EXPERIENCES WITH TYPING OF TYPHOID BACILLI BY MEANS OF Vi BACTERIOPHAGE*

BY

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The latest development in the study of the epidemiology of typhoid fever is the discovery made by Craigie and Yen (1938a, 1938b) of type-specific typhoid Vi bacteriophages. Until then the typhoid bacillus had been regarded as a single species without varietal subdivision. By the use of the new technique devised by Craigie and Yen it is now possible to divide typhoid bacilli into a number of well-defined types. The story of this discovery may be briefly summarized here.

Hadley (1925-6), Burnet (1927, 1929), and Levine and Frisch (1934) were the first to show that the action of bacteriophages on the various *Salmonella* species is closely related to the presence of certain heat-stable "O" antigens in the bacterial cell. Later studies on dysentery bacilli (Burnet and McKie, 1930), the cholera vibrio (Bruce White, 1936), and other pathogenic organisms also showed a close relationship between antigenic structure and phage susceptibility. As soon as the special importance of the so-called "Vi" antigen of the typhoid bacillus was recognized (Felix and Pitt, 1934) a number of authors, working independently of each other in different countries, established the existence of bacteriophages that were specific for the Vi form of the typhoid bacillus (Sertic and Boulgakov, 1936; Scholtens, 1936; Craigie and Brandon, 1936). These phages attacked typhoid strains irrespective of their origin, provided the cultures contained an adequate quantity of the Vi antigen. By propagating a particular anti-Vi phage on typhoid strains isolated from different sources Craigie and Yen (1938a) obtained bacteriophages which had developed a high degree of specificity for the particular strain on which they were grown. When such adapted phage preparations were tested against a great number of typhoid strains from different localities it was found that the strains could be divided according to their response to the different phage preparations and that strains that were related epidemiologically belonged to the same phage type. On this basis Craigie and Yen identified a number of distinct types of *Bact. typhosum*. A small proportion of Vi cultures cannot be typed in this way, for reasons that are not yet understood. Such cultures are referred to as "imperfect" Vi forms.

The phage type of a strain is for all practical purposes a permanent character, and typing of the typhoid bacillus by this means gives results as reliable as those obtained in streptococcal or pneumococcal infections by the use of serological tests. It may be stated in passing that the Vi antigen of typhoid strains belonging to different phage types cannot be differentiated by any of the customary serological methods (Felix and Pitt, unpublished results). The few reports on the application of the new method that have been published so far from Canada (Brandon, 1940), the United States of America (Lazarus, 1941), and China (Yen, 1939) have all corroborated the observations of Craigie and Yen. In this country Boyd (1939) drew attention to the potential value of this discovery.

The method of phage typing has been employed in the Emergency Public Health Laboratory Service since 1940.

Cultures of *Bact. typhosum* isolated in the constituent laboratories of the E.P.H.L. Service and in some other public health or clinical laboratories in England, Wales, and Scotland have been submitted to a reference laboratory for typing by the bacteriophage technique. The object of this paper is to give an account of the results so far obtained from this material.

Technique

The technique of the test followed as closely as possible that described in the original paper by Craigie and Yen (1938a), and need not be discussed here in detail. Early in 1940 Dr. J. Craigie of the Connaught Laboratories, Toronto, very kindly supplied the complete collection of his standard phage preparations and standard type cultures, together with a copy of his "Notes on the Typing of *B. typhosus* with Vi Phage," dated March, 1940, and embodying further technical details. The number of standard phage preparations recommended at that time by Craigie for use in routine typing was 18, representing the following types and subtypes: A, B1, B2, B3, B4, C, D1, D2, D3, E1, E2, F1, F2, G, H, J, L, and M. All the cultures examined in the course of the present investigation have been tested against these 18 type and subtype phages. For reasons of economy only the phages corresponding to the more common types were employed singly; the others were used in appropriately pooled mixtures.

Cultures of *Bact. typhosum* isolated in the course of the routine examination of typhoid patients or carriers were submitted for typing as soon as the organism had been identified by the customary cultural and serological methods. Some of the laboratories sent the subcultures on Dorset egg medium, others on plain agar slants or in plain agar stabs. The cultures were sent by post, and usually reached our laboratory within 4 or 5 days from the date of isolation. In many instances much older cultures were received, and these, too, were usually found to be suitable for typing. The only precaution taken was that the bacteriologists in charge of the various laboratories were asked to prepare the subcultures not from a single colony but, whenever possible, from about half a dozen selected "smooth" colonies which were seeded together on a slant of Dorset egg medium or plain agar. The pooling of a number of colonies is a useful safeguard against the chance selection of a single colony, which, though smooth-looking (Arkwright, 1930), may be composed of bacteria that are deficient in the Vi antigen or do not contain any Vi antigen ("O" forms).

The great majority of typhoid strains handled in this way gave a clear-cut result in the phage typing when the subculture required for the test was prepared directly from the original culture tube received. The result of the typing could thus be reported within 24 or 36 hours. If the phage tests on the original culture failed to give a clear-cut result, or if the strain was found to be an untypable "imperfect" Vi form, the tests were repeated. The culture was plated out on plain agar, and a number of single-colony cultures or a pooled culture that had been derived from at least half a dozen carefully selected "smooth" colonies were retested against the type phages. If the strain still remained untypable another culture was, when possible, obtained from the patient or carrier, and some of

* A report to the Medical Research Council.

these repeat cultures, especially those from chronic carriers of long standing, were found to be typable.

Typhoid Vi-phage Types Prevalent in England, Wales, and Scotland during 1940-2

The total number of cultures submitted to the phage typing test was 643. The number of typhoid patients and carriers from whom the strains had been derived was 440. In 87 cases the cultures were isolated from the blood; in a few instances from the urine or from post-mortem material; and the majority were obtained from faecal specimens. Thirty-five cultures had been isolated from samples of polluted water and sewage effluents. The remaining 168 cultures were obtained in the course of repeated examinations of the excreta of some of the patients and carriers. These repeat tests were carried out with a view to collecting further evidence regarding stability of the types, or as part of certain special investigations which are to be reported elsewhere. Table I shows the number of types and subtypes of the typhoid bacillus that have been met with in this country, and indicates their prevalence during the past three years.

TABLE I.—Showing the Vi-phage Types of *Bact. typhosum* found in England, Wales, and Scotland during 1940-2

Craigie's Original Vi-phage Types	Number of Patients and Carriers from whom Typhoid Strains belonging to the Various Vi-phage Types have been Isolated					
	England and Wales			Scotland		Total 1940-2
	1940	1941	1942	1941	1942	
	<i>Part I</i>					
A	15	18	50	3	7	93
B2			5			5
C	2	2	39		1	44
D1	1	4	10	1		16
D2	1	16				17
E1	17	19	57		16	109
F1	4	3	9			16
F2	2	1		2	7	12
J		2				2
"Imperfect" Vi forms	30	30	57	1		118
"O" form (Vi-negative variant)	1		6		1	8
Total	73	95	233	7	32	440
New Vi-phage Types	<i>Part II</i>					
	Some of the "Imperfect" Vi Forms recorded in Part I were Successfully Typed by Means of Four New Vi-type Phages					
D4		11	8			19
D5			1			1
L2	5			1		6
No. 91	2	10	11			23
Total	7	21	20	1		49

Part I of the table shows that 9 of the 18 types and subtypes of Craigie's original schema were found among the strains isolated in the United Kingdom. Type J has been included in the list for the sake of completeness, although that type is not prevalent in this country. One of the two Type J cases recorded in the table was a faecal carrier who had contracted typhoid fever in Egypt during 1940 and returned here in 1941. Six weeks later he passed on the infection to his brother-in-law, who died from his attack of typhoid fever. The remaining 8 types and subtypes, however, must all be considered as indigenous to this country. These 8 types were also represented among the late W. M. Scott's collection of English strains that had been examined by Craigie and Yen (1938b). Scott's collection comprised 74 cultures isolated from most of the major outbreaks of typhoid fever that occurred in England and Wales during the period 1932 to 1938.

It is too early to draw any conclusion on the relative frequency of the various types, because the figures shown in Table I represent only a fraction of the total number of typhoid cases notified during the three years under review. Most of the strains recorded in Table I were isolated from patients belonging to small groups of cases or from sporadic cases scattered throughout the country; but a few somewhat larger outbreaks also occurred, and some of these resulted in 10 or 20 cultures from the same outbreak being typed and included in the list. Finally, some of the laboratories that were supplying the cultures were able to examine most of the typhoid cases

that occurred in their area, whereas other laboratories elsewhere were not in a similar position. The figures shown in Table I therefore afford no more than a rough indication of the distribution of foci due to typhoid strains of the various Vi-phage types.

It is seen from Part I of the table that the strains isolated from 126 patients or carriers could not be typed with the original type phages of Craigie. Only 8 of these strains were "O" forms, which did not contain any Vi antigen. The remaining 118 strains, or 26.8% of the total number examined, were "imperfect" Vi forms—that is to say, the strains were resistant to the action of the 18 specific type phages in spite of the fact that the cultures contained the Vi antigen and were readily lysed by the concentrated non-specific Vi phages that are employed as control reagents in the test.

Part II of Table I shows that 49 strains, or 41.5% of the "imperfect" Vi forms, were later successfully typed by means of four new Vi-type phages. The new phage preparations were obtained by a process of adaptation similar to that employed by Craigie and Yen (1938a). The new types were provisionally labelled D4, D5, L2, and No. 91. Type D5 has been encountered on only one occasion, and may possibly have been introduced from abroad. The patient was an Indian soldier who suffered from an attack of typhoid fever a few months after he had arrived in this country. The other new Vi types are firmly established here. Type L2 was found in Scotland as well as in England. Type 91 has already been identified in eight different counties, widely separated from each other, and a strain belonging to this type has been isolated from a chronic carrier of long standing—a woman aged 70. A strain of Type D4 has also been found in a chronic carrier—a man aged 66 who has never lived abroad.

From a practical point of view the result of the typing of this series of cases is very satisfactory. If the 8 strains that were available only in the "O" form are disregarded, it is seen that the number of "imperfect" Vi forms which could not be typed was 69, or 15.9% of the total number of Vi forms examined. More than half were strains derived from three outbreaks, and these might have been typed successfully had special efforts been made at the time the outbreaks occurred. Thus the number of separate foci of infection in which the phage type remained unidentified was very small indeed.

A few typhoid strains from patients and carriers who contracted the infection abroad were also examined in the course of this investigation, and it may be of interest to record these cases here. The carrier of Type J bacilli of Egyptian origin has been already mentioned. The other cases belonged to the following types: Type A—from Chile and Russia; Type D1—from Eire and the Faroe Islands; Type E1—from Belgium and Malta.

It is obvious that workers in different countries who are employing the bacteriophage typing method should maintain close contact and exchange cultures and phage preparations before new types or subtypes are recognized and added to the original schema of Craigie and Yen. At the present time it is rather difficult to adhere to this rule, and some confusion concerning nomenclature is likely to arise. So far only Type D4 cultures and the corresponding phage preparations have been sent to Dr. Craigie, who reported that in his opinion D4 was a new Vi-phage type. The designations chosen for the four new types must necessarily be provisional, pending revision after the war, when co-operation between workers in different countries will again be possible. Craigie (1942) suggested a revised and simplified typing schema, but his investigations regarding the required phage preparations have apparently not yet been completed.

Epidemiological Significance of Typhoid Vi-phage Types

The observations of Craigie and Yen on the epidemiological significance of Vi-phage types of typhoid bacilli have been amply corroborated in the course of the present investigation. Patients from any given outbreak of typhoid fever yielded cultures belonging to the same Vi-phage type. Strains from chronic carriers, when repeatedly isolated over long periods, did not show any change from one type to another. Carrier strains do, however, often show antigenic degradation leading

to the appearance of "imperfect" Vi forms and of "O" forms, neither of which can be typed. Of the 8 strains recorded in Table I as "O" forms, 6 had been isolated from chronic carriers of many years' standing and only 2 from typhoid patients.

It is not proposed to state here in detail the number of outbreaks investigated or the number of patients examined during each outbreak. The cases summarized in Table I include a considerable number of small outbreaks, each affecting from 3 to 6 members of a family or household; cases of direct contact infection of nurses attending typhoid patients; outbreaks in mental hospitals, hostels, and camps, affecting groups of about 20 patients, from whom typhoid cultures have been examined. It is noteworthy that the results of the typing were in accord with the epidemiological observations; that is to say, all cases infected from the same source were found to have been caused by the same Vi-phage type of typhoid bacillus. Similarly each of the chronic carriers tested, who according to epidemiological evidence appeared to be responsible for an outbreak, was harbouring typhoid bacilli of the same Vi type as that isolated from the patients.

There were in this series two apparent exceptions to this rule, one of which, however, proved to confirm it. The first concerned an outbreak in a mental hospital, which was investigated by Dr. R. M. Fry of the E.P.H. Laboratory, Carmarthen. In this case most of the convalescent patients were excreting Type E1 bacilli, but a few yielded Type C. Dr. Fry succeeded in detecting among the mental patients chronic carriers of the two different strains, thus proving the presence of the separate sources of infection. The other instance of apparent disharmony between epidemiological data and laboratory findings concerned an outbreak in a large industrial city. All the patients had evidently contracted the infection from the same source, a butcher's shop, yet the cultures belonged to two bacteriophage types. The patients had been treated in a number of hospitals, and the cultures were received for typing from three different laboratories several weeks after the outbreak had occurred. Two of the laboratories sent only cultures of Type E1, and the third laboratory only cultures of Type F2. It was unfortunately not possible to obtain repeat specimens, as all the patients had been discharged from hospital by the time the cultures were typed. In view of the existing evidence as to stability of Vi types it seems justifiable to assume that this quite exceptional finding was due to an accident during the handling of the cultures.

The Practical Value of the Typing Method

Some of the cases recorded in Table I have been the object of special epidemiological investigations conducted by the Ministry of Health, in co-operation with the E.P.H.L. Service. The story of one of these investigations is told by Dr. W. H. Bradley at page 438. This fascinating story ended with the unmasking of the chronic carrier who was responsible for a score of typhoid cases of Type D4 that occurred over a wide area during a period of nearly two years. Bradley's report and my own related experiences in the course of a number of similar inquiries indicate clearly that the value of the typhoid typing method cannot be emphasized too strongly. Without this new laboratory aid there can be little hope of determining the source of infection of many of the sporadic cases, and small groups of cases, that occur throughout the country.

To illustrate this point Table II has been compiled, showing the sequence of typhoid cases that were examined in one single county during a period of 16 months. The list has been arranged in chronological order, though in abridged form. Most of the cultures were isolated by Dr. J. C. Cruickshank of the E.P.H. Laboratory, Exeter.

The table shows that cases belonging to at least six typhoid Vi types occurred in Devonshire during the period under review. The cases therefore originated from at least six different sources of infection. The topographical distribution makes it very likely that the number of active foci was considerably greater. It is clear that the medical officers of health who are called on to investigate sporadic cases in these circumstances are at present faced with an almost impossible task. The epidemiological investigations cannot lead in the right direction unless the infections that arise concurrently from a number of different

sources are first separated one from another. The bacteriophage test is at present the only laboratory method that makes this separation possible.

TABLE II.—List of Typed Typhoid Cases from Devonshire, Sept., 1941, to Dec., 1942*

Date of Isolation of Bact. typhosum	Locality	Number of Cases	Vi-phage Type
Sept., 1941	Honiton Clyst	2	E1
	Ottery St. Mary	1	F1
	Woodbury	1	C
Oct., 1941	Sidmouth	1	F1
	Torrington R.D.	1	F1
	Shaldon, nr. Teignmouth	4 (1 fatal)	No. 91
Nov., 1941	Ipplepen, nr. Newton Abbot	1	A
	Honiton Clyst	1	No. 91
	Tavistock	1 (carrier)	No. 91
Dec., 1941	Exeter	2	No. 91
	Sampford Peverell	1	No. 91
	Brixham	2	D1
Jan., 1942	Exeter	1	No. 91
	Exminster	24 { 1 fatal } 1 { carrier }	C
	Bideford	1	Imperf. Vi form
Feb., 1942	Honiton Clyst	1	E1
	Dunsford	1	C
	Bideford	1	Imperf. Vi form
March, 1942	Exeter	1 (fatal)	E1
	Sampford Peverell	1	No. 91
	Honiton Clyst	1 (fatal)	E1
April, 1942	Exminster	1	F1
June, 1942	Honiton Clyst	1	E1
July, 1942	Sampford Peverell	1	No. 91
Aug., 1942	Okehampton	1	D1
	Exeter	1	E1
	Tavistock	1	A
Sept., 1942	Exeter	1 (fatal)	A
	Sidmouth	4	E1
	Sampford Peverell	1	No. 91
Dec., 1942			
Total		62	

* Typhoid cases or carriers among mental patients are not included in the list.

Typhoid fever is now an uncommon disease in this country, but it would be wrong to assume that it is already in process of extinction, as some recent writings suggest. Endemic foci of the infection, maintained by chronic carriers, still exist in many parts of the country, and the case of Devonshire may serve as an example. How numerous these foci are can only be guessed at the present time. The diagnosis of the enteric fevers has not been satisfactory in rural districts, and it is still not uncommon for typhoid cases to be missed, the diagnosis having been made only at the post-mortem examination or not at all. Since the outbreak of the present war some of these areas have been provided with facilities for diagnosing enteric fevers by means of adequate laboratory methods. If these facilities could be made available throughout country districts generally, the number of enteric cases that remain undiagnosed would soon decrease very considerably.

Although bacteriological differentiation into typhoid and paratyphoid fever has been possible in some areas for many years, the enteric fevers have, for purposes of notification, been regarded as a single entity until recently. In July, 1941, it became practicable to separate the two diseases in the weekly returns of the General Register Office. The following table has been reconstructed from this source.

Notified Cases of Enteric Fevers in England and Wales

	1938	1939	1940	1941		1942
				Jan.-June	July-Dec.	
Typhoid fever ..					677	464
Paratyphoid fever ..					2,941	404
Total typhoid and paratyphoid fevers	1,322	1,479	2,833	1,145	3,618	868

In 1940 and 1941 numerous outbreaks of paratyphoid B fever occurred, and the table shows that during the last six months of 1941 the number of notified cases of paratyphoid was four times that of typhoid. In 1942, on the other hand, there were no large outbreaks of either of the two diseases, and the total number of cases was the lowest ever recorded in England and Wales. The interesting point is that during this rather exceptional "non-epidemic" year the numbers of notified cases of typhoid and paratyphoid were about equal, and this year probably indicates the relative numbers of endemic foci of the two diseases more accurately than does a year in which larger outbreaks occurred. It would be premature, however, to draw any conclusion from the figures so far available.

The control of typhoid fever depends ultimately on the control of the chronic carrier. The detection of a chronic carrier who is responsible for the infection of sporadic cases or of small groups of cases is usually a very difficult task. The introduction of refined culture media, particularly the bismuth sulphite medium of Wilson and Blair (1931), and of the Vi agglutination test, has made the problem less formidable than it was in earlier days. The new typing method devised by Craigie and Yen (1938a) has provided another weapon of great efficacy. It now seems possible to organize the fight against the chronic typhoid carrier in a manner that promises to lead far towards the ultimate goal, the eradication of the disease.

Summary

During 1940-2 440 strains of typhoid bacilli isolated in England, Wales, and Scotland were examined by the Vi bacteriophage method of Craigie and Yen. Of 432 strains that were Vi forms 363 (84.1%) were successfully typed, while 69 (15.9%) were untypable, or "imperfect" Vi forms. The remaining 8 strains, including 6 from chronic carriers, could not be typed because the cultures were "O" forms.

Most of the strains belonged to 8 of Craigie's original phage types. In addition 4 new Vi-phage types were identified.

The bacteriophage test is 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.

Unquestionably the new method will soon be recognized as an indispensable laboratory aid to the control of typhoid fever.

This investigation was made possible by the co-operation of many pathologists, medical officers of health, and superintendents of fever hospitals, and by the constant aid of Dr. J. R. Hutchinson and Dr. W. H. Bradley of the Ministry of Health. I wish to thank all of them for their help. My thanks are also due to Miss B. R. Callow for help in maintaining the cultures.

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AN ENDEMIOLOGICAL STUDY OF BACT. TYPHOSUM TYPE D4

BY

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During the period 1871-5 enteric fever killed annually 371 persons per million living in England and Wales. The "complex armour of modern sanitation" now protects us from the major epidemics of 70 years ago; but, even so, the mortality rate during the past 10 years has remained constant at about 6 per million living, and this residuum appears to be due to causes resistant to existing preventive measures. The final extinction of enteric fever may be possible when these resistant causes are better understood; for this reason studies of endemicity as distinct from epidemicity are of special interest, and the detailed investigation of a series of apparently solitary cases, although all too disappointing, may on occasion prove unexpectedly fertile.

The importance of sporadic cases and the frequency with which they occur are not always recognized. For instance, approximately one-half of the total number of towns in which enteric fever appeared in the first six months of 1940 had

only one case apiece. We may, with some justification, attempt to measure the efficacy of the "complex armour" by counting the number of sporadic cases which fail to initiate outbreaks, but at the same time it is well to remember that each such case—and they are for the most part of unknown origin—is a warning that chinks in the armour exist. Nowadays an explosive outbreak, particularly of paratyphoid fever, can generally be attributed to the undetected sporadic case which, by virtue of opportunities presented by the subject's employment, infects a widely consumed aliment (Savage, 1942). Presumably the chronic carrier is always somewhere in the background, but his relation to a series of cases may be remote, and he all too frequently evades detection.

The late W. M. Scott (1941) had written in this vein a short while before his death in April, 1941. Scott realized the epidemiological significance of the discovery by Craigie and Yen (1938) that typhoid bacilli could be typed according to their sensitiveness to certain Vi bacteriophages.

Phage Typing of *Bact. typhosum*

During 1940 the Emergency Public Health Laboratory Service began to concentrate investigations of typhoid cultures in a special laboratory, where Felix (see p. 435 of this issue) was able to build up a representative collection of the various phage types of *Bact. typhosum*. Up to the present 12 types have been recognized as giving rise to disease in this country, including four types or subtypes not previously described. The original strain of one of these, D4, was recovered from the blood of Case 1 in the series here described. This case was solitary, sporadic, and entirely unexplained at the time: it is distinguished as Case 1 only because it gave the first strain of Type D4 *Bact. typhosum*. We know nothing concerning the ancestry of D4 beyond the fact that it was apparently not represented in Scott's collection of 70 strains found in the country during the previous decade nor by Felix among 80 strains freshly isolated during 1940. Presumably uncommon, it promised to be a suitable subject for a sustained attempt to exploit phage typing in a field investigation; and subsequently, wherever and whenever Type D4 was recovered from a patient or a carrier exhaustive epidemiological investigations were made personally. Prof. G. S. Wilson collaborated throughout the inquiries, and a note on the preliminary observations appeared in the *Bulletin of the Emergency Public Health Laboratory Service* (March, 1941).

Chronology of Cases of Type D4 Typhoid identified in England and Wales, 1941-2

Nineteen D4 excretors have been detected, and all are listed in the accompanying table, which also includes five persons believed to have been infected with this organism but from whom no cultures were submitted to typing. It is significant that this list contains every case of typhoid fever notified in Buckinghamshire during 1941 and most of those occurring in the county during 1942, although in this year another type also appeared.

Cases in 1941

Case 1.—An itinerant merchant, living in Hertfordshire, who disliked milk was in bed intermittently from Jan. 4 to Jan. 10, 1941, when his doctor diagnosed pneumonia. By Jan. 17 he was able to take solid food. On Jan. 20 his temperature began to rise again, and reached 104.0° by Jan. 27, when he was admitted to hospital in coma. A petechial rash appeared; septicaemia was diagnosed, and blood was taken for culture on Jan. 28 and Jan. 31. Death occurred on Feb. 4. Enteric fever did not enter the minds of the medical officers performing the necropsy, but it was subsequently reported that typhoid bacilli were recovered from his blood. When this man first took to his bed he was put on a diet of raw milk purchased from retailer "A"; it is conjectured, for reasons which will appear subsequently, that this milk contained Type D4 *Bact. typhosum* on or about Jan. 8.

Cases 2 to 9.—Between Feb. 10 and Feb. 23 typhoid fever attacked 8 persons residing in seven households scattered over a wide area covering perhaps 100 square miles of Buckinghamshire and representing entirely different social and economic groups. There was an interval of 8 days between the onset of illness in the two cases in one house—father (Case 4) and son (Case 9)—and it is possible that the one was secondary to the other. Case 3 was an employee in a textile factory owned by Case 4, and Case 9 also worked in the factory. An attempt to recover *Bact. typhosum* from Case 3 (which was confirmed by Widal reaction) failed and she has not been proved