Isolation of *Vibrio cholerae* from Nightsoil During Epidemics of Classical and El Tor Cholera in East Pakistan*

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A clear difference has been observed between the classical Inaba V. cholerae and the El Tor Ogawa V. cholerae in relation to the ability to isolate the organism from the environment.

An early attempt to utilize nightsoil sampling as a tool to measure the extent of infection in the community during an epidemic of classical Inaba cholera in Dacca, East Pakistan, in the spring and fall of 1968 proved unsuccessful. During an epidemic caused by both the classical Inaba and the El Tor Ogawa vibrios in Chittagong between July 1968 and March 1969 the reasons for this failure became apparent. In Dacca, only 2 isolations of classical Inaba were made from 9906 individual latrine and pooled communal nightsoil samples, whereas in Chittagong, from 62,588 similar samples in which 2 classical Inaba isolations were also made, there were 52 El Tor Ogawa isolations. In areas where cases due to both biotypes were occurring simultaneously, El Tor Ogawa vibrios were isolated 10 times more frequently than the classical Inaba.

It remains unclear whether the differences observed between El Tor Ogawa and classical Inaba are related to the biotype or to the serotype of the organism, or to both. An extrapolation of nightsoil sampling, therefore, to the incidence and prevalence of infection in a community must consider both the biotype and the serotype.

Since 1961 (Van de Linde & Forbes, 1965; Forbes et al., 1967; Sinha et al., 1967), nightsoil and latrine sampling has been shown to be useful in assessing the presence and extent of El Tor Ogawa *Vibrio cholerae* in the community. Based on these reports, individual latrine and pooled communal nightsoil sampling was established in Dacca, East Pakistan, in 1968 during an epidemic of classical Inaba cholera, but without success. In the summer of 1968, epidemics caused by both classical Inaba and El Tor Ogawa *V. cholerae* appeared in Chittagong simultaneously, providing an opportunity for renewed efforts to test and extend this epidemiological tool. Both methods—individual latrine and pooled communal nightsoil sampling—were found to be very useful for detecting El Tor Ogawa infections but only of limited value in assessing the extent of infection in the community due to classical Inaba vibrios. These observed differences in ability to recover the classical Inaba and El Tor Ogawa vibrios from individual latrines and pooled communal nightsoil indicate a major biological distinction between the two biotypes which has broad epidemiological significance.
METHODS

Hospital surveillance

Dacca. Between January and December 1968, daily rectal swab cultures were obtained from all patients with acute gastroenteritis admitted to the Pakistan–SEATO Cholera Research Laboratory hospital (PSCRL). Swabs were immediately streaked on tellurite taurocholate gelatin agar (TTGA) (Monsur, 1963) and gelatin agar and then placed in 5 ml of tellurite bile peptone and delivered to the bacteriology laboratory.

Chittagong. The 5 hospitals which admit cases of acute gastroenteritis were visited daily and a rectal swab culture was taken from each patient. Swabs were immediately streaked on TTGA media, placed in 5 ml of bile peptone and delivered to the bacteriology laboratory.

Nightsoil surveillance

Dacca. In Dacca, three communities, Rajnaryandhar, Goalnagar and Rayer Bazar, having a total population of approximately 7000, were chosen for daily individual latrine sampling. Cases of cholera had occurred with high frequency in these communities during previous epidemics. Daily nightsoil sampling was also undertaken in the old city of Dacca at 11 nightsoil dumping centres. The population served by these dumping centres was approximately 50000. Sampling of the community areas and the dumping centres was undertaken on alternate days during the spring and fall epidemics of 1968. Of the 11 dumping centres, 6 were sampled only during the fall epidemic.

Chittagong. In Chittagong, daily nightsoil sampling was undertaken at 13 dumping centres for 10 consecutive months. The population served by these dumping stations is approximately 250000. In addition, individual latrine sampling was carried out every fifth day in two communities, Wireless and Port Trust Colonies, with a total population of approximately 15000.

FIG. 1
AREAS OF NIGHTSOIL COLLECTION, DACCA

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# Diagram

- **A** Dumping centre
- **ZZ** Area served by dumping centres
- **B** Area studied by individual latrine sampling

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*Further details and annotations for the diagram are not provided in the text.*
Collection of nightsoil specimens

The Conservancy system. Individual house latrines at fixed points on a route are emptied daily or on alternate days by a male sweeper employed by the municipality; he empties household latrine pails into a 60-gal (ca 270-litre) push cart or into 5-gal (ca 22-litre) buckets.

Dacca. Each bucket or cart brought by a sweeper to a dumping station for discharge was sampled by taking 10 ml of nightsoil and adding it to 90 ml of tellurite bile peptone and these were delivered daily to the PSCRL bacteriology laboratory. Sampling of individual latrines in the three communities under study was carried out in the same fashion.

Chittagong. Each bucket or cart brought by a sweeper to a dumping centre for discharge was sampled by dipping a swab into the nightsoil (the average size of the nightsoil samples was about 1 g). The swabs were immediately placed in 2 ml of sea salt transport medium (Venkatraman et al., 1941) and delivered daily to the bacteriology laboratory. Sampling of individual latrines in the community under study was carried out in the same fashion.

Bacteriological methods

On arrival at the laboratory the sea salt and swab were transferred together to tellurite bile peptone and incubated for 6 hours. Specimens collected directly into tellurite bile peptone were incubated for 6 hours. The samples were then plated on gelatin and TTGA agar and examined for V. cholerae as described by Monsur (1963). Serotyping was performed with type-specific Inaba and Ogawa antisera.

V. cholerae were classified as the El Tor biotype by 3 methods: on the basis of chicken cell agglutination (Finkelstein & Mukerjee, 1963), polymyxin-B resistance (Han & Khie, 1965) and resistance to lysis by Mukerjee's phage IV (Mukerjee, 1963).

RESULTS

Dacca

The sites of the communities studied by individual latrine sampling, the union councils served by that part of the conservancy from which pooled communal nightsoil sampling was carried out, and the sites of the dumping stations from which pooled communal nightsoil was obtained are shown in Fig. 1. Pooled communal nightsoil sampling in Dacca was limited to the old city where, in the past, most of the cholera cases have occurred.

Fig. 2 shows the epidemic curve of cholera cases in Dacca from January to December 1968 in the upper panel, and the isolations of V. cholerae from nightsoil in the lower panel. All cholera cases during
the period under study were *V. cholerae* of the classical biotype and Inaba serotype. During the time 735 cases were admitted to the hospital there were only 2 isolations of *V. cholerae* from individual latrines of one of the communities in which cholera has been endemic in the past. Both were of the classical biotype and Inaba serotype. None of the samples of pooled communal nightsoil from dumping stations was positive during these two epidemics.

Table 1 shows the 3 communities which were studied by individual latrine sampling. During the spring and fall epidemics, 5498 samples were taken from 243 latrines used by approximately 7000 people. On only 2 occasions was classical Inaba isolated, even though there were 7 classical Inaba cholera cases admitted to the hospital from these areas.

Table 2 shows the results of sampling pooled communal nightsoil from the 11 dumping stations that served approximately 50 000 people; 4408 samples were taken. No isolations of *V. cholerae* were made. During this period, 28 cholera cases were admitted to PSCRL from these same areas.

**Chittagong**

The areas under surveillance in Chittagong are shown in Fig. 3. This map shows the sites of the communities studied by individual latrine sampling, the union councils served by the conservancy system, and the sites of the dumping stations from which pooled communal nightsoil was sampled. Fig. 4 shows the temporal relationship between hospitalized cases of cholera and the isolation of *V. cholerae* from the nightsoil. During June, July and August, all hospital and nightsoil isolations were El Tor Ogawa; however, between September and January, El Tor Ogawa and classical Inaba cholera cases appeared simultaneously. El Tor Ogawa was isolated almost continuously from the pooled communal nightsoil during the time cases were occurring. There were 52 isolations of El Tor Ogawa (14 from individual latrines and 38 from pooled communal nightsoil) during the time that

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### Table 1

**NUMBER OF CHOLERA CASES AND NUMBER OF NIGHTSOIL ISOLATIONS FROM INDIVIDUAL LATRINES, DACCA, FEBRUARY TO JULY AND SEPTEMBER TO DECEMBER 1968**

<table>
<thead>
<tr>
<th>Area</th>
<th>Population</th>
<th>No. of latrines</th>
<th>No. of nightsoil samples</th>
<th>No. of cholera cases</th>
<th>No. of nightsoil isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajnarayandhar</td>
<td>1 200</td>
<td>45</td>
<td>2 267</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Goalnagar</td>
<td>1 300</td>
<td>84</td>
<td>1 211</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rayer Bazar</td>
<td>4 500</td>
<td>114</td>
<td>2 020</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7 000</strong></td>
<td><strong>243</strong></td>
<td><strong>5 498</strong></td>
<td><strong>7</strong></td>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>

*a* All isolations from cases and nightsoil were *V. cholerae* classical Inaba.

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### Table 2

**NUMBER OF CHOLERA CASES AND NUMBER OF NIGHTSOIL ISOLATIONS FROM THE DACCA CONSERVANCY SYSTEM, FEBRUARY TO JULY AND SEPTEMBER TO DECEMBER 1968**

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of cholera cases</th>
<th>No. of nightsoil samples</th>
<th>No. of nightsoil isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sutrapur</td>
<td>2</td>
<td>737</td>
<td>0</td>
</tr>
<tr>
<td>Narinda</td>
<td>0</td>
<td>277</td>
<td>0</td>
</tr>
<tr>
<td>Lalbagh</td>
<td>23</td>
<td>1 552</td>
<td>0</td>
</tr>
<tr>
<td>Almalka Road</td>
<td>0</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Siddique Bazar</td>
<td>1</td>
<td>288</td>
<td>0</td>
</tr>
<tr>
<td>Purna Bannerjee Lane</td>
<td>1</td>
<td>207</td>
<td>0</td>
</tr>
<tr>
<td>Hare Street</td>
<td>1</td>
<td>124</td>
<td>0</td>
</tr>
<tr>
<td>English Road</td>
<td>0</td>
<td>108</td>
<td>0</td>
</tr>
<tr>
<td>Mokin Bazar</td>
<td>0</td>
<td>469</td>
<td>0</td>
</tr>
<tr>
<td>Rupchand Lane</td>
<td>0</td>
<td>233</td>
<td>0</td>
</tr>
<tr>
<td>Mali Galli</td>
<td>0</td>
<td>376</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>28</strong></td>
<td><strong>4 408</strong></td>
<td><strong>0</strong></td>
</tr>
</tbody>
</table>

*a* All isolations from cases were *V. cholerae* classical Inaba.
82 El Tor Ogawa cases were hospitalized. By contrast, although 38 classical Inaba cholera cases were admitted to the hospital, only 2 isolations of classical Inaba were made from the pooled communal nightsoil. No isolations of classical Inaba were made from individual latrines.

Of 30,919 samples from the 2,666 individual latrines in the two housing communities under surveillance, 14 El Tor Ogawa isolations were made (Table 3). During the same period there were 3 El Tor Ogawa cases admitted to the hospital from these communities.

Table 4 shows the number of cholera cases and the number of isolations of *V. cholerae* from pooled communal nightsoil from those areas in which nightsoil surveillance was carried out. Samples were taken from the thirteen dumping stations serving approximately 250,000 people. There were 21 El Tor Ogawa cases and 9 classical Inaba cases, and 38 El Tor Ogawa isolations but only 2 classical Inaba isolations from the pooled communal nightsoil of these same areas. Of the 38 nightsoil isolations of El Tor Ogawa, 21 (55%) were made from areas where no hospital cases occurred. Isolations of classical Inaba were made only in areas where there were cases.

Nightsoil surveillance was continued in Chittagong for 4 months after the last case of cholera was
admitted to the hospital, totalling 30,604 samples. No isolations were made from either pooled communal nightsoil or individual latrines.

**DISCUSSION**

The infrequent isolations of classical Inaba *V. cholerae* from nightsoil sampled in Dacca and Chittagong at a time when classical Inaba cholera cases were being admitted to the hospital were strikingly different from the results found in Hong Kong, Calcutta and Chittagong, where the nightsoil isolations of El Tor Ogawa were frequent when El Tor Ogawa cases were being admitted to the hospital. In Chittagong, unlike Calcutta (Sinha et al., 1967, 1968), no isolations of either El Tor Ogawa or classical Inaba were made from pooled communal nightsoil or individual latrines when cases were no longer being admitted to the hospital.

These observations suggest a clear difference between the classical Inaba and the El Tor Ogawa *V. cholerae* in relation to the ability to isolate the

**TABLE 3**

<table>
<thead>
<tr>
<th>Area</th>
<th>Population</th>
<th>No. of latrines</th>
<th>No. of nightsoil samples</th>
<th>No. of cholera cases</th>
<th>No. of nightsoil isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>El Tor Ogawa</td>
<td>Classical Inaba</td>
</tr>
<tr>
<td>Wireless</td>
<td>7,000</td>
<td>1,263</td>
<td>20,478</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Port Trust</td>
<td>8,000</td>
<td>1,403</td>
<td>10,441</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15,000</td>
<td>2,666</td>
<td>30,919</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>
organism from the environment. Previous reviews have suggested that the differences observed between classical Inaba and El Tor Ogawa have been related not to the biotype or serotype of the organism but to the ecology of the communities and locality (Shrivastava, 1966). On the basis of the present observations, the epidemiology of El Tor cholera may need reinterpretation.

El Tor vibrios have frequently been isolated from nightsoil, water, and food during cholera epidemics (Taylor, 1941; Read & Pandit, 1941; Panja & Ghosh, 1947; DeMoor, 1949; Van de Linde & Forbes, 1965; Forbes et al., 1967; Konchady et al., 1969), as well as in communities where cholera could not be demonstrated (Taylor & Ahuja, 1938; Read & Pandit, 1941; Taylor, 1941; Venkatraman et al., 1941; Roy, 1959; Forbes et al., 1967) or was said to have been absent for a long time (Taylor, 1941; Spevak, 1961; Bykov, 1962; Sinha et al., 1967, 1968). By contrast, classical vibrios have been found in water and in nightsoil associated only with active cases or in communities in which there were active cases (Taylor, 1941; Kamal, 1951; Benenson et al., 1965; McCormack et al., 1969). In the past, these differences have been interpreted as indicating that El Tor had variable pathogenicity.

Viability studies of *V. cholerae* outside the body have demonstrated differences between the biotypes in survival in water (Felsenfeld, 1963) and in food (Felsenfeld, 1965): the El Tor vibrio persists as much as 2 1/2 times longer than the classical vibrio. Studies of the viability of vibrios in stools were carried out by early workers (Greig, 1914; Gildemeister & Baehrlein, 1915; Shoda et al., 1934) who demonstrated that the viability was a function of temperature; however, no data comparing the viability of El Tor and classical vibrios in stools are available. It is possible that the El Tor Ogawa has a longer viability than the classical Inaba in nightsoil. This is now being studied.

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1 See also an unpublished document: WHO Regional Office for the Western Pacific, Manila (1962) *Report on the meeting for the exchange of information on El Tor vibrio parachoerla*, 16-19 April 1962.

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### Table 4

**NUMBER OF CHOLERA CASES AND NUMBER OF NIGHTSOIL ISOLATIONS FROM THE CHITTAGONG CONSERVANCY SYSTEM, JULY 1968 TO MAY 1969**

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of cholera cases</th>
<th>No. of nightsoil samples</th>
<th>No. of nightsoil isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>El Tor Ogawa</td>
<td>Classical Inaba</td>
<td>El Tor Ogawa</td>
</tr>
<tr>
<td>Bandel</td>
<td>2</td>
<td>0</td>
<td>5889</td>
</tr>
<tr>
<td>Chaktal</td>
<td>5</td>
<td>1</td>
<td>2211</td>
</tr>
<tr>
<td>Chawk Bazar</td>
<td>2</td>
<td>1</td>
<td>752</td>
</tr>
<tr>
<td>Mader Bari</td>
<td>0</td>
<td>0</td>
<td>2128</td>
</tr>
<tr>
<td>Feringeebazar</td>
<td>2</td>
<td>0</td>
<td>3439</td>
</tr>
<tr>
<td>Zautala</td>
<td>0</td>
<td>1</td>
<td>4547</td>
</tr>
<tr>
<td>Dampara</td>
<td>4</td>
<td>4</td>
<td>2782</td>
</tr>
<tr>
<td>Railway</td>
<td>0</td>
<td>1</td>
<td>4199</td>
</tr>
<tr>
<td>Terribazar</td>
<td>1</td>
<td>1</td>
<td>3456</td>
</tr>
<tr>
<td>Dewanbazar</td>
<td>0</td>
<td>0</td>
<td>837</td>
</tr>
<tr>
<td>Rly. Kadamtali</td>
<td>0</td>
<td>0</td>
<td>326</td>
</tr>
<tr>
<td>Namunabazar</td>
<td>0</td>
<td>0</td>
<td>1281</td>
</tr>
<tr>
<td>Goalpara</td>
<td>5</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21</strong></td>
<td><strong>9</strong></td>
<td><strong>31669</strong></td>
</tr>
</tbody>
</table>

*The conservancy system in Chittagong Municipality serves approximately 50% of the population. Only cases that occurred in those areas where nightsoil surveillance was undertaken are included in this table.*
A large number of mildly symptomatic and inapparent infections surrounding the sporadically occurring El Tor Ogawa cholera case (DeMoor, 1949; Tamayo et al., 1965; Van de Linde & Forbes, 1965; Forbes et al., 1967; Sinha et al., 1968; Bart et al., 1970) may, by contamination of the environment, play a role in the higher number of nightsoil isolations observed. The relative importance of the increased viability of El Tor Owaga in the environment and of the high proportion of mild or asymptomatic infections contributing to the frequency of El Tor Ogawa isolations requires further study.

It is evident that bacteriological sampling of nightsoil is not a useful method for surveillance of classical Inaba cholera infection in the community. In areas where cases due to both El Tor Ogawa and classical Inaba were occurring simultaneously, the El Tor Ogawa was isolated 10 times more frequently from the nightsoil than the classical Inaba. It appears that the ability to recover the vibrio from nightsoil is related either to the biotype or serotype of the organism, or both. Whether the biotype or serotype, alone or in combination, determines the ability to isolate the organism from the environment requires further investigation. Extrapolation of the results of nightsoil sampling to the incidence and prevalence of infection in a community must take into consideration the biotype and serotype of the *V. cholerae* infecting the community.

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RÉSUMÉ

**ISOLEMENT DE *VIBRIO CHOLERAE* À PARTIR DE VIDANGES DURANT DES ÉPIDÉMIES DE CHOLÉRA CLASSIQUE ET DE CHOLÉRA EL TOR AU PAKISTAN ORIENTAL**

Les villes de Dacca et de Chittagong ont servi de cadre à une enquête sur l'efficacité des examens d'échantillons de vidanges en tant que moyen d'apprécier l'extension de l'infection cholérique dans une collectivité.

De janvier à décembre 1968, Dacca a connu une épidémie de choléra due à *Vibrio cholerae* classique sérotype Inaba, au cours de laquelle 735 malades furent hospitalisés. Les 9906 prélèvements effectués pendant cette période dans des fosses d'aisance communes et dans des latrines privées n'ont conduit qu'à deux isolments du vibrio responsable de l'épidémie. La même méthode a été utilisée au cours de l'épidémie de choléra qui a atteint Chittagong de juillet 1968 à mars 1969 et qui était causée à la fois par *V. cholerae* classique sérotype Inaba et par *V. cholera* biotype El Tor sérotype Ogawa. L'examen de 62 588 échantillons de vidanges a permis d'isoler à deux reprises le vibrio classique mais 52 isolments de vibrons El Tor Ogawa ont été réalisés. Dans les régions où les deux biotypes étaient présents simultanément, le vibrio El Tor Ogawa a été identifié dix fois plus fréquemment que le vibrio classique.

D'autres recherches sont nécessaires pour déterminer le facteur — biotype, sérotype ou les deux — qui est à l'origine de cette différence dans les possibilités d'isolement des vibrons. Toute tentative de convertir en taux d'incidence et de prévalence les données recueillies par la méthode d'échantillonnage des vidanges doit nécessairement tenir compte du biotype et du sérotype des vibrons responsables de l'infection dans une collectivité.
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