THE PERSISTENCE OF THE VI ANTIGEN OF *SALMONELLA TYPHOSA* IN SOIL AND WATER

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INTRODUCTION

For many centuries typhoid fever has been one of the most dreaded and deadly diseases of mankind. It is now an uncommon disease in this country, but it would be wrong to assume that it is already in the process of extinction. Endemic loci of the infection, maintained by chronic carriers, still exist in many parts of the country. Only one death from typhoid fever was reported in Michigan during 1949, however, there were forty-eight recognized and reported cases according to Leeder (1950).

The control of typhoid fever depends ultimately on the detection and control of the chronic carrier. The detection of a carrier is usually a difficult task, however, the new bacteriophage typing method devised by Craigie and Yen (1938) has provided a reliable guide to the epidemiological investigation of outbreaks or sporadic cases of typhoid fever. Its application greatly facilitates the detection of chronic carriers, especially those responsible for sporadic cases in endemic areas.

The bacteriophage typing method reveals strain differences which cannot be demonstrated by any serological method. Because these strain differences remain unaltered on human passage, they provide a means of identification whereby transmission in man may be traced. A carrier may be absolved from responsibility for a certain outbreak if he is excreting a strain of Salmonella typhosa which differs in Vi-bacteriophage type from those isolated from the diseased cases. The bacteriophage typing method permits the epidemiologist to trace the connections between sporadic cases of the disease. This is a task which cannot be accomplished without the use of this new laboratory aid.

In the past, the major epidemics have been caused by contaminated food or polluted water, the carrier being the indirect source of the organism. Even though improvement in the proper disposal of sewage has greatly helped to improve conditions, the rural areas in many sections of the world are still sources of danger.

If the fecal material is deposited on the soil or used as fertilizer, the soil may serve as a medium upon which the organisms may survive. Vegetables grown on the infected soil may harbor the organisms and when the vegetables are marketed, the typhoid bacillus may serve as the source of a new outbreak of the disease. Wells, rivers, and streams, which are polluted with S. typhosa may also serve as the source of infection. A number of studies have been carried out on the longevity of the typhoid bacillus in soil and water, but as Beard (1940) has pointed out, the results show no close correlation.

Felix and Pitt (1934) were the first to recognize that the type of S. typhosa which occurs in human infection is characterized by antigens which are distinct from the O and H antigens, namely the Vi antigen.

Since the Vi antigen of S. typhosa is present, practically without exception, in cultures isolated from patients with typhoid, Felix and others have reasoned that the Vi antigen is intimately associated with virulence or invasive ability. This theory has been substantiated by animal tests and by observations in vitro, wherein it has been shown that loss of Vi antigen is one of the first steps in the degradation of a culture. It is noteworthy that W forms of S. typhosa are found with considerable frequency in old carriers in whom, it has been conjectured, the immune forces which hold the organism in check bring about the V W changes.

Because the presence of Vi antigen is so closely related to virulence, a test for the antigenic component constitutes a rough qualitative method of determining virulence. In the present study an attempt has been made to determine the persistence of Vi antigen in typhoid organisms inoculated into soil and water. It is felt that experimental data resulting from this study will be of value both to the sanitary engineer and to the epidemiologist.

The method used to determine the presence of the Vi antigen on the organisms from soil and water was that of bacteriophage typing, which was described by Craigie and Yen in their original work in 1938.

HISTORY

During the past twenty years considerable new knowledge has been accumulated concerning the antigenic structure and antigenic variations of S. typhosa. Until 1930, it was generally accepted that the typhoid bacillus consisted of a somatic and flagellar antigens, but few, if any theories had been advanced to explain the relationship of virulence and resistance to O agglutination by O antiserum.

In 1934, Felix and Pitt published a report of their work which revealed that virulence was closely associated with resistance to the action of the O antibody. They were the first to show that the inagglutinable strains were highly virulent, whereas, agglutinable strains were less virulent. Both types of the organism had the general characteristics of smoothness and did not differ in their contant of smooth O antigen. This indicated that the mere presence of smooth O antigen did not of itself determine high virulence, but that some unknown factor was required to render this antigen resistant to the action of the O antibody.

In a later report, during the same year, Felix and Pitt (1934) concluded that virulence and resistance to O agglutination by O antiserum were correlated by the presence of an antigen, separate and distinct from the long-established O and H antigens. They called the newly discovered antigen the Vi antigen or Virulence antigen.

Kauffmann, in 1935, made an extensive study of the typhoid bacillus primarily from the point of view of the agglutination reactions and particularly the presence of the Vi antigen. He introduced the terms "V form" and "W form" to denote, respectively, the form of S. typhosa which contained

the Vi antigen and the form which did not. An organism with the Vi antigen was agglutinated by the pure anti-Vi serum but not by a pure anti-O serum. Those organisms which did not show the presence of the Vi antigen in agglutination reactions were classified as "W form", that is, organisms which were not agglutinated by anti-Vi serum, but were agglutinated by the anti-O serum. Kauffmann found that the organism may also exist in an intermediate form. The intermediate form showed partial agglutination in both a pure anti-Vi and a pure anti-O serum, and thus he called it the "V-W form".

Craigie and Brandon, in 1936, found that certain strains of bacteriophage would lyse the typhoid bacteria bearing the Vi antigen. This served as the foundation which lead to the typing of S. typhosa by bacteriophage.

In 1938 Craigie and Yen isolated four Vi-specific bacteriophages. They observed that one of these bacteriophages had the unusual ability to adapt itself to certain strains of S. typhosa containing the Vi antigen. Realizing the highly selective, lytic activity that bacteriophage type II was capable of developing, Craigie and Yen (1938) set up a series of experiments in which they conditioned the bacteriophage to particular strains of S. typhosa. Rawlings, Watson, and Ty2 were the first typical strains studied. Bacteriophage type II was propagated on the Rawlings and Watson strains and the bacteriophage developed an equally high lytic activity for both. When the bacteriophage, grown on these two strains, was applied to Ty2, it was found that it had lost its lytic ability except in very high concentrations. The original bacteriophage type II was then applied to strain Ty2. It was observed that lysis occured, and that the bacteriophage

had developed a high specificity for the Ty2 strain and lost its ability to lyse the Rawlings and Watson strains. Several preparations of bacteriophage, showing selective affinity, were made by adapting the bacteriophage type II to different strains of S. typhosa.

Craigie and Yen (1938) made the supposition that there are different varieties of S. typhosa which differ only in their Vi antigen and the variations or differences in the Vi antigen could be shown only by the action of adapted bacteriophage type II.

This meant that the bacteriophage appeared as a number of mutants,, differing in their affinity for different types of typhoid bacilli. Differences in the strains caused differences in environment which favored mutation. The dominant mutants continued to propagate and the others did not. By growing the mutant on the strain capable of supporting it, which is really selective propagation of the mutant, a series of phages were obtained showing a high relative affinity for the homologous strain of S. typhosa. Eleven phages were originally produced by this method of adaption and selective propagation and since that time the number has increased to twenty-five known types.

The eleven original phages were designated as A, B_1, B_2, C, D_1, D_2, E, F, G, H and L. The letter "I" was intentionally omitted in the designation of types to avoid possible confusion with Phage Type I. The complete list of known types and subtypes include A, B_1, B_2, B_3, C, D_1, D_2, D_4HP_{56}, D_5, D_6, E_1, E_2, F_1, F_2, G, H, J, K, L_1, L_2, M, N, O and T. The reaction of these phages when tested against the corresponding series of Vi type strains of S. typhosa are given in the following Table I.

THE FOUR ORIGINAL VI PHAGES

Vi Phage	Relative Particle Size	Thermal Death Point	Neutralization by antiphage serum I II III IV	Lytic activity for V forms of S. typhosa
Type I	Large phage	67-70°C.		Lyses all V forms
Type II	Medium phage	69-72°C.		Develops a highly selective lytic activity for the type of S. typhosa on which it is propagated.
Type III	Small phage	61-64°C.		Lyses majority of V forms
Type IV	Medium phage	59-62°C.		Lyses majority of V forms with exceptions of type F and D_1.

From the data in this table, Craigie and Yen (1938) concluded that:

1. The four phages are specific for the V form of S. typhosa.
2. Serologically, the four types of phage are distinct.
3. A variation occurs in the lytic action on V form strain of S. typhosa, particularly in the case of Type II phage.

TABLE I

VI PHAGE PREPARATIONS

Vi type Strains	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
A	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
B_1	2+S	Cl	2+S	+S	+S	–	–	–	+S	+m	+S	+S	–	+S	+S	+S	–	+S	+S	+S	+S	+S
B_2	+S	–	Cl	–	–	–	–	+m	–	–	+m	–	+m	–	+m	+m	+m	–	–	±m	±m	+S
B_3	3+S	–	3+S	Cl	3+S	2+m	2+m	–	–	–	3+S	3+S	3+S	2+m	3+m	3+m	3+m	3+S	3+S	3+S	3+S	3+S
C	±n	±n	±n	±n	Cl	±m	±m	±m	±m	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n
D_1	–	–	–	–	–	Cl	Cl	Cl	2+m	2+m	–	–	–	–	–	–	–	–	–	–	–	–
D_2	–	–	–	–	–	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D_4HP_{56}	–	–	–	–	–	–	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D_5	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–	–	–
D_6	–	–	–	–	–	–	–	–	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–
E_1	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
E_2	–	–	–	–	–	–	–	–	–	–	–	Cl	–	–	–	–	–	–	–	–	–	–
F_1	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
F_2	–	–	–	–	–	–	–	–	–	–	–	–	±n	Cl	–	–	–	–	–	–	–	–
G	–	–	–	–	±m	–	–	–	–	–	–	–	–	±n	Cl	–	–	–	–	–	–	–
H	–	–	–	–	–	–	–	–	–	–	±S	–	±n	±n	–	Cl	–	–	–	–	–	–
J	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Cl	–	–	–	–	–
K	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Cl	–	–	±m	–
L_1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–
L_2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±m	Cl	–	–
M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Cl	–
N	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Cl

Cl = Confluent lysis – = No plaques ± = a few plaques usually present

n = normal plaques S = Small plaquesy visible to the naked eye m = minute plaques, visible only by means of magnification (X10)

+, 2+, 3+ Increasingly numerous plaques

MATERIALS

The twenty-two standard Vi phage preparations used in this study were obtained in concentrated form from the Laboratories of the Michigan State Department of Health, Lansing, Michigan. Originally they were obtained from the Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada. The phages used included: A, B_1, B_2, B_3, C, D_1, D_2, D_4HP_{56}, D_5, D_6, E_1, E_2, F_1, F_2, G, H, J, K, L_1, L_2, M and N. Phages O and T were not included in the group. The four Vi-type cultures of S. typhosa (A, D_4HP_{56}, E_1 and F_1), used in this work, were supplied from the same source.

The soil samples used for determining the persistence of the Vi antigens were obtained from the Soils Department of Michigan State College. The soil samples were of four types, namely: Oshtemo sand, Miami sand, Brookston clay and muck. By taking four different types of soil, a wider range in the amount of organic matter present could be obtained. The water samples for this study were taken from the Red Cedar River.

The concentrated bacteriophages were diluted to their "Critical Test Concentration" (C.T.C.) before they were used for the typing. "C.T.C." is defined as the greatest dilution of bacteriophage that will produce lysis on the homologous strain of S. typhosa. The proper dilution for the above mentioned bacteriophages was found to be 1-100.

The media used for typing were prepared according to the work of Craigie and Yen (1938).

Agar medium

'Bacto' nutrient broth (dehydrated)	20.00 gms.
Sodium chloride	7.50 gms.
'Bacto' agar	20.00 gms.
Distilled water to	1000.00 ml.

Phage dilution broth

'Bacto' nutrient broth (dehydrated)	8.00 gms.
Distilled water to	1000.00 ml.

Phage culture broth

'Bacto' nutrient broth (dehydrated)	15.00 gms.
Sodium chloride	7.00 gms.
Distilled water to	1000.00 ml.

Craigie and Felix (1947) stated that any good meat digest agar may be used as an alternative medium providing that the standard reactions are reproducible. The media used in this study, however, were prepared according to the above mentioned formulae. The desired results could not be obtained by substituting different agar.

'Bacto' tryptose agar was used for growing the organisms to seed the soil and water; saline solution (.85 percent) was used to wash the organisms from the agar. 'Bacto' tetrathionate broth base was employed as the selective liquid enrichment medium; MacConkey, S.S., and bismuth sulfite as the differential media for the isolation of S. typhosa, and Kligler's iron agar slants for further identification of the organisms.

The cultures were stored on Dorset Egg medium, which retains the Vi antigen. The Dorset Egg medium consists of three parts of whole egg, one part of distilled water. It was slanted and inspissated. The stock strains were transferred to fresh slants at four-month intervals following passage on nutrient agar and tests for specificity.

METHOD

The four soils, Oshtemo sand, Miami clay, Brookston clay, and muck, were each divided into four samples of 300 gms. By taking four soils. a representative sample of various soils was obtained, each differing in the amount of organic matter present. Moisture content tests were performed on each of the samples at the beginning of the study and the results obtained are shown in Table II. The optimum water-holding capacity of the various soils according to Lohnis and Fred (1923) is: Oshtemo sand- 6%, Miami clay- 10 to 11%, Brookston clay- 25%, and muck- varies up to 200%. The moisture content of the samples was adjusted to approximately that of standard conditions and kept at the optimum throughout the experiment. Moisture content tests were conducted by weighing out 10 gm samples of the soil, drying until a constant weight (usually 48 hours) was obtained and then calculating the percent of moisture from the loss in weight.

A sample of each soil was seeded with one strain of S. typhosa. The four strains of S. typhosa used for the seeding were A, D_4HP_{56}, E_1, F_1. This made a total of 16 jars. Ordinary quart fruit jars were employed. They were covered with Mason lids, but not sealed air tight. The temperature was that of the room in which they were kept. (approximately 22°C.)

Before seeding the soil with the organisms, a 1:10 dilution of soil in tetrathionate broth was made and incubated for twenty-four hours. From the tetrathionate broth, plates of bismuth sulfite, MacConkey's and S.S. agar were streaked. A few colonies which might be confused with those of S. typhosa were picked, stabbed into and streaked on Kligler's iron agar slants to verify the fact that no salmonella-like organisms were present.

Table II

OSHTEMO SAND

Typhoid types	Wt. of the flask 10 gm. of soil before drying	Wt. of the flask soil after drying at 115°C.	%Moisture
A	31.7 gm.	30.8 gm.	9%
D_4HP_{56}	31.7 gm.	30.92 gm.	7.8%
E E	32.0 gm.	31.01 gm.	9.9%
F	32.0 gm.	30.30 gm.	7.9%
	MIAMI SAND		
A	31.2 gm.	29.6 gm.	16%
D_4HP_{56}	31.8 gm.	30.4 gm.	14%
E	31.5 gm.	30.0 gm.	14%
F	31.7 gm.	30.3 gm.	14%
	BROOKSTON CLAY		
A	32.0 gm.	30.35 gm.	16.5%
D_4HP_{56}	31.2 gm.	29.5 gm.	17%
E	31.0 gm.	29.2 gm.	18%
F	31.7 gm.	30.0 gm.	17%
	MUCK		
A	32.0 gm.	26.3 gm.	56.9%
D_4HP_{56}	31.7 gm.	26.3 gm.	43.2%
E_1	31.6 gm.	26.1 gm.	55.0%
F_1	32.3 gm.	26.7 gm.	56.0%

in the soil before seeding. The four typhoid strains were typed by bacteriophage before seeding the soil to make sure the Vi antigen was present.

8 The organisms to be seeded were taken from the stock cultures, broth suspensions made, incubated for twenty-four hours, plated in Kolle flasks containing tryptose agar, incubated for twenty four hours and then washed from the agar with .85 percent saline solution. The saline suspension of the organisms was used to seed the soil. This suspension contained some nutrient which would be added to the soil and water, and which might help to maintain the organisms in the samples.

Five-tenths ml. of an .85 percent saline suspension of the organism was added to 30.00 ml. of distilled water and this was added to the 300.00 gm. of soil for each of the sixteen samples. By adding a 30.00 ml. suspension of the organisms, the organisms could be more uniformly distributed throughout the sample.

At weekly intervals, a 2 gm. sample of soil was taken from the respective jars and tested for longevity of the typhoid bacillus and the presence of the Vi antigen by bacteriophage typing. The soil sample was added to tetrathionate broth, incubated for 12-18 hours, then streaked on bismuth sulfite, MacConkey's and S.S. agar and incubated for 48 hours. Colonies were picked from the differential media, stabbed into and streaked on Kligler's iron agar slants and incubated for 24 hours. The characteristic reaction of S. typhosa in Kligler's iron agar is acid butt, alkaline slant, and H_2S production. The organisms identified on Kligler's iron agar were then ready for typing for the presence of the Vi antigen.

The procedure used for typing was essentially the same as described by Craigie and Yen (1938). A small amount of the growth on Kligler's iron agar was inoculated into 2 ml. of nutrient broth and the tube incubated at 37°C. for 2 hours. The inoculation sites were then made on nutrient agar plates in the following manner: A loopholder, containing a platinum loop (2.75 mm. internal diameter) was taken between the thumb and first 2 fingers like a pen and a full and uniform drop of broth culture was obtained by quickly breaking the surface of the broth. With the forearm and side of the hand resting on the table the loop was lowered until the drop touched the surface of the agar, then the loop was given a horizontal twist by moving the fingers in an area approximately 15 mm. in diameter. The loop should never touch the agar because it may cause an area which may be confused with lysis. The loopholder should be used merely to direct the spread of the culture. After the agar had absorbed the fluid, usually five to ten minutes, the bacteriophage was applied to the center of the inoculated area. Two loopholders may be used alternately to conserve time. In applying the bacteriophage the loopholder should be held between the thumb and fingers in a vertical position. The loopholder was then lowered until the loop almost touched the surface of the agar and then rotated in a vertical position and the contents allowed to flow onto the center of the inoculated area. The bacteriophage was allowed to spread naturally on the sited area. The plated were then inverted and incubated at 37°C. for two hours and stored in the refrigerator at 4-6°C. overnight. In the morning the plates were returned to the incubator for four to six hours before reading the results.

It is important that the plates never be stacked, because S. typhosa will grow, but will not show lysis below 37°C. The plates should be prepared at least one day before being used because excess moisture delays absorption of broth and bacteriophage. The plates, however, should not be prepared too far in advance because excessive drying causes uneven distribution of the culture and bacteriophage.

Uninterrupted incubation can be used with success, but the interrupted method has proved to be more advantageous:

1. The interrupted incubation method permits phage reactions to be observed at their optimum and before they are obscured by late growth.
2. The intermediate period in the cold room permits diffusion of the phage into the surrounding normal culture.

TABLE III

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE A IN OSHTEMO SAND

PHATE PREPARATION

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
1 Wk.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE IV

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE D_4HP56 IN OSHTEMO SAND

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP56	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

– = No lysis and no growth

± = Semi-confluent lysis

TABLE V

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE E_1 IN OSHTEMO SAND

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE VI

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE F_1 IN OSHTEMO SAND

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	⌐	⌐	■	⌐	⌐	⌐	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	⌐	⌐	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	⌐	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE VII

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE A IN BROOKSTON CLAY

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
1 Wk.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
2 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
3 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE VIII

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE D_4HP_{56} IN BROOKSTON CLAY

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6 Wks.	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7 Wks.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
8 Wks.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

− = No lysis and no growth

± = Semi-confluent lysis

TABLE IX

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE E_1 IN BROOKSTON CLAY

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE X

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE F_1 IN BROOKSTON CLAY

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
1 Wk.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
2 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
3 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
4 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Cl = Confluent lysis

— = No lysis and no growth

TABLE XI

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE A IN MIAMI CLAY LOAM

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	C_1	Cl	Cl	Cl	Cl	Cl	Cl
1 Wk.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
2 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
3 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
4 Wks.	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
5 Wks.	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

± = Semi-confluent lysis

TABLE XII

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE D_4HP_{56} IN MIAMI CLAY LOAM

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 Wk.	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 Wks.	-	-	-	-	-	-	-	-G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 Wks.	-	-	-	-	-	-	-	-G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4 Wks.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5 Wks.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6 Wks.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7 Wks.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8 Wks.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = No lysis and no growth

-G = Growth, but no lysis

± = Semi-confluent lysis

TABLE XIII

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE E_1 IN MIAMI CLAY LOAM

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE XIV

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE F_1 IN MIAMI CLAY LOAM

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE XV

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE A IN MUCK

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
1 Wk.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
2 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
3 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
4 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
5 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE XVI

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE D_4HP_{56} IN MUCK

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 Wks.	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

– = No lysis and no growth

± = Semi-confluent lysis

TABLE XVII

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE E_1 IN MUCK

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—	—	—
1 Wk.	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—	—	—
2 Wks.	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—	—	—
3 Wks.	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—	—	—
4 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Cl = Confluent lysis

— = No lysis and no growth

TABLE XVIII

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA

TYPE F_1 IN MUCK

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	E_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
0 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
1 Wk.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
2 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
3 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
4 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	Cl	Cl	—	—	—	—	—	—	—	—
5 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8 Wks.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Cl = Confluent lysis

— = No lysis and no growth

TABLE XIX

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA IN WATER

PHAGE PREPARATIONS

TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
TYPE A																						
0 Wks.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
1 Wk.	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TYPE D_4HP_{56}																						
0 Wks.	–	–	–	–	–	–	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	Cl	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE XX

PERSISTENCE OF THE VI ANTIGEN OF S. TYPHOSA IN WATER

PHAGE PREPARATIONS

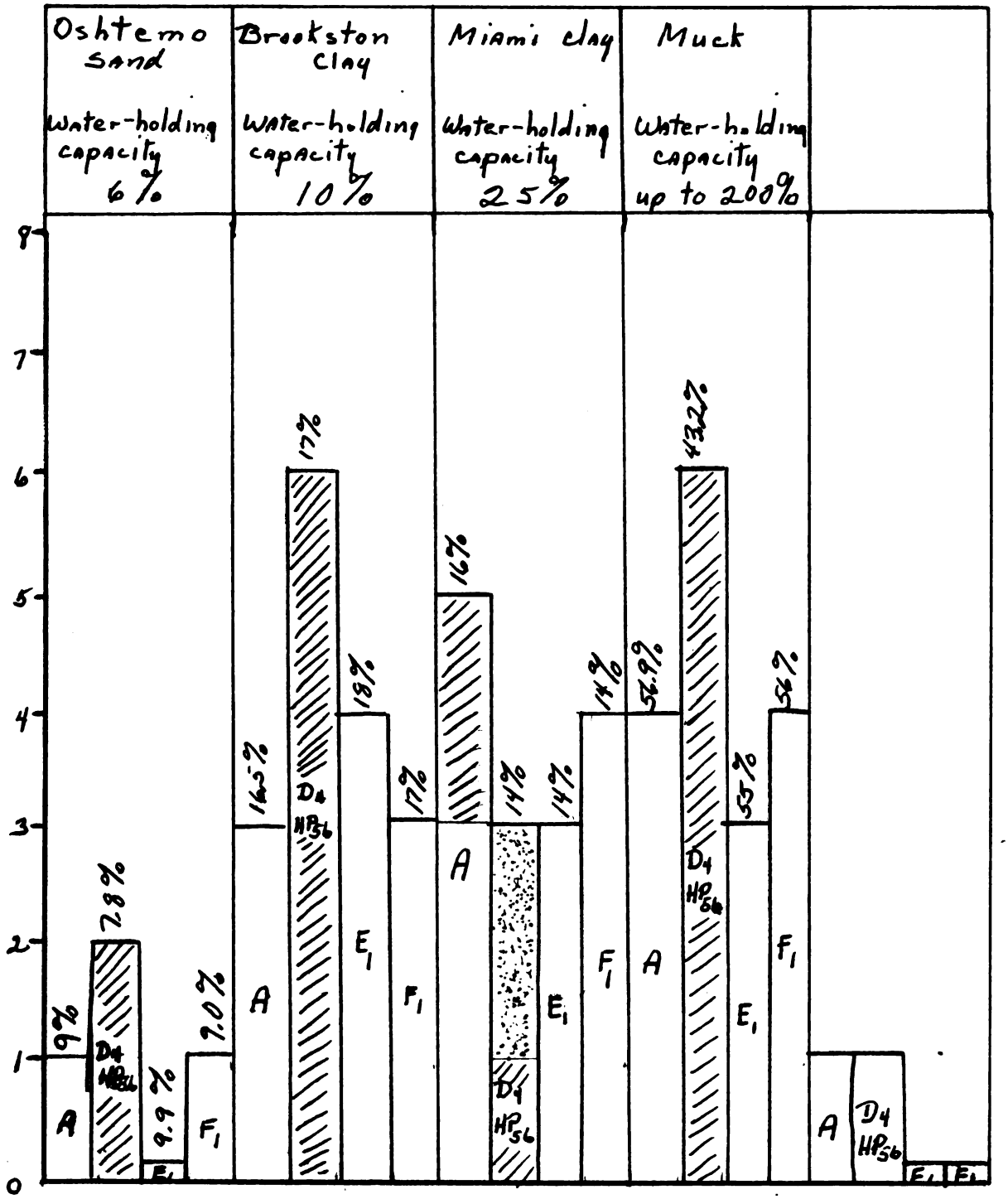
TIME	A	B_1	B_2	B_3	C	D_1	D_2	D_4HP_{56}	D_5	D_6	E_1	E_2	F_1	F_2	G	H	J	K	L_1	L_2	M	N
TYPE E_1																						
0 Wks.	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TYPE F_1																						
0 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	Cl	Cl	–	–	–	–	–	–	–	–
1 Wk.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 Wks.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cl = Confluent lysis

– = No lysis and no growth

TABLE XXI

Summary of Persistence of *S. typhosa* and its Vi antigen in soil and water



DISCUSSION OF RESULTS

The results given in Tables III-XX show that S. typhosa survives in Oshtemo sand, Miami clay, Brookston clay, muck, and water from the Red Cedar River for varying periods of time. In addition to showing the survival of the organism, the tables also show the persistence of the Vi antigen. This latter point is of special interest since the Vi antigen is related to virulence. Table XXI summarizes the results reported in the preceeding eighteen tables.

The Oshtemo sand did not support the organism for more than two weeks and only type D_4HP_{56} survived for this period. After the first typing following seeding, type D_4HP_{56} showed only semi-confluent lysis. More will be said about this type and its reactions later. Types A and F_1 survived only one week, but showed equal growth and presence of the Vi antigen. Type E_1 did not survive through the first week after seeding into the soil sample.

Brookston clay provided conditions necessary for survival through six weeks. Here again type D_4HP_{56} outlived the other types by two weeks, but it did not show confluent lysis after the first typing which followed seeding. Type E_1 survived through the fourth week and the others i.e. A and F_1 survived three weeks but not four. The latter three types showed good confluent lysis when typed with bacteriophage.

In Miami clay, type A survived through the fifth week, however, during the last two weeks of its survival, no clear confluent lysis was obtained. The semi-confluent lysis which was produced indicated that the organism was losing its Vi antigen. Confluent lysis was obtained only

through the third week. Type D_4HP_{56} organism grew through the third week but was not typable after the first week. Apparently the organism had completely lost it Vi antigen after this short period. Types E_1 and F_1 survived and gave confluent lysis through three and four weeks respectively.

The muck soil gave results closely parallel to the Brookston clay. Type D_4HP_{56} was recovered through six weeks and as in the Brookston clay sample it showed confluent lysis after seeding the soil. At the end of one week and until the organism died out, it produced only semi-confluent lysis. Types A, E_1, and F_1 survived four, three and four weeks respectively. For all three types, the Vi antigen remained as long as the organism survived.

In the water samples all the typhoid organisms died out at the end of one week. All four types (A, D_4HP_{56}, E_1 and F_1) gave confluent lysis until the death of the organism, which indicated that the Vi antigen was present as long as the organisms remained alive.

Type D_4HP_{56} persisted for a greater period of time in all samples except the Miami clay and as long as type A in water. In soils where this type of organism survived the longest, it lost its ability to produce confluent lysis in less than one week. The consistent appearance of semi-confluent lysis which occurred following the first week indicates that this type differs from the other types. The specific bacteriophage, D_4HP_{56}, was tested with the original stock culture of type D_4HP_{56} so as to make sure that the bacteriophage was not contaminated. Several factors may be involved which may account for the organism losing its ability

to exhibit confluent lysis. It is believed that when the organism begins to lose its Vi antigen, it will not show confluent lysis. Another explanation of this phenomenon, as is indicated in Table I, is that, when the bacteriophage is at its critical test concentration, a cross between closely related organisms and bacteriophage may show semi-confluent lysis. The most likely explanation, however, is that this bacteriophage D_4HP_{56} carries a latent bacteriophage which interferes with the typing.

SUMMARY

In this work with four types of soil and one water sample, the longevity of the four types of S. typhosa and the persistence of their Vi antigen were determined by using bacteriophage typing technique.

In general, the survival of the organisms in Brookston clay, Miami clay and muck, when the three were maintained at their optimum water-holding capacity and a temperature of 22°C., varied from three to six weeks. In Oshtemo sand and in river water the organism did not survive for three weeks. S. typhosa types A, E_1 , and F_1 showed no consistency in longevity in any of the soil and water samples, but the Vi antigen was found as long as the organism remained alive.

S. typhosa type D_4HP_{56} gave semi-confluent lysis in the four soil samples used. This type of the organism also survived longer than the other three types in Oshtemo sand, Brookston clay and muck. S. typhosa type D_4HP_{56} showed confluent lysis in all samples in the first typing after seeding, but after the first typing it produced semi-confluent lysis. Possible explanations for the appearance of semi-confluent lysis are given in the discussion.

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