Duration of Viability and the Growth and Expiration Rates of Group E Streptococci in Soil

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In irradiated and nonirradiated feedlot and pasture soils inoculated with group E streptococci, the organism was not recovered 17 days postinoculation from either the irradiated or nonirradiated feedlot soils incubated at 37 C, but survived in the irradiated pasture soils for 24 and 31 days postinoculation. The streptococci survived in irradiated and nonirradiated soils incubated at 4 C for 116 days and in one irradiated feedlot soil for 165 days. The population of streptococci did not increase in either irradiated or nonirradiated soil, and the expiration rate was greater in the soils incubated at 37 and 25 C than at 4 C. With the relatively prolonged duration of viability of group E streptococci in soil at 4 C, it is suggested that soil contaminated with exudate from draining abscesses of infected swine could act as a source of infection during the colder season.

Recently, it was reported that swine which carry group E streptococci (GES) do exist and possibly are a major factor in the propagation of streptococcal lymphadenitis in swine (SLS) (3, 4; J. A. Schmitz, Ph.D. thesis, Univ. of Missouri, 1971). However, it is also possible that the contamination of soil with exudate from draining abscesses containing GES could constitute a method of infecting a swine herd. This phenomenon could have occurred on a farm where the disease was not eradicated by depopulation of infected swine, disinfection of premises, or the introduction of specific pathogen-free swine (2). It had been observed previously that streptococci, such as Streptococcus agalactiae, S. dysgalactiae, S. pyogenes, and S. uberis, did survive in damp soil for more than a year (1). This study was designed to determine the duration of viability and the growth and expiration rate of GES in sterilized and unsterilized soil at several temperatures.

MATERIALS AND METHODS

Experiment 1: Duration of viability of GES in soil. (i) Soils. Irradiated and nonirradiated samples of pasture and feedlot soil from two farms in Missouri designated A and B were used (Table 1).

(ii) Sterilization. Soil samples weighing 100 g and air dried were twice irradiated with 170,000 R of gamma radiation per hr for 4 hr from a cobalt 60 cylinder. The interval between irradiation treatments was 72 hr.

(iii) Inoculation and incubation. Soil samples, in 500-ml plastic containers without lids, were inoculated with approximately 5 × 10⁸ colony-forming units (CFU) of GES (strain 3X29a) and hydrated to 80% moisture holding capacity (MHC) with sterile isotonic saline solution (ISS). (Strain 3X29a from R. D. Shuman, National Animal Disease Laboratory, Ames, Iowa. Cells were washed twice in isotonic saline after growth for 24 hr at 37 C in Todd-Hewitt broth.) Three irradiated and three nonirradiated samples of each of the four soils were incubated at 4 C and 37 C. Humidity was maintained in the incubator at 37 C by bubbling air entering the chamber at approximately 2 liters/min through a reservoir of water in the chamber. Humidity was maintained in the incubator at 4 C by keeping a pan of water on a lower shelf.

Table 1. Characteristics of the soils used in determining the rate of expiration and duration of viability of group E Streptococci

| Soil          | Soil texture | pH  | Organic matter |
|---------------|--------------|-----|----------------|
| Sarpy*        | Loamy sand   | 7.5 | 1.8            |
| Huntington*   | Loam         | 6.7 | 2.7            |
| Feedlot A     | Silt loam    | 6.4 | 9.6            |
| Pasture A     | Silt loam    | 7.6 | 2.2            |
| Feedlot B     | Silt loam    | 6.2 | 9.0            |
| Pasture B     | Silt loam    | 7.7 | 2.7            |

* Soil types.
(iv) Recovery of GES. Each sample was cultured for GES at various intervals postinoculation (PI) by inoculating each of three tubes containing 30 ml of blood azide-crystal violet (BACV) broth (8) with 2 g of soil (Table 2). The broth cultures were incubated at 37 C for 24 hr and then centrifuged at 600 × g for 15 min. The sediment was streaked on BACV agar (8) and incubated for 24 to 48 hr at 37 C. One characteristic colony from each plate was used to confirm the serologic grouping of GES (7, 9).

Experiment 2: Growth and expiration rate of GES in soil. (i) Soils. Irradiated and nonirradiated soils of the Sarpy and Huntington types obtained in Missouri were used (Table 1).

(ii) Sterilization. Three 80-g, oven-dried samples of each soil were irradiated twice with 450,000 R of gamma radiation per hr for 2.5 hr at an interval of 96 hr in a cobalt 60 cylinder.

(iii) Inoculation and incubation of soils. Soil samples were inoculated with approximately 10² CFU of GES (strain 3X29a) and hydrated to 80% MHC with sterile ISS. Irradiated and nonirradiated specimens of each soil type were incubated at 37 C, 25 C, and 4 C in 100-ml glass containers with lids ajar.

(iv) Quantitation of GES. The number of CFU of GES per gram of soil was determined by streak plate colony counts performed on BACV agar. Counts were made on days 0, 1, 2, 4, 8, 16, 23, 30, and 37. Incubation of the plates for 24 to 48 hr at 4 C after the initial incubation at 37 C for 24 to 36 hr enhanced beta-hemolysis and facilitated counting the GES colonies. One characteristic colony from each plate was used to confirm the serologic grouping of GES (7, 9).

RESULTS

Experiment 1. With the feedlot soil incubated at 37 C, GES was detected in the nonirradiated specimens at 4 days PI and in the irradiated specimens at 10 days PI; however, GES could not be isolated from either irradiated or nonirradiated samples at 17 days PI (Table 2).

With the pasture soil at 37 C, GES was isolated from nonirradiated samples at 4 days PI but not at 17 days PI. The GES was isolated from all irradiated pasture samples at 24 days PI and from the pasture A sample at 31 days PI. This sample was negative for GES at 52 days PI (Table 2).

The shortest survival time for GES at 4 C was in the soils of feedlot B where the final isolations were made at 31 days PI from the nonirradiated samples and at 59 days PI from the irradiated samples (Table 2). The GES was isolated from all of the remaining three soil types, both irradiated and nonirradiated, at 116 days PI. At 165 days PI, the irradiated soil of feedlot A was positive for GES while all other samples were negative. The feedlot A sample was negative for GES at 200 days PI.

Experiment 2. The number of CFU of GES per gram of soil was between 10⁴ and 10⁵ in all samples on the day of inoculation. The number of GES in the soil samples incubated at 37 C decreased rapidly, and at 37 days PI only the irradiated Huntington soil contained viable organisms. At 25 C, the expiration rate of GES was slower, with viable organisms present in all but the nonirradiated Huntington soil at 37 days PI. The expiration rate of GES in soil incubated at 4 C was significantly less (P < 0.05, analysis of variance of means) than at 37 or 25 C, with high concentrations of GES in all specimens at 37 days PI (Fig. 1).

At each temperature of incubation, there was no significant difference (P > 0.05) in the expiration rates of GES between Sarpy or Huntington soils or between irradiated or nonirradiated soil.

| Soil       | Survival at 4 C on days postinoculation: | Survival at 37 C on days postinoculation: |
|------------|----------------------------------------|----------------------------------------|
|            | 31 45 59 74 99 116 165 200             | 4 10 17 24 31 52                       |
| Irradiated |                                        |                                        |
| Feedlot A  | + + + + + + -                          | + -                                    |
| Feedlot B  | + + - + + - +                         | + -                                    |
| Pasture A  | + + + + + + +                         | + + + + + + +                         |
| Pasture B  | + + + + + + +                         | + + + + + + +                         |
| Nonirradiated |                                    |                                        |
| Feedlot A  | + + + + + + -                          | + -                                    |
| Feedlot B  | + - + + + + +                         | + -                                    |
| Pasture A  | + + + + + + +                         | + + + + + + +                         |
| Pasture B  | + + + + + + +                         | + + + + + + +                         |

* + and -, Days on which soil specimens were cultured for group E Streptococcus (GES). +, GES isolated from the soil; -, GES not isolated.
DISCUSSION

The significant finding in this study was that GES survived longer in soil, specifically at lower temperatures, than these investigators had anticipated. It appeared that there was little or no multiplication of GES in soil, but rather the population of organisms decreased at a rate which was related to temperature. Unquestionably, the technique used in quantitating the GES in experiment 2 resulted in considerable error; however, all specimens were quantitated in the same manner, thus justifying comparisons within the experiment. Irradiation of soil, which was designed to sterilize the soil without greatly altering its physical qualities (5), had less influence on the expiration rate on GES in experiment 2 than was anticipated, although there was an appreciable difference in the duration of viability in experiment 1. It is possible that oven-drying the soil samples in experiment 2 reduced the microflora significantly whereas air-drying in experiment 1 did not alter the microflora, thus accounting for the comparable rates of expiration of GES in irradiated and nonirradiated soils in experiment 2 and also for the marked difference in the duration of viability of GES at 37 C between the two experiments. However, even within experiment 1, it appeared that GES survived better in some soils than in others.

This variability in the survival time of bacteria in different soils has been observed previously (6). The relatively short survival of GES in both irradiated and nonirradiated soils from feedlot B at 4 C cannot be explained. However, it would appear that it was due to factors other
than pH or organic matter since the survival time of GES was considerably shorter than in the remaining soils of comparable composition. The greater survival time of GES in the pasture soils than in the feedlot soils at 37 °C does not agree with a previous report in which survival of enteric bacteria was enhanced by increasing the organic content of soil (6). This disparity may be related to differences in the physiological properties of GES and the enteric bacteria or to the higher incubation temperature. The latter appeared likely considering the prolonged GES survival time of GES in the irradiated soil of feedlot A at 4 °C.

The isolation of GES from soils incubated at 4 °C for 116 and 165 days, in this study, suggests that soil contaminated with exudate from draining abscesses of swine infected with GES could serve as a source of infection for swine during the late fall, winter, and early spring.

There is the possibility that the sodium azide and crystal violet in the BACV, which was used as a selective medium, could have had an inhibitory effect on the growth of GES. In a previous study, there was a reduction of approximately 20% in the isolation of GES from BACV as compared to blood agar (unpublished data). However, in the same study, GES was isolated from BACV agar that had been streaked with an inoculum that contained less than 10 CFU on blood agar.

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LITERATURE CITED
1. Ashoub, M. A., El-R, A. A. El-Naasan, and M. Attia. 1967. Viability of mastitic streptococci outside the udder. Med. J. Giza. 14:119-123.
2. Collier, J. R. 1954. Swine jowl abscesses. Iowa Vet. 25:16-18.
3. Collier, J. R., and J. Noel. 1971. Streptococcic lymphadenitis of swine: a contagious disease. Amer. J. Vet. Res. 32:1501.
4. Collier, J. R., and J. Noel. 1971. Streptococcic lymphadenitis of swine. An immune carrier of Streptococcus suis. Amer. J. Vet. Res. 32:1507.
5. Eno, F. C., and H. Popenoe. 1964. Gamma radiation compared with steam and methylbromide as a soil sterilizing agent. Soil Sci. Soc. Amer. Proc. 28:533-535.
6. Mallmann, W. L., and W. Litsky. 1951. Survival of selected enteric organisms in various types of soil. Amer. J. Pub. Health. 41:38.
7. Moody, M. C. 1970. Streptococcus, p. 65-68. In J. E. Blair, E. H. Lenette, and J. P. Truant (ed.), Manual of Clinical Microbiology. Williams & Wilkins Company, Baltimore, Md.
8. Packer, R. A. 1943. The use of sodium azide and crystal violet in a selective medium for streptococci and Erysipelothrix rhusiopathiae. J. Bacteriol. 46:343-349.
9. Rantz, L. A., and E. Randall. 1955. Use of autoclave extracts of hemolytic streptococci for serologic grouping. Stanford Med. Bull. 13:290-291.