

The Relationship of Vibriocidal Antibody Titre to Susceptibility to Cholera in Family Contacts of Cholera Patients*

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A bacteriological and serological study of family contacts of 81 cholera patients was carried out in Dacca, East Pakistan. In the 10-day follow-up, 78 (16.7%) of 466 contacts were found to be infected with Vibrio cholerae; half of them were symptomatic and 29 had to be admitted to hospital. A study of serum pairs revealed a 4-fold, or greater, rise in titre in 86% of those infected in whom diarrhoea was present, and in 77% of individuals with inapparent infections. The infection rate fell markedly with age. Serological tests for vibriocidal antibody on blood specimens collected at the initiation of the follow-up revealed higher titres with increasing age. Vibrio cholerae infection, whether symptomatic or asymptomatic, predominantly occurred in individuals with low titres.

Serological surveys on inhabitants of the Pakistan-SEATO Cholera Research Laboratory vaccine trial area in rural East Pakistan have demonstrated a rise in the level of vibriocidal antibody with age. This could be correlated with a fall in the cholera case rate with age (Mosley et al., 1968a). In addition, the reduction in the cholera case rate produced by vaccine in the population could be correlated with the rise in antibody titre produced by the vaccine (Mosley et al., 1968b). While those studies indicated that the vibriocidal antibody level was a measure of immunity in the population, direct evidence was lacking that a higher level of immunity in individuals

was actually associated with a lower susceptibility to cholera.

The present study is a combined serological and bacteriological study of family contacts of cholera cases. In this study it was possible to demonstrate that individual immunity, as measured by the vibriocidal titre, was directly related to the susceptibility of the individual to infection with *Vibrio cholerae*.

METHODS

From October 1965 to the end of March 1966, family contacts of bacteriologically confirmed cholera cases admitted to the hospital of the Pakistan-SEATO Cholera Research Laboratory were followed for 10 consecutive days. Since there were often multiple cases from the same family admitted to hospital, the "index case" has been defined as the hospitalized case with the earliest onset of diarrhoea. Family contacts were defined as those individuals who lived and ate with the index case. The initial house visit was made within 1 to 3 days of admission of the index case. A family roster including vaccine history and history of recent diarrhoeas was completed, and a rectal swab culture and the initial finger-tip serological sample were obtained. On daily follow-up examinations, rectal swab cultures were col-

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lected and the individuals were questioned about any symptoms of diarrhoea. On the tenth day, the last rectal swab and a second serological specimen were collected.

The rectal swab cultures were collected with a tellurite-impregnated swab and placed in liquid alkaline taurocholate peptone transport media and identified by a code number. These were subcultured the following morning to gelatin agar and alkaline taurocholate tellurite gelatin agar (TTGA) (Monsur, 1963; Monsur et al., 1965). Suspicious colonies were identified by agglutination with specific absorbed antisera.

Finger-tip blood specimens were collected in a 0.05-ml capillary tube and discharged into 0.45 ml

of saline in a sterile screw-cap vial making a 1:10 dilution. They were identified by a code number and immediately stored in wet ice. In the laboratory the cells were separated and the diluted plasma was frozen at -20°C until the titrations were performed.

Vibriocidal titres against both Ogawa and Inaba organisms were determined with the microtechnique previously described (Benenson, Saad & Mosely, 1968) utilizing serial doubling dilutions beginning at 1:20. In this report only the titre against Inaba organisms will be presented since the infecting organisms were predominantly of the Inaba serotype. The Ogawa titres gave essentially comparable results.

RESULTS

TABLE 1
AGE AND SEX DISTRIBUTION OF INDEX CASES
AND FAMILY CONTACTS

Age-group (years)	No. of index cases			No. of contacts		
	Male	Female	Total	Male	Female	Total
0-4	13	14	27	43	46	89
5-9	14	8	22	54	52	106
10-29	10	12	22	96	77	173
≥ 30	2	8	10	76	62	138
Total	39	42	81	269	237	506

From October 1965 to the end of March 1966, 81 families were followed. *Vibrio cholerae*, Inaba, was isolated from 80 of the index cases; 1 isolate was of the Ogawa serotype. Table 1 gives the age and sex distribution of the index cases and of the family contacts. A total of 60% of the index cases were under the age of 10 years with an almost equal number of males and females. There were 506 contacts, or an average of 6.2 contacts per case. Only 39% of the contacts were under the age of 10 years. Both sexes were almost equally represented.

Table 2 gives the clinical and bacteriological findings in the family contacts by age-group. A 10-day follow-up including a minimum of 5 rectal swab cultures was completed on 466 (92%) of the

TABLE 2
BACTERIOLOGICAL AND CLINICAL FINDINGS IN FAMILY CONTACTS OF CHOLERA CASES BY AGE

Age-group (years)	<i>Vibrio cholerae</i> present				<i>Vibrio cholerae</i> absent				Total No. studied	No. not studied ^a	Percentage infected ^b
	No. of symptomatic diarrhoeas				No. of symptomatic diarrhoeas						
	Hospital	Field	Asymptomatic	Total	Hospital	Field	Asymptomatic	Total			
0-4	13	5	13	31	1	3	51	55	86	3	36.0
5-9	9	1	13	23	1	3	74	78	101	5	22.8
10-29	6	3	8	17	1		134	135	152	21	11.2
≥ 30	1	1	5	7		1	119	120	127	11	5.5
Total	29	10	39	78	3	7	378	388	466	40	16.7

^a All asymptomatic; refused cultures or not available for culture.

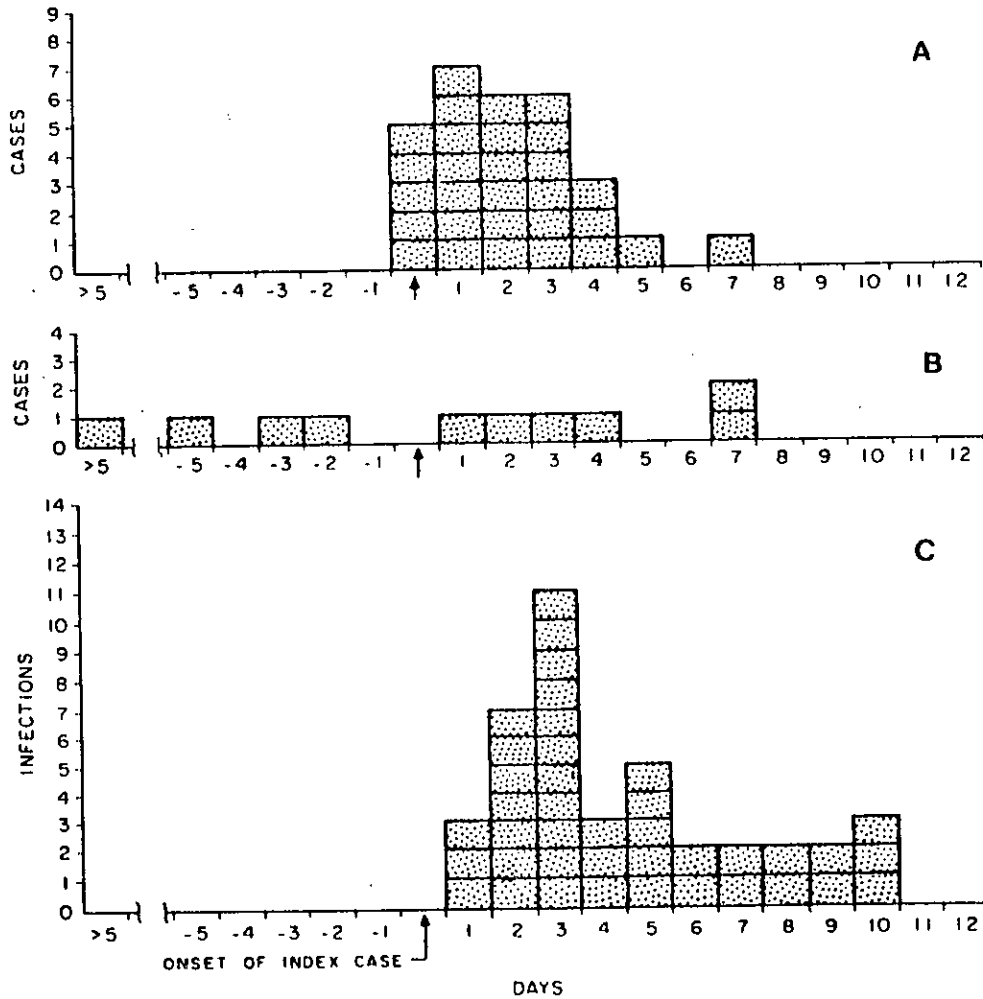
^b Calculated only for those studied.

306 contacts. Infection with *Vibrio cholerae* was found in 78 (16.7%) of the contacts; diarrhoea was present in 39 (50%) of these and three-fourths had to be admitted to hospital. Only 10 of the 388 contacts with negative cultures reported diarrhoea; 3 had to be admitted. The infection rate was 36% in children under 5 years and fell progressively to 5.5% in adults over 30 years of age.

Fig. 1 illustrates the time relationships of the onset of subsequent cases and of positive cultures in asymptomatic contacts.

Five hospitalized cases occurred on the same day as the index cases, and the majority occurred within 4 days. In the field there were 3 individuals who reported diarrhoea 2-5 days prior to the onset of the index case, and 1 child who had had intermittent recurrent diarrhoea for 30 days. All were positive for *Vibrio cholerae*. The 2 cases of field diarrhoea with onset of symptoms 7 days after the index case had positive cultures 3 and 4 days prior to the development of diarrhoea. In 13 (33%) of the persons with asymptomatic infection the first culture taken was positive, and the

FIG. 1
RELATIONSHIP OF ONSET OF SUBSEQUENT HOSPITALIZED CASES AND FIELD DIARRHOEAS, AND OF FIRST POSITIVE CULTURES IN ASYMPTOMATIC INFECTIONS TO ONSET OF INDEX CASE



A = Subsequent hospitalized cases (day of onset).
 B = Field diarrhoeas (day of onset).
 C = Asymptomatic infections (day of first positive culture).
 Arrows indicate onset of index case.

majority of infections were detected within 5 days of onset of the index case. There were 11 (28%), however, who did not have a positive culture until 5 or more days after the onset of the index case. This study does not include 2 individuals, from whom cultures were not obtained, who died of acute diarrhoea and vomiting; one died 2 days prior to, and the other 2 days after, the onset of the index case.

TABLE 3
NUMBER OF CULTURES POSITIVE FOR *VIBRIO CHOLERAE*
PER CASE FROM FIELD DIARRHOEA
AND ASYMPTOMATIC INFECTIONS FOUND BY DAILY
RECTAL SWAB CULTURES FOR 10 DAYS

No. of positive cultures	No. of cases of symptomatic field diarrhoeas ^a	No. of asymptomatic cases ^b
1	—	15
2	3	7
3	1	4
4	2	5
5	3	1
6	1	4
7	—	3
Total	10	39

^a An average of 9.5 daily cultures per person obtained.

^b An average of 9.2 daily cultures per person obtained.

Table 3 illustrates the number of positive cultures found by successive swabbings of the individuals with asymptomatic infections and those with mild diarrhoea detected in the field. Individuals with diarrhoea had from 2 to 6 positive cultures during the 10-day follow-up with a median of 4. By contrast, 38% of those with asymptomatic infections had only a single positive culture.

An initial blood specimen was available for titration from 421 (90%) of the 466 family contacts who were followed for the 10-day period. Table 4 shows the distribution of vibriocidal titres from these initial specimens by age-group. The progressive increase with age in the percentage with antibodies is evident. Among children under 5 years, only 16% had titres of 1:20 or greater, while among adults over 30 years, 73% had titres of 1:20 or greater.

A second serum specimen was obtained after 10 days from 316 (75%) of the 421 contacts who submitted the first specimen. The vibriocidal antibody response in these serum pairs in relation to the bacteriological and clinical findings is summarized in Table 5. Over-all, 82% of those contacts with a positive rectal swab culture for *Vibrio cholerae* had a 4-fold or greater rise in vibriocidal titre. A positive antibody response was seen rather more frequently when there was associated diarrhoea; however, 77% of those with asymptomatic infection had a significant vibriocidal response. A 4-fold rise in titre was present in 67% of the asymptomatic contacts with only a single positive culture. Among the 10 infected individuals without a rise in titre, 4 did not have positive cultures until at least the sixth day of follow-up, and 2 were under 1 year of age.

Among those contacts with negative rectal swab cultures, 17 had a significant rise in vibriocidal antibody titre; however, 8 of these had taken cholera vaccine between the collection of the first and second blood specimens. The other 9 are considered to represent infections which were missed by multiple rectal swab cultures, since in earlier studies (Benenson, Saad & Mosley, 1968) a significant increase in vibriocidal antibody was observed in only 1.6% of 191 vibrio-negative hospitalized patients who had had no known contact with confirmed cases of cholera. In addition, 2 asymptomatic individuals had a greater than 4-fold fall in titre from very high initial levels which probably represented recent infections in patients who were first examined in the early convalescent period.

The distribution of the initial vibriocidal titres in the contacts with bacteriologically confirmed cholera infection, with and without diarrhoea, as well as those with negative cultures is presented in Table 6. This reveals that in all age-groups, the majority of persons infected with *Vibrio cholerae* initially had low or non-detectable titres. Over-all, only 31% had a titre of 1:20 or greater. Among the bacteriologically negative contacts, 65% had titres of 1:20 or greater with a progressive increase in the antibody levels with age.

Based on the data in Table 6, a summary of the cholera infection rate among the family contacts by the level of initial vibriocidal antibody titre is given in Table 7. These data are also illustrated in Fig. 2. There is a progressive fall in the infection rate as the antibody titre increases. This approximates to a 50% reduction in case rate for each doubling of the vibriocidal antibody titre.

TABLE 4
VIBRIOCIDAL TITRES IN INITIAL BLOOD SPECIMENS OF FAMILY CONTACTS ARRANGED BY AGE-GROUPS

Age-group (years)	No. of titres (reciprocal)										No. not tested ^a	Percentage with titre >20
	<20	20	40	80	160	320	640	1 280	>2 560	Total		
0-4	67	7	2		1	2	1			80	6	16.3
5-9	50	9	11	10	6	1	3	1	4	95	6	47.4
10-29	42	24	32	12	13	5	3	1		132	20	68.2
>30	31	25	20	20	13	4	1			114	13	72.8
Total	190	65	65	42	33	12	8	2	4	421	45	54.9

^a Refused blood collection.

TABLE 5
VIBRIOCIDAL ANTIBODY RESPONSE IN RELATION TO BACTERIOLOGICAL AND CLINICAL FINDINGS IN FAMILY CONTACTS OF CHOLERA CASES

Vibriocidal response	<i>Vibrio cholerae</i> present			<i>Vibrio cholerae</i> absent		
	No. of symptomatic diarrhoeas	No. asymptomatic	Total	No. of symptomatic diarrhoeas	No. asymptomatic	Total
≥4-fold rise	30	23	53	1(1) ^a	8(7) ^a	9(8) ^a
2-fold rise	2		2	1	21	22
None	3	7	10	5	192	197
2-fold fall				1	12	13
≥4-fold fall					2	2
Total	35	30	65	8(1) ^a	235(7) ^a	243(8) ^a
Not tested ^b	4	9	13	1	136	137
Percentage with ≥4-fold rise in titre ^c	85.7	76.7	81.5	12.5	3.4	3.7

^a Values in parentheses represent individuals who were vaccinated between collection of first and second specimen and are excluded from totals.

^b First or second, or both, blood specimens not obtained.

^c Calculated on the basis of total tested.

TABLE 6
VIBRIOCIDAL TITRES OF INITIAL BLOOD SPECIMEN OF FAMILY CONTACTS OF CHOLERA PATIENTS
ACCORDING TO CLINICAL ILLNESS, BACTERIOLOGICAL FINDINGS BY AGE

A. Diarrhoea—*V. cholerae* present

Age-group (years)	No. of initial vibriocidal titres (reciprocal)										No. not tested	
	<20	20	40	80	160	320	640	1 280	≥2 560	Total		
0-4	15	3									18	
5-9	7		2								9	1
10-29	4	1	2	1	1						9	
≥30	2										2	
Total	28	4	4	1	1						38	1

B. Asymptomatic—*V. cholerae* present

Age-group	<20	20	40	80	160	320	640	1 280	≥2 560	Total	Not tested
0-4	10	1	1							12	1
5-9	8	1	2	1			1			13	
10-29	4	2	1	1						8	
≥30	2	1	1							4	1
Total	24	5	5	2			1			37	2

C. *Vibrio cholerae* absent

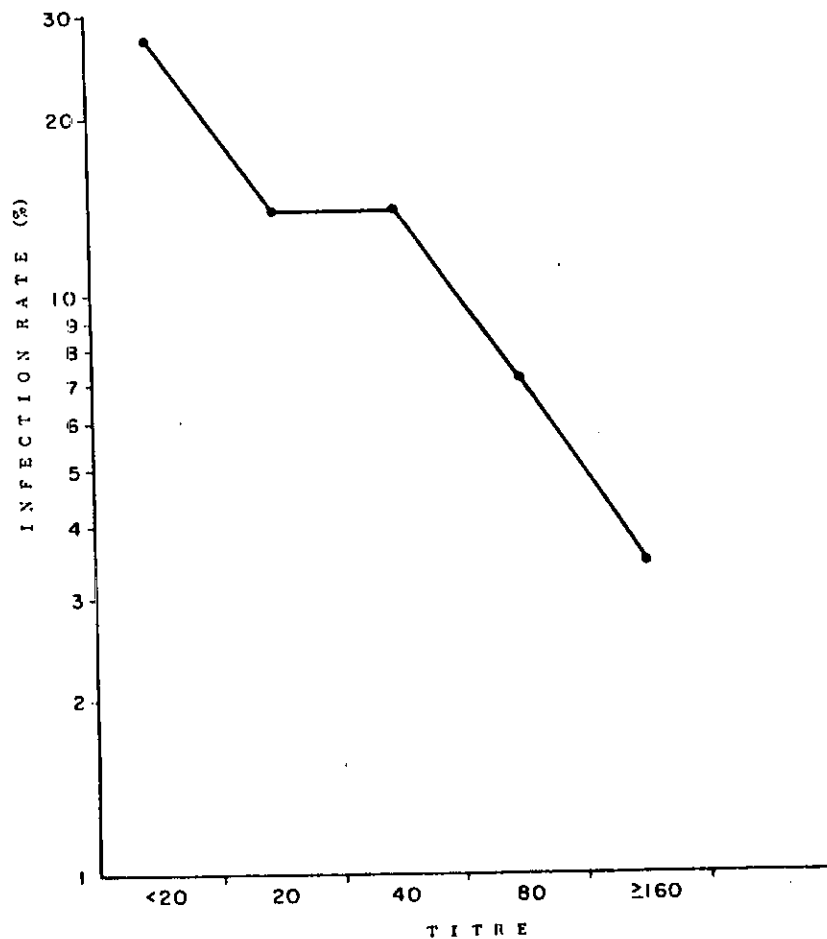
Age-group	<20	20	40	80	160	320	640	1 280	≥2 560	Total	Not tested
0-4	42	3	1		1	2	1			50	5
5-9	35	8	7	9	6	1	2	1	4	73	5
10-29	34	21	29	10	12	5	3	1		115	20
>30	27	24	19	20	13	4	1			108	12
Total	138	56	56	39	32	12	7	2	4	346	42

TABLE 7
CHOLERA INFECTION RATES AMONG FAMILY CONTACTS OF CHOLERA PATIENTS
BY INITIAL VIBRIOCIDAL ANTIBODY TITRE

Initial vibriocidal titres	No. of total contacts	<i>Vibrio cholerae</i> present			Rate (%)		
		No. with diarrhoea	No. asymptomatic	Total	No. with diarrhoea	No. asymptomatic	Total
<20	190	28	24	52	14.7	12.6	27.4
20	65	4	5	9	6.2	7.7	13.8
40	65	4	5	9	6.2	7.7	13.8
80	42	1	2	3	2.4	4.8	7.1
≥160	59	1	1	2	1.7	1.7	3.4
Total	421	38	37	75	9.0	8.8	17.8

FIG. 2

INFECTION RATE AMONG FAMILY CONTACTS OF CHOLERA CASES BY INITIAL VIBRIOCIDAL ANTIBODY TITRE



DISCUSSION

The serological surveys in the vaccine field-trial population correlating a low cholera case rate with high antibody titres suggested that vibriocidal antibody was a measure of immunity (Mosley et al., 1968a, 1968b). A direct study of the relationship of antibody to susceptibility to infection in individuals in the general population is difficult because of the low attack rate. Among family contacts, however, higher infection rates permit such direct observations to be made (Oseasohn et al., 1966).

This study of family contacts of cholera patients has provided direct evidence that the vibriocidal titre can be used as a measure of immunity to cholera in man. In this study it has been possible to demonstrate that cholera infection occurred more frequently in those individuals with a lower level of immunity as measured by the vibriocidal antibody

titre. Thus, in this endemic area, the impressive fall in the cholera case rate among family contacts with increasing age can be largely attributed to the development of serologically demonstrable immunity with increasing age.

The use of serological techniques has provided additional data on the pattern of cholera in family contacts of cholera cases. The distribution of antibody titres found on the initial blood specimens (Table 4) suggests that possibly 3 or 4 children in the 0-4-years age-group and 4 or 5 children in the 5-9-years age-group with unusually high titres had an infection shortly before the onset of symptoms in the index case. Further evidence for this is suggested by the fact that 2 of these individuals had a 4-fold, or greater, fall in vibriocidal titre in the second blood specimens. Additionally, there were 9 individuals with multiple negative rectal swab cultures who had

a 4-fold or greater rise in vibriocidal titre, 8 of whom were asymptomatic. This response is indicative of inapparent infection that has been missed by multiple rectal swabs. If this is true, then daily rectal swab culturing detected only 39 (81%) out of at least 47 individuals with inapparent infection.

A 4-fold or greater rise in titre was seen in 82% of the infected contacts in this study. This is considerably lower than the 96% conversion reported by Benenson et al. (1968) for hospitalized cholera cases. Two factors may be responsible. First, the serological conversion rate for hospitalized cases is reported only for those cases where the second specimen was collected 6 or more days after the onset of illness—at a time when a significant serological response is expected to be present. In the family contacts, the serum pairs collected at a 10-day interval would only be expected to show a significant antibody response in those persons infected in the first 4 or 5 days of the study. These persons with positive cultures after the fifth day may not show an

antibody response. A second possibility is that a milder intestinal infection with *Vibrio cholerae* may not always provide the same antigenic stimulus to the host as the clinical disease. This is suggested by the somewhat lower frequency of serological conversion seen in persons with inapparent infection than in those with diarrhoea.

This study has indicated the usefulness of the vibriocidal antibody test as an epidemiological tool in the study of the pattern of cholera. Since there is a vibriocidal antibody response with inapparent infection approximating to the frequency seen in patients with symptomatic infection, this technique can be useful in tracing the patterns of spread of cholera and the frequency of inapparent infections. More importantly, because of the relationship of the vibriocidal antibody titre to susceptibility to cholera this technique can be used both to survey the immunity in populations as well as to provide preliminary evaluations of the protective efficacy of whole-cell cholera vaccines.

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RÉSUMÉ

Une étude bactériologique et sérologique des contacts familiaux de 81 malades atteints de choléra a été effectuée à Dacca, Pakistan oriental. Au cours d'une enquête de contrôle d'une durée de dix jours pendant laquelle au moins cinq cultures d'écouvillonnages rectaux ont été faites, on a constaté que 78 (16,7%) des 466 contacts examinés présentaient une infection à *Vibrio cholerae*; sur ces 78 sujets, 39 (50%) souffraient de diarrhée et les trois quarts d'entre eux ont dû être hospitalisés. Parmi les 388 contacts dont les cultures étaient négatives, 10 seulement ont signalé de la diarrhée et 3 d'entre eux ont été hospitalisés. Le taux d'infection était de 36% chez les enfants de moins de 5 ans et diminuait progressivement jusqu'à 5,5% chez les adultes de plus de 30 ans. L'examen de paires de sérums prélevés sur un certain nombre des 466 contacts a montré que le titre des anticorps vibriocides s'était trouvé multiplié par quatre ou plus chez 82% des sujets porteurs d'une infection à *V. cholerae*.

La réponse immunitaire était plus fréquemment positive lorsque l'infection s'accompagnait de diarrhée; cependant, 77% des contacts atteints d'une infection asymptomatique ont présenté une réponse immunitaire forte. Le titre des anticorps avait quadruplé chez 67% des contacts asymptomatiques pour lesquels une seule culture avait été trouvée positive. Chez 4 des 10 sujets infectés sans hausse concomitante des titres d'anticorps, on n'a trouvé de culture positive qu'à partir du sixième jour de l'enquête de contrôle. Deux de ces malades avaient moins d'un an.

Les tests sérologiques de recherche des anticorps vibriocides pratiqués sur des échantillons de sang prélevés au début de l'enquête ont montré que les titres augmentaient avec l'âge des sujets. L'infection à *V. cholerae*, symptomatique ou non, prédominait chez les sujets dont les titres d'anticorps étaient faibles.

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