 0.1 ml) on selective medium. Airborne insects (predominantly flies) were collected on commercial adhesive fly paper hung above the two stalls. Pooled samples of insects (0.1–1.0 g) were diluted 10-fold in SBS and macerated with a glass grinder prior to plating.

Less than 10 cfu/g was detected by homogenization of 25 g of sample in 225 ml of MAC broth with Tc and Cm. Following aeration at 37°C for 24 hr, the broth was plated on the corresponding selective agar. This enrichment method was initiated on day 39 for the animals and environmental samples.

To distinguish feces of pigs within the same pen, 0.2 g of an inert blue tracer dye (FD & C no. 2, gift of Crompton and Knowles) was fed orally in gelatin capsules to one of the two animals 1–2 days prior to sampling. This imparted a blue-green tinge to fecal specimens of pigs receiving the dye.

Air and a variety of environmental elements, including horizontal and vertical surfaces, as well as animal and human skin and clothing were monitored for test bacteria. Sampling was performed with the Andersen microbial air sampler, gravitational settling cultures, and contact plates (11) using the same selective media described above.

Confirmation of Test Bacteria. Test bacteria recovered on primary isolation media were replica-plated to Na- or Rf-containing agar for confirmation. One or more of the isolates obtained from each animal species was selected for plasmid evaluation and restriction enzyme analysis of chromosomal DNA using at least two endonucleases (*Eco*RI, *Bam*HI, *Hin*dIII, and *Bgl* II). Patterns identical to those of the inoculated strains confirmed the presence of test bacteria in the new host. These data were further confirmed by multilocus enzyme electrophoresis (see above). Representative colonies from all primary selective media (i.e., Tc and Cm or Cm and Kn) that were not Rf^r or Na^r were selected for analysis of plasmid DNA by gel electrophoresis. Any strains containing large plasmids that migrated similarly to pSL222-1 were digested with *Eco*RI for further confirmation.

RESULTS

Spread from Bovine Hosts in a Closed Environment. The first study examined colonization and spread of marked bovine *E. coli* to potential recipients within the pen of an inoculated heifer (pen B) as well as to a bull and mice maintained in an adjacent closed environment (pen A). The heifer received approximately equal numbers of *E. coli* SLC1-R and SLC2-R (pen B, Fig. 1) orally in evenly divided doses on days 0, 2, and 3. The inoculated animal showed immediate colonization, primarily with SLC1-R (Fig. 1). High levels (up to 10^6 cfu/g, ≈10–50% of the total coliforms) were excreted through 10 days postinoculation. Intermittently, low levels were recovered for the duration of the study (≈4 months). Titers up to 10^5 cfu/g were found in all bedding materials, but only sporadically and at low titers (≤10^3 cfu/g) from drinking water.

Test organisms (predominantly SLC1-R) were recovered from mice during the first 16 days and again on day 42, but only from those housed in the donor pen. Test organisms occasionally appeared in enrichment cultures of bedding materials from the bull cage (Fig. 1) but not from the bull feces. Nineteen days after inoculation, SLC1-R was excreted by the single caretaker having direct animal contact (Fig. 1). Titers were as high as 10^5 cfu/g (1–3% of all coliforms) and persisted for ≈1 month before declining to undetectable levels.

On day 111, the heifer and bull each received chlortetracycline (12 g over a 5-day period; Fig. 1). In both animals the frequency of Tc^r indigenous flora increased from ≤15% to a maximum of 63–92% of the total Enterobacteriaceae; however, there was little effect on recovery of test organisms from the heifer (Figs. 1 and 2), and none was detected in the bull.

Spread from Bovine Hosts in an Open Environment. In the second study, designed to test spread in an open environment, the bull was inoculated with a combined total of 2.7 × 10^{11} SLC1-R and SLC2-R (Fig. 2) and procedures preventing cross-contamination were eliminated. The organisms (chiefly SLC1-R) readily colonized the bull's intestinal tract (Fig. 2). Within 5–11 days, both caretakers who had direct contact

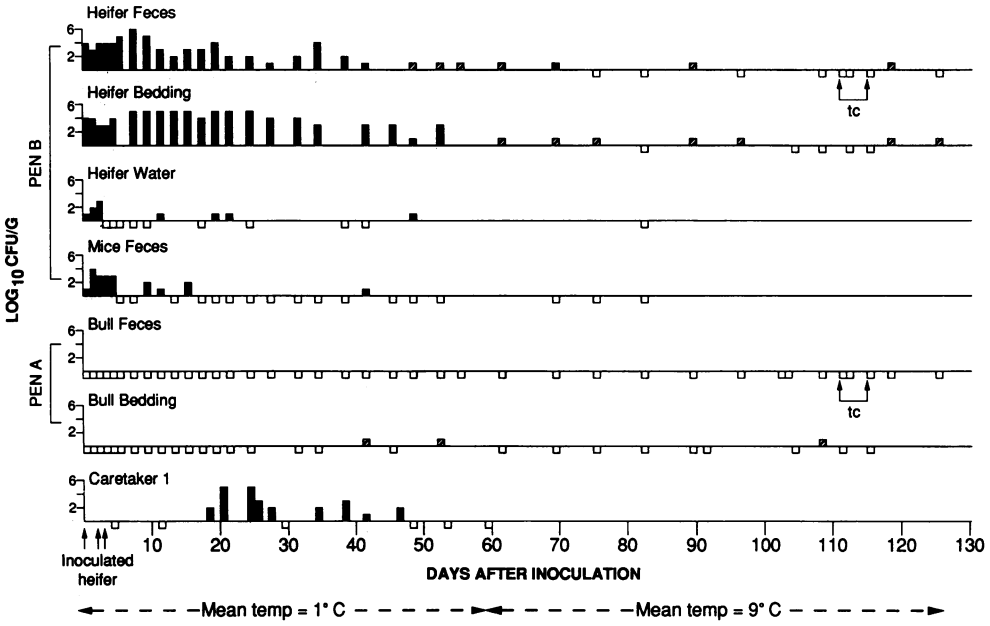

FIG. 1. Recovery of marked bovine *E. coli* in a closed environment. The heifer was fed SLC1-R (5.4×10^{10}) and SLC2-R (4.7×10^{10}) bovine *E. coli* in three divided doses on days 0, 2, and 3. The animal was maintained in isolation (see text). Data present only the titers of the Na^r mutant (SLC1-R) per g of sample (solid bars), as this strain represented >95% of the test organisms recovered on primary isolation medium. Hatched bars represent qualitative recovery by enrichment (see text); negative samples are indicated by open bars. Tests of the caretaker were terminated on day 60, at which time feces were negative by dilution and enrichment methods. Samples from caged chickens on days 28, 46, and 53 were negative by dilution and enrichment. Pigs were not available for monitoring during this phase. Arrows indicate the administration of 12 g of chlortetracycline over a 5-day period.

with the bull began to excrete test organisms (>95% SLC1-R) at titers of 10^4–10^6 cfu/g. Excretion ceased in caretaker 1, who had animal contact for only 2 weeks, but persisted for at least 37 days in caretaker 2, who maintained regular contact. Mice excreted low levels of SLC1-R for 2 weeks only. Beginning on day 10, substantial numbers of flies (about 10–35; i.e., 0.2–1.0 g) were obtained and these bore SLC1-R at titers of 10^1–10^3 cfu/g for ≈30 days.

Within 3 days postinoculation, test organisms were recovered from bedding materials in a pig stall adjacent to the bull pen. By at least day 10 (Fig. 2), pigs began excreting test bacteria at titers of 10^2–10^5 cfu/g (0.001–0.01% of the total Enterobacteriaceae). The heifer showed no increased excretion but sporadically shed the test bacteria (presumably from the initial ingestion) for a minimum of 13 additional days, when testing was stopped. Chickens confined to wire cages 6.1 m away began excreting SLC1-R within 25 days and continued to shed them at titers up to 10^4 cfu/g for ≈1.5 months (Fig. 2). In addition, fresh feces from a free-roaming rooster harbored SLC1-R at 10^4 cfu/g. This and other free-roaming fowl had been observed within the confines of the pig pen as well as around the chicken cages.

In both studies, densities of test organisms from air samples were consistently low (0–3 cfu/1.68 m^3). Recovery from gravitational settling cultures and contact plates indicated organisms were present on surfaces inside and outside of donor and recipient pens, whether or not containment procedures were employed. The highest concentrations were recovered from horizontal wooden surfaces within the test pens, including those of the mouse cages. As in the other samples, the Na^r mutant was the major organism detected; the Rf^r mutant was rarely recovered from these cultures.

Spread from Porcine Hosts. In a third study, two pigs were placed in each isolation cage and each pig in pen B received 5.4×10^9 SLP1-R and 6.7×10^9 SLP2-R (Fig. 3). Although containment procedures for a "closed" environment were followed (as described in experiment 1), flies were present which had access to both pens. The titers of indigenous and test bacteria were examined in the most recently deposited pig feces. Test bacteria (generally >99% SLP1-R) colonized the porcine intestinal tract up to 10^6 cfu/g and were consistently recovered from water and bedding materials. SLP1-R were also excreted by mice in the same pen as the inoculated pigs but not by mice in the recipient side (Fig. 3). Test bacteria (>90% SLP1-R) were recovered regularly from flies and occasionally from bedding materials on the recipient side, but only once (1 cfu/0.4 g) from pigs in the adjacent pen (Fig. 3). SLP1-R were detected once in a single caretaker (<10 cfu/g). No caged chickens were available for examination; however, test organisms were recovered from 1 of 10 cultures of free-roaming turkeys (10^2 cfu/g).

To determine the effect of direct physical contact, we exchanged one pig from each of the two cages (Fig. 4). The positive pig in each cage continued to excrete low levels (≤10^1 cfu/g) of Na^r and Rf^r test organisms. The negative "recipient" animals occasionally shed detectable levels, but this trend did not persist. To evaluate the effect of antibiotic ingestion, each pig in pen A was given 4.5 g of oxytetracycline (Sigma) (Fig. 4). In at least one pig, the antibiotic increased the relative excretion of Tc^r bacteria (as compared to total Enterobacteriaceae) from ≈50% to 100% but did not increase the overall numbers of Tc^r bacteria or the titers of test organisms. An additional dose (2 g) to the original donor pig (pen A) also failed to increase excretion of test bacteria.

On day 116, the donor pig in pen B was reinoculated with 7.2×10^9 SLP1-R and 9.6×10^9 SLP2-R (Fig. 4). The titers of each organism increased in both cagemates; however, recovery from the adjacent pen showed no change. As in the bovine experiments, the Na^r derivative (SLP1-R) was detected in low titer in aerosols (<3 cfu/1.68 m^3) and in various environmental samples throughout the test period.

Relative Recovery of Na^r and Rf^r Marked Strains. In all studies, the Rf^r mutant showed a markedly decreased survival as compared to the Na^r mutant. Whereas the initial Rf^r/Na^r ratio of ingested organisms was approximately equal (0.87 and 0.69 for the bovine studies; 1.2 and 1.3 for the porcine experiments), the subsequent ratio in feces was ≤0.07. Although the Rf^r bovine mutant was detectable for a maximum of 7–9 days, the Na^r mutant persisted much longer (at least 70 days in the heifer and pigs). The Na^r, but not the Rf^r, bovine mutant spread to flies, pigs, and chickens. The Rf^r porcine *E. coli* was detectable throughout most of the sampling period, but only at low levels.

Spread of Plasmid to Indigenous Strains. Organisms recovered on primary isolation media that were not resistant to Na or Rf were indigenous flora and assumed to be potential recipients of the test plasmid. However, none of those representative phenotypes bearing large plasmids produced *Eco*RI digestion patterns similar to the test plasmids. Thus

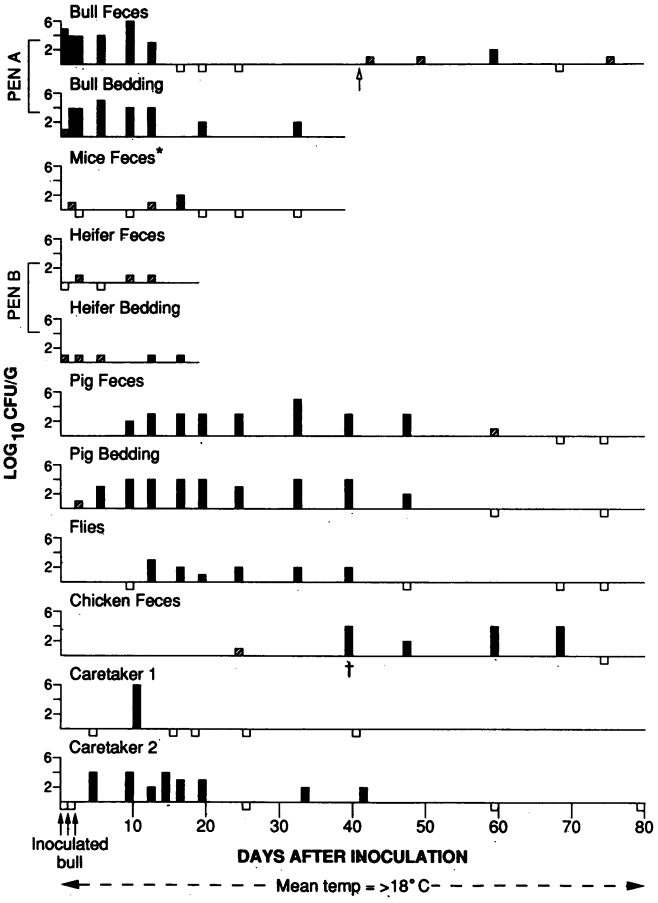

FIG. 2. Recovery of marked bovine *E. coli* in an open environment. The bull was inoculated with marked *E. coli* (1.6×10^{11} SLC1-R; 1.1×10^{11} SLC2-R). No containment procedures were observed during this phase. (Data are presented as described in the legend to Fig. 1.) The open arrow indicates time when the bull was released into an adjacent field. Flies first appeared on day 10. *, At least one of the positive fecal specimens was derived from mice in pen A, but it was not determined whether mice in pen B were excreting test bacteria as well. †, Fresh feces retrieved from a free-roaming rooster contained 1×10^4 cfu/g of SLC1-R.

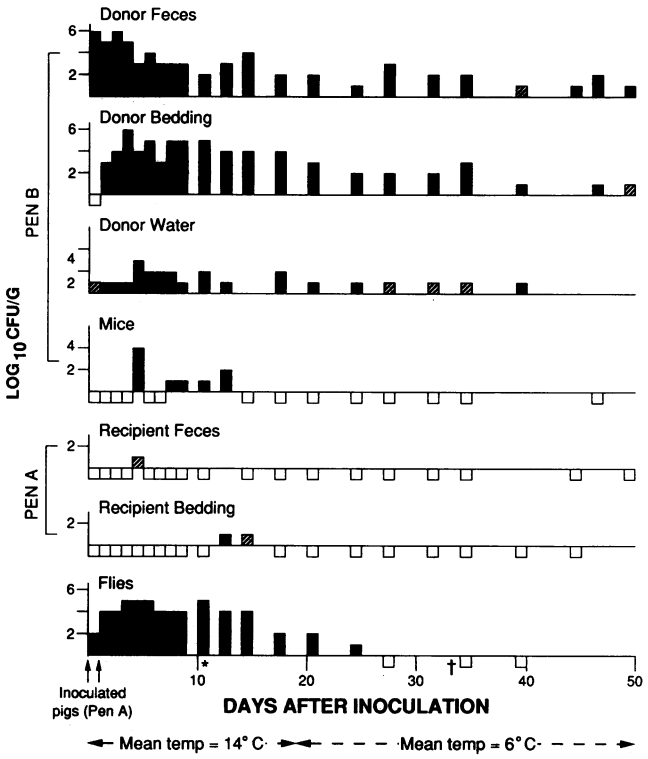

FIG. 3. Recovery of marked porcine *E. coli* in a closed environment. Two pigs housed in pen B each received SLP1-R (5.4×10^9) and SLP2-R (6.7×10^9) porcine *E. coli* bearing pSL222-1::Tn*5* (see text). Survival and spread of test bacteria were followed as described in the legend to Fig. 1, maintaining isolation conditions for animals in pen A. Bars represent titers of SLP1-R, which was the predominant organism recovered. No caged chickens were available during this study and no flies were recovered after day 40 due to decreasing ambient temperatures. *, SLP1-R found at 10^3 cfu/g in 1 of 10 samples of fresh feces taken between days 3 and 54 from free-roaming turkeys. †, SLP1-R found in a fecal enrichment culture of one caretaker on day 33.

transfer of the test plasmids was not detected in the indigenous flora of new animal hosts.

DISCUSSION

In previous studies we traced an *E. coli* from chickens to two human handlers, but the chicken strain was only transiently found in their fecal flora (6). Other investigators have also demonstrated transient carriage or poor colonization of *E. coli* ingested by humans (7–9, 12) or picked up from animals or their carcasses (4), with or without concurrent antibiotic ingestion. In the present experiments, *E. coli* of bovine or porcine gut origin spread readily into human and other host species in the immediate interactive environments, resulting, in some cases, in prolonged colonization of these secondary recipients. Such exchange was maximal when open contact among the animal hosts was permitted and minimal when animals were kept in isolation. Test organisms were isolated from humans in direct contact with inoculated bovines and pigs, from pigs having indirect contact through humans and flies, and from mice and chickens having indirect contact through aerosols, flies, or other free-roaming barnyard animals. The reason for the more efficient spread in this study as compared to others is not clear but may relate to intrinsic characteristics (including the marker used) of the strains selected or to the routes of acquisition.

When reintroduced into their respective hosts, *E. coli* recolonized the gut for months in the absence of any antibiotic consumption. Although colonization did not persist indefinitely at high titers, the test strains did remain for many weeks before gradually decreasing to undetectable or occasionally detectable levels. This gradual loss was presumably due to the inherent transient nature of gut flora as has been noted previously (1, 13). Although colonization may have persisted because of reingestion from environmental sources (14), there were periods when fecal titers were low despite high concentration in the immediate environments (e.g., bedding) (Fig. 1). Moreover, colonization occurred in humans and wild animals having limited or intermittent exposure to the inoculated pen (Fig. 2).

No colonization occurred in isolated animals of the same species despite detectable titers of test organisms in their immediate environment (i.e., bedding materials, air samples, and various surfaces). The low levels of organisms present under isolation conditions were apparently insufficient to result in colonization. It was surprising, therefore, that animals of different species outside the confines of the pens were colonized by the test organisms (e.g., turkeys by the porcine strain; chickens and pigs by the bovine strain). Presumably factors other than environmental density alone enhanced this intraspecies colonization.

There was a large difference in the colonization competency of the inoculated marked strains. Except for the Rf^r and

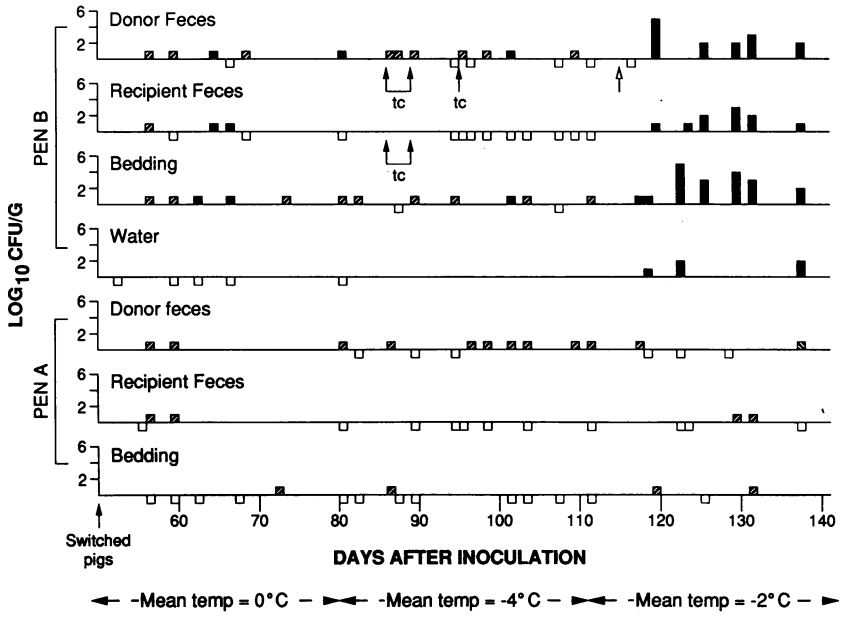

FIG. 4. Recovery of marked porcine *E. coli* in an open environment. On day 50 (see Fig. 3) one pig in pen A was exchanged with a pig from pen B. Samples were taken as before, but containment procedures were eliminated. Recovery of test bacteria (SLP1-R) was followed as described in the legend to Fig. 1. On days 87–89, each pig in pen B received a total of 1.5 g of oxytetracycline per day in drinking water; on day 96, the donor pig in pen B was refed an additional dose of 2 g. On day 116 (open arrow), this pig was reinoculated with 1.7×10^{10} of an equal mixture of SLP1-R and SLP2-R. At this time the mean ambient temperature of the barn was 1°C, and flies and other insects were not present.

Na^r markers, the mutants were otherwise identical as determined by morphology, growth rates, restriction endonuclease digestion patterns of chromosomal DNA, and multilocus enzyme electrophoresis. The Rf^r mutant showed a marked decrease in ability to survive and persist, suggesting that the mutation introduced may have somehow affected survival. We noted a similar phenomenon previously in which Rf^r *E. coli* K-12 failed to compete efficiently in germ-free mice that were colonized with an otherwise isogenic Na^r derivative (15). More recently we observed that certain Rf^r mutants of a *Pseudomonas fluorescens* strain showed decreased competitive fitness in soil assays as compared to the wild-type parental strain (16); however, this change could be linked to altered growth rate and an altered membrane protein profile. Such changes were not found in the wild-type *E. coli* studied here (data not shown).

The bovine derivative appeared to colonize a variety of hosts more efficiently than the porcine strain. Whereas the porcine strains were readily recoverable from mice and flies, they were recovered only transiently from avian species and one human caretaker. These findings indicated that the porcine strains could survive and grow but less successfully colonize the gut of these latter hosts. We also noted that the plasmid was somewhat unstable in the porcine hosts, so that its spread may be underestimated. We checked this possibility by testing a limited number of negative fecal samples on Rf- or Na-containing agar. This procedure did not reveal any plasmid-less derivatives of the inoculated strains.

Transfer of the pSL222 derivative plasmids to indigenous wild-type bacteria was not detected in any of these studies. This finding probably relates to its low transfer from the wild-type *E. coli* hosts ($\leq 10^{-8}$ transconjugants per donor bacterium from bovine and porcine strains). Despite lack of observed transfer, the plasmids were carried with their bacterial host into multiple animal species, providing the potential for considerable spread should a conjugation event occur. That resistance genes can transfer widely has been demonstrated in studies of nourseothricin resistance. The gene initially emerged on a transposon among *E. coli* in pigs treated with the antibiotic. It was subsequently found on different plasmids in *E. coli* associated with farm workers, farm family members, and people in the community (17).

This study documents and characterizes prospectively the natural colonization of the intestinal tract of different animal hosts by *E. coli* from another animal. The results agree with studies that found *E. coli* of the same multilocus enzyme electrophoretic type in fecal samples from different animal hosts (18). They extend these findings and others that have shown transient transfer (4, 6) by demonstrating the rapidity of spread and the persistence in several different domestic and wild animals without antibiotic selection. The findings, therefore, show that *E. coli* are not restricted to one animal host and that they, with their plasmids, can spread naturally from one animal host to colonize the intestinal tract of humans and other animal species.

We thank Mr. and Mrs. Richard Downing for the use of their farm facilities, T. Whittam for performing electrophoretic typing, Pauline Flynn for help in initial isolation of mutants, and Allison McAteer for assistance with chromosomal DNA analyses. These studies were supported by a grant from the Environmental Protection Agency (EPA) and through an EPA collaborative agreement with the Center on Environmental Management of Tufts University.

1. Levy, S. B., Marshall, B. M., Schluederberg, S., Rowse, D. & Davis, J. (1988) *Antimicrob. Agents Chemother.* **32**, 1801–1806.
2. Linton, A. H. (1984) *Br. Med. Bull.* **40**, 91–95.
3. Levy, S. B. & Marshall, B. M. (1988) in *The Release of Genetically Engineered Microorganisms*, eds. Sussman, M., Collins, C. H., Skinner, F. A. & Stewart-Tull, D. E. (Academic, New York), pp. 61–76.
4. Linton, A. H., Howe, K., Bennett, P. M. & Richmond, N. H. (1977) *J. Appl. Bacteriol.* **43**, 465–469.
5. Dorn, R. K., Tsutakawa, R. K., Fein, D., Burton, G. C. & Blendon, D. C. (1975) *Am. J. Epidemiol.* **102**, 319–326.
6. Levy, S. B., Fitzgerald, G. B. & Macone, A. B. (1976) *Nature (London)* **260**, 40–42.
7. Smith, H. W. (1969) *Lancet* **i**, 1174–1176.
8. Anderson, J. D., Gillespie, W. A. & Richmond, M. H. (1973) *J. Med. Microbiol.* **6**, 461–473.
9. Hirsch, D. C., Burton, G. C. & Blenden, D. C. (1974) *J. Appl. Bacteriol.* **37**, 327–333.
10. Stanier, R. Y., Palleroni, N. J. & Douderoff, M. (1965) *J. Gen. Microbiol.* **43**, 159–271.
11. Marshall, B. M., Flynn, P. A., Kamely, D. & Levy, S. B. (1988) *Appl. Env. Microbiol.* **54**, 1776–1783.
12. Cooke, E. M., Hettiaratchy, I. G. T. & Buck, A. C. (1972) *J. Med. Microbiol.* **5**, 361–369.
13. Cooke, E. M., Edwins, S. & Shooter, R. A. (1969) *Br. Med. J.* **4**, 593–595.
14. Levy, S. B. (1986) in *Antibiotic Resistance Genes: Ecology, Transfer and Expression*, eds. Levy, S. B. & Novick, R. P. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 17–29.
15. Onderdonk, A. B., Marshall, B. M., Cisneros, R. & Levy, S. B. (1981) *Infect. Immun.* **32**, 74–79.
16. Compeau, G., Al-achi, B. J., Platsouka, E. & Levy, S. B. (1988) *Appl. Environ. Microbiol.* **54**, 2432–2438.
17. Hummel, R., Tschape, H. & Witte, W. (1986) *J. Basic Microbiol.* **8**, 461–466.
18. Caugant, D. A., Levin, B. R. & Selander, R. K. (1984) *J. Hygiene* **92**, 377–384.