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Survival of Two Enterobacteria in Feces Buried in Soil Under Field Conditions

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Feces samples, inoculated with 10^6 *Escherichia coli* resistant to streptomycin and nalidixic acid and with 10^5 *Salmonella typhimurium* per g, were buried at five mountain field sites ranging from 2,005 to 2,730 m in elevation. Counts of each bacterium rose initially and then declined to 10^3 or 10^4 per g of feces in 8 weeks. The survival pattern was similar at all sites regardless of marked differences in elevation, soil, moisture, exposure, and vegetation. *S. typhimurium* numbers were consistently higher than *E. coli* numbers after week 3. The test encompassed most of the time that the area is snow-free and accessible for hiking. The results were judged to discredit the recommendation for shallow burial of feces and to indicate a potential health hazard under intensive use.

The rapidly increasing use of areas designated as wilderness under the National Wilderness Preservation System administered by the U.S. Forest Service has created a number of management problems (8). Among these is a heavy local concentration of people and the consequent problem of human waste disposal at particular sites, e.g., on mountain climbing routes and at the limited camping spots on float trips, which are engendered by the nature of the terrain. Many of these areas are too remote or inaccessible for an organized waste disposal system (7). The potential survival of intestinal pathogens at these sites has not been investigated. Background information on survival of enteric bacteria is available from numerous studies related to the use of sewage products as soil amendments, sanitary landfill, and the disposal of feedlot wastes. Most recent studies have employed sterilized soil (4, 6, 11) but others have used unsterilized soil (1, 9, 12). Survival of enteric bacteria is more easily studied in sterilized soil but has more applicability if unsterilized soils are used. Most soils have been agricultural or lowland soils. The present study was undertaken to test the hypothesis that enteric bacteria disappear rapidly after shallow burial of feces. This hypothesis is the basis of present management. The study was limited to a high mountain area. A technique was devised for incubating an unsterilized, inoculated soil-feces mixture under field conditions somewhat similar to those frequently recommended for hikers. The method was tested at several field sites characterized by different plant communities and having different types of exposure.

MATERIALS AND METHODS

Bacteria. *Escherichia coli*, resistant to streptomycin (100 μg/ml) and nalidixic acid (50 μg/ml), and *Salmonella typhimurium* were grown in tryptic soy broth (Difco Laboratories) for 18 h, centrifuged, and suspended in sterile reagent-grade water (resistance > 10 MΩ cm) at a concentration of 10^9 cells per ml. Each bacterial suspension was diluted so that 1 ml added to 1 g of feces gave a count of that bacterium of 10^6 per g of feces. Counts were made on Levine eosin-methylene blue agar containing streptomycin and nalidixic acid and on salmonella-shigella agar respectively.

Inoculated fecal suspension. Feces not containing organisms similar to those above were blended with an equal weight of sterile water for 5 min in a Sorvall blender. A 3-g sample was removed as a control. Bacterial suspensions prepared as described above were added at a rate of 0.1 g of bacterial suspension per g of feces.

Sample holders. Coulter Counter plastic sample holders (Acuvettes) were adapted by piercing the sides and bottom with a hot wire to give four holes approximately 3 mm in diameter. An aluminum strip welded to the top of the Acuvette assisted in retrieving buried sample holders.

Sample preparation. Soil was collected from each field site. Three grams of soil was placed in the bottom of an Acuvette, 3 g of inoculated fecal suspension was added, and an additional 3 g of soil was put on top. Samples were prepared on the same day and refrigerated for 2 days before burial.

Field sites. Five sites were selected in the Bridger Mountains north of Bozeman, Mont., differing in elevation, exposure, and plant community. All were close to a hiking trail which is heavily used during the brief snow-free season which extends from early or mid-July until early September. Site 1 (elevation, ca. 2,730 m) was above the timberline and had a western exposure dominated by *Sibbaldia*, *Carex*, *Frasera*, *Orthocarpus*, *Cerastium*, and *Potentilla*. The vegetation was a dense mat rarely more than 10 cm high. Site 2 (elevation, ca. 2,730 m) was also above the timberline and close to site 1, but with an eastern exposure and mostly bare soil and rock. A mat of *Dryas* occupied part of the site. Site 3 (elevation, ca. 2,500 m) was at the timberline with a pure stand of severely stunted

Abies lasiocarpa. Site 4 (elevation, ca. 2,377 m) was an *Abies-Picea* forest with *Thalictrum*, *Ribes*, *Arnica*, and *Senecio* in the understory (a so-called "dry-limestone" site). Site 5 (elevation, ca. 2,005 m) was a steeply pitching meadow dominated by *Phleum*, *Delphineum*, *Rudbeckia*, *Heracleum*, *Agropyron*, and *Bromus* but containing numerous other grasses and forbs. Sites 1, 2, and 5 were mineral soils, and sites 3 and 4 were organic duff layers composed largely of slightly decomposed conifer needles.

Soil moisture. Soil moisture readings were made weekly at the time of sample retrieval by using a soil moisture meter (Soil Test, Inc., Evanston, Ill.) and gypsum blocks (13) buried at the same depths as the samples.

Experimental procedures. Eight replicate soil-feces samples were buried at each site at a depth of 5 to 10 cm. One sample was retrieved each subsequent week for 8 weeks. Samples were processed in the lab on the same day that they were retrieved. The entire sample was mixed with 300 ml of water in a Waring blender for 5 min, and 0.1 ml of suitable dilutions was spread on each of the two counting media. No overlay was used. Counts were made in duplicate with dilutions chosen on the basis of preliminary experiments. Results were expressed as the number of bacteria per gram of starting weight of feces.

RESULTS AND DISCUSSION

Table 1 presents the survival data as the logarithm to the base 10 of the numbers of bacteria per gram of starting fecal material. Field moisture data are given in Table 2. The initial increase in numbers agreed with some earlier experiments (1, 9). For the most part, site differences could not be detected; an analysis of variance gave a significant value for only site 4 ($P = 0.01$). This was in spite of much greater differences in our sites than existed in the different soils used by some previous investigators. Our sites included two organic soils where the duff layer showed only minor decomposition, with an abundance of fungal mycelium visible (sites 3 and 4). Site 1 had dense vegetation, an abundance of insect life, a heavy population of small burrowing rodents, and a largely mineral soil which, because of its exposure, remained moist (Table 2). Site 2, with soil derived from the same rock formations and only a few yards distant, was almost devoid of vegetation and frequently became dry, again because of the nature of its exposure. Site 5, a meadow in a drainage basin, became very wet but also dried quickly and had a large and diverse plant community. The general similarity of survival data in these diverse environments is striking. Tate found much greater survival in organic soils than in mineral soils (12), but his Florida Everglades muck and fine sand are very different from our northern Rocky Mountain soils. Papavassiliou and Leon-

TABLE 1. *Survival of E. coli (EC) and S. typhimurium (ST) at five field sites, $\log_{10}$ of cell numbers versus time*

Week	Site 1		Site 2		Site 3		Site 4		Site 5	
	EC	ST	EC	ST	EC	ST	EC	ST	EC	ST
0	6.91	5.99	6.91	5.99	6.91	5.99	6.91	5.99	6.91	5.99
1	7.08	6.26	8.10	7.14	7.04	6.10	7.32	6.59	8.16	7.34
2	7.27	6.53	7.10	7.25	6.97	7.59	7.27	8.04	7.11	8.00
3	5.26	6.39	6.74	6.50	6.92	6.60	6.50	7.55	7.90	7.89
4	4.63	5.51	5.70	5.56	5.92	5.80	5.53	6.60	5.59	7.61
5	4.60	5.53	5.79	5.59	5.92	5.78	5.06	6.60	6.85	6.71
6	4.23	4.54	4.59	5.25	4.92	4.92	4.70	6.34	4.53	6.76
7	3.95	4.55	4.51	5.20	4.84	4.91	4.52	5.26	3.92	5.28
8	3.87	4.84	4.37	4.95	4.49	4.65	3.86	4.41	3.51	3.94

TABLE 2. *Soil moisture at five field sites in actual meter readings and in minus bars*[a]

Week	Site 1		Site 2		Site 3		Site 4		Site 5	
	Meter reading	−bars	Meter reading	−bars	Meter reading	−bars	Meter reading	−bars	Meter reading	−bars
0	178	<1	45	6	72	4	43	6	1	>15
1	187	<1	26	9	55	5	20	15	153	1
2	186	<1	108	2	0	>15	18	>15	150	1
3	151	<1	0	>15	0	>15	5	>15	0	>15
4	180	<1	130	1	0	>15	198	<1	156	<1
5	186	<1	180	<1	183	<1	185	<1	186	<1
6	187	<1	183	<1	185	<1	187	<1	185	<1
7	186	<1	185	<1	183	<1	185	<1	186	<1
8	162	<1	131	<1	180	<1	188	<1	132	<1

[a] One bar equals 10^5 Pa.

ardopoulos (11) observed a survival difference between loam and sandy loam, but their soils were sterilized and may not be comparable to ours for that reason alone. Other experimenters usually inoculated soil directly or mechanically mixed sewage or a fecal material with soil. Our procedure was closer to the shallow burial frequently recommended by the U.S. Forest Service. Most other studies had a higher and more constant water level than our field soils. Apparently, these or other unidentified experimental or environmental factors were more important than soil or vegetation in our experiment. A lysimeter study of Michigan soils with added sewage or bacteria (9) gave results roughly comparable to ours in that the differences among sand, loam, sandy loam, clay loam, and muck were not consistent with time or consistently related to soil texture.

A paired *t* test comparing *E. coli* and *S. typhimurium* on the basis of the combined data from all five sites was highly significant for a difference in numbers. *S. typhimurium* showed somewhat greater survival, which agrees with numerous studies indicating that *S. typhimurium* is one of the more resistant species of its genus and of enteric bacteria in general (11), but that conclusion is not universal (4). The important result from our study is that both organisms survived in appreciable numbers for the entire period during which that area is available for recreational hiking use. The use of actual pathogens in field studies of this sort is unwise and some extrapolations must be made from other research. In dried and sterile sandy loam, reported survival rates of *S. typhimurium*, *Salmonella enteritidis*, and *Salmonella paratyphi* approached that of lyophilized cultures (11), whereas *Salmonella typhi* died out within a few days. But many studies indicated long survival times even for *S. typhi* in soil. Mallmann and Litsky (9) found survival of virulent *S. typhi* for 12, 19, and 50 days in three different soils. Several much older studies in which virulent, freshly isolated *S. typhi* was added to normal soils found survival times of 65, 31, and 58 days (2, 3, 10). But some *Salmonella* serotypes survived in dried feces for 3 years (5). The survival pattern which we found, and the fact that it was essentially independent of the burial location, strongly suggests that the survival of pathogenic bacteria in locations where campers, hikers, boaters, or climbers are forced to congregate by the physical nature of the terrain should be a matter of concern in recreational management of wilderness and other backcountry.

Soil moisture readings in Table 2 showed real differences in the water regimes at different sites, but this was not reflected by any site difference in bacterial numbers. Perhaps the water present in the fecal slurry nullified soil water differences in the early part of the experiment. This would also be a factor in the natural burial of feces, especially since diarrhea does occur among campers and hikers.

The survival of *E. coli* and *S. typhimurium* at 10^3 and more often at 10^4 organisms per g of feces lasted for the duration of the time during which this field site is accessible. The absence of site differences indicated that this survival may be general, but it is quite possible that warmer or wetter sites will have different survival patterns and rates. The presence of intestinal pathogens at some wilderness sites may become a health hazard in the near future. In particular, the recommendation of shallow burial of feces is not substantiated by the results of this study, although such a practice is presumably preferable to surface disposal.

The use of a multiply resistant strain of *E. coli*, instead of the normal *E. coli* of the feces, has obvious advantages. It made counting simpler and eliminated the question of contamination from animal sources. However, this raises the possibility that the survival was related to this enhanced resistance. If the *E. coli* had survived much better than the *S. typhimurium*, this would have been a serious point. However, the *S. typhimurium*, which was not resistant, showed slightly better survival than the resistant *E. coli*, which supports the conclusion that the survival found in this experiment is general for these organisms and conditions.

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LITERATURE CITED

1. **Brandon, J. R., W. D. Burge, and N. K. Enkiri.** 1977. Inactivation by ionizing radiation of *Salmonella enteritidis* serotype montevideo grown in composted sewage sludge. Appl. Environ. Microbiol. **33**:1011-1012.
2. **Creel, R. H.** 1912. Vegetables as a possible factor in the dissemination of typhoid fever. Public Health. Rep. **27**: 187-193.
3. **Firth, R. H., and W. H. Horrocks.** 1902. An inquiry into the influence of soil, fabric and flies in the dissemination of enteric infection. Br. Med. J. **2**:936-943.
4. **Hirte, W. F.** 1977. Untersuchungen zur Tenazität von Gullenbakterien im Boden. Arch. Acker Pflanzenbau Bodenkd. **21**:379-386.
5. **Hyslop, N. St. G.** 1978. Observations on the survival of

pathogens in water and air at ambient temperatures and relative humidity, p. 197–205. *In* M. W. Loutit and J. A. R. Miles (ed.), Microbial ecology. Springer-Verlag, New York.
6. **Kibbey, H. J., C. Hagedorn, and E. L. McCoy.** 1978. Use of fecal streptococci as indicators of pollution in soil. Appl. Environ. Microbiol. **35**:711–717.
7. **Leonard, R. E., and H. J. Plumley.** 1979. Human waste disposal in eastern backcountry. J. For. **77**:349–352.
8. **Lucas, R. C.** 1973. Wilderness: a management framework. J. Soil Water Conserv. **28**:150–154.
9. **Mallmann, W. L., and W. Litsky.** 1951. Survival of selected enteric organisms in various types of soil. Am. J. Public Health **41**:38–44.
10. **Melick, C. O.** 1917. The possibility of typhoid infection through vegetables. J. Infect. Dis. **21**:28–38.
11. **Papavassiliou, J., and J. Leonardopoulos.** 1978. Survival of enterobacteria in two different types of sterile soil, p. 206–210. *In* M. W. Loutit and J. A. R. Miles (ed.), Microbial ecology. Springer-Verlag, New York.
12. **Tate, R. L., III.** 1978. Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. Appl. Environ. Microbiol. **35**:925–929.
13. **Wilde, S. A., and G. K. Voigt.** 1955. Analysis of soils for foresters and horticulturists, p. 18–21. J. W. Edwards, Inc., Ann Arbor, Mich.