

3. Title: Asymptomatic Cholera Carrier Survey in an Urban Slum Area

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Objective To determine the rate of asymptomatic cholera carriers in a high risk population and to get information about the carriers.

Description In January 1968, Dr. Asoka Sunthornsaratul, Bureau of Public Health, Bangkok Municipality requested that this department collaborate in a study of a slum area of Makkasan in metropolitan Bangkok. This area of about 10,000 residents contains about 2,000 small houses in approximately 0.2 km<sup>2</sup>. Most of heads of families are laborers and tricycle taxi drivers. Because of the low level of this area, there is always undrained water under the houses and raised planks serve as sidewalks. Municipal water is available but few houses have spigots. Occupants of these houses sell water to their neighbors and distribute the water by means of nylon hoses to their neighbors' jars. The sewage disposal system used by all families is concrete slab latrines connected to septic tanks but young children frequently defecate on the ground. There was one case of cholera in this area in January 1968.

Teams of Bangkok Municipal health workers were sent to take census and simultaneously to distribute stool specimen cups to residents who were asked to collect stool defecated after midnight. The next morning the teams collected the cups, coded them, and delivered them to this laboratory before noon each day. Within one hour of arrival swabs of stools were placed in alkaline peptone broth and incubated at 37 C. After 4-5 hours of incubation, the alkaline peptone broths were streaked on MEA and alkaline lauryl sulphate tellurite (T) plates; reincubated overnight, and again streaked on T plates. Colonies on MEA plates were examined under a stereoscopic microscope using transmitted illumination. Suspect colonies on MEA and T plates were tested for agglutination with V. cholerae "O" antiserum by the slide test, and concurrently picked to KIA slants. Cultures which produced an alkaline slant, acid butt and no H<sub>2</sub>S on KIA were tested for motility and fermentation of mannitol, sucrose, mannose and arabinose; production of indole, and liquefaction of gelatin. Later in the study water samples were collected from the spigot hoses or storage jars for examination for total bacteria, coliforms and vibrios.

Progress The survey began on 30 January and was completed on 18 March 1968, a period during which there was no reported case of cholera in the area. About 30% of the population cooperated in the stool specimen collection. Their age and sex distributions are shown in Table 8. No V. cholerae was isolated. There were 53 isolates of non-agglutinable (NAG) vibrios from 52 individuals. Age and sex distribution of NAG positive individuals are shown in Table 9. It was noted that there were no females positive for NAG vibrios in age groups 5-9, 10-14 and 15-19 years. The total number of positive males was double that of females. NAG vibrios were classified by the Heiberg system. Frequency distribution is shown in Table 10 which shows that 75% of the NAG vibrio isolates were in group I. There was only one instance of a NAG positive stool being watery. Stool cultures of NAG positive individuals were repeated one week later. Eight of 48 specimens received were positive for NAG vibrios but only 5 were in the same Heiberg group found initially.

Coliforms were found in 22 of 26 water samples tested with counts varying from 4.5/100 ml to 2400/100 ml. In addition one Heiberg group I and one Heiberg group V NAG were found in water samples taken from storage jars.

**Summary** A survey for asymptomatic cholera carriers was carried out in an urban slum area of Bangkok. Almost three thousand people participated. There was no reported case of cholera from the study area during that period and no agglutinable vibrio carrier was found. The incidence of NAG vibrio carriers was 1.79% in this population. The NAG vibrio most frequently isolated was of Heiberg group I. Although this area is supplied by chlorinated municipal water, 85% of the samples taken from storage jars in home was considered contaminated with fecal material. NAG vibrios were isolated from 2 water samples.

**References:**

1. Gohar, M.A. et al, *J. Trop. Med. & Hyg.*, 55: 241-245, 1952.
2. Bryson, V., and Szybalski, W., *Science*, 116: 45-51, 1952.
3. Powell, C.J., Jr., and Finkelstein, R.A., *J. Bact.* 87: (5) 1249-1250, 1964.
4. Felsenfeld, O., and Watanabe, Y., *U.S. Armed Forces Med. J.* 9: 975, 1958.
5. Finkelstein, R.A., and Gomez, C.Z., *Bull. Wld. Hlth. Org.* 28: 327-332, 1963.
6. Finkelstein, R.A., and Mukerjee, S., *Proc. Soc. Exp. Biol. & Med.* 112: 355-359, 1963.

Table 1. Comparison of Activity of Regular Purified Cholera with Disc Electrophoretically Purified Cholera

Test System	Dose (mcg)	Regular Purified Cholera	Disc Purified Cholera
Infant Rabbit	40	9/9*	4/4
	20	11/15	5/5
	10	5/12	2/2
	4	n.t.	0/3
Rabbit Loop	10	2.46**	2.6
	2	0.0	0.0
	0.4	0.0	0.0
Rabbit Skin	1.0	erythema only***	erythema only***
Ouchterlony	10.0	ppt.	ppt.

\* No. with choleraic manifestations/total

\*\* ml/cm of intestinal loop. Mean of 4 loops

\*\*\* The rabbits tested were apparently refractory to intra-dermal cholera

Table 2. Anti-bacterial Antibodies in Rabbits Immunized with Cholerae

A. Agglutinating Antibody (reciprocal of titer)

Rabbit No.	Pre-immunization	Post-immunization		
		3 wks	5 wks	8 wks
1*	<20	320	640	160
2**	<20	±@20	20	20
3*	<20	1280	1280	160
T-V 19*	<20	320	640	80
T-V 21*	<20	2560	2560	640

B. Vibriocidal antibody (Neg Log<sub>10</sub> of highest dilution killing 100% of inoculum)

Rabbit No.	Pre-immunization	Post-immunization		
		3 wks	5 wks	8 wks
1*	3	6	5	5
2**	4	5	5	5
3*	2	7	7	6
T-V 19*	2	5	5	5
T-V 21*	2	7	7	7

\* Immunized with regular purified cholerae

\*\* Immunized with disc electrophoretic purified cholerae

Table 3. Anti-cholera Antibodies in Rabbits Immunized with Cholera

A. Precipitating antibody with 25 ug cholera (reciprocal of dilution)

Rabbit No.	Post-immunization		
	3 wks	5 wks	8 wks
1*	8	8	16
2**	16	32	32
3*	8	32	32
T-V 19*	4	8	16
T-V 21*	8	16	128

B. Neutralization of cholera in infant rabbits (1:10 dilution of serum + 25 ug cholera). All 3 wk sera neutralized 25 ug cholera.

C. Passive protection of 5 wk sera for infant rabbits fed with cholera (1 ml of serum I.P. 18 hours before feeding of cholera)

Rabbit No.	No. diarrhea/total	Protection (%)	No. dead/total	Protection (%)
1*	2/2	0	2/2	0
2**	3/5	40	1/5	80
3*	1/3	67	1/3	67
T-V 19*	3/3	0	0/3	100
T-V 21*	0/3	100	0/3	100
Control (25 ug ch)	5/5		5/5	

D. Intradermal immunity

Rabbit No.	Day of Immunization	3 wks post
1*	0.25***	0.50
2**	0.06	0.50
3*	0.06	0.50
T-V 19*	0.002	0.50
T-V 21*	0.06	0.25

\* Immunized with regular purified cholera

\*\* Immunized with disc electrophoretic purified cholera

\*\*\* ug of cholera that produced positive reaction

Table 4. Cholerae and V. cholerae in Thiry-Vella Loops

Rabbit No.	Length of Loop (cms)	Volume* (ml)	Loop Secretion				Minimal skin response dose (mcg of cholerae)
			Na (in Eq/L)	K (in Eq/L)	Cl (in Eq/L)	CO <sub>2</sub> (in M/L)	
3	22.5	Control	0.6 (1h)	—	—	—	—
		Cholerae I	20.2	153.8	4.4	71	79.3
		Cholerae II	24.7	N.D.	N.D.	N.D.	N.D.
7	21	Control	2.0 (2h)	159	3.4	58	—
		Cholerae I	35.5 (17h)	155	4.4	59	—
		Cholerae II	38.2	148.7	5.1	67.1	78
		<u>V. cholerae</u>	34.7 (23h)	155	3.9	87	90.6
12	12	Control	2.3 (1h)	158	4.8	—	83.0
		Cholerae I	16.5	167.5	5.2	72.8	95.0
		Cholerae II	14.9	157.8	4.9	70.2	79.7
		<u>V. cholerae</u>	10.7	163.9	4.9	84	67.1
		2 wks pos <u>V. cholerae</u>	8.7	159.4	5.8	59.7	89.5
14	18	Control	7.6 (6h)	159.5	4.7	59.5	87.5
		Cholerae I	31.9	156	4.6	66.6	86.9
		Cholerae II	13.1	161.4	5.2	86.3	72.3
		<u>V. cholerae</u>	11.9	161.4	4.4	69.8	81.6
16	24.5	Control	13.1 (7h)	157.8	4.4	64.2	83.8
		Cholerae I	17.1	155.4	4.5	69.7	76.0
		Cholerae II**	9.1	156.9	4.3	66.3	66.0
		<u>V. cholerae</u> ***	1.7	152	4.0	53	85.8
19	20	Control	4.7	162.5	5.7	72.2	85.1
		Cholerae I	16.5	159.1	4.7	78.6	78
		Cholerae II	8.0	153.1	3.8	63.3	70.3

\* ml per 8 hours unless stated otherwise  
 \*\* The loop could not hold 5 ml of cholerae solution, had to reduce to 2.5 ml.  
 \*\*\* The loop could not hold any amount of fluid.

Table 5. Sensitivity of Enteric Bacteria to Entero-Vioform<sup>R\*</sup>

<u>Organism</u>	<u>Sensitive**</u>	<u>Slightly Sensitive***</u>	<u>Resistant</u>
<u>V. cholerae</u>	15	0	0
El Tor vibrio	22	0	0
NAG vibrio	1	0	0
<u>Salmonella</u>			
paratyphi A	0	0	1
paratyphi B	0	0	1
typhimurium	0	0	1
saint paul	0	0	1
newport	0	0	1
typhi	0	0	1
anatum	0	0	1
<u>Shigella</u>			
dysenteriae 1	1	0	0
dysenteriae 2	1	0	0
flexneri 1	1	0	0
flexneri 2	1	0	0
flexneri 3	1	0	0
flexneri 4	1	0	0
flexneri 6	1	0	0
boydii 4	1	0	0
sonnei 1	1	0	0
<u>E. coli</u>			
025:B 19	0	1	0
0128:B 12	0	1	0
0127:B 8	0	1	0
0119:B 14	0	1	0
0126:B 16	0	0	1
untyped	0	5	0
<u>Paracolon</u>			
aerogenoides	0	1	1
coliforme	0	1	1
intermediate	0	0	1
<u>Aerobacter</u>			
cloacae	0	0	1
aerogenes	0	0	4
<u>Proteus</u>			
vulgaris	0	0	1
mirabilis	0	0	2
morganii	0	0	1
<u>Pseudomonas</u>			
aeruginosa	0	2	2
pseudomallei	1	0	0
<u>Bethesda</u>	0	1	0
<u>Providencia</u>	0	5	0

\* Pieces of 250 mg tablets applied on the surface of streaked agar plates

\*\* Marked zones of inhibition ranging from 5 to 19 mm diameter

\*\*\* Very slight zones of inhibition in some cases obtained only with Sapsamine-containing tablets

Table 6. Effect of Quixaline<sup>R</sup> and Entero-Vioform<sup>R</sup> on Experimental Cholera in Infant Rabbits

Start of Treatment	Observations at times after inoculation of <i>V. cholerae</i>								
	22 hrs			26 hrs			46 hrs		
	Q	EV	Control	Q	EV	Control	Q	EV	Control
20 hours before Vibrio inoculation	0/2/6*	0/0/7	1/3/6	1/2/6	1/1/7	3/5/6	4/4/6	5/3/7**	6/6/6
6 hours before Vibrio inoculation	0/1/7	0/2/8	2/5/8	1/3/7	2/4/8	6/8/8	N.O.***	N.O.	N.O.
1 hour after Vibrio inoculation	0/2/6	0/2/8	0/3/7	0/3/6	1/4/8	3/5/7	4/6/6	7/7/8	7/7/7

- \* No. dead/diarrhea/total tested
- \*\* Two animals died before diarrhea started
- \*\*\* Not observed

Table 7. Effect of Iodochlorohydroxyquinoline on Vibrio Excretion of Cholera Patients

Treatment Group	Total Patients	Patients Excreting Vibrios through Day								
		1	2	3	4	5	6	7	8	9
A*	15	1	2	2	2	3	3	0	2	0
B**	8	0	0	0	0	2	3	0	1	2

- \* Iodochlorohydroxyquinoline (Entero-Vioform, Ciba) 250 mg q 4 h
- \*\* Placebo (starch) 1 cap q 4 h

Table 8. Age and Sex Distribution of Participants in Asymptomatic Cholera Carrier Survey, Makkasan Slum, Bangkok

Age group (year)	Male		Female		Both Sexes	
	No.	% of total	No.	% of total	No.	% of total
0-1	63	2.12	37	1.25	100	3.37
1-4	253	8.51	222	7.47	475	15.98
5-9	254	8.55	266	8.95	520	17.50
10-14	262	8.82	193	6.49	455	15.31
15-19	91	3.06	111	3.74	202	6.80
20-29	137	4.61	221	7.43	358	12.04
30-39	193	6.49	219	7.37	412	13.86
40-49	123	4.14	111	3.73	234	7.87
50-59	58	1.96	67	2.25	125	4.21
60 and over	33	1.11	58	1.95	91	3.06
Total	1467	49.37	1505	50.63	2972	100.00

Table 9. NAG Vibrio by Age and Sex, Makkasan Slum Survey 30 January-18 March 1968

Age group (year)	Male		Female	
	No. participant	No. NAG positive	No. participant	No. NAG positive
0-1	63	2	37	1
1-4	253	4	222	4
5-9	254	4	266	0
10-14	262	6	193	0
15-19	91	6	111	0
20-29	137	4	221	3
30-39	193	5	219	5
40-49	123	4	111	1
50-59	58	0	67	1
60 and over	33	1	58	2
Total	1467	36	1505	17

Table 10. Frequency Distribution of NAG Vibrio Isolates, Makkasan Slum Survey  
30 January— 18 March 1968

Heiberg groups	No. Isolates	Percent
I	40	75.47
II	2	3.78
III	5	9.43
IV	1	1.89
V	5	9.43
VI	0	0.00
<b>Total</b>	<b>53</b>	<b>100.00</b>