

SEROLOGICAL STUDY ON CHOLERA IN THAILAND†

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INTRODUCTION

Isolation of vibrio from rectal swabs or from stools of cholera carriers has not been entirely satisfactory because of the irregularity in the excretion of vibrios, which often were lodged in the gall bladder and became detectable in the stool only after purgation (Wallace *et al*, 1967, Gangarosa, *et al*, 1966; Paguio and Pesigan, 1966). Furthermore, the daily output of vibrio excretion in the stool is so small, in the order of 10^2 – 10^5 organisms per gram feces (Dizon *et al*, 1967), that detection of vibrios by means of rectal swabbing is not always possible. Laboratory procedure in isolating vibrios from a large number of specimens as in a survey of carriers is very costly in terms of manpower, transportation, media, chemicals and expendable supplies.

It was decided therefore to apply a serological method to see whether such a technique would be applicable to the study of cholera carriers. If a less costly serological method as used by Benenson, *et al*, (1968) could be successfully applied for the detection of cholera carriers in a community, then a large scale surveillance of cholera could be undertaken with little expenses, which a country with limited budgetary resources like Thailand could better afford.

In the present study, the examination of plasma for vibriocidal antibody was conducted in a selected community throughout the year.

This serological test was also carried out in contact carriers, blood donors and patients with non-choleraic diarrhea, to see if such a test would lead to a detection of inapparent cholera infection in an endemic area for cholera in Thailand.

MATERIALS AND METHODS

Monthly determination of vibriocidal antibody in the blood was carried out in 85 villagers in Damnoen Saduak District (Community K), Ratchaburi Province from December 1968 to November 1969. These people had not been vaccinated against either cholera or against any other communicable diseases.

Another study was conducted in a Home for the Infirm, Phra Pradaeng District, 30 kilometers south of Bangkok. The study period lasted from December 1968 to March 1969. In December, two cholera cases were observed in House No. 2, in which there were 120 male inmates. The general health condition of these people were very poor, many of them having skin infections of one form or another. Some of them were of psychopathic personality and had been sent there by the police because of misdemeanours. Out of the 8 remaining houses in this settlement, House No. 9 was selected for comparative study. There were approximately 60 men in apparently good health in this house and were much more co-operative than those in House No. 2 in allowing their blood samples to be taken.

Blood samples were taken by the finger prick method. A volume of 0.05 ml. was collected in microcapillary tubes (Drummond

† The work was partly supported by the World Health Organization and partly by the National Research Council of Thailand.

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Scientific Co., Broomall, Pa) and diluted in 0.45 ml. of physiological saline to give 1:10 dilution of blood or 1:20 dilution of serum. The specimens were brought to the laboratory without chilling. Preliminary study had shown that there was no significant difference in the vibriocidal antibody titers between the sera kept at -20°C and the same sera diluted 1:10 in physiological saline and kept at 32°C for 12 and 24 hours.

Vibriocidal antibody titer was determined by the micromethod as described by Benenson, Saad and Mosley (1968), using *Vibrio cholerae Inaba* strain CRL 22463 and *V. cholerae Ogawa* strain CRL 465 as the test organism.

In addition, the following sera were used

1. Ten paired samples from cholera patients taken at an interval of 7 days.
2. Ninety seven samples from 83 cholera patients, in fourteen of whom, the samples were taken twice at the interval of less than 7 days.
3. Two hundred and seventy patients with non-choleraic diarrhea, 74 of whom did not have cholera vaccination in the past 6 months, and in the remaining 196

patients, it was not definitely known if they have had cholera vaccination or not. All these patients were admitted to the Bamrasnaradura Hospital.

4. Three hundred and fifteen blood donors at the Red Cross Institute from whom blood were taken between October 1969 and January 1970, during which period vaccination against cholera had not been imposed by the Ministry of Health.
5. Eleven contact carriers in whom *V. cholerae El Tor Inaba* were isolated.

Samples of the first five groups were brought to the laboratory as serum, and the titer determined was finally multiplied by two for convenience to give a titer comparable to that of the whole blood.

Samples from group 5 were collected by the finger prick method.

RESULTS

1. Vibriocidal antibody in the blood of the villagers of Community K.

Results of vibriocidal antibody titer in each month from December 1968 to November

Table 1a

Showing monthly determination of vibriocidal antibody titer (anti-Inaba) among the villagers of community K.

Month	< 1:20	1:20-1:160	1:320-1:2560	> 1:2560	Total	Number not tested	% Villagers with titers more than 1:160
Dec. 68	22	44	5	-	71	14	7.0
Jan. 69	21	41	6	1	74	11	9.5
Feb.	17	43	13	1	74	11	18.9
March	20	46	5	-	71	15	7.0
April	20	50	10	-	80	5	12.5
May	20	44	12	-	76	9	15.8
June	17	41	18	-	76	9	23.7
July	18	48	11	-	77	8	14.3
Aug.	16	30	21	1	68	17	32.4
Sept.	15	34	15	1	65	20	24.6
Oct.	15	61	4	-	72	13	5.6
Nov.	16	50	3	-	69	16	4.3

Table 1b

Showing monthly determination of vibriocidal antibody titer (anti-Ogawa) among the villagers of community K.

Month	< 1:20	1:20-1:160	1:320-1:2560	> 1:2560	Total	Number not tested	%Villagers with titers more than 1:160
Dec. 68	18	49	4	-	71	14	5.6
Jan. 69	22	44	4	-	70	15	5.6
Feb.	15	41	15	1	72	13	22.2
March	16	41	7	-	64	21	10.9
April	18	52	10	-	80	5	12.5
May	21	49	6	-	76	9	7.9
June	16	47	13	-	76	9	17.1
July	17	53	7	-	77	8	9.1
Aug.	16	36	16	-	68	17	23.5
Sept.	17	33	15	-	65	20	23.1
Oct.	16	44	12	-	72	13	16.7
Nov.	13	47	9	-	69	16	13.0

1969 are shown in Table 1a & b. It was found that while 60 villagers did not show any significant change in antibody titer during the period of observation, 25 individuals (29.4%) showed definitely more than a 4-fold rise of antibody titer. Of particular interest is the increase in the number of individuals having titers more than 1:160 that formed three distinct peaks in February, in June and in the two month period August and September (Fig.1). From the results, it was apparent that there was no significant difference between anti-Inaba and anti-Ogawa titers.

It was also found that among individuals, whose blood showed more than a 4-fold rise in antibody titers, some of them were members of the same family and appeared to have a similar chronological sequence of antibody response. (Fig. 2a, b, c, d). In another 2 families, (22k & 23k), the antibody response in each member of the family appeared to follow a similar trend but was less pronounced. (Fig. 2e, 2f).

2. Vibriocidal antibody in the blood of the infirm.

The results are presented in Table 2. It was shown that the percentage with titers of more than 1:160 was higher among the contact carriers than among their companions living in the same or separate houses.

Follow-up study on 11 contact carriers over the next four months showed that antibody titers declined in 5 carriers, rose in 4 others and 2 showed practically no change. (Fig.2). The other carrier escaped from the home, and consequently follow-up could not be done.

3. Vibriocidal antibody in patients with cholera.

Vibriocidal antibody titers in 97 samples from 83 patients showed large variations. Nevertheless there appeared to be a certain degree of correlation between the titer and the time at which samples were taken (Table 3).

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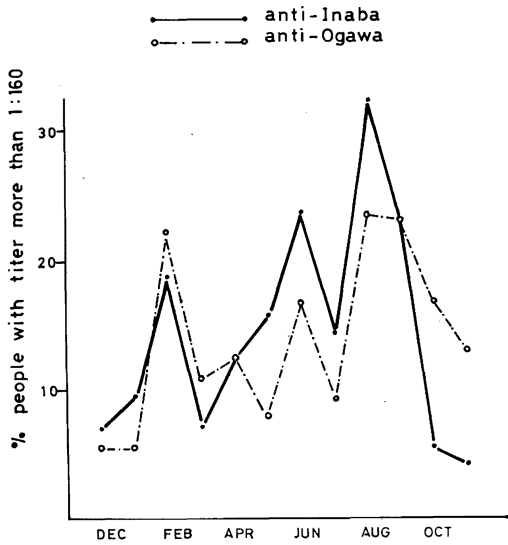


Fig. 1—Showing percentage of villagers in the Community K with vibriocidal antibody titers more than 1:160 from December 1968 - November 1969.

Hence, only 5/40 such samples (12.5%) taken in the first 3 days after the onset of diarrhea were shown to have titers of more than 1:160. In contrast, 37/57 samples (64.9%) taken after the 3rd day of the onset of diarrhea had titers of more than 1:160.

Vibriocidal antibody titers in 10 paired serum samples are presented in Table 4. All but one showed definitely a fourfold or greater rise in antibody titer.

4. Vibriocidal antibody in contact carriers, in patients with non-choleraic diarrhea and in blood donors.

The results are given in Table 5. In one other contact carrier (not included in the table), paired samples were obtained on Jan. 27 and Feb. 7, 1969. The titer rose from 1:40 in the first sample to 1:10240 in the second sample. When only single specimens were tested, 3 out of 10 contact carriers had

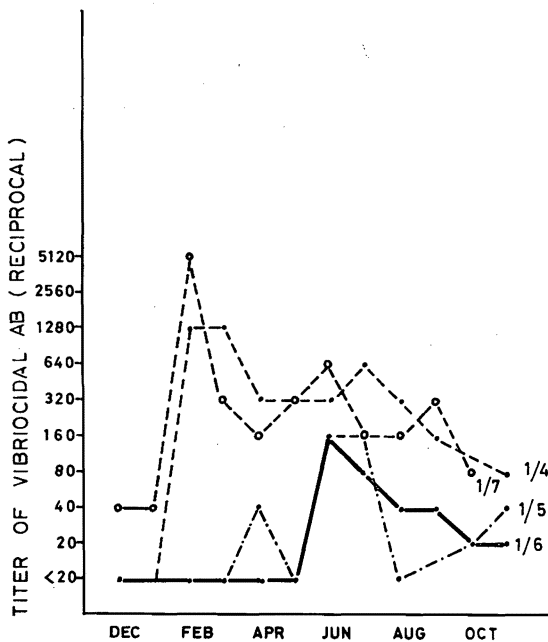


Fig. 2a—Showing vibriocidal antibody titers in blood of 4 persons in the family 1 K.

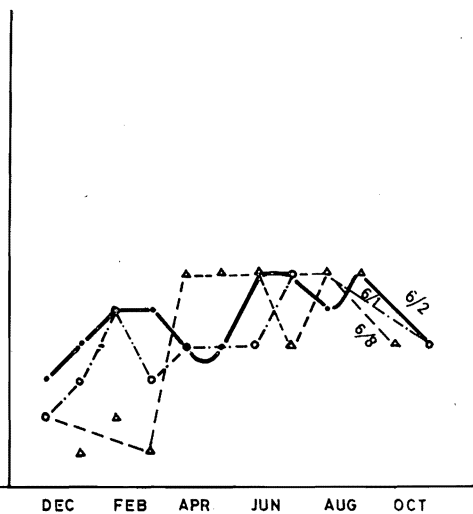


Fig. 2b—Showing vibriocidal antibody titers in blood of 3 persons in the family 6 K.

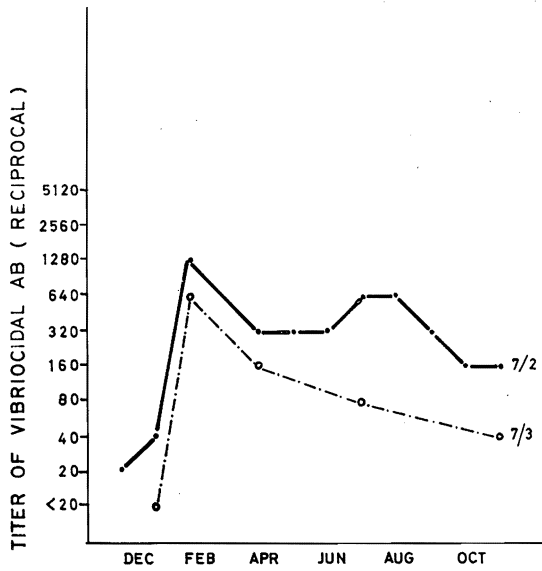


Fig. 2c—Showing vibriocidal antibody titers in blood of 2 persons in the family 7K.

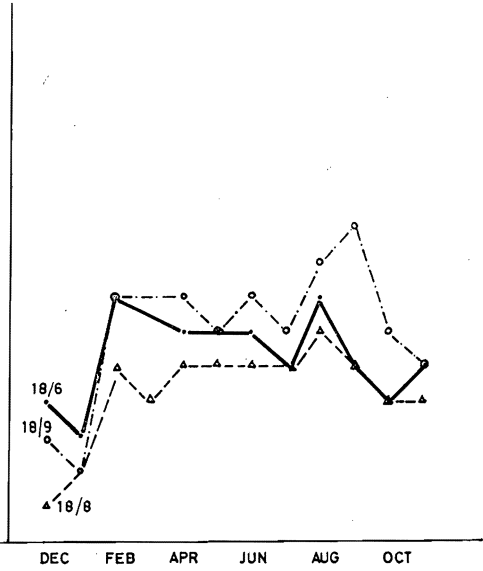


Fig. 2d—Showing vibriocidal antibody titers in blood of persons in the family 18 K.

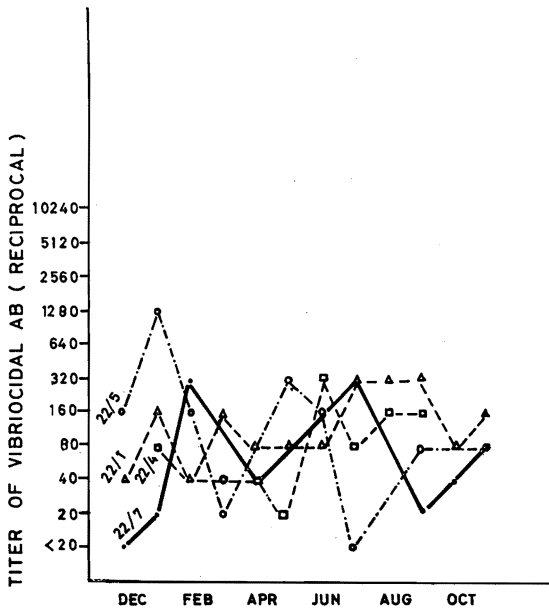


Fig. 2e—Showing vibriocidal antibody titers in blood of 4 persons in the family 22 k.

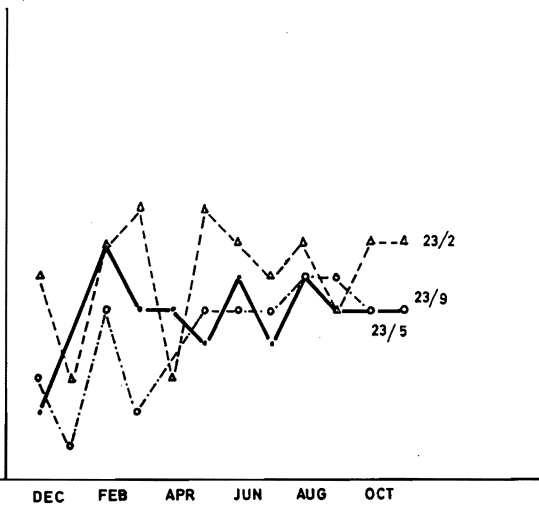


Fig. 2f—Showing vibriocidal antibody titers in blood of 4 persons in the family 23 K.

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Table 2

Vibriocidal antibody (anti-Inaba) among the inhabitants of the Home for the Infirm, Prapadaeng.

	< 1:20	1:20-1:160	1:320-1:2560	> 1:2560	Total	%people with titers more than 1:160
Contact carriers in House No. 2	-	2	6	4	12	83.3
Infirm in House No. 2	1	39	38	3	81	50.6
Infirm in House No. 9	-	20	25	13	58	65.5

Table 3

Showing vibriocidal antibody titer (anti-Inaba) among cholera patients in relation to day of onset of diarrhea.

Day after onset of diarrhea	< 1:20	1:20-1:160	1:320-1:2560	> 1:2560
0	3	5	3	-
1	1	7	-	-
2	1	11	-	-
3	1	6	2	-
4	-	8	11	1
5	2	4	8	-
6	-	6	6	-
7	-	-	2	1
8	-	-	2	1
9	-	-	-	-
10	-	-	1	-
10	-	-	4	-

Table 4

Showing vibriocidal antibody titers (anti-Inaba) in paired sera of cholera patients taken at the interval of 7 days.

No.	Titer of the first specimen	Titer of the second specimen
1	1:20	1:1280
2	< 1:20	1:640
3	< 1:20	1:2560
4	< 1:20	1:10240
5	1:640	1:640
6	1:80	1:640
7	1:160	1:640
8	1:640	1:20480
9	1:80	1:5120
10	< 1:20	1:160

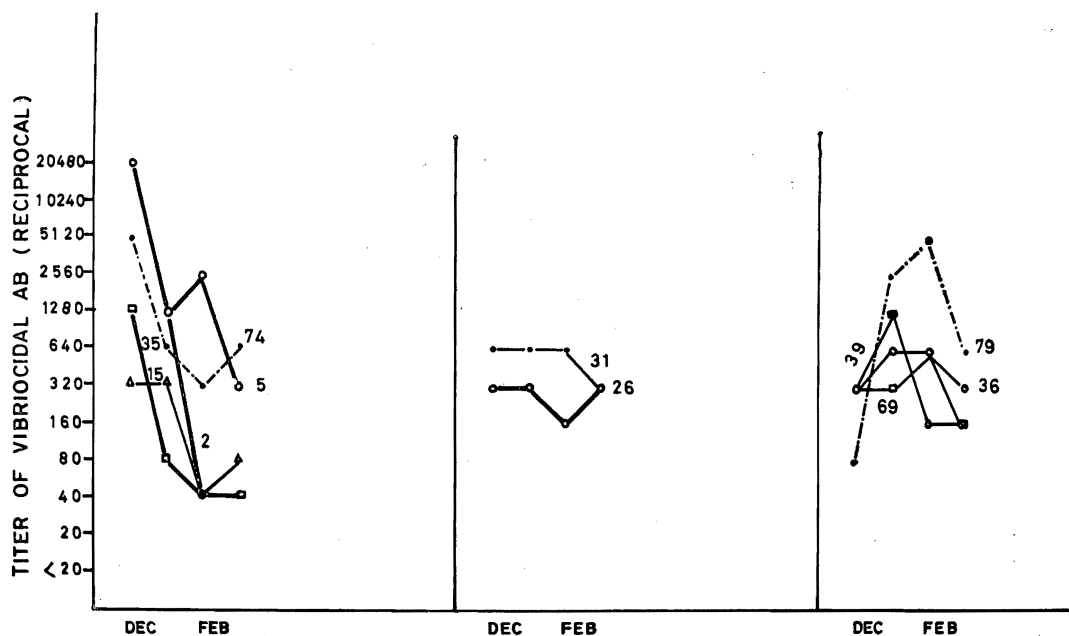


Fig. 3—Follow up of vibriocidal antibody titers in contact carriers in the Home for the Infirm (From December 1968 to March 1969).

titers of more than 1:160. It should be noted that vibriocidal antibody titer was also high

in blood donors and in patients with non-choleraic diarrhea.

Table 5

Showing vibriocidal antibody titers(anti-Inaba)among contact carriers, patients with non-choleraic diarrhea and blood donors (single specimen).

	< 1:20	1:20-1:160	1:320-1:2560	> 1:2560	Total	%people with titers over 1:160
Contact carrier	1	6	2	1	10	30.0
Patients with non-choleraic diarrhea						
a. No. vaccination in the past 6 months	13	49	12	-	74	16.2
b. No. definite history of vaccination	28	128	40	-	196	20.4
Blood donors	4	210	101	-	315	32.0

DISCUSSION

Mosley and co-workers (1968) followed serological responses in household cholera contacts at intervals of 10 days. It was shown that 23 out of 30 contacts without diarrhea (76.7%) had more than a fourfold rise in vibriocidal antibody titers. This increase of antibody titer was also found in 8 out of 9 contacts with negative multiple rectal swab cultures, and it was believed that inapparent cholera infections were likely to be the cause of this conversion.

A serological follow-up in community K showed that the vibriocidal antibody titer was increased more than fourfold above normal in 25 villagers during the study period while that in the remaining villagers did not change. These increases in antibody titers appeared to occur three times during the year. The reasons for this fluctuation were not known with certainty. It is possible that inapparent cholera infections were responsible as suggested by Mosley *et al.* (1968). Alternatively, this could be due to infections by other organisms such as *Proteus* and *Escherichia coli* sharing common antigen or antigens with cholera vibrios (Pollitzer, 1959). If the first contention was true, it could then be assumed that during the inter-epidemic period, the cholera organisms might have circulated freely among the population, but their infectivity was so mild as to cause no clinical illnesses. This idea is further strengthened by our own finding (Savanat *et al.*, 1970) that during the inter-epidemic period, *Vibrio cholerae El Tor* was found in 3 villagers, 2 in one community and 1 in another community.

Determination of vibriocidal antibody alone appears to be unsatisfactory in the detection of cholera carriers when a single specimen is used, since the antibody titers among the Thai are normally very high (Table 5), even among the blood donors. This finding could also be interpreted to indicate

endemicity of cholera vibrios in Thailand.

Whilst vibriocidal test on single specimens seems to be of limited usefulness, such a test on paired samples taken at an interval of approximately 10 days was promising either as confirmation of the disease in patients with cholera (Table 4) or in the detection of carriers (Mosley *et al.*, 1968). If such a test were applied in the epidemiological study on cholera during the inter-epidemic period, a sharp increase in vibriocidal antibody titer in the second specimen of an individual would prompt the epidemiologist to actively search for the vibrio regardless of the frequent negative rectal swab cultures.

SUMMARY

A serological survey of cholera was carried out from December 1968 to November 1969 in a village in a rural area with 85 inhabitants. It was found that 25 villagers (29.4%) showed more than a fourfold fluctuations in blood vibriocidal antibody titers, whilst the titers in the remaining 60 villagers showed no significant changes. As a whole, the percentage of the population studied with titers more than 1:160 was high in the months of February, June, and the period of August and September and when it formed 3 distinct peaks. This fluctuation of antibody titer is taken as an indication of the continued circulation of cholera vibrios in the community.

Serological examination of single specimens of blood was found to be unsatisfactory for the detection of inapparent infection in a country like Thailand, where cholera is endemic and where the antibody titer is very high even in blood donors and in people having non-choleraic diarrhea.

ACKNOWLEDGEMENTS

We are indebted to Dr. Y. Zinnaka, Dr. Z. Bencic and Dr. D. Barua for their guidance,

encouragement and criticism during the course of this study. Our thanks are due to the World Health Organization and the National Research Council of Thailand for the financial support. Thanks also to Dr. Sommai Sri-ngarm of the Thai Red Cross Institute for the generous supply of sera.

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