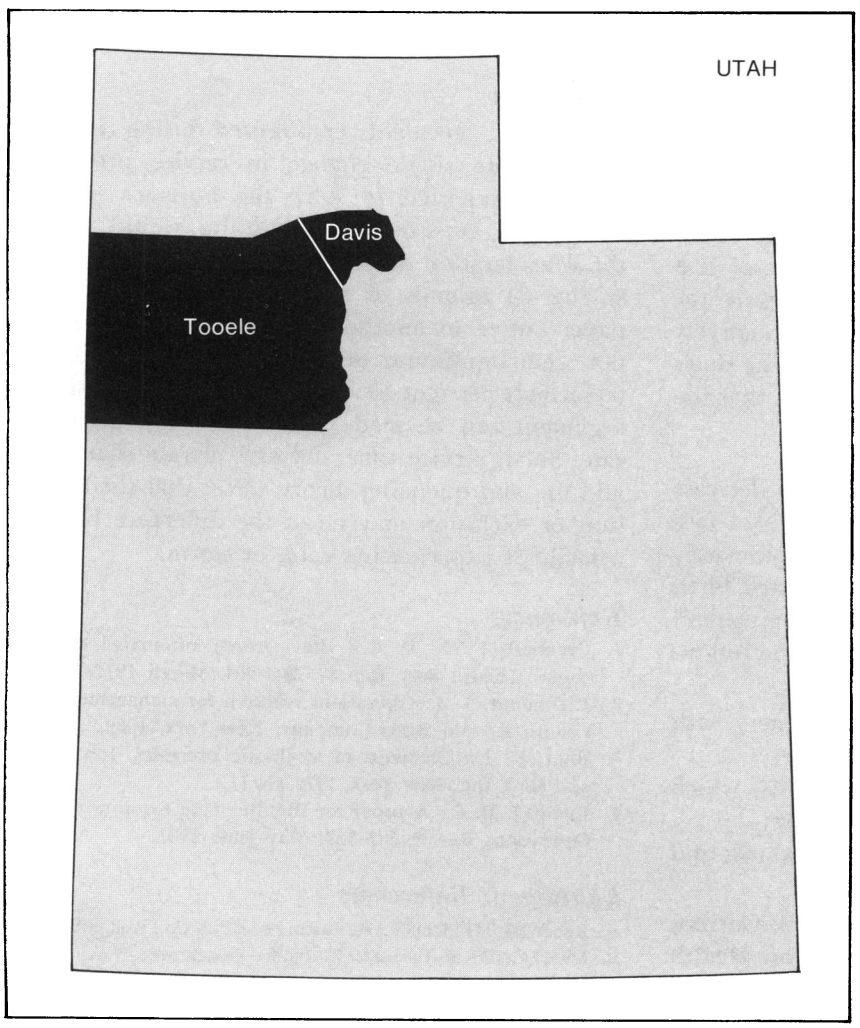
Epidemiologic and Laboratory Investigations of Bovine Anthrax in Two Utah Counties in 1975

TWO EPIZOOTICS OF BOVINE ANTHRAX occurred in Tooele and Davis Counties, Utah, in the summer and fall of 1975. The last previously reported case of bovine anthrax in Utah had occurred in Summit County in 1944 (*1*). Previous outbreaks had also been reported for Tooele County in 1922 (*2*) and for Davis County in 1928 (*3*). Although the soil ecology in these two counties appeared to coincide closely with that in the area in which bovine anthrax was reported by Van Ness (*4*), the long apparent absence of the disease from these two Utah counties prompted investigations to determine the etiology of the 1975 epizootics.



Epidemiology

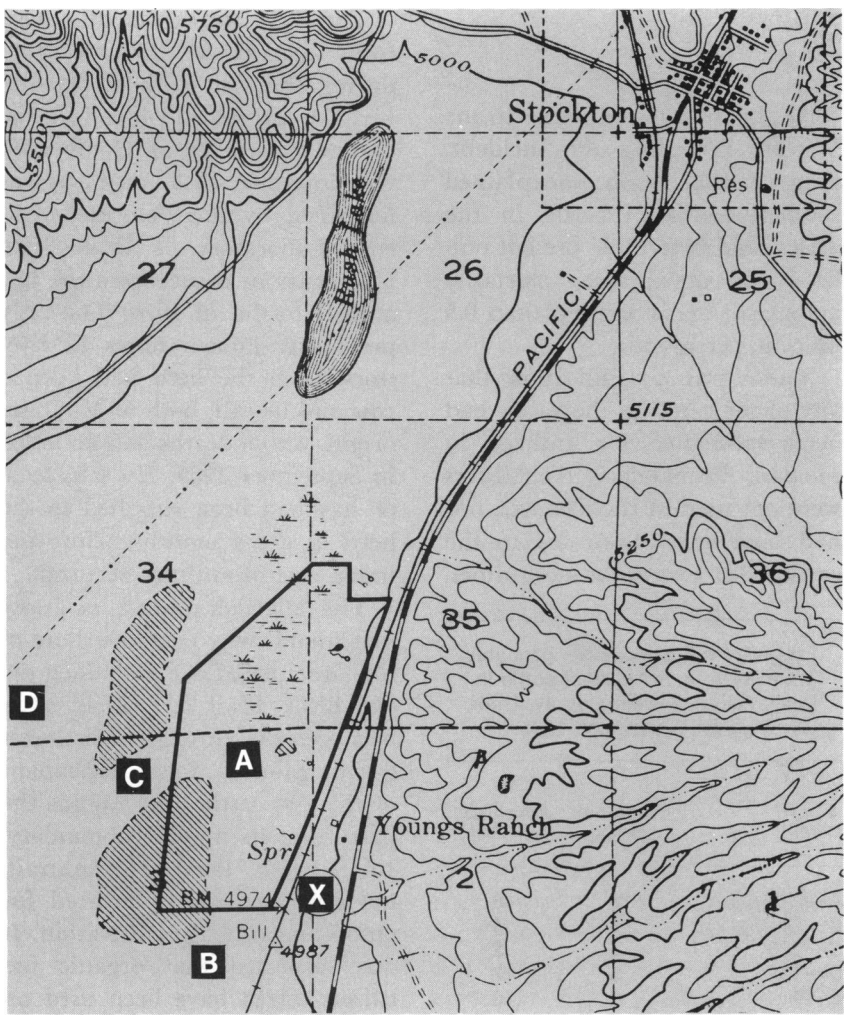
Tooele County. The first reported outbreak of bovine anthrax in Tooele County in 1975 was in the narrow confines of Rush Valley, 35 miles southeast of Salt Lake City (fig. 1). Ranches incorporate all of the land in the central and northern sections of this valley, which is used primarily for grazing livestock. Between August 4 and September 13, 1975, 25 head of cattle in 4 of the affected herds in Rush Valley died suddenly—within 12 to 48 hours after the onset of illness (fig. 2).

Our investigations were directed initially at arsenic poisoning, because the first cattle deaths oc-

□ *Dr. Rees is a microbiologist and Dr. Spendlove is chief. Environmental and Life Sciences Division, U.S. Army Dugway Proving Ground. Mr. Smith, Mr.*

H. B. REES, Jr, PhD, M. A. SMITH, MS,
J. C. SPENDLOVE, PhD, R. S. FRASER, MS,
T. FUKUSHIMA, MD, MPH, A. G. BARBOUR, Jr, MD,
and F. J. SCHOENFELD, DVM

Figure 1. Detailed map of area of Tooele County, Utah, affected by bovine anthrax in 1975



NOTE: Letters show relation of affected herds to primary herd A. X indicates sampling site where *Bacillus anthracis* was isolated from surface soil.

Fraser, Dr. Fukushima, and Dr. Barbour are in the Utah State Division of Health, Salt Lake City: Mr. Smith is chief of environmental microbiology, Mr. Fraser is director of the Bureau of Laboratories, Dr. Fukushima is deputy director of health, Bureau of Disease Prevention, and Dr. Barbour is an Epidemiologic Intelligence Service officer, Public Health Service, assigned to the Bureau of Epidemiology. Dr. Schoenfeld is the State veterinarian, Utah State Department of Agriculture, Salt Lake City.

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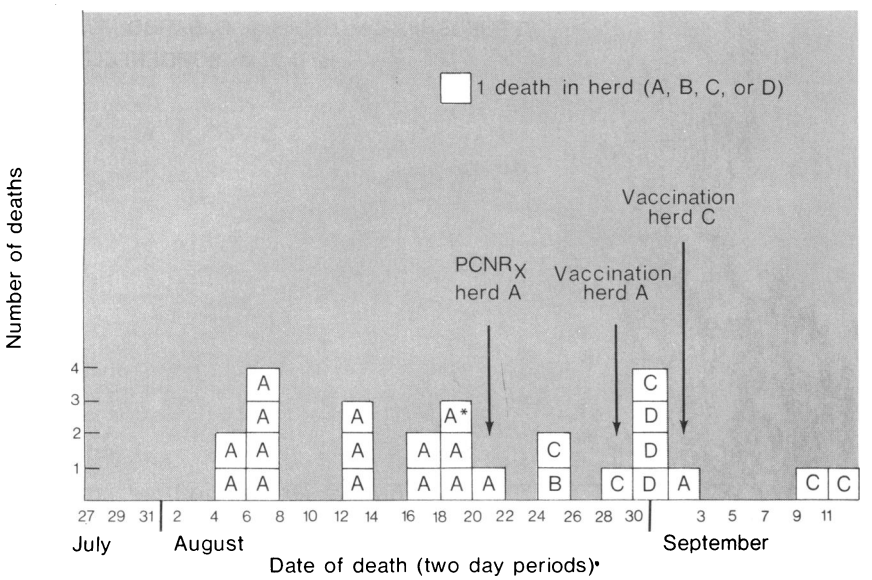
Tearsheet requests to Dr. Horace B. Rees, Jr., Environmental and Life Sciences Division, U.S. Army Dugway Proving Ground, Dugway, Utah 84022.

Figure 2. Chronology of sudden deaths from anthrax among cattle in Rush Valley, Tooele County, Utah, July-September 1975



NOTE: PCNR$_X$ indicates clostridial vaccine and penicillin therapy.
(*) indicates a cow from which *Bacillus anthracis* was isolated.

curred in a pasture close to an old abandoned mine that had produced arsenic ores. However, chemical analyses of tissue and blood samples indicated low levels of arsenic, thus eliminating the possibility of such poisoning. Also, clostridial infection was considered when the pronounced hypocoagulability of the dead animals' blood was noted. Consequently, on August 21 the cattle in herd A (fig. 1) were treated with penicillin and given a clostridial vaccine. On August 25 *Bacillus anthracis* was identified from autopsied material by the Veterinary Science Laboratory of the Utah State University at Logan. On August 29 the cattle of herd A and on September 3 the cattle of herd C were injected with 1 ml of a nonencapsulated anthrax vaccine.

Interviews with all who owned land or grazed cattle in Rush Valley established that the attack rates ranged from 0 to 17 percent (table 1). There were no deaths among the few horses, sheep, and swine nor among the suckling calves in the affected areas. In the 5 years preceding the incident, there had been no unexplained sudden deaths of cattle in the valley, and before the present outbreak the unexplained mortality among all herds was less than 0.5 percent per month.

There was no indication that any of the cattle in the valley had been vaccinated for anthrax in the past. Animal-derived fertilizers were not used in the pastures, nor had bone meal been fed to the cattle. Also, except for mosquitoes, flies and other biting insects were not present in large numbers.

Table 1. Sudden deaths, probably from anthrax, in herds of cattle in Rush Valley, Tooele County, Utah, August-September 1975

Size of herd [1]	Number of deaths	Percentage attacked
A—276	16	6
B—24	1	4
C—220	5	2
D—18	3	17
E—9	0	0

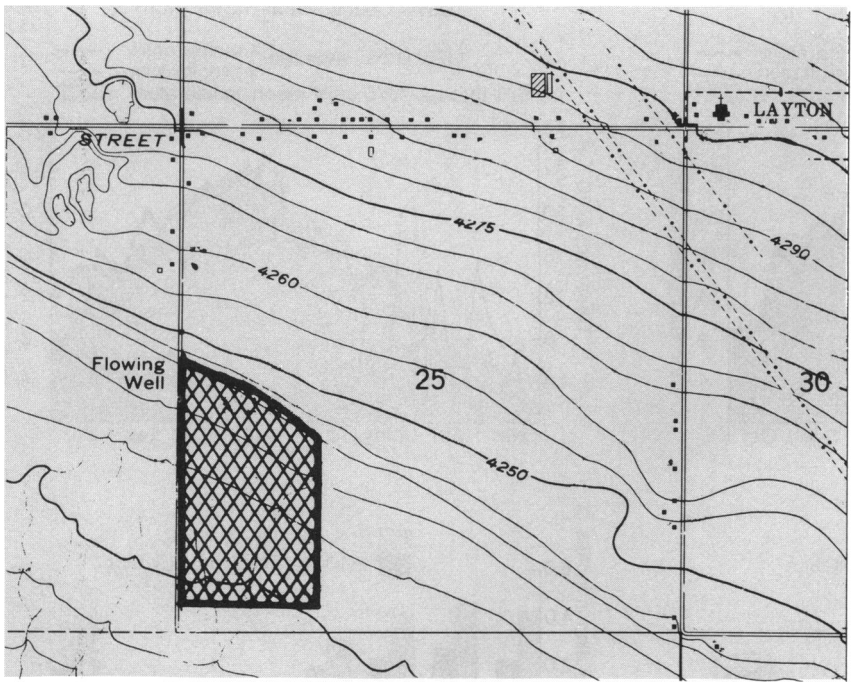
[1] Location of herds is shown in figure 1.

Davis County. The investigation in Davis County began west of the town of Layton with an interview with the owner of the herd that was in the center of the epizootic area (fig. 3), approximately 30 miles north northwest of Salt Lake City. Management of this long-established herd of approximately 90 head of purebred Aberdeen Angus had begun approximately 13 years before the current outbreaks of bovine anthrax. The last cattle that had been added to the herd were 10 animals purchased from a ranch in Jerome, Idaho, in 1969.

The first death in this herd from anthrax in 1975 occurred during the last week of September; the second and last death in that year occurred on October 10. On November 12 *B. anthracis* was identified by the Veterinary Science Laboratory of Utah State University in tissues from the last animal to die in 1975. The only previously known losses in livestock from the herd had been a cow and its calf, both of Montana origin, whose deaths had occurred in September 1969. No salt, feed, or hay had been supplied to the herd in the 4 months before the index case of anthrax occurred.

The affected pasture is grassy marshland, very near the shore of the Great Salt Lake and along the old Bluff Trail on which cattle had been driven to the Northwest during pioneer times. A remnant of this old cattle trail adjoins the pasture at its northern boundary. Immediately north of the trail, the farm acreage is terraced for onion and cabbage cultivation. It was suspected that organic fertilizers might have been used on these farms, but definitive information could not be obtained.

Figure 3. Stippling indicates acreage in Davis County , Utah, primarily affected in outbreak of anthrax in 1975



NOTE: 3" = 1 mile

Runoff water from the farm acreage flowed directly onto the affected pasture. Water to irrigate the 80-acre pasture came from a rapidly flowing drainage ditch, which was dammed as frequently as every 10 days for this purpose.

Livestock losses by other owners in the area included one bull and three cows from the land immediately to the east and at least five animals from the herds grazing on pastures to the south and west of the reference pasture. Earlier, approximately 20 abortion-related deaths had occurred in the herd pastured to the south of the primary site. The herd immediately to the west of the site had lost at least one bull, whose carcass was visible from the access road. Owners of these other herds did not respond to questions.

Soil surveys. The soil types in both areas of the epizootics are similar. They are generally deep, wet, and clayey loam soils that are characteristic of arid and semiarid valley bottoms, low lake plains, and lake terraces. The rock formations from the surrounding mountains provided the parent materials—sandstone shale, quartzitic sandstone, and volcanic tuff—materials deposited by streams which once had fed the ancient Lake Bonneville. Wave action of the lake had sorted the finer particles and produced a mixing and layering of sand, silt, and clay. This material, in conjunction with the deposits of stream flood plains and alluvial fans, comprises the soils of the affected areas.

These silty, clayey loam soils, whose characteristics are detailed in table 2, have been classified as Warm Springs, Ford, and Arave. They are deep, strongly alkaline (pH 8.1 to 9.9), and poorly drained and have slow to moderately slow permeability. Water tables are at or near the surface and rarely lower than 20 inches.

The dominant vegetation in these soils is salt-tolerant and alkaline-tolerant grass, such as alkaline sacaton, salt grass, wiregrass, sedges, rushes, greasewood, foxtail barley, and rabbit brush.

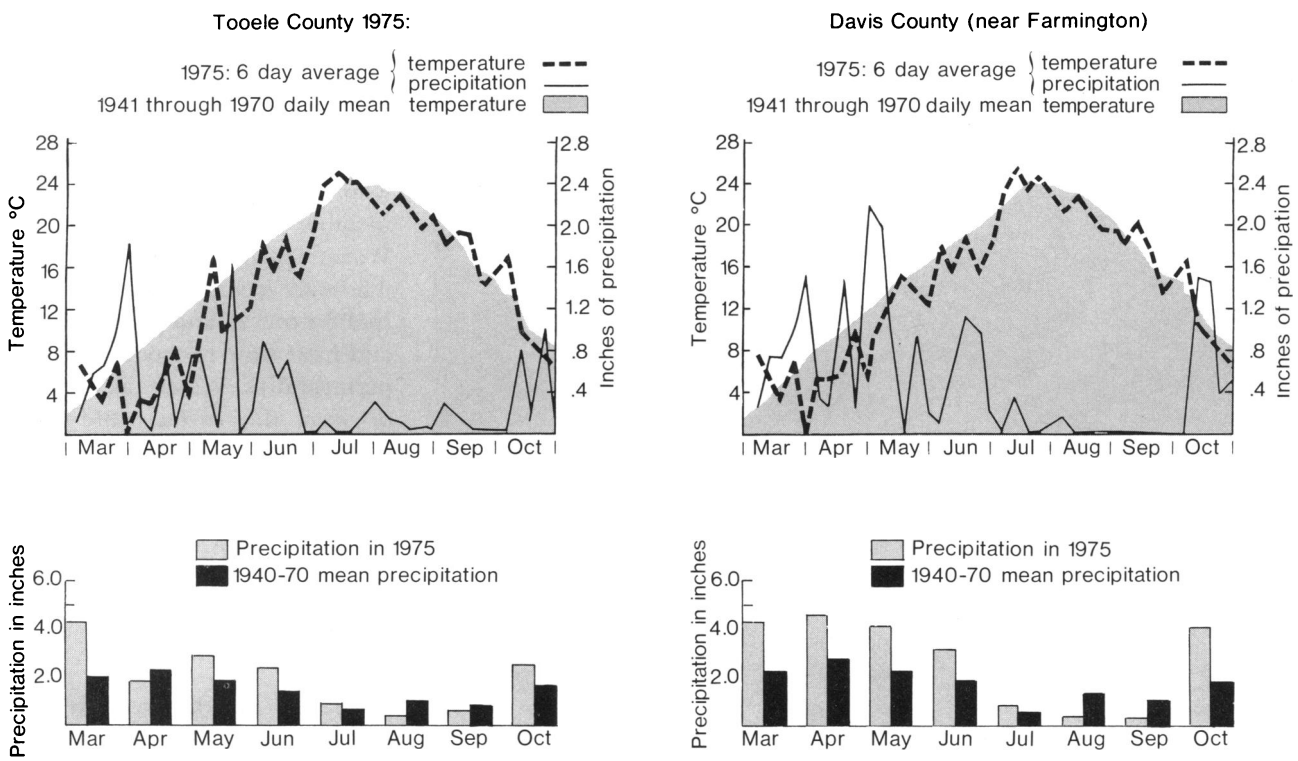
Climate. The areas affected by anthrax are characterized by warm dry summers, cold wet winters, and cool moist springs. Differences in elevation between the two areas is approximately 800 ft (4,225 ft for Layton in Davis County versus 5,000 ft in Rush Valley, Tooele County). The range of temperatures for both areas is similar. The daily mean temperature for the coldest month, December, was 1.83° C for a 30-

Table 2. Composition, in percentages, of soil types encountered in two outbreaks of bovine anthrax in Utah during 1975

Soil components	*Warm Springs*	*Ford*	*Arave*
Sand	70.0	54.0	33.00
Silt	14.0	26.0	56.00
Clay	16.0	20.0	11.00
$CaCO_3$ (equivalents)	24.0	18.0	30.00
Organic C	0.8	1.4	1.4
N	0	0	0.12
Range of pH	7.5–10.4	8.8–10.4	8.6–9.1

NOTE: pH is measured from surface to subsurface.

Figure 4. Daily mean temperatures and precipitation in 1975 for areas in Utah affected by bovine anthrax



year period ending in 1970 (fig. 4) and for the hottest month, July, was 23.3° C. Normally Davis County receives approximately twice the annual precipitation that Tooele County does (20 inches versus 8 to 10).

Temperatures in the two counties during the spring of 1975 were cooler by approximately 3 to 5° C than the mean record for the 30-year period (fig. 4). Rainfall for the spring of 1975 was markedly greater than the recorded mean for that season. Precipitation values in Tooele County totaled 11.0 inches for March through June 1975, as compared with the long-term average precipitation of 7 inches. Davis County recorded 16 inches for the same period as compared with the average precipitation for these months of 8.2 inches. Despite the excess moisture recorded in 1975, both areas experienced 45 percent less rainfall during July and August 1975 than was normal.

Laboratory Investigations

Sample collection. On August 27, we collected samples of soil and water and a burlap bag from Rush Valley in Tooele County, and on August 29 we took a second group of soil and water samples from this valley. The water samples came from the two largest springs in the pasture. One spring was at the northeast side of the pasture; the other was near the center of the affected area (fig. 1). The soil samples were collected from three potential incubator areas—one in the northeast portion of the pasture, one in the central portion, and one in the salty southern portion. (Incubator areas, as defined by Van Ness (*4*), are lowland depressions in which water has stood long enough to kill the grass and provide increased nutrients for micro-organisms.) At two sites east of the railroad track, near carcasses of dead cattle that had been moved there from the index pasture, we took additional soil samples. We removed the burlap bag for culture because the owner of the pasture had observed some cattle chewing it. Subsequently, another 82 soil samples, 4 water samples, and a bone were collected. This latter group of samples was collected systematically along five north-to-south traversals of the affected pasture.

Only one set of samples was collected from the affected area of Davis County. Four traversals of the length of the pasture in this county on November 14 (fig. 3) provided 76 soil and 2 water samples.

All samples were held at 4° C. For assay, they were grouped according to type and geographic

collection point to reduce the number of laboratory animals required for the initial screening. These groupings or pools were made by taking 5 gm of each soil sample or 200 ml of each water sample and combining each with four or five other samples with siimlar characteristics.

Materials

Bacterial strains. Bovine isolates of *B. anthracis* that established each of the two specific epizootics were confirmed to be susceptible to gamma phage by the Center for Disease Control, Atlanta, Ga. As an adjunct to isolating similar strains from the native soil samples on bacterial media, each confirmed culture was tested and found to grow well on the selective PLET (polymyxin, lysozyme, EDTA, thallous acetate) medium of Knisely (*5*).

Spores of *Bacillus subtilis* var. *niger* were used as control suspensions while the sensitivity of the isolation method was being determined.

Media. PLET medium and 5 percent sheep-blood agar were used in attempting isolation of *B. anthracis* from environmental samples. Nutrient agar was employed for growing *B. subtilis.*

Animals. Guinea pigs of the Hartley strain, weighing 250 to 400 grams, were used.

Methods

Review of the method recommended by Weaver and associates (*6a*) led us to doubt the degree of concentration of biological material that might be achieved when relatively light centrifugal force (10 minutes at 2,000 rpm or 650 × *g* for a 5.75-inch rotor radius) was applied. In addition, the streaking of 6 percent or less of the final preparation to surfaces of a nonselective bacterial medium appeared to offer a minimal probability of isolating *B. anthracis* successfully. The procedure detailed in the succeeding paragraphs of this section, therefore, was substituted for the published one (*6a*) in order to provide greater concentration of soil suspension and to increase the possibility that anthrax spores, if present, could be isolated from soil samples.

Tared 20-gram to 40-gram pools of soil were suspended to approximately 20 percent and homogenized in 200 ml of 0.25 percent nonionic detergent (*A*) in 0.3 millimolar KH_2PO_4-buffered water with a blender (*B*) for 90 seconds at maximal speed. The suspensions were decanted into 250 ml bottles and centrifuged at 400 × *g* for 5 minutes (low-speed step). Supernatant fluids from this centrifugation were decanted to fresh bottles and centrifuged at 6,000 × *g* for 30 minutes (high-speed step). The sediments from the high-speed centrifugation were resuspended in 10 ml of 0.3 millimolar KH_2PO_4-buffer by repeatedly pipetting and mixing the samples on a mixer (*C*) for 2 to 3 minutes.

Suspensions were placed in screw-capped tubes, which were tightly closed and sealed with waterproof tape before being submerged in a 70° C waterbath. Timing for heat-shocking (10 minutes) began when the contents of the control tube, which was fitted with a thermometer, reached the desired temperature (after approximately 13 minutes). The tubes were then chilled immediately in ice before further processing.

Water samples also were pooled for the screening procedure so that the size of each would be approximately 200 ml. However, instead of applying two steps of centrifugation, only low-speed centrifugation was used. Subsequently, the supernatant fluids were passed through membrane filters (*D*) with pores having a mean diameter of 0.45 micron. Filter pads were removed aseptically, placed in tubes containing 5 ml of buffer, and then heat-shocked as described.

Bone samples were placed in sterile paper bags and crushed with a hammer. Fragments were then placed in widemouthed bottles, which were shaken vigorously 3 to 5 minutes. Subsequently, fluids from the washing process were treated in the same way as the soil samples.

Concentrated suspensions were streaked to the surfaces of each of two PLET and two blood-agar plates (0.2 ml per plate). The remaining suspension was inoculated subcutaneously at several sites on the abdomen of the guinea pigs. No more than 5 ml were administered to each animal. Inoculated plates were incubated at 37° C and examined after 24 hours and 48 hours for the presence of colonies exhibiting the typical morphology of *B. anthracis.* The guinea pigs were examined several times a day for 7 to 10 days. Dead animals were autopsied, and their spleens were removed. Portions of spleen were used to prepare smears for microscopic examination and to streak to blood agar for cultural identification.

pH measurement. The sediments of pooled soil samples from the low-speed centrifugation were resuspended with approximately 100 ml of distilled water and measured potentiometrically. Water samples were measured directly.

Sensitivity of method used. To determine the degree of sensitivity afforded by the modified procedure we used, portions of pooled negative soils collected in Rush Valley were seeded with a *B. anthracis* spore suspension of known concentration and then processed by the procedure just described with certain exceptions, as described in the two following paragraphs.

The spore suspension was prepared by harvesting the growth of a month-old agar slant in 8 ml of 0.25 percent nonionic detergent in 0.3 millimolar KH_2PO_4 buffer. Glass beads were added to the tube containing the suspension, and the suspension was shaken for 2 minutes on a mixer. After the suspension was heat-shocked for 10 minutes at 65° C and chilled, serial dilutions were plated to blood agar. The spore concentration was approximately 1.2×10^8 cells per milliliter.

Ten-gram samples of pooled soil that earlier had been found to be negative for *B. anthracis* were seeded with approximately 40, 20, 10, 5, 2, or 1 viable spores. Two other 10-gm samples of soil were seeded with 1.9×10^9 spores of *B. subtilis* var. *niger*. The latter suspensions (which served as duplicate controls) were used to map the relative percentages of spore recovery for each step in the procedure. They were plated to nutrient agar, and the resulting colonies were counted after 24 hours of incubation at 37° C. In contrast to the procedure described previously, the low-speed centrifugation was increased to 650 × *g*, as recommended by Weaver and Associates (*6a*), so that we could compare the relative efficiencies of the two methods. Assurance that the specified centrifugal forces were exact was attained by adjusting the revolutions per minutes with a calibrated stroboscope.

Results

Environmental samples. Only a single pool of all specimens processed from Tooele and Davis Counties yielded *B. anthracis*. The isolation of *B. anthracis* was made from colonies arising on PLET medium as well as from inoculation of guinea pigs. The Center for Disease Control found both isolates to be susceptible to gamma phage. Reexamination of the two soil samples from this one pool revealed that the isolation of *B. anthracis* was made from a surface sample of soil taken near the carcass of one of the first animals in Rush Valley to die (the approximate location is marked with an X in figure 1). Subsequent samples taken from the same site after the owner of the herd had incinerated the carcass yielded no spores. Nor were any spores found in ashes from the burning pit in which many of the carcasses in Tooele County had been burned along with old tires and fuel oil.

Comparison of methods. The following table shows the mean recoveries of *B. subtilis* var. *niger* from an alkaline soil of approximately 8.2 pH after a one-step and after a two-step centrifugation procedure. The original 20 percent soil suspension represents 100.0 percent, and the mean recoveries are based on duplicate treatments.

Treatment	*Mean recovery in percent*
A. Sediment after 650 × *g* for 10 minutes	5.3
B. Sediment after supernatant A was subjected to 6,000 × *g* for 30 minutes	61.4
C. Supernatant fluid after step B	8.0
Total (A + B + C)	74.7

A comparison of the two methods for concentration revealed that with a centrifugal force of 650 × *g*, only 5.3 percent of the *B. subtilis* spores from the control suspensions were sedimented. In contrast, when a centrifugal force of 6,000 × *g* was applied a second time for 30 minutes, approximately two-thirds of the spores remaining in the treated suspensions were sedimented. The supernatant fluids after the second centrifugation contained 8 percent of the spore inoculum. Surprisingly, the total recovery of spores was only 74.7 percent. The amount of spore agglomeration during the procedure was not determined. This factor, however, would reduce the apparent, but not the real recovery.

The sensitivity of the two-step centrifugation procedure in recovering and detecting spores of *B. anthracis* seemed excellent (table 3). When 25 viable spores were added to 10 gm of soil and the sediment was resuspended in 10 ml of buffered water after high-speed centrifugation (6,000 × *g*), we were able to detect as few as 13 spores in one instance (one-half of the actual spore content used as a 5 ml inoculum for a guinea pig). In a smaller inoculum of 13 spores per 10 gm of soil, 7 spores were found. The probability that a spore would be present on media plates that were inoculated was small, and we found no colonies of *B. anthracis* on them.

Discussion

One of the most perplexing aspects of the ecology of anthrax is its recrudescence in a given area after long periods of absence. Presently, two theories have been advanced to explain this phenomenon. The earlier one purports that new epizootics arise

Table 3. Sensitivity of two-step centrifugation method in recovering *Bacillus anthracis* spores from an alkaline soil of pH 8.2

Theoretical number of B. anthracis *spores per 10 gm of soil* [1]	*Actual number of* B. anthracis *spores per 10 gm of soil* [2]	*Number of isolations of viable spores per 2 guinea pigs inoculated*
41	25	1
21	13	1
14	9	0
5	3	0
3	2	0
1	<1	0

[1] Calculated from stock dilution of spores.
[2] Product of theoretical value multiplied by 0.614; 0.614 is the mean recovery found for *Bacillus subtilis* after step B, text table, p. 182.

from static, persistent spores present in the soil. A later and more plausible theory suggests that there is a dynamic state in which anthrax bacilli undergo cycles of propagation in the soil, but that the appearance of the disease is limited to periods when the environment has been altered specifically and drastically. Although anthrax spores maintain viability almost indefinitely—as long as 68 years according to Novel and Pongratz (*7*), it is difficult to imagine that spores once present in the soil would fail to infect exposed and susceptible hosts for many decades. Van Ness (*4*) indicates that anthrax spores germinate and that new spores are formed in suitable soils without the micro-organisms necessarily infecting susceptible animals. Certain severe deviations from normal environmental conditions appear to give the anthrax bacilli a competitive advantage. Whenever these conditions exist, the potential for epizootics increases.

The fact that soils are among the natural habitats for *B. anthracis* seems well documented. In addition to our isolation of this micro-organism from soil near a carcass of an animal that had died of anthrax, Fox and co-workers (*8*) made three isolations from some 300 samples of soil taken from affected areas in Louisiana during a 1971 outbreak of bovine anthrax. Similarly, in Europe, Federic and co-workers (*9*) isolated three strains from soil of an anthrax district of Kosická Belá, Czechoslovakia, and Hugh-Jones and Hussaini (*10*) isolated *B. anthracis* from the soil of Berkshire, England, in 1972.

Evidence that *B. anthracis* propagates in certain soils has been provided by at least two groups of investigators. Maly and coworkers (*11*) found that these bacilli grew exponentially in "humusgley" soils (pH 7.2) of East Slovakia when the soils were moistened and incubated at 20° C. Lomakovskaya (*12*) found that sterilization of the soil favored the growth of these bacilli, but that the organisms also grew in 24 hours at 37° C in one nonsterile soil from a muddy swamp.

The literature on the spread of anthrax by carrion appears to be dogmatic with regard to the size of the carcass of the infected animal. Toschkoff and Veljanov (*13*) state that sporulation occurs in the carcasses of smaller animals that succumb to anthrax infection, specifically the guinea pig and rabbit, and thereby the immediate environment is contaminated. In contrast, they found that the bacilli in the carcasses of sheep and cattle did not sporulate, but were lysed instead; thus, the carcasses of these larger animals posed little threat of contamination. Similarly, Van Ness (*4*) reported that bovine carcasses infected with anthrax were of little epidemiologic significance after the remains had been exposed to a tropical environment for 3 days. Evidence to the contrary was reported by Hugh-Jones and Hussaini (*10*), who isolated anthrax spores from soil contaminated with sanguineous effluent from an infected cow 236 days after its death, but not 300 days after. Likewise, our isolation of anthrax spores was made in a soil sample taken near a carcass that had been exposed for more than 21 days to an air temperature of 35° C.

The process by which the soil is contaminated with anthrax by a carcass is undoubtedly too complex to be explained entirely by the variables of extremes of temperature and initial size of the infected animal. The presence of atmospheric oxygen, rather than the size of the carcass per se, may play a significant physiological role in determining whether sporulation will occur in the decaying tissues. The belief that carcass size determined whether sporulation would occur may have been no more than an obscure recognition that certain oxygen and temperature relationships were required for sporulation. Davies (*14*) has documented how critical temperature and humidity are. For him, optimal sporulation occurred in the laboratory at 37° C and at very high relative humidity (100 percent). At a temperature below 30° C, sporulation was minimal. He postulates that the anthrax

bacilli have little chance for survival in soils in temperate climates. Minett (*15*) found that the optimal sporulation of the bacilli occurred in blood from carcasses at temperatures between 32 and 38° C. In the absence of these ideal conditions favoring sporulation (15 and 21° C), the saprophytic contaminants quickly outgrew the anthrax bacilli, which disappeared completely in time.

The entry of anthrax bacilli into Utah soil is implicated historically, since the geographic spread of anthrax in the western United States seems coupled unquestionably with the cattle drives of the Texas Longhorn stock (*4*). Once the organism was introduced into the area, its chances for survival were enhanced by the nature of the soil. Both areas in Utah that have been affected recently have soils with the characteristics described by Van Ness and Stein (*16*) as being the types in which anthrax bacilli would be maintained.

The area around Rush Lake, Tooele County, has been used to graze livestock since 1854 (*17a*). On September 2 of that year, the U.S. Army established Camp Relief on the eastern shore of the lake to protect and feed the 450 mules and 300 horses in Col. E. J. Steptoe's command. After the camp was closed the following year, Rush Valley became pasture land for Longhorns and other breeds of cattle. In 1862, the Second Cavalry, California Volunteers, under the command of Gen. Patrick Connor, pastured their stock in the valley (*17b*).

The affected acreage of Davis County is near the old Bluff Trail, a route used by early pioneers to move livestock to the West (via the Humboldt Route) and to the North and Northwest around the Great Salt Lake to the Oregon Trail. The predominance of the Longhorn stock during the settlement of Utah and the movement of these animals along these trails provided the best possibility for the initial introduction of anthrax bacilli into Utah soils.

The resemblance between the soils of Tooele and Davis Counties and the soils that supported the Louisiana epizootic of 1971 (*8*) is noteworthy. The soils of the levees and back swamps of Louisiana developed from loam, silt, and clayey-type sediments deposited by the Mississippi River and its tributaries. The Louisiana soils also were poorly drained and had a surface pH of 5.6 to 7.8 and underlying layers of very alkaline soil.

The duration of, and the sequence in which, climatic events occur appear to be of major importance in determining whether the bacilli will express their presence as bovine disease, since very similar conditions have been recorded for years when anthrax was not reported. If the exact sequence of these events were known, the information would affect veterinary economics profoundly. Early vaccination of herds, for example, would reduce losses markedly during the year of an anticipated occurrence of the disease, and conversely, the expense of immunization could be avoided in years in which conditions favorable for the bacilli failed to materialize.

There is apparently an unexplainable contradiction between the number of spores needed to infect cattle by the oral route—10^7 spores (*18*)—and our failure to find abundant spores in the Utah soil with a concentration method sensitive enough to detect as few as 13 spores per 10 gm of soil. Heretofore, possible explanations for this dichotomy have included the large quantities of soil eaten by cattle as opposed to the small amounts of soil processed in the laboratory, the insensitivity of the method in detecting spores, and the usually long delay between the cattle's ingestion of infected soil and the investigators' sampling. Our investigations in the Rush Valley epizootic, however, were prompt enough to reduce the probable effect of delay between the soil ingestion by cattle and the collection of soil samples. Soil had been sampled 1 week before the last bovine death occurred. Also, although the concentration method used was shown to be sensitive in detecting anthrax spores, no spores were found in any of the soil samples examined that came from potential "incubator areas."

The number of spores needed to infect a cow orally with laboratory-grown material seems to differ markedly from the dose needed to infect an equally susceptible cow with spores grown in the natural environment. We believe that this difference exists despite the fact that cattle in New Zealand have been shown to consume as much as 1.3 kg of soil per day (*19*). If the minimal infectious dose by the oral route is indeed 10^7 spores, as indicated for example by Schlingman and co-workers (*18*), each 10 gm portion of soil would have to contain approximately 10^5 spores (based on the reported soil consumption of cattle), or a value 10,000 times greater than the sensitivity of our detection method. In these calculations, however, no consideration is given to the possibility that cattle may become infected by ingesting numerous doses of spores that taken singly are less than the minimum infectious dose or that an extended

incubation may be required for infection when the number of spores is less than the infectious dose reported in the literature.

Conclusions

A cool, unusually moist spring followed by drought in early summer appears to have fomented the two outbreaks of bovine anthrax in central Utah during 1975 that resulted in at least 37 deaths in cattle. The anthrax is believed to have originated in the soil because of (*a*) the historical likelihood that anthrax had existed previously in the specific localities affected, (*b*) the stability of the infected herds—no new animals having been added to them for at least 90 days before the outbreaks, (*c*) the fact that the herds had not been given commercial feed or feed supplements and that no fertilizers had been applied directly to the pasture land, (*d*) the presence of all the known climatic and soil characteristics required in the ecology of bovine anthrax, (*e*) the presence of only a small population of flies and other biting insects, and (*f*) our failure to find any evidence of deaths occurring among the large number of suckling calves in the affected herds. Since soil composition and climate were similar throughout the affected regions, we believe that other foci of bovine anthrax (possibly in Summit and Utah Counties) might have existed in the State in 1975 but were not reported.

We failed to isolate spores of *B. anthracis* from the soils of the index pasture even though the detection method used was most sensitive and samples were collected while the epizootic in Tooele County was occurring. The reason may have been that fewer spores propagated in nature are required for an oral infectious dose in cattle as compared with the number needed to initiate infections by the oral route with spores grown in the laboratory. Alternatively, it is possible that the vegetative bacilli may play a far greater role in the epidemiology of anthrax than has heretofore been ascribed to them.

Successful isolation of anthrax spores from soil and other environmental samples requires that the material collected be processed with as much sensitivity and selectivity as existing techniques permit. The technique recommended by Weaver and associates (*6*a) does little to meet either of these criteria. Inoculation of 6 percent or less of the "concentrated" suspension (5.3 percent of the spore content in our comparison) to a nonselective medium such as blood agar will permit the isolation of *B. anthracis* only from grossly contaminated samples. The insensitivity of the method notwithstanding, Fox and co-workers (*8*) used it to find *B. anthracis* in 1 percent of their 300 samples. A summary of an accelerated method for detecting anthrax bacilli reported by Tomov and Todorov (*19*) is so short that the method is described inadequately. The method, in which staining with fluorescent-labeled antibody is used, must remain dubious, because it is not known whether Tomov and Todorov gave adequate consideration to the nonspecificity of fluorescent-labeled antibody in differentiating a variety of *Bacillus* species (*6b*).

A selective medium such as that of Morris (*20*) or PLET is recommended for environmental samples whenever the index strains of *B. anthracis* can be shown to grow in the presence of the inhibitors incorporated in the medium. Our experience indicates that the presence of inhibitors in PLET medium markedly enhances recognition of anthrax colonies. In soils of the affected areas of Utah, numerous spores germinated and produced *B. cereus*-like colonies, which were partially or completely inhibited when plated to PLET medium.

Use of large laboratory animals, such as guinea pigs, proved to be a practical means of achieving greater sensitivity in isolating anthrax spores because a large inoculum could be employed. The choice of the Hartley strain of guinea pigs was fortuitous since this breed appeared refractory to the toxigenic strains of *Bacillus cereus* that are frequently lethal for mice.

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Equipment References

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B. Commercial single-speed blender. Catalog No. 700. Waring Products Division, Dynamics Corporation of America, New Hartford, Conn.
C. Mechanical mixer, model K-500-J. Scientific Industries Incorporated, Queens Village, N.Y.
D. Membrane filter, type HAWG 047 50. Millipore Corporation, Bedford, Mass.

SYNOPSIS

REES, H. B., Jr. (U.S. Army Dugway Proving Ground), SMITH, M. A., SPENDLOVE, J. C., FRASER, R. S., FUKUSHIMA, T., BARBOUR, A. G., Jr., and SCHOENFELD, F. J.: *Epidemiologic and laboratory investigations of bovine anthrax in two Utah counties in 1975. Public Health Reports, Vol. 92, March–April 1977, pp. 176–186.*

Bovine anthrax broke out in Tooele and Davis Counties of Utah during the summer and fall of 1975. The disease had last been reported in Utah in 1944 and in the same areas in 1928. At least 37 cattle deaths were recorded in the two counties in 1975, but no deaths or infections were reported for men, suckling calves, sheep, horses, or swine. Vaccination of the affected herds terminated the anthrax epizootics. Both epizootic areas, which were separated by approximately 50 miles, possessed the alkaline soils requisite for the ecological maintenance of anthrax. Soil moisture and temperatures paralleled those that have been reported in the literature as fostering the propagation of the anthrax bacilli.

Soil and water samples taken from the primary sites of infection, however, yielded but one isolation of *Bacillis anthracis,* from a sample of soil taken near a carcass; no isolations were made from soil samples taken from potential "incubator areas" (lowland depressions where standing water had killed the grasses at some previous time). The absence of additional isolations was surprising because soil samples of sufficient size for analysis had been collected before the last cattle deaths of 1975 occurred. Moreover, to recover anthrax spores, the samples had been processed by a newly devised, very sensitive two-step centrifugation method.

This new two-step centrifugation method concentrates the spore content of the soil approximately fourfold (based on the number of grams of soil extracted into milliliters of suspension). The entire volume of concentrated suspension is assayed. In experiments designed to compare the recommended method with the new technique, it was found that only 5.3 percent of the spores of *B. subtilis* var. *niger* seeded to the soil experimentally could be recovered, as compared with 61.4 percent by the two-step centrifugation technique. Other soil, initially examined and found to contain no anthrax spores, was pooled and seeded with known concentrations of *B. anthracis* spores to determine the sensitivity of the new technique in recovering anthrax spores from the seeded soil samples. Results showed that with the new method as few as 13 spores of *B. anthracis* could be detected in 10 gm of soil.

Lack of success in isolating anthrax spores has caused questions to be raised as to what the minimal dose needed to infect cattle is. Either the infectious dose reported in the literature, 10^4 laboratory-grown anthrax spores per gram of soil ingested by a susceptible cow, is unrealistically excessive in comparison with the dose required for anthrax spores grown in the soil, or else the vegetative form of the bacillus plays a far greater role in the epidemiology of bovine anthrax than heretofore has been ascribed to it. Evidence was found to question the belief that the size per se of an animal species that becomes infected with anthrax is a significant factor in determining whether the environment will be contaminated.