Longevity of Eberthella Typhosus in Various Soils*

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WITH increasing congestion of population, the steady decrease in unpolluted water supply sources, and with a growing tendency to reclaim polluted waters from sewage plants, and to use more or less completely purified sewage for irrigation, the longevity of intestinal pathogens becomes a question of growing concern.

The present studies report the results of investigations carried on at intervals during the past 4 years upon the length of time that *Eberthella typhosus* is able to maintain itself in soil subjected as closely as possible to conditions approximating those prevailing in nature.

This subject has been a matter of interest almost from the time of Eberth's original report of the causal organism of typhoid fever.

Grancher and Deschamps [1] in 1889 claim to have recovered it from soil after 5½ months. Karlinski [2] in the same year reported a 3 months' survival in natural soil. Dempster [3] reported survivals of from 12 to 42 days in various types of soil. Pfuhl found a survival of 28 days in dry soil and 88 days in moist.

Robertson [4] added bouillon cultures to unsterilized soil and claimed to have isolated the organisms after 315 days and from natural soil after 86 days. Martin's [5] data indicated a possible survival of 404 days. Death was more rapid in sterilized soil. In pure peat soil the organisms could not be recovered after 24 hours. Firth and Horrocks [6] found that laboratory and freshly isolated strains survived 55 and 32 days, respectively, in unsterilized soil.

Rullmann [7] recovered viable organisms from organically polluted soils after 100 days when the soil was unsterilized and after 16 months in similar sterilized soil.

Sedgwick and Winslow [8] reported over 99 per cent destruction in dry soil in 2 weeks with a longer survival in moist soil. Smith [9] believed *Eberthella typhosus* would live only a short time in unsterilized soil and a little longer in sterile. Mair [10] reported to the contrary claiming survivals of 42 and 74 days in unsterilized and 9 days in sterile soil. Kligler [11] in perhaps the best organized and most exhaustive series of experiments of all reported survivals of 80 days under certain conditions. His experiments, however, appear to have been carried out under controlled rather than under natural conditions.

On one point the investigators are in accord, that moisture plays an important rôle. With the exception of Kligler's work it is difficult to evaluate

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TABLE 1

Eberthella Typhosus per Gram Soil

Rainy Season 3.2 Inches of Rain

Soil	Initial	1 Day	2 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days	49 Days
Adobe	16,000,000	12,000,000	8,200,000	1,000,000	80,000	22,000	3,000	650	40	0
Adobe peat	15,000,000	10,000,000	9,500,000	2,000,000	200,000	50,000	7,000	800	250	0
Loam	16,000,000	11,500,000	9,000,000	3,500,000	600,000	75,000	10,000	950	300	95
Sand	22,000,000	5,000,000	50,000	10	0	0	0	0	0	0
Peat	14,000,000	100	0	0	0	0	0	0	0	0

Dry Season 2.0 Inches of Rain

Soil	Initial	1 Day	2 Days	7 Days	14 Days	21 Days	28 Days
Adobe	25,000,000	11,950,000	7,000,000	800,000	50,000	10	
Adobe peat	23,000,000	11,500,000	8,000,000	900,000	75,000	10,000	50
Loam	23,000,000	11,650,000	7,500,000	500,000	10,000	75	
Sand	20,000,000	1,000,000	1,100				
Peat	26,000,000	25	0				

much of the earlier work. Technics in many cases were still comparatively new and crude and the conditions of the experiments have not always been clearly discernible.

The observations reported in this paper constitute part of a program of studies on the longevity of typhoid organism exposed to a variety of natural conditions. Survivals in sea water and in sewage have been previously reported.[12, 13]

The data that follow are derived from our attempts to gain an approximation at least of the length of time massive inocula of typhoid organisms would survive in four types of soil during rainy seasons and during seasons practically free from rain, together with certain other related factors.

Adobe, adobe-peat-moss mixture garden loam, sand and straight peat moss were first investigated. Portions of the soil were placed in unglazed clay flower pots suspended over containers of 5 per cent lysol and placed out of doors in such a position that they were exposed to rains at any time and to sun about two-thirds of the day. Initially the soils were adjusted to approximately the same moisture content (20 per cent) and inoculated with saline suspensions from 24 hour agar slants. The organisms were distributed as evenly as possible through the upper 4 inch stratum. At intervals, 5 gm. of soil were removed, shaken up in 95 cc. of saline filtered and the organisms per gm. of soil determined.

In investigations of this nature we have found the Wilson-Blair bismuth sulfite [14] agar to be of great assistance, since *Eberthella typhosus* stands out as a startling jet black colony and the majority of other types are inhibited or eliminated altogether. Counts of typhi on this medium and plain agar are comparable. Originally, we prepared our own media with which at times we had difficulty, but in the present studies the Digestive Ferments Company * dehydrated product was used and found to be entirely satisfactory.

Equal weights of the various soil types were held at a constant temperature of 20° C. for 48 hours to observe the rate at which moisture was lost. All soils were then adjusted to approximately the same initial moisture content. There was probably some error in the results due to differences in volume and area. This would be more noticeable in case of the peat which oc-

* The author wishes to acknowledge the courtesy of the Digestive Ferments Company for their generous contribution of the media used in these studies.

TABLE 2

Soil	Per cent Death First 24 Hours Season: Wet	Per cent Death First 24 Hours Season: Dry	Per cent Loss * Moisture 48 Hours 20° C.	Days Known Survival: Wet	Days Known Survival: Dry
Adobe	25	45	5.0	42	21
Adobe peat	30	50	5.3	42	28
Loam	28	45	6.0	49	21
Sand	70	95	12.0	8	4
Peat	99.99		9.5	1	2

* Initial moisture = 20%

Average Maximum temperature
Wet Season 58
Dry Season 74

Average Minimum temperature
Wet Season 39
Dry Season 54

cupied a considerably larger volume, for example, than the adobe. Initially, the adobe suffered the least moisture loss, but as drying continues, this soil shows a great tendency to open up cracks and fissures.

The data from four sets of observations, two during California rainy seasons and two during dry seasons, are shown in Tables 1 and 3. The behavior in the first three soils is quite consistent throughout the series.

SUMMARIZING THESE DATA

The survival in all types of soil was greatest during the rainy season when moisture was occasionally added by the rains and when the rate of evaporation was low. The adobe-peat and loam held their moisture with greater tenacity than the adobe. In sand where drying was rapid the survival time was very short—between 4 and 7 days The rapid death in peat cannot be explained upon the same basis. Infusions of this peat indicated a pH of between 3 and 4, so that may be the answer. The experiments during the dry season demonstrate the same tendency, the essential difference lying in the shorter survival time. The effect of moisture on survival is brought out sharply in the second experiment (Table 3) where with a longer rainy season the survival is almost tripled. The effect of moisture retaining capacity is indicated by comparing the survivals in sand, sand-loam mixture, and loam.

Table 2 indicates certain additional observations having a bearing on soil properties and organism behavior during the first experiment.

TABLE 3

Eberthella Typhosus per Gram of Soil

Rainy Season 10.1 Inches of Rain

Soil	Initial	1 Day	15 Days	30 Days	45 - 60 Days	75 Days	90 Days	105 Days	120 Days
Adobe	20,000,000	17,000,000	3,000,000	1,000,000	500,000	25,000	5,000	900	15	0
Adobe peat	21,000,000	17,800,000	3,200,000	900,000	500,000	17,000	3,000	400	0	0
Loam	20,500,000	17,200,000	2,900,000	950,000	480,000	20,000	4,500	500	30	3
Sand	21,000,000	7,000,000	250	0						
Loam sand	20,300,000	15,000,000	2,000,000	300,000	25,000	125	0	0	0	0

Dry Season 0.7 Inches of Rain

Soil	Initial	1 Day	2 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Adobe	23,000,000	13,000,000	5,000,000	600,000	30,000	450	25	0	0
Adobe peat	22,000,000	11,000,000	5,500,000	700,000	42,000	7,000	300	75	2
Loam	22,500,000	11,800,000	6,000,000	600,000	45,000	2,500	150	23	15
Sand	22,000,000	1,200,000	950	2	0				
Loam sand	22,000,000	8,000,000	2,000,000	100,000	75	0	0	0	0

Effort was also made to learn what rôle if any was played by sunlight in the destruction of the organisms. Pots with a finely pulverized loam-peat mixture were prepared as before but this time a layer of the top soil approximately ¼ inch deep was separated from the remainder by a sheet of thin gauze so that this top layer could be lifted off for examination without carrying over any soil from the deeper layers. The pots were exposed outside with one exposed to the full rays of the sun and the other shaded by a dark cloth 3 inches above the surface of the soil. Table 4 shows the results of this experiment: While the moisture loss was essentially the same in both pots the death rate was 36.0 per cent higher in the unshaded than in the control.

Various workers have investigated or commented on the bearing that other organisms in the soil might have on the survival of intestinal pathogens. Martin [5] reported *Bacillus typhosus* survival was longer in sterile than in normal soil. Rullmann's [7] investigations led to similar conclusions, and Smith,[9] although somewhat vague, appeared to concur. Mair,[11] on the other hand, disagrees with these conclusions. Frost,[13] using pure cultures of *Eberthella typhosus* and soil organisms in broth, showed that the metabolic products of a number of the latter had a definitely inhibitory effect on the typhoid organisms. Kligler,[12] in the course of his extensive researches, confirmed the observations of Frost but did not determine whether this inhibitive effect might be an expectancy in the soil itself.

To investigate this possibility six pots were prepared, inoculated, and exposed as before. Three strains of *Eberthella typhosus* were inoculated in duplicate, giving 3 pairs of inoculated pots. One pot of each pair was inoculated also with a washed culture of *Pseudomonas fluorescens* suspended in saline. The ratio of *Eberthella typhi* to *Pseudomonas fluorescens* was approximately 4 to 1. The other pots inoculated with *Eberthella typhosus* alone served as controls. The third pot count was slightly lower than in the control pot. The experiment was repeated three times with this last strain, but no real antagonistic effect could be demonstrated. Sometimes the control was a trifle higher, sometimes lower than in the other pot. A second series of experiments employing *Proteus vulgaris* in place of Pseudomonas yielded entirely similar results. Tests with the filtrates of these two genera according to Kligler's method demonstrated their "antagonistic" action in broth cultures of *Eberthella typhosus*.

Experiments with sterilized soils were not carried out inasmuch as that condition would never be obtained in nature.

When unbuffered loam was inoculated with *Eberthella typhi* in fecal suspension, the maximum survival was 52 days as compared with a survival period of 100 days in controls inoculated with saline suspensions and exposed during a rainy season with 10.1 inches of rain.

TABLE 4

Effect of Direct Sunlight on the Survival of Eberthella Typhosus

	Per cent Moisture Loss	*Eberthella typhi per Gram Soil*		*Per cent Death*
		Initial	*24 Hours*	
Top soil:				
Sun	41			
Shade	40	25,000,000	3,500,000	86
Lower soil:				
Sun	30	25,000,000	12,500,000	
Shade	30	25,000,000	12,500,000	50

A duplicate experiment carried on during the dry season with 0.3 inches of rain showed less difference, the fecal suspension dying in 27 days and the saline in 33 days.

Pursuing this possibility further, an inoculated soil was thoroughly wet with raw settled sewage and exposed with a control. No viable typhoid organisms could be recovered from the pot wet with sewage after the 45th day, whereas they were recoverable from the control pot up to the 85th day. The rainfall during this experiment totaled 8.1 inches. The pH of the soil in pot wet with sewage rose to 8.8 during the experiment, while that of the control remained at 7.8. Microscopic examination showed the presence of numerous protozoan types of organisms. No attempt was made to evaluate the factors of pH and protozoa. It is possible that those two factors may be too intimately related to consider separately.

The experiment was varied by inoculating solid feces heavily with typhoid organisms. These were lightly covered with earth and exposed outside. Viable typhoid bacilli could be recovered on the 30th day but not on the 35th day during a rainy season, and the 15th day, but not on the 20th day during the dry season.

In studying the significance of hydrogen ion concentration garden loam buffered to pH values of 6.5, 7.0, 7.5, and 8.0 were set up in pots and exposed out of door as before. Table 5 shows the results of experiments.

In general it is seen that variations between pH 6.5 and 7.5 are of little significance, that the loam provided a slightly better menstruum for survival than adobe peat mixture, and that the pH effect was relatively less pronounced in the dry season.

Loam at pH 7.0 was inoculated with approximately 18 million *Eberthella typhi* per gm., was sealed in a series of tubes, and placed in the freezing compartment of an electric refrigerator and examined at intervals of 6 months. Counts after 6 months showed a 70 per cent reduction, after 12 months a 95 per cent reduction, after 18 months over 99 per cent reduction. An occasional viable organism could be recovered after 24 months. In feces similarly stored no viable typhi could be demonstrated after 12 months.

SUMMARY

The longevity of *Eberthella typhosus* in soils exposed to natural conditions has been studied over a period of 4 years.

The most important apparent factor determining survival was moisture. This confirms the conclusions of other earlier investigators. This is directly related to the amount of rainfall, the moisture retaining power of the soil, and to temperature. Fifty per cent of typhoid bacilli will probably die during the first 48 hours. Survival of the remainder may extend over a period of months depending upon the conditions noted above.

TABLE 5

Effect of Soil pH on Survival of Eberthella Typhosus

	Days Survival			
	Rainy Season 6.3 Inches Rain		*Dry Season 0.3 Inches Rain*	
pH	*Loam*	*Adobe-peat*	*Loam*	*Adobe-peat*
6.5	80	75	29	25
7.0	87	77	30	27
7.5	105	100	36	30
8.0	90	81	25	21

Sunlight also exerts a definite lethal action, although its effect will be limited to surface pollution.

The survival is longer in natural soil than in sewage polluted soil. Death occurs more quickly in soil impregnated with fecal suspensions than with saline suspensions.

There was no evidence of antagonistic action by soil organisms such as *Pseudomonas fluorescens* and *Proteus vulgaris* in a soil environment.

The pH of soil can affect survival adversely, but does not seem to be important in the pH range of ordinary fillable soils.

Eberthella typhosus stored in moist soil at freezing temperature may survive as long as 24 months. In feces similarly stored the survival time is much shorter, but may, nevertheless, extend over several months.

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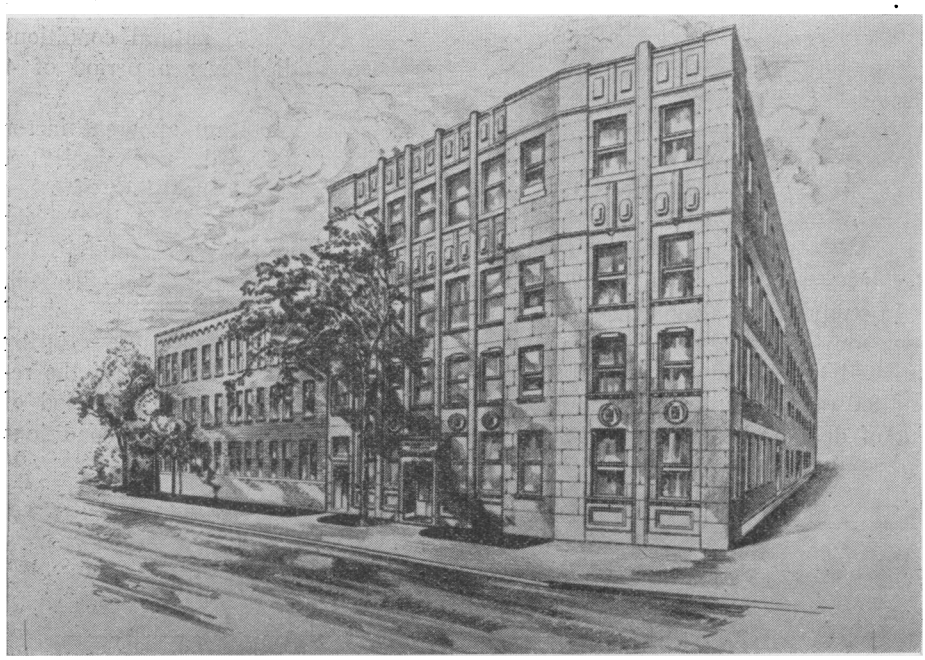

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