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Serological Studies on Cholera Patients and their Household Contacts in Calcutta in 1968*

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Vibriocidal and agglutination tests have been performed, using a microtechnique, on 170 pairs of sera obtained, at intervals of 13–26 days, from bacteriologically proven cholera patients and their contacts, carriers and vibrio-negative contacts. Of the carriers, 44 %–46 % of those with low initial vibriocidin titres (< 1 : 80) and 28 %–37 % of those having high initial titres (< 1 : 160) showed a 4-fold or greater rise in vibriocidal titres. Carriers and negative contacts exhibited almost similar pictures. With an increase in the number of carriers per household, a larger number of negative contacts developed significant titres in their second samples. In general, initial titres increased with age, but were highest in the 10–25-years age-group: however, 30 % of children below 10 years of age had titres ≥ 1 : 640. The results indicated that individuals with high titres might become carriers but may not suffer from overt cholera.

The investigation also showed that retrospective diagnosis of cholera infection in a highly endemic area should not depend on serology alone.

Immuno-epidemiological patterns in a cholera endemic area are of great importance in the elucidation of the epidemiology of cholera. A serological study has, therefore, been carried out simultaneously with the cholera carrier studies in Calcutta since 1966 (Sinha et al., 1967, 1968). Blood samples were collected from cases, carriers and their *Vibrio cholerae* negative contacts in the community and agglutination, vibriocidal and antitoxin titres were determined. Results of the study during 1966–67 will be published in a separate communication.

It was observed during the earlier part of the cholera carrier studies in Calcutta (Sinha et al., 1967, 1968) that, in spite of the abundance of infection in the community, clinical cases of cholera were not very common. Accordingly an intensive investigation was carried out during 1968 to determine the incidence and persistence of infection in households in which cases of cholera occurred. Serological studies were made at the same time as the epidemiological studies (Joint ICMR–GWB–WHO Cholera Study Group, Calcutta, India, 1970a, 1970b) to determine the immunological behaviour of the members of the households. Results of agglutination and vibriocidal tests on these sera are discussed in the present communication.

MATERIALS AND METHODS

When a cholera patient was admitted to the Infectious Diseases Hospital, Calcutta, and found to be *Vibrio cholerae* positive, blood samples were immediately collected from the other members of his household. Samples of blood were also collected from the patients within 2–5 days of their admission, except in the cases of two patients whose first serum samples were collected 8 and 13 days after their admission.

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[1] This is a joint project under the auspices of the Indian Council of Medical Research (represented by the Cholera Research Centre), the Health Department of West Bengal Government and the World Health Organization. These groups were represented by the workers listed below:

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TABLE 1
DISTRIBUTION OF PERSONS FROM WHOM SERA WERE COLLECTED BY AGE, SEX AND INFECTION STATUS

Age-group (years)	Sex	Cases			Carriers			Negative contacts		
		No. of persons bled	No. of paired sera tested	% coverage	No. of persons bled	No. of paired sera tested	%	No. of persons bled	No. of paired sera tested	% coverage
<10	Male	3	2	66.7	9	6	66.7	14	10	71.4
	Female	1	1	100.0	10	7	70.0	9	7	77.8
10–25	Male	2	1	50.0	18	16	88.9	24	16	66.7
	Female	1	—	0.0	13	10	76.9	47	32	68.0
>25	Male	2	2	100.0	19	10	52.6	38	22	57.9
	Female	8	5	62.5	16	10	62.5	17	13	76.5

The categories of persons from whom blood was collected included (*a*) cases (index cholera patients), (*b*) carriers—members of the households from whom cholera vibrios were isolated on one or more occasions during the study but who did not show clinical symptoms of cholera and (*c*) negative contacts—members of the household from whose stools *V. cholerae* was not isolated despite daily sampling for a minimum period of 10 days after the onset of the index case. Some of them were sampled for a longer period but remained vibrio negative. Out of a total of 251 persons belonging to the different categories mentioned above, from whom sera were obtained, 170 were bled for a second time after an interval of about 13–26 days, except for one case and one carrier who were bled 7 and 37 days, respectively, after their initial bleedings. Only the paired sera obtained from these 170 persons are described in the present communication. The number of sera obtained from cases was, of course, very small.

Venous blood was obtained in each case with the help of Vacutainers. After separation, sera were filtered through Millipore membrane filters (0.45 μ) and aliquots (about 0.5 ml) were preserved in sealed ampoules at $-20°C$.

Vibriocidal tests on the sera were carried out in duplicate using the microtitration method (Benenson et al., 1968a) as this method was found advantageous in handling large numbers of sera, in requiring smaller amounts (0.05 ml) and because of its convenience (Deb et al., 1969). Anti-cholera serum, raised in rabbits and of known vibriocidal titre, was included in each experiment as a positive control. This antiserum was prepared by hyper-immunizing rabbits with a live whole-cell suspension of *V. cholerae*, Inaba ASM Kuki. Commercial lyophilized guinea-pig complement was obtained from Messrs Markham Laboratories, Chicago, Ill., USA and was reconstituted with normal saline during the experiment. Complement, bacterial and sterility controls were carried out each time. Vibriocidin titres were expressed as the reciprocals of the highest dilutions of serum which lysed the test organisms.

The agglutination test was also performed by the microtechnique described by Benenson et al. (1968b), with the following modifications. To provide the antigen in the current study, an 18-hour surface culture on heart-infusion-agar slants was harvested in normal saline, and adjusted to a reading of 30 on the scale of the Klett-Summerson colorimeter [1] using green filter No. 54. Sera were not inactivated before the experiments.

For both tests two *V. cholerae* strains,[2] Ogawa 465 and Inaba 22463, were used.

RESULTS

The age- and sex-distributions of the different categories of persons bled once and those from whom paired sera were collected are given in Table 1. Of the 170 sera investigated in the current study, 85 belonged to males and 85 to females and the over-all coverage in the second bleeding was 67.7%.

[1] Klett Manufacturing Co., New York, N.Y., USA.

[2] Obtained from C.R.L., Dacca, through the courtesy of Dr Y. Zinnaka.

Replicate vibriocidal tests were performed, using the anti-cholera rabbit serum to establish the standard deviation of the microtitration method. Results of these replicate determinations are given in Table 2. A total of 66 tests was performed with each serotype. The standard deviation in the 132 tests, including both serotypes, is slightly greater than 1 cup dilution (1.09), and this indicates that the method compares favourably with most other serological tests in which serial dilutions of serum are used for end-point determinations. An analysis of these data also indicates that, with the immune rabbit serum employed, there is no significant difference between the vibriocidin titres against the two serotypes of *V. cholerae*.

On the other hand the data shown in Table 3 may indicate that under natural conditions there might be preferential stimulation of antibody towards a single serotype. While most of the observed Ogawa/Inaba vibriocidin titres are within plus or minus 1 dilution cup, in carriers and in negative contacts there is a small number of sera that show greater differences in titre against these two serotypes. There was a high observed correlation between the Ogawa and Inaba titres of either the first or the second samples of the different categories of individuals, except for the first samples from cases. Almost similar results were obtained with the agglutination tests.

TABLE 2

STANDARD DEVIATIONS OF REPLICATE VIBRIOCIDAL TESTS USING A SINGLE ANTI-CHOLERA RABBIT SERUM

Test organism	Number of tests	Mean titre [a]	S.D.	*t*	5% *t*
V. cholerae, Ogawa	66	9.57	1.20		
V. cholerae, Inaba	66	9.85	0.94	0.471	1.96
Total or mean	132	9.71	1.09		

[a] Dilution cup number showing complete lysis of the test organism (two-fold dilutions starting from 1 : 20 in the first cup).

The distribution of persons in the three categories according to the vibriocidal titres of their paired sera is shown in Table 4. For the purposes of this comparison the higher of the titres against Ogawa or Inaba has been used. A good correlation may be observed between the titres of the first and second samples from the hospital cases, but for the carriers and negative contacts the observed correlation is low.

A similar presentation of the distribution according to the agglutinin titres of the paired sera of the three groups can be seen in Table 5. The figures in the table indicate that the carriers showed a relatively

TABLE 3

RATIO OF OGAWA/INABA VIBRIOCIDIN TITRES IN CASES, CARRIERS AND NEGATIVE CONTACTS: PERCENTAGE DISTRIBUTION [a]

Category	Serum collection	Ratio: Ogawa/Inaba titres [b]						
		⩽0.0625	0.125	0.25	0.5–2.0 [c]	4.0	8.0	⩾16.0
Cases	1st	—	—	—	82 (9)	—	—	18 (2)
	2nd	—	—	9 (1)	91 (10)	—	—	—
Carriers	1st	—	2 (1)	8 (5)	83 (49)	5 (3)	—	2 (1)
	2nd	2 (1)	2 (1)	—	92 (55)	—	2 (1)	2 (1)
Negative contacts	1st	1 (1)	1 (1)	1 (1)	83 (83)	6 (6)	3 (3)	5 (5)
	2nd	1 (1)	—	3 (3)	87 (87)	6 (6)	—	3 (3)

[a] Percentage of total in each subject category for each serum collection.

[b] The values of the correlation coefficients (*r*) between the Ogawa and Inaba titres of the sera:

(1) Cases — 1st sample — 0.04; 2nd sample — 0.73
(2) Carriers — 1st sample — 0.91; 2nd sample — 0.95
(3) Negative contacts — 1st sample — 0.47; 2nd sample — 0.73

[c] The 0.5–2.0 dilution tube range is considered to be within the experimental error.

good response in their second samples compared with the negative contacts.

Table 6 shows the vibriocidal response of the paired sera of the cases, the two categories of carriers detected before or after their first bleeding and the negative contacts, on the basis of their low or high initial titres. Since a 2-fold rise or fall in titre by the microtitration method may be considered to be within the experimental error, only 4-fold rises or falls were considered as significant changes in titre. Of the 2 cases having initial titres of 1 : 80 or less, one showed a 4-fold rise in the second sample and the other did not show any change, but neither showed a fall in titre. Among the 9 cases having initial titres of 1: 160 or greater, none showed a rise in titre; in 4 cases the titre fell and in 5 others there was no change. It may be expected that carriers detected before the first bleeding will already have raised titres in their sera as compared with those detected after the first bleeding. The latter were presumably negative contacts during their first bleeding. It is in the case of this second group that a more pronounced

TABLE 4

DISTRIBUTION OF VIBRIOCIDAL TITRES OF PAIRED SERA FROM CASES, CARRIERS AND NEGATIVE CONTACTS

Titre	Cases		Carriers		Negative contacts	
	1st Sample	2nd Sample	1st Sample	2nd Sample	1st Sample	2nd Sample
0	1	5	19	18	29	28
20	1	—	—	—	1	1
40	—	—	1	1	7	3
80	—	—	2	3	9	3
160	2	1	4	5	13	11
320	1	3	6	2	7	15
640	5	1	9	9	12	10
1 280	1	1	4	6	9	10
2 560	—	—	4	7	9	9
5 120	—	—	5	3	3	4
10 240	—	—	2	2	—	4
20 480	—	—	3	2	1	2
40 960	—	—	—	1	—	—
Total	11	11	59	59	100	100

TABLE 5

DISTRIBUTION OF AGGLUTININ TITRES OF PAIRED SERA FROM CASES, CARRIERS AND NEGATIVE CONTACTS

Titre	Case		Carriers		Negative contacts	
	1st Sample	2nd Sample	1st Sample	2nd Sample	1st Sample	2nd Sample
0	6	3	16	4	31	23
20	3	1	14	9	15	17
40	1	3	6	9	16	23
80	—	—	6	9	15	5
160	—	4	7	5	7	11
320	—	—	5	8	6	6
640	—	—	2	6	5	8
1 280	1	—	2	3	3	4
2 560	—	—	—	5	2	1
5 120	—	—	1	1	—	1
10 240	—	—	—	—	—	—
20 480	—	—	—	—	—	—
40 960	—	—	—	—	—	—
Total	11	11	59	59	100	100

TABLE 6

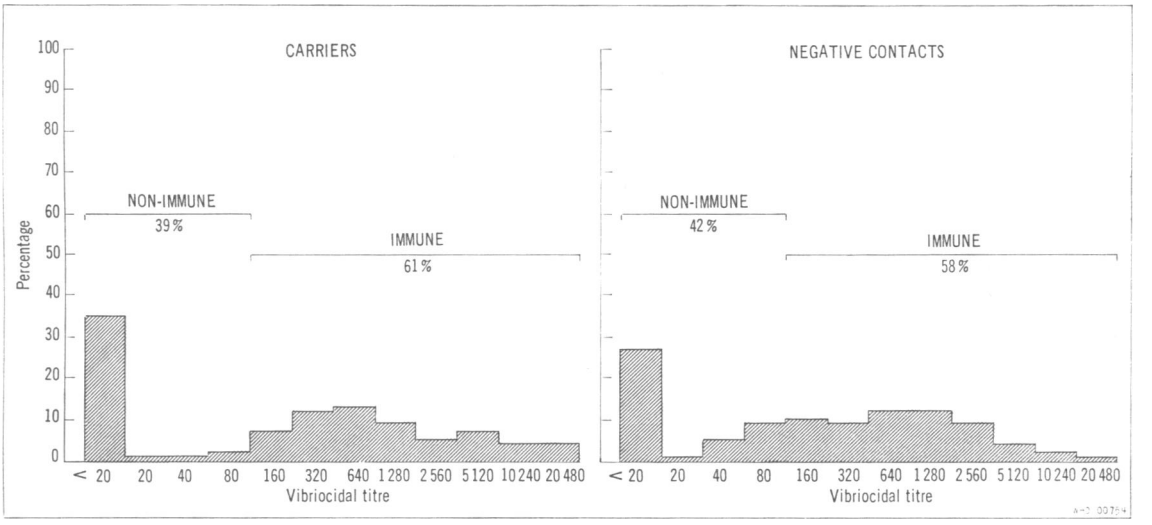
PERCENTAGE DISTRIBUTION OF THE VIBRIOCIDAL RESPONSES OF PAIRED SERA FROM CASES, TWO CATEGORIES OF CARRIERS AND NEGATIVE CONTACTS HAVING LOW OR HIGH INITIAL TITRES [a]

Vibriocidal response	Cases	Carriers		Negative contacts
		Detected before 1st bleeding	Detected after 1st bleeding	
Persons with initial titre of ⩽ 1 : 80				
⩾ 4-fold rise	50 (1)	46 (6)	44 (4)	52 (24)
None	50 (1)	46 (6)	56 (5)	39 (18)
⩾ 4-fold fall	—	8 (1)	—	9 (4)
Persons with initial titre of ⩾ 1 : 160				
⩾ 4-fold rise	—	37 (7)	28 (5)	24 (13)
None	56 (5)	16 (3)	33 (6)	43 (23)
⩾ 4-fold fall	44 (4)	47 (9)	39 (7)	33 (18)

[a] The figures in parentheses indicate number of sera examined.

FIG. 1

INITIAL VIBRIOCIDAL ANTIBODY PATTERNS OF BOTH CARRIERS AND NEGATIVE CONTACTS



rise of titre would be expected in their second samples. But from the data available in Table 6 it appears that there is no significant difference between the two categories of carriers, nor between carriers and negative contacts. Persons having low initial titres (< 1 : 80) show a greater tendency towards rise of titre than those with high initial titres (> 1 : 160) who in contrast exhibited a higher percentage of titre reductions. The over-all picture in the table suggests that the initial serological patterns in a highly endemic area such as Calcutta are essentially the same in negative contacts and carriers. Mosley et al. (1968) observed that *Vibrio cholerae* infection, whether symptomatic or asymptomatic, predominantly occurred in individuals with low titres.

Fig. 1 shows the initial vibriocidal antibody patterns of both the carriers and the negative contacts. It can be seen that there is hardly any difference in the initial antibody levels of the two groups. It is difficult to explain why only the carriers showed subsequent infection when both groups had an almost similar number of susceptible non-immunes.

Table 7 shows the agglutination response of paired sera similar to those presented in Table 6. Of the 11 cases, 10 had low initial agglutinin titres and 40% of this group showed a 4-fold or greater rise in titre. Both categories of carriers in the lower initial titre group showed considerable rise in titre, but it is interesting to note that in the group with higher initial titres (>1 : 80) the carriers detected before their first bleeding showed fewer rises (7%) of titre in their second sample than did those detected after their first bleeding (56%). Negative contacts also

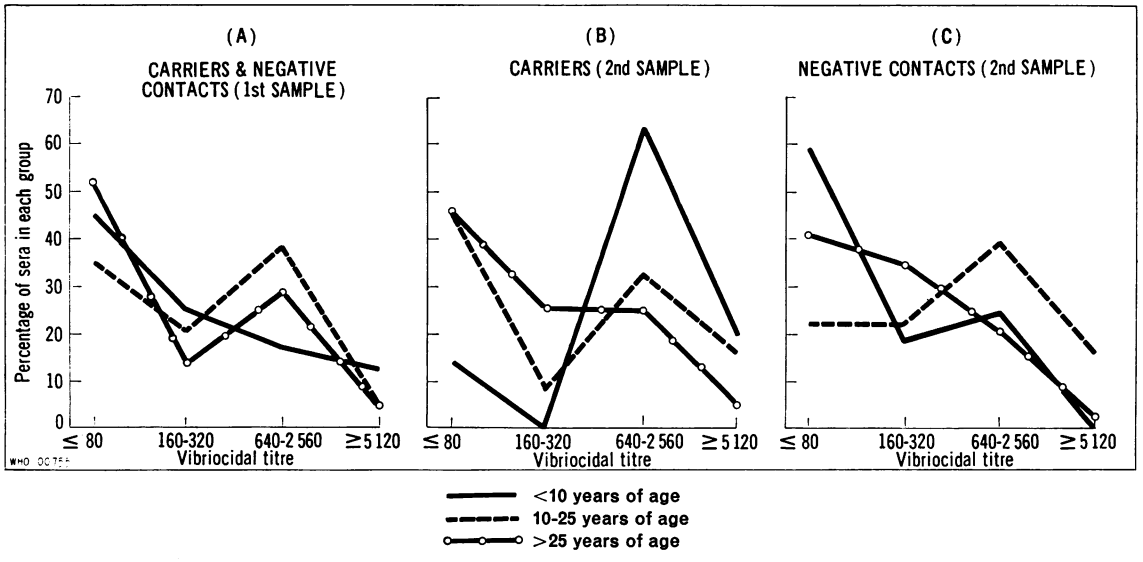
TABLE 7

PERCENTAGE DISTRIBUTION OF THE AGGLUTININ RESPONSE OF PAIRED SERA FROM CASES, TWO CATEGORIES OF CARRIERS AND NEGATIVE CONTACTS HAVING LOW OR HIGH INITIAL TITRES[a]

Agglutination response	Cases	Carriers		Negative contacts
		Detected before 1st bleeding	Detected after 1st bleeding	
Persons with initial titre of ⩽ 1 : 40				
⩾ 4-fold rise	40 (4)	56 (10)	44 (8)	34 (21)
None	60 (6)	44 (8)	56 (10)	61 (38)
⩾ 4-fold fall	—	—	—	5 (3)
Persons with initial titre of ⩾ 1 : 80				
⩾ 4-fold rise	—	7 (1)	56 (5)	13 (5)
None	—	86 (12)	33 (3)	47 (18)
⩾ 4-fold fall	100 (1)	7 (1)	11 (1)	40 (15)

[a] The figures in parentheses indicate number of sera examined.

FIG. 2
VIBRIOCIDAL ANTIBODY PATTERN BY AGE-GROUP



exhibited relatively fewer rises and more falls of titre compared with carriers, both in the low and high initial titre groups.

As the level of vibriocidins in contacts may be related to the probability of contact with the vibrios, an attempt was made to show in Table 8 the titres in each category when grouped according to the number of established carriers within the household. In the first group of households with no carriers, it can be seen that at the time of the first bleeding, 81% of the household contacts had no significant antibody titres. As the number of carriers within the household increased, a definite shift towards a significant antibody level can be observed. In households having 1–4 carriers, the percentage of contacts with insignificant antibody levels was reduced to 48% and in households having 5 or more carriers, this figure was further reduced to 29%. The carriers in these households also showed a similar tendency to raised antibody levels in their sera with a rise in the number of carriers per household. These findings also indicate that there is not much difference in titres between carriers and negative contacts in a highly endemic situation, where there is the possibility that individuals of the latter group may become infected any time prior to the second bleeding. There is ample evidence of a rise in titre in individuals along with a rise in the number of infected persons in the household. Moreover, in the group of houses in which no carrier could be detected, none showed a titre higher than 1 : 2560, whereas in the other groups of houses with carriers there were many individuals with titres beyond that level. This may point towards frequent circulation of vibrios among the persons in houses with multiple infections.

The vibriocidal antibody pattern according to age-group is shown in Fig. 2, the higher of the titres against either Ogawa or Inaba having been used. The initial titres of carriers and negative contacts of the three age-groups (<10 years, 10–25 years and >25 years) are shown together in the first part (A) of the figure. During their initial bleeding these carriers were actually negative contacts as they were detected after their first bleedings. The titres in the second samples from carriers and negative contacts, by age-group, are shown in the second and third parts (B and C) of the figure, respectively. It appears from the figure (part A) that 42% of the children below 10 years of age had low titres (≤1 : 80) in the first sample but that higher titres were also present in the group. In the second part of the figure (B), however, the carriers in this age-group (<10 years) showed a peak (64%) in the 1 : 640–1 : 2560 titre region, clearly indicating that this age-group responded better to the antigenic stimulus. The third part of the figure (C) shows that among the negative contacts below 10 years of age the pattern is almost similar to that of their initial titre except in the

TABLE 8

PERCENTAGE OF INDIVIDUALS SHOWING DIFFERENT VIBRIOCIDIN TITRES AS INFLUENCED BY NUMBER OF CARRIERS IN THEIR RESPECTIVE HOUSEHOLDS

Number of carriers per household	Subject category	Serum collection	Percentage of individuals having vibriocidin titres [a]			
			⩾80	160–320	640–2560	⩾5120
None	Cases	1st	—	—	—	—
		2nd				
	Carriers	1st	—	—	—	—
		2nd	—	—	—	—
	Negative contacts	1st	81 (17)	10 (2)	10 (2)	—
		2nd	33 (7)	43 (9)	24 (5)	—
1–4	Cases	1st	—	33 (2)	67 (4)	—
		2nd	17 (1)	50 (3)	33 (2)	—
	Carriers	1st	43 (6)	14 (2)	43 (6)	—
		2nd	14 (2)	14 (2)	43 (6)	29 (4)
	Negative contacts	1st	48 (15)	23 (7)	23 (7)	6 (2)
		2nd	42 (13)	23 (7)	19 (6)	16 (5)
⩾5	Cases	1st	40 (2)	20 (1)	40 (2)	—
		2nd	80 (4)	20 (1)	—	—
	Carriers	1st	36 (16)	18 (8)	24 (11)	22 (10)
		2nd	44 (20)	9 (4)	38 (17)	9 (4)
	Negative contacts	1st	29 (14)	25 (12)	42 (20)	4 (2)
		2nd	29 (14)	23 (11)	38 (18)	10 (5)

[a] The higher of the titres against either Ogawa or Inaba has been used and the values are shown as reciprocals. The figures in parentheses indicate the number of sera examined.

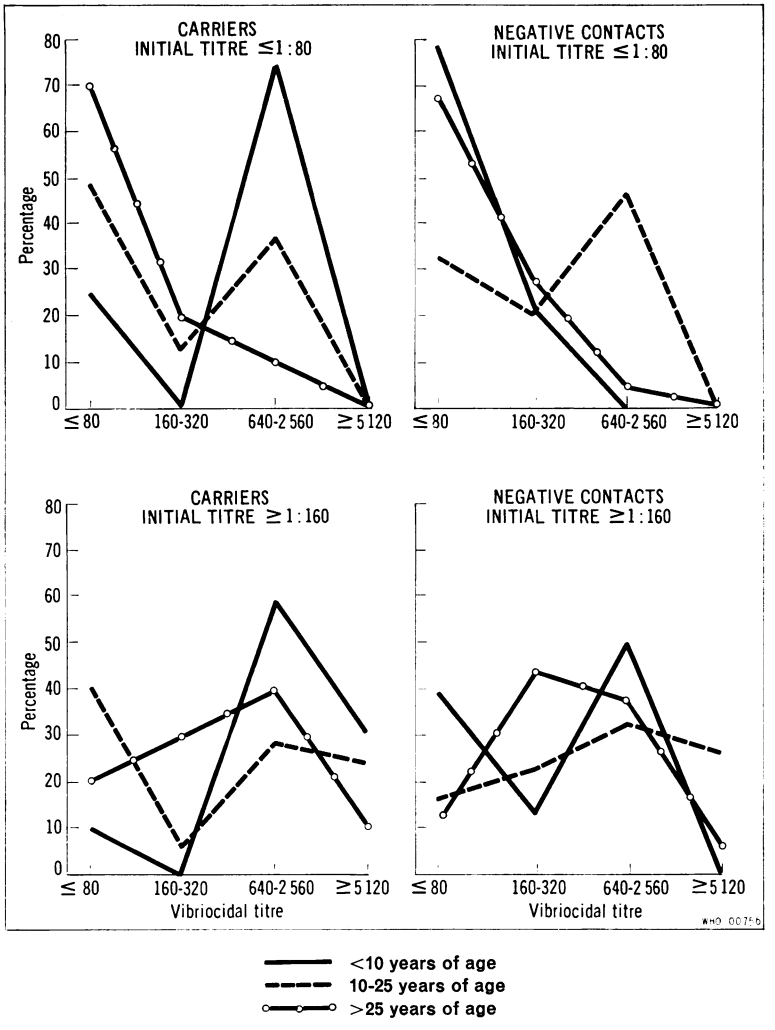
1 : 640–1 : 2560 titre region where a slight increase is apparent: this probably indicates that they were not altogether free from infection. Antibody response in individuals over 25 years of age is not well marked compared with that of the individuals between 10–25 years of age.

Fig. 3 gives the vibriocidal response of carriers and negative contacts in their second samples by age-groups depending on their low (⩽1 : 80) or high (⩾1 : 160) initial antibody titre. It is clear from the figure that there is a difference in the pattern of vibriocidal response amongst those having low or high initial titres. None of those having a low initial titre showed a rise of titre to 1 : 5120 or higher, as did those with high initial titre. Carriers and negative contacts who had high initial titres showed an almost similar picture and thereby indicated that the negative contacts were also infected at some stage: this infection was missed or it took place before the period of follow-up.

The distribution of vibriocidin titres by sex is presented in Table 9. No significant influence of sex on the vibriocidin titres of carriers and negative contacts was observed.

FIG. 3

VIBRIOCIDAL RESPONSE BY AGE-GROUP ACCORDING TO LEVEL OF INITIAL TITRE



The temporal aspects of the vibriocidin titres are shown in Table 10 for the entire study population including those from whom only a single serum sample was taken. Similar tabulations for the carriers and negative contacts did not demonstrate appreciable differences from the total population and are not, therefore, shown here. Inspection of this table reveals that there is a definite seasonal variation in the antibody titres of the population. In general, an increase in titre coincides with the peak of the cholera season. Titres were generally low in April, began to increase in early May and reached peak in mid- to late-June. Although the population sample was small during July, there appears to have been a definite fall in titre level coincident with the decline of clinical disease in the population.

DISCUSSION

Recent reports from East Pakistan (Mosley et al., 1968, 1969) indicate that 77% of individuals with inapparent infection showed a 4-fold or greater rise in vibriocidal titres in their second blood samples

TABLE 9

PERCENTAGE DISTRIBUTION OF VIBRIOCIDIN TITRES ACCORDING TO SEX

Category	Sex	Serum collection	Vibriocidin titre [a]			
			⩽80	160–320	640–2560	⩾5120
Cases	Male	1st	40 (2)	20 (1)	40 (2)	—
		2nd	60 (3)	40 (2)	—	—
	Female	1st	—	33 (2)	67 (4)	—
		2nd	33 (2)	33 (2)	33 (2)	—
Carriers	Male	1st	38 (12)	16 (5)	25 (8)	22 (7)
		2nd	41 (13)	13 (4)	38 (12)	9 (3)
	Female	1st	37 (10)	19 (5)	33 (9)	11 (3)
		2nd	33 (9)	11 (3)	37 (10)	19 (5)
Negative contacts	Male	1st	54 (26)	17 (8)	25 (12)	4 (2)
		2nd	40 (19)	21 (10)	33 (16)	6 (3)
	Female	1st	38 20)	23 (12)	35 (18)	4 (2)
		2nd	31 (16)	31 (16)	27 (14)	12 (6)

[a] The higher of the titres against either Ogawa or Inaba has been used and the values are shown as reciprocals. The figures in parentheses indicate the number of sera examined.

TABLE 10

PERCENTAGE DISTRIBUTION OF RELATIVE VIBRIOCIDIN TITRES IN THE TOTAL STUDY POPULATION BY DATE OF COLLECTION

Sera collected between	Vibriocidin titres [a]			
	⩽ 80	160–320	640–2560	⩾ 5120
3–17 April	83 (25)	7 (2)	10 (3)	—
18 April–2 May	40 (37)	28 (26)	24 (22)	8 (7)
3–17 May	30 (26)	20 (17)	36 (31)	15 (13)
18 May–1 June	38 (33)	16 (14)	40 (35)	6 (5)
2–16 June	33 (17)	26 (13)	33 (17)	8 (4)
17 June–1 July	18 (8)	13 (6)	47 (21)	22 (10)
2–14 July	67 (18)	26 (7)	4 (1)	4 (1)

[a] The higher of the titres against either Ogawa or Inaba has been used and the values are shown as reciprocals. The figures in parentheses indicate the number of sera examined.

obtained after an interval of 10 days. They also found that initial vibriocidal antibody titres in blood increased with increasing age, and that the infection, symptomatic or asymptomatic, occurred in individuals with low initial titres.

The present serological study in the highly endemic area of Calcutta, has, however, indicated a different picture. It has been found that only 44%–46% of carriers with low initial vibriocidin titres (⩽1 : 80) and 28%–37% of those with high initial titres (⩾1 : 160) showed a 4-fold or greater rise of vibriocidin titre in their second serum specimens taken after an interval of 13–26 days. Negative contacts showed rises in antibody titre almost similar to those of the carriers (Table 6). This study has also shown that the vibriocidal antibody patterns of both carriers and negative contacts are almost the same (Fig. 1). Although 61% of the carriers had high titres (⩾1 : 160) during their initial bleedings they were later found to be infected with *V. cholerae*, but none developed clinical symptoms of cholera. It appears from the above findings that an individual with high vibriocidin titre might become a carrier but

may not suffer from an attack of clinical cholera. In this connection it is interesting to recall the observation of McCormack et al. (1969) that 13 of their 58 admitted cholera patients had vibriocidal titres above 1 : 40.

Vibriocidin titres were found to increase with age (Fig. 2, part A) the rise being greater at the titre level of 1 : 640–1 : 2560. A slightly greater effect was seen in the 10–25-years age-group than in persons over 25 years of age. Although 46% of the children below 10 years of age showed low titres ($\leqslant$1 : 80) in their initial blood samples, 30% of them had titres above 1 : 640. This may have been due to the high degree of infection in Calcutta and the frequent circulation of vibrios amongst children. It may be mentioned here that during the cholera carrier studies in Calcutta in 1968, two newborn (twin) babies were found infected with *V. cholerae* within 1 month of their discharge from the maternity hospital; their parents were established carriers, the father being a long-term carrier. This shows that children among the shanty dwellers in Calcutta are exposed to cholera infection very early in life, obviously due to the presence of heavy infection in their households.

That the infection is circulating freely in the communities is further illustrated by the fact that with increase in the number of carriers within the households, a larger number of negative contacts developed significant antibody titres in their second blood samples (Table 8). This also suggests that the negative contacts had at some stage been infected with *V. cholerae*, which could not be isolated.

It is rather interesting to note that the agglutination titres by the microtitration method on the other hand showed a more significant difference between the carriers and the negative contacts, the former showing better response than the latter. It was also observed that 10 out of 11 cases (Table 5) had low initial titres ($\leqslant$1 : 40) on admission which may have contributed towards their becoming cholera patients, if it is presumed that agglutinating antibodies have any protective action against cholera. No similar indications were, however, obtained from vibriocidal antibody titrations, although these are usually believed to be more sensitive and specific (Ganguly et al., 1969). In any case the nature of protective antibodies against cholera is still not clear (WHO Scientific Group on Cholera Immunology, 1969).

The data available from the present study indicate that probably one cannot depend solely on serological findings in a highly endemic situation like that in Calcutta, for retrospective diagnosis of cholera infection. The serological picture is almost similar in carriers and negative contacts, and this may suggest that bacteriological techniques available at present are not adequately sensitive to detect very low-grade infections. Mosley et al. (1968) also suggested that some isolations might be missed. Another possibility is that the negative contacts may have been infected with vibrios within the 10-days period after the first sample was taken. Annual anticholera inoculation may have also accounted for the higher titres amongst the negative contacts, but authentic information regarding this could not be obtained.

On the basis of the above data it may be said that a more intensive study of the serological behaviour of a cross-section of the community at different times would probably throw more light on the problem of immunity against cholera.

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RÉSUMÉ

ENQUÊTE SÉROLOGIQUE DANS DES FAMILLES ATTEINTES PAR L'INFECTION CHOLÉRIQUE À CALCUTTA EN 1968

Des investigations sérologiques ont été menées dans des familles où était survenu un cas de choléra avéré. Elles ont porté sur les malades eux-mêmes et sur les contacts familiaux qu'ils aient été, à l'examen bactériologique, reconnus porteurs de germes ou exempts de toute infection à *Vibrio cholerae*. Au total, 170 paires de sérums prélevés à intervalle de 13–26 jours ont été examinées, une microtechnique étant utilisée pour la recherche des agglutinines et des anticorps vibriocides.

On a relevé une plus forte proportion de hausses des titres d'anticorps chez les sujets faiblement positifs (titres $<1:80$) que parmi ceux dont le sérum réagissait fortement (titres >160) lors du premier examen. Chez 44%–46% des porteurs de germes à faibles titres d'anticorps vibriocides et chez 28%–37% des porteurs présentant des titres élevés, l'examen du second échantillon de sérum a montré une élévation de quatre fois des titres. Une réponse quasi similaire a été observée chez les contacts bactériologiquement négatifs. On a constaté que lorsqu'un contact présentait à l'origine des titres élevés, il pouvait par la suite devenir porteur de germes du choléra sans être atteint d'une forme clinique de l'affection. L'enquête a également révélé que l'augmentation du nombre des porteurs au sein des familles avait pour conséquence d'accroître la proportion des contacts bactériologiquement négatifs dont le second échantillon de sérum renfermait des anticorps à des titres élevés.

Dans les premiers échantillons de sérum, les titres d'anticorps vibriocides augmentaient en fonction de l'âge des sujets; cependant, après 25 ans, les titres élevés étaient moins nombreux que dans le groupe d'âge 10-25 ans. Des titres de 1 : 640 ou plus ont été trouvés chez 30% des enfants âgés de moins de 10 ans.

Les auteurs estiment qu'il n'est pas indiqué de fonder un diagnostic rétrospectif de l'infection à *V. cholerae* dans une région de forte endémicité sur les seules données sérologiques.

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