STUDY ON CHOLERA CARRIERS IN THAILAND†

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INTRODUCTION

In 1958-1959, an epidemic of cholera occurred in Thailand for which classical vibrios were mainly responsible. The epidemic then waned, though sporadic occurrence of cholera was recorded in the following year (Morgan, *et al.*, 1960), and with the exception of focal epidemic of non-fatal cholera *El Tor* in Ubol in 1961 (Felsenfeld, *et al.*, 1961), no further case was reported until June 1963. The June 1963 outbreak was due to *Vibrio cholerae El Tor* and since then this organism has replaced the classical vibrios and has established firmly its endemicity in Thailand. One of the factors believed to be responsible for the endemicity of *El Tor* cholera is the presence in communities of carriers, in whom the organisms can survive for extended period varying from a few days to a few months (Dizon, *et al.*, 1963; Wallace, *et al.*, 1967; Sinha, *et al.*, 1967). In an exceptional case e.g. that of cholera *Dolores*, the organism was found to be present in the host for more than 4 years (Azurin, *et al.*, 1967). The most likely organ which harbours the organism is the gall bladder (Wallace *et al.*,1967; Paguio and Pesigan, 1966). The prevalence rate of *El Tor* carriers among the contacts was shown by Dizon *et al.* (1967) to be as high as 21.7% whereas in the general population it was only 0.34%.

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The objective of the current study was to determine the incidence and seasonal variations of *Vibrio cholerae El Tor* in selected communities throughout the year and to find out whether there was any relationship between the carrier rate and the actual number of cholera cases. It was hoped to establish an index, which might serve during the inter-epidemic period as a guide-line to predict the possible forthcoming outbreaks of cholera when the number of the carriers detected exceeded the index figure.

MATERIALS AND METHODS

Place of study

Three villages in rural areas in Thailand were selected for study during the period from April 17, 1967 to September 1968.

1. Srisurat Village, Ratchaburi Provicne (Community A)with a population of 500.
2. Donmanorah Village, Samut Songkhram Province (Community B) with a population of 120.
3. Pangpuay Village, Ratchaburi Province (Community K) with a population of 85.

Living conditions of the people and the sanitation in these villages were extremely poor. The main source of water supply was derived from the canals passing through the areas. The people used canal water for

drinking and other domestic purposes. Such conditions were theoretically highly favorable for the epidemic spread of cholera.

In addition, 9 other communities (C,D,E, F,G,H,I,J and X) in which 10 cholera cases were notified were also studied during the first 4 month-period.

Another area, Arjnarong Canton, a suburban slum area of Bangkok with approximately 20,000 inhabitants was studied for a period of 5 months beginning in September 1967.

Collection of specimens

Rectal swabs were taken from individuals in the communities at various time intervals depending on the discovery of cholera vibrios in the area. When cholera vibrios were present, rectal swabs were taken weekly until all swabs from people in the community became negative for 2 consecutive weeks. Thereafter, two more swabs were taken at fortnightly intervals and if still negative, swabs were then taken only once every month, until isolation of cholera vibrios became positive again. This was then automatically followed by a regular weekly swabbing of all the people in that community.

Rectal swabs were dipped in 25 ml. screw capped bottles containing 5 ml. of alkaline peptone water, pH 8.6 and then carried to the laboratory. The swabs usually arrived late at night and were left at room temperature (28-34°C) and processed in the morning.

Specimens from Arjnarong Canton were collected either as rectal swabs or as fresh stools.

Laboratory work

Specimens in alkaline peptone water were processed by 2 stages, i.e. primary and secondary plating on Aronson medium or on TCBS agar (Eiken) and on meat extract agar (MEA). For the secondary plating, a sample of the alkaline peptone water, of approximately 0.1 ml., was inoculated into the same medium, incubated at 37°C for 6 hours and then plated. For stool specimens, an aliquot was first inoculated into alkaline peptone water and then incubated overnight at 37°C. This was then processed by 2 stages as with the rectal swabs. The plates were incubated at 37°C overnight, and thereafter examined by direct visual observation (Aronson and TCBS agar) or by oblique light under a stereomicroscope (MEA plates) as described by Finkelstein and Gomez (1963). Suspected colonies were picked up and subjected to slide agglutination with polyvalent anticholera serum. If positive, the colony was again tested by monospecific antisera, followed by subsequent inoculation into Kligler agar slant (KIA). In case the colonies were not discretely isolated but confluent bacterial growth gave agglutination with polyvalent antiserum, the culture was reprocessed, either by replating on MEA medium or by reinoculation into fresh alkaline peptone water and then replated on MEA medium.

Colonies in KIA were subjected to confirmatory slide agglutination and if still positive were tested for the following reactions.

1. Sugar fermentation, sucrose, mannose and arabinose
2. Indol test
3. Voges-Proskauer (VP) test
4. Haemagglutination of 2.5 % chicken red blood cell
5. Sensitivity to cholera phage 4 of Mukerjee
6. Sensitivity to polymyxin B
7. Production of kappa type phage.

The details concerning these tests, except item 7 were reported elsewhere (WHO/BD/cholera/67:12cholera, Bacteriological Diagnosis). For item 7, the method of Takeya and Shimodori (1963) was used.

Examination of water

One volume of 10 per cent peptone water pH 8.6 was mixed with nine volume of water, so that the final peptone concentration was 1 per cent. The subsequent procedure for isolation of vibrios was the same as that described for rectal swabs.

RESULTS

1. Isolation of *V. cholerae* from three selected communities A, B & K.

Results of the survey of cholera carriers in communitiy A are shown in Fig. 1, and Table 1. During the period of surveillance from April 11, 1967 to the end of September 1968, 42 organisms were isolated from 40 healthy individuals. Two persons were found positive twice. One of them, a man aged 76 was positive on March 5 and April 17, 1967, and another a woman of 37 was positive on April 21, 1967 and on March 26, 1968. The majority of the organisms isolated (37) were detected in April, during which time 3 cholera cases were found. Two of the carriers were members of the same family as one of the index cases. There were 5 persons in this family, and hence the carrier rate in this family was 40%. Another carrier was a member of a family of 12, in which another index case was found. Hence the carrier rate in this family was 8.3%. The overall carrier rate in this community in April was 7.4%. During May 1967 and September 1968, there were no more cholera cases, and the number of carriers declined sharply to 3 in March 1967 and thereafter became undetectable until February and March 1968, when during each month one carrier was detected.

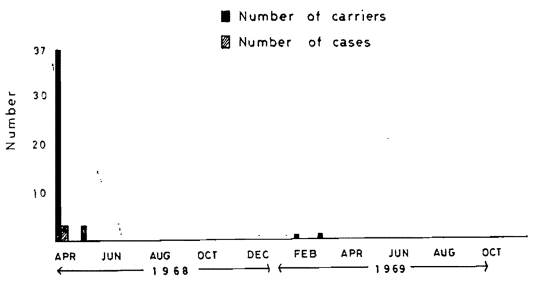

Fig. 1 — Incidence of cholera carriers in community A during April 1968 - September 1969.

In community B, a total of 2,656 specimens were examined during May 1967 and September 1968 but only one carrier was detected and this was in December 1967. During this period, no cholera case was found.

In community K, a survey of cholera carriers was carried out from July 1967 to September 1968, during which time 4504 samples were examined. Six carriers were detected, three in July 1967 and three more in August 1967. Only one cholera case was found. Two carriers were members of the same family of 7 as the index case. The ratio of carriers to cholera cases in July was 4:1 and the carrier rates in the whole community in July and August 1967 were 5% and 3.7% respectively.

2. Isolation of *V. cholerae* in other communities.

A survey of cholera carriers was made during the period April 1967 and October 1967 in 9 other communities (C,D,E,F,G,H, I, J and X) where 10 cholera cases were found and 9 carriers were detected. The results are shown in Table 2. It should be noted that the 4 carriers in community X and the 3 carriers in community G were all in the same family as the index cholera case of that community.

In addition, examination of 1706 specimens from people in Arjnarong Canton during September 1967 and January 1968 yielded negative results.

3. Age distribution of the carriers.

The results are presented in Table 3.

Table 1

Isolation of *V.cholerae* from community A (with 500 inhabitants) during April 1967-September 1968.

Month	Total number of specimens taken **a**	Frequency of collections per month **b**	Number positive **c**	Biotype isolated		Number cholera cases	Ratio carrier to cholera case	% carrier in the community $\frac{c \times b}{a} \times 100$
				El Tor Ogawa	*El Tor Inaba*			
April (17-29th)	1042	8	37	-	37	3	37:3	7.4*
May	1835	4	3	-	3	-	-	0.65
June	1141	3	-	-	-	-	-	-
July	265	1	-	-	-	-	-	-
August	1097	3	-	-	-	-	-	-
September	686	2	-	-	-	-	-	-
October	680	2	-	-	-	-	-	-
November	694	2	-	-	-	-	-	-
December	702	2	-	-	-	-	-	-
January	707	2	-	-	-	-	-	-
February	989	3	1	-	1	-	-	0.3
March	1208	3	1	-	1	-	-	0.25
April	1195	3	-	-	-	-	-	-
May	454	1	-	-	-	-	-	-
June	470	1	-	-	-	-	-	-
July	470	1	-	-	-	-	-	-
August	499	2	-	-	-	-	-	-
September	474	1	-	-	-	-	-	-

* Since the number of people from whom rectal swabs were taken were rather selective in this month the percentage carrier was calculated using a formula, % carrier = $\frac{c \times 100}{500}$

Most of carriers were children. 28 out of 47 cases in communities A, B and K, and 6 out of 9 cases in other communities were under 10 years of age. The youngest was a girl of 2 months old. The ratio of male to female carriers in communities A, B and K was 1:1 whereas in other communities, it was 7:2.

4. Characteristics of the organisms isolated.

Altogether 58 vibrios were isolated from 56 carriers, two of whom were positive twice.

Only 18 organisms were completely studied. All gave strong agglutinating reactions with mono-specific anti-Inaba serum. Three of them, however, gave also weak agglutination with anti-Ogawa serum. They all belong to Heiberg group I, agglutinated 2.5% chicken red blood cells, were resistant to polymyxin B, and also resistant to lysis by Mukerjee phage IV. They liberated kappa type phages which lysed an indicator strain *V. cholerae H* 218. Indol and V.P. reactions were positive. On the basis of these reactions, the vibrios

Table 2

Isolation of *V. cholerae* from 9 other communities during April-October 1967.

Month	Community	Population	Total number of specimen examined a	Frequency of collections b	Number positive c	Biotype isolated		Number cholera of cases	Ratio of carrier to cholera case	%carrier in the community $\frac{c \times b}{a} \times 100$
						El Tor Ogawa	*El Tor Inaba*			
April	X	28	16	1	4	-	4	1	4:1	25.0
May	C	96	153	2	0	-	-	1	0:1	-
	D	212	311	2	0	-	-	1	0:1	-
	E	45	37	1	0	-	-	1	0:1	-
	F	21	19	1	0	-	-	1	0:1	-
	G	108	86	1	3	-	3	1	3:1	3.5
June	G	108	101	1	0	-	-	-	-	-
	H	197	324	2	1	-	1	2	1:2	0.62
	I	248	214	1	1	-	1	2	1:2	0.47
July	I	248	179	1	-	-	-	-	-	-
	J	224	196	1	-	-	-	-	-	-
October	C	96	28	1	-	-	-	-	-	-

Table 3

Showing age group distribution of carriers.

Age Group	Communities A, B, K		Other communities		Total	
	Male	Female	Male	Female	Male	Female
1-5 mon.	-	1	-	1	-	2
6-11 ,,	2	-	-	-	2	-
1-4 yr.	8	3	2	-	10	3
5-9 ,,	7	7	3	-	10	7
10-19 ,,	1	7	2	1	3	8
20-29 ,,	2	2	-	-	2	2
30-39 ,,	-	2	-	-	-	2
40-49 ,,	1	-	-	-	1	-
50-59 ,,	1	1	-	-	1	1
60-69 ,,	1	-	-	-	1	-
70-79 ,,	1	-	-	-	1	-
	24	23	7	2	31	25

isolated would be classified as *V. cholerae El Tor, Inaba*, "Celebes" type.

Of the remaining organisms, twenty three were isolated in the laboratory of the Bamrasnaradura Disease Hospital between April 17-25, 1967, and were reported as *V. cholerae El Tor, Inaba* biotype. The remaining 17 organisms were lost during storage, before a complete study could be made. However, all gave strong agglutinating reactions with mono-specific anti-Inaba serum. Four of them gave, in addition, weak agglutination

with mono-specific anti-Ogawa serum. The organisms agglutinated 2.5% chicken red blood cells and belong to Heiberg group I. Indol reactions were all positive.

5. Isolation of *V. cholerae* from water.

During August 1967 and September 1968, water was collected from canals and drinking from communities A, B, K and Arjnarong Canton. A total of 174 water samples were cultured for *V. cholerae* but all were negative.

DISCUSSION

In this study on cholera carriers, as in other epidemiological studies, selection of appropriate communities was of prime importance. The community should consist of appropriate number of people who are willing to co-operate with the authorities concerned. The study should be undertaken continuously over a long period of time to allow for a meaningful assessment of the results. Such places should be prone to cholera outbreak by virtue of their unsanitary conditions, such as poor living conditions and inadequte supply of clean water.

The communities which met the above criteria were communities A, B and K, which were then selected for intensive study.

To avoid any confusion and to obviate any unnecessary semantic argument which may invariably arise from misunderstanding of the terminology, it is thought necessary to clarify the meaning of the word "carrier". According to the sense used in this study, a carrier refers to person who harbours *V. cholerae* in the bowel for a certain period of time without having any clinical manifestation of the disease i.e. diarrhea. Thus the carrier can be either a person who has recovered from the disease (convalescent carrier) or a person who has never developed clinical disease despite the presence of cholera vibrios in the bowel (asymptomatic carrier). The length of the carrier state may vary. Study on carriers of *cholera El Tor* in the Philippines showed that the organisms remained in the body for an average period of 10 days in the asymptomatic carrier and 51 days in the convalescent carrier (Dizon, *et al*, 1963). In Taiwan, 99% of 237 asymptomatic carriers became negative by the 9th day of the date of detection (Yen, 1964). The study of Sinha and co-workers (1967) in Calcutta, showed that the organisms persisted in the carriers from 2 to 45 days with the average of 15 days. Most interesting was the discovery of a long term carrier in a Philippine housewife, in whom the organisms existed for a period of not less than 4 years (Azurin *et al*, 1967). In the present study, all but 2 carriers became negative within a week. This was interpreted to mean that the carrier state among the Thais was of short duration. These carriers may be no more than persons who receive a sub-clinical form of infection. Among the two carriers whose excretion of vibrios lasted more than 7 days, one, a man aged 76, was positive on 2 occasions namely March 18 and April 7, 1967, and another, a woman aged 37 was positive on April 21, 1967 and once again in March 26, 1968. Since no vibrio excretion was found in these two individuals during the intervening period, despite regular rectal swabbing, it could be interpreted that they received re-infection, or alternatively they were cases of long term carrier. If the latter possibility is true, failure to isolate the organisms during the period of quiescence could be due to too small a number of the vibrios being excreted to be detected by the method used as demonstrated by Dizon *et al*, (1967), or that the organisms were lodged in the gall bladder and thus would become detectable only after receiving cholagogues or cathartics (Wallace *et al*, 1967). To solve this problem, it would have been necessary to prove the identity of the organisms isolated at the two different times,

by using a more sensitive method such as complete typing with Mukerjee phages. Unfortunately, this was not done.

Rapid disappearance of cholera vibrios from the carrier may be interpreted to mean that immunity in these individuals is developed very quickly to combat the invading organisms resulting in their complete elimination. Alternatively, this may be attributable to antibiotics, since all but one of the carriers received tetracycline hydrochloride orally at a dosage of 30-50 mg/kg per day for 2 consecutive days. However, antibiotics alone were unlikely to be the sole factor, because the rectal swab of one carrier (82/7B) was negative when tested a week after it was previously proved positive, though no antibiotics or chemotherapeutic agents were given.

Another interesting finding which emerges as a result of this study is the apparent correlation between the carrier rate and the number of the actual cholera cases. In community A, for instance, the ratio of carriers to cholera cases was as high as 37:3 in April, while the carrier rate was 7.4. Continued observation showed a sharp drop of the carrier rate to 0.65% in May and thereafter to zero. Coinciding with this drop in the carrier rate, was the disappearance of cholera cases. Similar findings were apparent in communities K & G. The carrier rate among the contacts who lived in the same house as the cholera case was very high, i, e. 50% in one family of 5 in community A, 44% in a family of 10 in community X, and 25% in a family of 9 in community G. In the series of Dizon *et al.* (1967) and Forbes *et al.* (1968), the percentage of carriers among the household contacts was only 21.7% and 6.4% respectively.

The whole point of the work was to try to establish an index figure to predict a forthcoming outbreak of cholera. Unfortunately, the absence of an epidemic of cholera during the period of study made it impossible to establish such an index to express the percentage number of carriers in the community.

SUMMARY

A survey of asymptomatic cholera carriers was made in selected communities in rural and urban areas in Thailand where cholera outbreaks had recently occurred. *Vibrio cholerae El Tor, Inaba* was isolated from 56 healthy persons, 2 of whom were positive twice. Thirtyfour carriers were children under 10 years of age. When the outbreak was at its peak the carrier rate was 7.4% in one community (community A), but this rate quickly declined and after a few months no carriers were found. During the inter-epidemic period, 2 carriers were detected in community A and one in community B indicating that sub-clinical infection was still circulating in the community. The carrier rate was high among the household contacts. Most of the individual carriers were shown to harbour the organisms no longer than a week. Owing to the relative quiescence of cholera during the period studied, it was not possible to establish an index figure based on the percentage of carriers during the inter-epidemic period that could be used to predict the size of a forthcoming cholera outbreak.

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