ANNOTATED BIBLIOGRAPHY ON

CLASSICAL VIBRIO CHOLERAE

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ANNOTATED BIBLIOGRAPHY ON
CLASSICAL *VIBRIO CHOLERAE*

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE RESEARCH, BANGLADESH
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PREFACE

The *Specialized Bibliography Series* is part of the larger effort to facilitate exchange of information and to establish an information network in the field of diarrhoeal diseases -- an effort being carried out by the International Diarrhoeal Disease Information Service and Documentation Centre (DISC) of the ICDDR,B. The present issue, the fifth of the Series, includes 213 papers (100 abstracted) on classical *Vibrio cholerae*. This is a subject of high current importance, and the reason for selecting the topic is explained in the introduction by Dr K M S Aziz, a scientist of the ICDDR,B.

This is not an exhaustive bibliography on the topic. The bibliography was compiled from the available resources, and it is possible that inadvertent omissions may have occurred.

We hope the present bibliography will contribute towards generating greater interest and awareness in this field, and will facilitate user access to existing knowledge. Copies of articles abstracted and cited in this bibliography are available from DISC to interested persons/organizations. We will consider this attempt successful if the bibliography helps diarrhoeal disease researchers and practitioners. Suggestions for improvement of a future edition will be appreciated.

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  and Communications
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  Diarrhoeal Disease Research, Bangladesh

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INTRODUCTION

As a killer disease, classical cholera was dreaded the world over and the six known pandemics caused by the ingestion of *Vibrio cholerae* have left frightening memories and impressions in the cholera-hit regions. The figures of death due to this illness have indeed been alarming: the case fatality rate used to be more than 60% due to the rapid loss of body fluid and the resultant dehydration. The episodes of cholera have been unique and remarkable: death has been found to occur within hours after the onset of acute watery diarrhoea. The manifestations of this disease have made the illness so unique that it can be traced to the Vedic literature of several thousand years back. Even though the disease is known as Asiatic cholera, classical *V. cholerae* was found to transcend continental boundaries as well.

This dreaded disease which spreads rapidly with such high case fatality rates was first tamed in the context of the hospital when case fatality rates came down to 4 per thousand with the advent and use of the electrolyte-containing intravenous solution (I.V.) to replenish the lost fluids and salts. The oral rehydration solution has now replaced the I.V. solution to a large extent and has taken the treatment of cholera to the doorsteps of people in remote areas and regions far from hospitals. Today, cholera outbreaks can be managed with ORS, I.V. and scalp vein needles in the field, and can reduce the case fatality rate to below 10 per thousand, with or without antibiotics.

Dramatically though, cholera caused by classical *V. cholerae*, got almost entirely replaced by El Tor cholera. The last stronghold of *V. cholerae* was finally conquered by El Tor *V. cholerae* only to be replaced in about a decade's time, at least in some places. We still do not know how classical *V. cholerae* conquered El Tor *V. cholerae*. More study is needed to gain proper insights into the major cycles of biotypes of the organism.

Another aspect of *V. cholerae* is its antibiotic resistance pattern. For a long time the organism remained sensitive to almost all antibiotics. Abruptly, however, a few years ago there was a surge for acquiring antibiotic resistance by this organism, a trend that disappeared as suddenly as its appearance, before the phenomenon could even be studied properly. This year, antibiotic resistance started to appear again, with even greater intensity, but for how long and why we do not know.

A recent landmark finding -- that it can survive in the environment -- has opened another chapter in the diverse behavioral pattern of this organism. The recent cholera outbreak in Louisiana, USA, and the report of the presence of this organism in the aquatic environment has put afloat the idea that *V. cholerae* is an environmental species. An exciting area of work continues to be the biology and ecology of *V. cholerae* in the environment and its inter-relationships and inter-action with other phylogenetically-related species and genera.

In the Ganges delta region of the Indian sub-continent, a popular myth is associated with this disease: "Ola Bibi" is the name of the Goddess of cholera.

"Ola Bibi" is still feared in the countryside as being omnipresent and ready to strike on the poor and the vulnerable. As long as clean water is not made available for all household purposes, and as long as malnutrition and poverty remain at their present level with the associated inadequacy of sanitation and personal hygiene, *V. cholerae* will continue to be a threat. The damage that can be caused by a multiple antibiotic resistant strain of *V. cholerae* with renewed virulence, should not be underestimated.

There has been already a lot of research on classical *V. cholerae*, but there still remains enormous potential for further work in this field. The completed studies have generated meaningful literature on this subject, and in this bibliography attempts have been made to highlight [mostly] the recent worldwide publications. It is hoped that this bibliography will serve as a handy reference for scientists and researchers in this field.

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  and Communication
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The bibliography is divided into subject and author sections. In the Subject
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and then by the title of paper. Co-authors' names also appear in alphabetical
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Efforts have been made to present abstracts with all available information
regarding the study's nature and objective, methods used, and the major findings
and conclusions.

The bibliography is in English. A title in parentheses indicates that the paper
is in another language.

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ULTRASTRUCTURE

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AUTHOR SECTION

Abrams GD see Jones GW

Agarwal MN see Khan S

Agarwal RK, Parija SC, Sanyal SC. Taxonomic studies on *Vibrio* and related genera. Indian J Med Res 1980 Mar;71:340-53

There exists differences in opinion about the real taxonomic position of some organisms, and this creates difficulties in maintaining and classifying culture collections by strain categories. A numerical taxonomic study was, therefore, done with 45 strains of *Vibrio* and related genera including reference strains, to examine: (i) whether oxidase negative *Vibrio proteus* is a biotype of *Vibrio cholerae* or an independent species; (ii) whether the group F organisms belong to *Aeromonas* or form a separate genus; (iii) the exact taxonomic positions of certain culture collection strains assigned to the genus *Vibrio;* (iv) whether the definition of the family *Vibrionaceae* needs to be modified in the light of the study results; and (v) the characteristics required for differentiating important genera and species of the family. Based on the study, it was recommended that: (i) *V. proteus* with 83% similarity with *V. cholerae,* should form a distinct species of the genus *Vibrio;* (ii) group F strains, having 75% similarity with *V. cholerae* and 72% with *Aeromonas,* should form a separate genus of the family *Vibrionaceae;* (iii) the 2 NCTC strains labelled as *Vibrio* species having 88 per cent similarity belong to *V. cholerae;* (iv) the NCTC strains, *Vibrio alcaligenes,* be excluded from the family *Vibrionaceae* as it is non-fermentative; and (v) the definition of the family *Vibrionaceae* needs amendment to include oxidase negative or otherwise highly similar organisms.

Agarwal S see Darbari BS

Ahmad N see Kabir S

Ahmed A see Benenson AS

Ahmed A see Mosley WH

Ahmed S see Martin AR

Ahmed S see Oseasohn R

Ahmed SZ see Benenson AS

Akbar R see Lindenbaum J

Akef QMA, Akhter J, Davies BI, Islam MA, Khan SA, Khan AQ, Rahman ASMM, Stockard JL, Oseasohn RO. Cholera in East Pakistan families, 1962-63. Bull WHO 1965;32 (2):205-9

Akhter J see Akef QMA

Al-Mahmud KA see Sanyal SC

Inaba. It remained unclear whether the differences observed between El Tor Ogawa and classical Inaba *V. cholerae* are related to the biotype or serotype of the organism, or both. However, it was suggested that bacteriologic or serologic methods alone are not adequate to detect infections of either biotype. Combined application of these techniques is recommended.

Barua D, Mukherjee AC. Direct bacterial haemagglutination test for differentiating El Tor vibrios from *V. cholerae*. Bull Calcutta Sch Trop Med 1963 Jul;11(3):85-6

Barua D. Laboratory diagnosis of cholera. Geneva: World Health Organization, 1964. 4 p. (Chol/WP/1.64)

Barua D. Review of the present situation. Geneva: World Health Organization, 1970. 6 p. (WHO/BD/CHOLERA/70.20)

Baselski V, Briggs R, Parker C. Intestinal fluid accumulation induced by oral challenge with *Vibrio cholerae* or cholera toxin in infant mice. Infect Immun 1977 Mar; 15(3):704-12

The study's aim was to develop a model in which quantitation of fluid secretion served as an index of *Vibrio cholerae* infection. The diarrhoeal response of orally inoculated infant mice to viable *V. cholerae* and purified cholera toxin was quantified by a fluid accumulation (FA) ratio, which is defined as the gut weight/remaining body weight. FA ratios were determined in relation to time of exposure and dose. With viable cells of strains CA401 and 569B, fluid accumulation onset occurred 8 h post-inoculation, and reached a near maximum at 16 h. A dose of 4 X 10^6 colony-forming units of strain CA401 was required for a positive response 16-18 h post-inoculation; and several other classical strains showed a similar dose-related response. However, 569B required a 100-fold higher dose to get a positive response. Fluid accumulation onset with cholera toxin occurred 6-8 post-inoculation, reaching a maximum by 10 h. A 0.5 μg dose was needed for a positive response 10-12 h post-inoculation. The positive response to toxin could be inhibited by pre-incubation with specific antitoxin.

Baselski VS, Medina RA, Parker CD. *In vivo* and *in vitro* characterization of virulence-deficient mutants of *Vibrio cholerae*. Infect Immun 1979 Apr;24(1):111-6

In vitro and *in vivo* interactions between *Vibrio cholerae* and the infant mouse intestinal environment were examined by using a number of virulence-deficient mutants of strain CA401, which were unable to induce a typical diarrhoeal response. *In vitro* interactions with upper bowel sections were evaluated by determining percent association of radiolabeled organisms with the sections. For most mutants, the percent recovery did not significantly differ from that of the CA401 strain. However, a significant reduction in recovery was noted for rough and toxin-deficient mutants. *In vivo* behavior was evaluated in the upper bowel early in infection with radiolabeled inocula. Four hours post-inoculation was chosen as the time for evaluating changes in specific activity in the upper bowel. The relative degree of mechanical clearance was indicated by the percent recovery of input label. The relative degree of multiplication and killing that occurred in upper bowel was determined by changes in the specific activities (counts per minute per colony forming unit) of inocula compared with recovered viable organisms. The various classes of virulence-deficient *V. cholerae* mutants showed characteristic altered patterns of *in vivo* behavior whereas some mutant types exhibited net multiplication in the upper bowel early in the infection. Other mutant types were rapidly killed and were susceptible to more rapid mechanical clearance from the upper bowel. The *in vitro* association patterns failed to correlate with *in vivo* upper bowel recovery.

Baselski VS, Upchurch S, Parker CD. Isolation and phenotypic characterization of virulence-deficient mutants of *Vibrio cholerae*. Infect Immun 1978 Oct;22(1):181-8

The isolation and preliminary characterization of virulence-deficient mutants of *V. cholerae* which do not elicit a diarrhoeal response in infant mice after oral challenge is reported. Nitrosoguanidine-treated clones were screened for low fluid accumulation ratios in individual mice, and the mutants were confirmed by conducting additional mouse tests. The mutants were examined for alterations in phage type, motility, toxin production, proteolytic activity, neuraminidase production, amylase production, morphology, growth requirements, carbohydrate fermentation, *in vitro* growth patterns, and cell surface alterations. The mutant types found included several types with previously recognized virulence-associated markers (round, non-motile, toxin deficient, protease deficient), several types with pleiotropic altera-tions (cell morphology, decreased extracellular products), and several with no previously recognized virulence-deficient phenotype (purine requiring, cell surface altered, rapid death *in vitro*, no defect found). Dose-response kinetics showed that most mutants could provoke diarrhoea if given at a higher dose (100-fold greater) than the dose used for screening. Recovery of viable organisms from the gut, late in infection, showed reduction of survival and/or multiplication capacity for the mutants. There was variation in the degree or reduction for different classes.

Baselski VS, Medina RA, Parker CD. Survival and multiplication of *Vibrio cholerae* in the upper bowel of infant mice. Infect Immun 1978 Nov;22(2):435-40

Several *Vibrio cholerae* strains with varying virulence was orally inoculated in the upper bowel of infant mice. Their survival and multiplication potential was evaluated soon after the challenge. Apparent specific activity changes (counts per minute per colony-forming unit) of the cell population after 4 h as compared with the inoculum, indicated that strain CA401 established a viable multiplying cell population, whereas strains VB12 (a rough variant) and 569B were subject to host bactericidal and bacteriolytic mechanisms. The increased specific activity observed with 569B and VB12 (which indicated the decreased ability to establish infection) was consistent with their inability to induce disease in the model system of the present study. Events which might have contributed to an increase or decrease in specific activity are analyzed. Based on the findings, the strains' infective potential were defined in terms of alterations in specific activity which were found to occur in the upper bowels of infant mice 4 h after oral inoculation. The relative infective potentials of the strains under investigation are: CA401 569B VB12.

Baselski VS see Sigel SP

Bashford DJ, Donovan TJ, Furniss AL, Lee JV. *Vibrio cholerae* in Kent (letter). Lancet 1979 Feb 24;1(8113):436-7

In a search for *Vibrio cholerae* in surface waters in Kent, UK, the organisms were found in a number of sites. Their incidence appeared to be related to water tempe-rature, isolation being more common in the summer months. Some isolates were from streams where there was a possibility of sewage contamination. One site, extensively studied in the preliminary work yielded strains of *V. cholerae* of several different serovars in large numbers. This site was an agricultural drainage ditch where the possibility of sewage contamination was negligible. Amongst the *V. cholerae* isolated, there were some of the 01 serovars - typical cholera vibrios of the Ogawa subtype. The phage-typing pattern of these cholera vibrios was similar to that of some of the old classical strains and thus differed from that of the El Tor strains usually

Classical *Vibrio cholerae*

Bowman RK see Forbes GI

Bradford H see Colwell RR

Bradford HB see Barrett TJ

Brestel C see Foster JW

Briggs R see Baselski V

Brown MA see Horsfall DJ

Burrows W see Husain SS

Bystryi NF, Drzhevkina MS. Basic criteria and classification scheme for bacterio-phages acting against cholera vibrios of the classical and El Tor biotypes. J Hyg Epidemiol Microbiol Immunol (Praha) 1979;23(1):85-94

Carpenter CCJ see Wallace CK

Carpenter KP. Isolation and identification of cholera vibrios. Monthly Bull Ministry Health Public Health Lab Ser 1966;25:58-63

Cash R see Hornick RB

Cash RA. Aspects of the current understanding of the pathophysiology of cholera. J Pak Med Assoc 1969 Aug;19(8):345-8

Cash RA, Music SI, Libonati JP, Snyder MJ, Wenzel RP, Hornick RB. Response of man to infection with *Vibrio cholerae*. I. Clinical, serologic, and bacteriologic res-ponses to a known inoculum. J Infect Dis 1974 Jan;129(1):45-52

The clinical manifestations and immunologic response in cholera induced by the classical Inaba 569B and Ogawa 395 strains of *Vibrio cholerae* were studied in 111 volunteers. Vibrios suspended in 1.0 ml of buffered saline at pH 7.2 ± 0.1 were given water and a base ($NaHCO_3$). The base, by neutralizing gastric acidity, was conducive to *Vibrio* survival. An oral dose of 10^8 organisms in buffered saline induced diarrhoea, whereas with $NaHCO_3$, a dose of 10^4 organisms was required. The illness spectrum was similar for those challenged with 10^6 organisms of either serotype. Most of the volunteers with diarrhoea had borborygmi several hours before the appearance of liquid stool. The stools were initially brown and partially formed. The appearance changed to yellow and later to the classical "rice water" stool when diarrhoea became increasingly liquid. In 45% of the cases stools were positive for *V. cholerae*, 7-24 hours before diarrhoea onset. The onset of severe diarrhoea was not abrupt but increased dramatically during a 24 h period. Seventy-three percent of the subjects had detectable vibriocidal antibodies. The antibody titers peaked 2 weeks after challenge but rapidly fell to levels slightly above the baseline by 8 weeks.

Chaicumpa W, Peungjesda U, Martinez B, Atthasishtha N. Soluble haemagglutinin of classical vibrios: isolation protection against cholera by its antibodies. Southeast Asian J Trop Med Public Health 1982 Dec;13(4):637-45

Soluble hemagglutinin was isolated from a late culture of *Vibrio cholerae*, biotype: classical, serotype: Inaba, and strain: 569B, in casein peptone broth with aeration by saturated ammonium sulphate precipitation, followed, respectively, by gel filtration, electrophoresis, either extraction and electrophoresis. The soluble hemagglutinin of the classical vibrios consists of three protein fractions with different electrophoretic mobilities. Antibodies to these fractions were prepared and their protective ability tested. These antibodies afforded low but significant protection against oral challenges with homologous classical *V. cholerae* in the experimental baby mouse cholera model. The low but significant vibriocidal titers detected in antibodies to the three fractions tend to indicate that reasonable amount of the soluble hemagglutinin are present on the intact vibrios.

Chakrabarti MK, Ganguly U. Adenylate cyclase activity in toxinogenic & non-toxinogenic strains of *Vibrio cholerae*. Indian J Exp Biol 1980 Mar;18(3):311-3

This study evaluates the cholera toxin's effects on the regulation of adenylate cyclase activity in *Vibrio cholerae*. Difference in the enzymatic activity of adenylate cyclase *in vivo* between toxinogenic and nontoxinogenic *V. cholerae* strains under different growth conditions was observed. Five different toxinogenic *V. cholerae*, strains e.g. biotype classical Inaba 2597, Ogawa 2615 and El Tor Inaba 2079, Ogawa 2019 were used. Three nontoxinogenic strains of EW_6, ME_7 and CRCV, were used as controls. The cells were grown in the syncase medium with desired glucose concentration at 35°C with shaking. Adenylate cyclase in intact cells was measured by pre-labelling the ATP with radioactive adenine. Both toxinogenic and nontoxinogenic strains possess an active adenylate cyclase system. The initial rate of cAMP synthesis at logarithmic growth phase was low in both toxinogenic and non-toxinogenic *V. cholerae* strains. The adenylate cyclase activity increased sharply at stationary phase in toxinogenic *V. cholerae* strains, whereas the activity remained unaltered in nontoxinogenic strains. No significant changes in the enzyme activity between the classical and El Tor was observed.

Chakrabarti MK, Pal SC, Ganguly U. Adenylate cyclase activity in toxigenic and nontoxigenic strains of *Vibrio cholerae:* effect of different carbohydrates. J Diar Dis Res 1983 Jun;1(2):82-6

Adenylate cyclase activity in intact cells of *V. cholerae* was measured by prelabelling ATP with radioactive adenine. In toxigenic strains, adenylate cyclase activity increased sharply at stationary phase. No such increase in adenylate cyclase activity occurred in nontoxigenic strains, and the enzyme activity remained almost the same at both logarithmic and stationary phases of growth. The increase in adenylate cyclase activity from logarithmic to stationary phase in toxigenic strains was in correlation with the increased secretion of toxin into the medium. High concentration of glucose (3gm%) inhibited adenylate cyclase activity. Maximum concentration of toxin and c-AMP formed varied considerably with the carbohydrate sources. In the presence of a lower concentration of glucose (0.25gm%), production of toxin and adenylate cyclase activity of the cells was highest compared to other carbohydrate sources. The presence of lactose and glycerol in the media decreased both adenylate cyclase activity and toxin secretion.

Chakraborty MK, Ganguly U. Comparison of extracellular and intracellular level of cyclic AMP between the toxinogenic & non-toxinogenic strains of *Vibrio cholerae*. Indian J Exp Biol 1981 Feb;19(2):179-80

Extracellular and intracellular cyclic AMP concentrations were measured at stationary phase of the growth medium in 5 toxinogenic (tox^+) and 3 nontoxinogenic (tox^-) strains of *Vibrio cholerae*. Cells were grown in 50 ml syncase medium supplemented with 0.25%

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glucose at 37°C. All the 5 tox[+] *V. cholerae* strains showed much higher level of extracellular cyclic AMP (range: 1040-1370 pmol/ml medium) than that of tox[-] strains (range: 192-252 pmol/ml medium). Intracellular cyclic AMP level also showed similar variations between the tox[+] and tox[-] *V. cholerae* strains. The range in tox[+] was 4.5-6.4 pmol/mg wet weight while that in tox[-] strain was 1.1-1.3 pmol/mg wet weight. A high glucose concentration (3%) lowered the intra- and extracellular cyclic AMP levels both in tox[+] and tox[-] *V. cholerae* strains. The effect of glucose was severe in the tox[+] strain and distinctly less severe in the tox[-] strain. These observations conformed to those reported earlier. The low level of cyclic AMP both within the cells and in the medium in tox[-] strains may be accounted for by the lower activity of adenyl cyclase observed earlier. It was indicated that the pattern of cyclic AMP release into the medium in tox[+] and tox[-] strains were also consistent with the synthetic capacity of those strains.

Charunmethu P see Finkelstein RA

Chatterjee DN see De SN

Chatterjee SN, Ghosh S. Mechanism of action of furazolidone: inter-strand cross-linking in DNA & liquid holding recovery of *Vibrio cholerae* cells. Indian J Biochem Biophys 1979 Jun;16(3):125-30

Chatterjee SN see Banerjee SK

Chatterjee SN see Maiti M

Chaudhuri K see Maiti M

Cholera and other vibrio-associated diarrhoeas. Report of a sub-group of the Scientific Working Group on Epidemiology and Etiology (Geneva, 24-27 Sep 1979). Geneva: Diarrhoeal Diseases Control Programme, World Health Organization, 1980. (WHO/DDC/EPE/80.3)

The cholera and the comma-bacillus. Br Med J 1885;1:1076-7

Cholera carrier studies in Calcutta, 1968. Bull WHO 1970;43(3):379-87

Chowdhury MK see Samadi AR

Chulasamaya M see Finkelstein RA

Colwell RR, Seidler RJ, Kaper J, Joseph SW, Garges S, Lockman H, Maneval D, Bradford H, Roberts N, Remmers E, Huq I, Huq A. Occurrence of *Vibrio cholerae* serotype 01 in Maryland and Louisiana Estuaries. Appl Environ Microbiol 1981 Feb;41(2):555-8

Thirty-three strains of *Vibrio cholerae* 01 (Inaba) were isolated from water samples collected from Chesapeake Bay and from Louisiana saltmarshes and sewers, between 1977 and 1978. *Vibrio cholerae* non-01 was isolated from both the areas, alongwith some isolations of *V. cholerae* 01 strains as well. The finding is important because, in September 1978, the first cholera outbreak in the United States, since 1911, occurred in Louisiana. Percent guanine plus cytosine (overall) DNA base composition was determined by the thermal denaturation method to confirm phenotypic identitication of *V. cholerae*. The occurrence of *V. cholerae* 01 in the aquatic environment in the absence of fecal contamination suggests that this organism survives and multiplies in the natural environment and sporadic outbreaks can be expected when proper food handling techniques are not used.

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Colwell RR. Polyphasic taxonomy of the genus *Vibrio:* numerical taxonomy of *Vibrio cholerae, Vibrio parahaemolyticus* and related *Vibrio* species. J Bacteriol 1970 Oct; 104(1):410-33

Colwell RR see Huq A

Colwell RR see Kaper J

Cook WL, Wachsmuth K, Johnson SR, Birkness KA, Samadi AR. Persistence of plasmids, cholera toxin genes, and prophage DNA in classical *Vibrio cholerae* 01. Infect Immun 1984 Jul;45(1):222-6

Plasmid profiles, the location of cholera toxin subunit A genes, and the presence of defective VcA1 prophage genome in classical *Vibrio cholerae* isolated from patients in Bangladesh in 1982 were compared with those in older classical strains isolated during the Sixth pandemic and those in selected El Tor and nontoxigenic 01 isolates. Of the 42 classical *V. cholerae* 01 strains, 34 contained a 3-and a 21-megadalton plasmid species. Only 1 of the 13 typical El Tor biotype isolates contained plasmid DNA. Some of the 13 nontoxigenic isolates contained a variety of plasmids which may reflect the heterogeneity of this group. The old and new classical *V. cholerae* isolates had two *Hind* 111 chromosomal digest fragments containing cholera toxin sub- unit A genes, whereas the El Tor strains from Eastern countries had one fragment. The El Tor strains from areas surrounding the Gulf of Mexico also had two subunit A gene fragments, which were smaller and readily distinguishable from the classical pattern. All classical strains had 8-10 *Hind* 111 fragements, and all hybridized with the phage VcA1. The strains were not lysed and were immune to superinfection or lysis by the phage, indicating the presence of the defective VcA1 in the prophage state. None of the Eastern El Tor strains had these genes, and the Gulf Coast El Tor strains contained a different array of weakly hybridizing genes (data not given). Two of the nontoxigenic 01 strains from the Gulf region also hybridized with VcA1 and had a fragment pattern similar to that of the Gulf region El Tor strains. The data suggest that the recent isolates of classical cholera in Bangladesh are closely related to those bacterial strain(s) which caused the sixth pandemic classical cholera. Based on the plasmid profiles, restriction endonuclease studies, and hybridization with toxin and phage genes, it seems likely that neither the El Tor nor the nontoxi- genic *Vibrio* strains are a direct precursor of the newly isolated classical strains.

Cook WL, Wachsmuth K, Feeley J, Huq I. The question of classical cholera. Lancet 1983 Apr 16;1(8329):879-80

A recent report from the International Centre for Diarrhoeal Disease Research, Bangladesh, indicates the return of the classical biotype of *Vibrio cholerae* in Bangladesh and casts doubt about earlier speculation that during the seventh pandemic, the classical biotype was completely replaced by the El Tor biotype in Asia. Between 1973 and Spetember 1982, over 5000 *V. cholerae* O group 1 were examined in Dhaka and only 5 classical strains were cultured from 5 cases occurring on one day in October 1979. From September 1982, there has been a gradual transition from the El Tor biotype to the classical biotype, which in January 1983, accounted for over 90% of the cholera cases. Whether these recently isolated classical.biotype strains were the same as or similar to the classical strains from the sixth pandemic, was an important epidemiological question. The authors attempted to answer it by using molecular techniques. Older isolates of the classical bioptye were acquired from geographically diverse areas. A total of 43 classical biotype (including 2 recent isolates) and 15 El Tor biotype strains were examined. . *V. cholerae* strains isolated from cases as widely separated as Hong Kong and Calcutta, from 1936 to 1982, possessed

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the same plasmid profile. This indicated that the classical strains from the sixth pandemic were not replaced by the El Tor biotype but have remained dormant or caused undetected illness over the past two decades.

Craig JO. The effect of cholera stool and culture filtrates on the skin of guinea pigs and rabbits. *In:* Proceedings of the Cholera Research Symposium, Honolulu, Hawaii, 24-29 Jan 1965. Washington, D.C.: U S Government Printing Office, 1965:153-8

This study determines whether sterile filtrate of stools obtained from bacteriologically confirmed cholera cases injected intracutaneously can evoke a skin response. Stools collected from patients admitted to the hospital were processed; for liquid stools the supernatant was filtered and the formed stools were diluted two-fold to ten-fold with sterile saline before being centrifuged, and then filtered. Initially the sterile stool filtrate was diluted 1:20 in sterile saline and subsequently injected at 1:5 dilution. Stool filtrates were given intracutaneously in 0.1 ml volumes in randomized sites on the back of albino guinea pigs and 24 injections were given in six rows of four each. Rabbits were given 60 to 70 injections. Marked induration and erythema beginning 6 to 8 h after injection reached a peak at 18 to 24 h in animals; this persisted for 4 to 5 days. Noncholera stools did not produce any induration but some showed transient erythema. The diameter of induration and erythema produced by sterile cholera stools bore a linear relationship to log dose of filtrate over a range of 6 to 5 mm. The effects of stool filtrates on skin capillary permeability was also investigated by intravenous injection. Filtrates that evoked induration also caused an increase in skin permeability of at least 24 h duration and the diameter of the blue lesion also bore a linear relationship to log dose of the filtrate within the range of 5 to 12 mm. The skin reactions evoked by culture filtrates of *Vibrio cholerae* grown in different media were also discussed.

Craig JP. Preparation of the vascular permeability factor of *Vibrio cholerae*. J Bacteriol 1966 Sep;92(3):793-5

Craig JP see Kusama H

Darbari BS, Tiwari HL, Agarwal S. Pattern of cholera in Raipur: a 12-year appraisal. J Indian Assoc Commun Dis 1982 Sep-Dec;5(3-4):83-7

Cholera outbreaks were recorded in Raipur, Madhya Pradesh, India, in 1970, 1975, 1977 and 1979-81. A total of 8,253 rectal swabs/stool samples were examined for *V. cholerae* during this period; and 144 strains were isolated. *V. cholerae* biotype classical, serotype Inaba persisted there until 1970, and a shift to biotype El Tor, serotype Ogawa was observed only in 1974. Since then, El Tor has been the principal strain in this area. In 1977, a temporary shift from Ogawa to Inaba was noted, which again reverted to Ogawa, and was responsible for the protracted outbreak in 1979-81. In 1975, 1977 and 1980, *V. cholerae* strains were isolated throughout the year, and no age or sex preponderance was seen. NAG vibrios also were isolated occasionally. The data suggested Raipur to be a cholera endemic area.

Das G see Roy NK

Das J see Balganes M

Das J see Roy NK

Datta A see Guhathakurta B

Datta A see Konchady D

Datta GC see Guhathakurta B

Davies BI see Akef QMA

Davis GHG, Park RWA. A taxonomic study of certain bacteria currently classified as *Vibrio* species. J Gen Microbiol 1962 Jan;27(1):101-19

The paper combined two independent studies by the two authors. In one, a group of vibrios were examined as part of a comparative study of the polarly flagellate bacteria commonly found in surface waters, and in the other, taxonomic relationship of a so-called *Vibrio* was determined. In these two independent studies, 25 strains currently classified into 14 serological types or species of the genus *Vibrio* were examined in detail for their morphological, cultural and biochemical characters. The results indicated that the strains under study included members of the distinct taxonomic groups or genera, *Vibrio pseudomonas* and *Comamonas*. The authors proposed the new generic name *Comamonas* to replace *Lophomonas* in bacteriology. The change in nomenclature was necessary under Rule 24 of the International Code of Nomenclature of Bacteria and Viruses (1958) where the name *Lophomonas* was proven invalid.

De SN. Enterotoxicity of bacteria free culture filtrates of *Vibrio cholerae*. Nature 1959;183:1533-4

De SN, Chatterjee DN. An experimental study of the mechanism of action of *Vibrio cholerae* on intestinal mucous membrane. J Pathol Bacteriol 1953;66:559-62

De SP, Lahiri MN. Laboratory diagnosis of cholera. Indian J Med Res 1964 Aug;52 (8):842-7

De SP see Konchady D

Deb BC see Niyogi SG

DeWitt WE, Gangarosa EJ, Huq I, Zarifi A. Holding media for the transport of *Vibrio cholerae* from field to laboratory. Am J Trop Med Hyg 1971 Sep;20(5):685-8

This study compares the efficacy of several media used commonly for transport of fecal specimens from the field to distant laboratories for *Vibrio* isolation. Alkaline bile salt-tellurite peptone broth commonly referred to as bile peptone broth, with pH 9.0, has proved to be the best holding medium for *Vibrio cholerae* at the Pakistan-SEATO Cholera Research Laboratory (now International Centre for Diarrhoeal Disease Research, Bangladesh). This medium is ideal for carrying specimens from field to laboratory for short-term enrichment. When long delays were anticipated, alkaline bile-salt peptone broth with tellurite-impregnated swabs, Cary-Blair medium (CB), and the sea-salt medium of Venkatraman and Ramakrishnan (VR) were used. The recovery rates of both biotypes of *V. cholerae* from specimens on either CB or VR media were found to be 77% or higher for as long as 4 weeks. The combination of CB and VR media resulted in a 100% recovery of *V. cholerae* after 4 weeks. Buffered glycerol saline solution was not found suitable for transport of *V. cholerae* even for shorter periods. This medium had a marked inhibitory effect within 24 hours, due to glycerol.

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DeWitt WE see Gangarosa EJ

Dey SN see Roy NK

Donovan TJ see Bashford DJ

Drasar B see Feachem R

Drozhevkina MS, Arutyunov YuI. Phage typing of *Vibrio cholerae* using a new collection of phages. J Hyg Epidemiol Microbiol Immunol 1979;23(3):340-7

This paper reports a new set of phages for typing of the classical and El Tor biotypes of *Vibrio cholerae*. The new collection consisted of seven virulent phages of different serotypes, including those of Mukerjee. This collection revealed a larger number of phage types as compared to the Mukerjee's phage types and differentiated the strains of both biotypes. This method enhances the epidemiological significance of *V. cholerae* phage typing and could be useful in tracing sources and ways of spread of infection within a relatively isolated focus.

Drozhevkina MS see Bystryi NF

D'Souza S see Levine RJ

Duffy TP see Wallace CK

Duran AP see Twedt RM

Dutta A see Sen AK

Dutta NK see Sanyal SC

Ehara M see Iwanaga M

Eubanks ER see Hranitzky KW

Eusof A see Khan MU

Eusof A see Samadi AR

Fahimuddin M see McCormack WM

Falkow S see Kaper JB

Farooki MA. The role of frogs and fish in the survival of *Vibrio cholerae* in water. Pak J Med Res 1965 Jul;4(3):281-309

To elucidate the role of frogs and fishes in enhancing the survival of vibrios in contaminated water, raw tap water (pH 7.6-7.8) was inoculated with large quantities of fresh *Vibrio cholerae* cultures. Plate subcultures of such contaminated water, at varying (half hourly) intervals revealed that *V. cholerae* survival in raw tap water was very short ($\frac{3}{4}$ to $4\frac{3}{4}$ h mean $2\frac{1}{4}$ h). Ordinary local species of frogs and toads, washed in tap water was added to the bottles containing tap water. This was followed by additions of vibrio cultures in quantities equal to those used for

the control experiments with plain water. *V. cholerae* survival in water increased significantly in association with frogs (range 2½ h to more than 7 h; mean 5½ h). Small fishes (4-5 inches in length) obtained from local river water were used in the same way as frogs. *V. cholerae* survival in water was significantly prolonged in association with fish (range 1½-6¾ h; mean 4¾ h). The enhanced vitality and viability of *V. cholerae*, in association with frogs or fishes, have significant implications in cholera endemicity.

Faruque ASG see Samadi AR

Feachem R, Miller C, Drasar B. Environmental aspects of cholera epidemiology. II. Occurrence and survival of *Vibrio cholerae* in the environment. Trop Dis Bull 1981 Oct;78(10):865-80

The occurrence and survival of *V. cholerae* in the environment is discussed on the basis of a review of available literature. Some traditionally held beliefs about cholera epidemiology may be challenged in light of recent discoveries. There now is strong evidence suggestive of the fact that an aquatic reservoir for *V. cholerae* exists. Some *V. cholerae* strains have been isolated from non-contaminated aquatic environments, and may be part of the permanent microflora. Moreover, *V. cholerae* appears to have prolonged existence in some environments, i.e. sewage. Although the evidence is limited, foods also act as one of the vehicles for spreading cholera. Possible inter-biotypic and intra-biotypic variability in environmental persistence of the El Tor and classical cholera biotypes remains to be documented.

Feachem RG. Environmental aspects of cholera epidemiology. I. A review of selected reports of endemic and epidemic situations during 1961-1980. Trop Dis Bull 1981 Aug; 78(8):675-98

Feachem RG. Environmental aspects of cholera epidemiology. III. Transmission and control. Trop Dis Bull 1982 Jan;79(1):1-47

Feachem RG. Is cholera primarily water-borne? (letter). Lancet 1976 Oct 30;2(7990): 957-8

Feeley JC. Classification of *Vibrio cholerae (Vibrio comma)*, including El Tor vibrios, by intrasubspecific characteristics. J Bacteriol 1965 Mar;89(3):665-70

Classification of *Vibrio cholerae* including El Tor vibrios was done by intra-subspecific characteristics. The properties of 220 serotype O group I vibrios most of which were obtained from an epidemic were studied. The strains were cultured on gelatin agar and colonial morphology was examined. All the 220 strains produced cloudy zones of strong gelatinase activity on gelatin agar. The colonial morphology of all strains was identical to that of vibrios. Microscopic examination revealed that all the strains were Gram-negative short rods with majority showing some tendency towards cellular curvature. Serologically all the strains were found to be agglutinated by vibrio O group I antiserum. However, on the basis of hemolytic activity, phage IV sensitivity test, agglutinated chicken red cells (CCA) and Voges-Proskaver (VP) reactivity, the strains were identified and classified into five types. Type 1 strains were phage IV sensitive, non-hemolytic by tube and plate tests, unable to agglutinate chicken red cells and showed Voges-Proskaver reaction weakly positive at 22°C. Type 2 strains were identical to type 1 except in that the strains gave positive CCA reactions. Type 3 strains were strongly hemolytic by the tube hemolysis test as well as by the plate hemolysis test and showed clear cut zones of hemolysis around well isolated colonies on both aerobic

and anaerobic sheep blood-agar plates. The strains were resistant to lysis by group IV-phage, positive to CCA reactions and strongly positive to VP reactions. Type 4 strains resemble type 3 except these strains were hemolytic only by plate method unless culture had undergone pronounced rugose variation. These strains produced weak but detectable hemolysis on aerobic and anaerobic blood agar plates, the zone of hemolysis was slightly larger than the colony and was easily identified. Type 5 strains were identical with types 3 and 4 except that these strains were completely non-hemolytic. Type 1 and 3 strains possess the same characteristics usually ascribed to classical and El Tor. The properties of type 3 strains and the original El Tor isolated from the El Tor quarantine station were also studied. With regard to geographical and chronological distribution of these types of strains the author recommends the recognition of a single species *V. cholerae* having several types.

Feeley J see Cook WL

Felsenfeld O. Notes on food, beverages and fomites contaminated with *Vibrio cholerae*. Bull WHO 1965;33(5):725-34

Felsenfeld O. Relationship of food and choleragenic vibrios. *In:* Proceedings of the Cholera Research Symposium, Honolulu, Hawaii, 24-29 Jan 1965. Washington, D.C.: U S Government Printing Office, 1965:313

The growth of vibrios were observed on the foods when they were inoculated with *Vibrio cholerae* biotypes classical and El Tor. Sixty-seven Thai fruits, vegetables, twenty-five types of cooked foods, fifteen milk products and eleven fomites were inoculated with 5 strains of each *V. cholerae* biotypes, using a mixture of vibrios, mucin and human feces adjusted to an alkaline pH. Six experiments were set up with each sample and each strain, three of them being inoculated at tropical room temperature and three in the icebox. None of the vibrios survived on acid fruits and on acid foods for more than few hours but the survival time was longer in the icebox than at room temperature. Cooked starchy foods, milk and milk products and the inside of watermelons permitted *Vibrio* survival for several weeks. Classical *V. cholerae* died off sooner than the El Tor biotype. Moisture, pH, sodium chloride content and sugar content considerably influenced the results of the experiments. All the strains produced mutant and variant colonies in a significantly higher proportion on certain foods than when cultured on conventional laboratory media. Thus, strain El Tor 2, when grown on conventional media, produced colonies of which 1 out of 200-250 were agglutinable by antisera diluted to one-fourth of their final titers, but not by the maximal dilutions of the antisera. The same strain after 7 days on milk pudding, produced one such colony for every 50-70 normal colonies. Loss of agglutinability and of the rapidity of acid formation was frequent after sojourn on fruits, meats, in water, and on metal fomites. Strain Ogawa 1, after one day on paper money, gave 9-10 times more colonies that were agglutinated only by concentrated sera than did the original strain. The toxicity, as measured in infant rabbits, decreased 3-4 times. The study confirmed that cholera vibrios may change their characteristics during their sojourn in nature.

Felsenfeld O see Gyr K

Finkelstein RA, Mukerjee S. Hemagglutination: a rapid method for differentiating *Vibrio cholerae* and El Tor vibrios. Proc Soc Exp Biol Med 1963 Feb;112(2):355-9

A rapid, simple and reliable method to distinguish between *Vibrio cholerae* and El Tor vibrios using a hemagglutination technique is described. A total of 327 laboratory strains of *V. cholerae* and 349 laboratory cultures of El Tor vibrios were

examined. In addition, a number of fresh isolates of *V. cholerae* from the 1962 cholera epidemic in Calcutta and the 1961 and 1962 epidemics of cholera El Tor in the Philippines were studied. A drop of physiological saline was applied on a previously divided microscopic slide. Bacterial growth from agar cultures was emulsified in the drop to make a suspension. A loopful of 2.5% chicken red blood cells washed in normal saline was then added. Clumping of the erythrocytes was observed with the El Tor vibrios whereas *V. cholerae* were non-hemagglutinating. Hemagglutination appears to be a more stable property than the hemolytic activity of El Tor vibrios. This test can be performed at the time of primary isolation to give immediate results visible to the naked eye.

Finkelstein RA, Atthasampunna P, Chulasamaya M, Charunmethee P. Pathogenesis of experimental cholera: biologic activities of purified Procholeragen A. J Immunol 1966 Mar;96(3):440-9

This paper describes procedures for the further purification of Procholeragen A employing an immunologic assay as a means of recognition. Procholeragen A, a choleragenic moiety elaborated by *Vibrio cholerae* 569 B Inaba, was concentrated and partially purified by gel filtration with Sephadex G-200. Purified Procholeragen A, which was essentially free of cholera endotoxin but still not pure, gave a single zone of precipitation with rabbit anti-choleragen antibody adsorbed with live vibrios This purified Procholeragen A when administered in quantities as little as 6.0 µg with Procholeragen B, *per os* in infant rabbits caused experimental cholera. When 0.1 to 0.2 µg amounts were inoculated intradermally in adult rabbits, Procholeragen A caused a delayed but sustained local erythematous oedematous induration which fixed intravenous Evans blue dye. The present work indicated that Procholeragen B can be replaced by a variety of buffers and serves no function in experimental cholera other than to protect Procholeragen A against destruction under the acid conditions in the stomach. Procholeragen A causes experimental cholera when inoculated by itself into the small bowel of infant rabbits, but Procholeragen B did not have any further biologic significance in experimental cholera. On the basis of the experimental observations, it was proposed, as an operational concept, that a major factor in the pathogenesis of cholera is an increase in villus capillary permeability, caused by a permeability factor similar or identical to Procholeragen A, elaborated by the cholera vibrios in the small intestine.

Finkelstein RA, Vasil ML, Holmes RK. Studies on toxinogenesis in *Vibrio cholerae*. I. Isolation of mutants with altered toxinogenicity. J Infect Dis 1974 Feb;129(2): 117-23

The study represents some methods designed to elucidate the genetic mechanism regulating toxigenesis in *Vibrio cholerae*. Methods were developed to identify toxin produced by individual colonies of *V. cholerae*. Strain 569B Inaba and mutants derived from it by treatment with nitrosoguanidine, other strains including El Tor Ogawa 3083-2 var and 3083-B, strains NIH35A3 Inaham NIH41 Ogawa, B1307 Ogawa, Inaba-16 and 12Rx-1 Ogawa were used in this study. Colonies of strain 569B produced a halo of toxin-antitoxin (T-AT) precipitate in antitoxin agar. Similarly, colonies of strains 3083-2 var and 3083-13 also produced T-AT halos, whereas strains NH35A3, NIH41, B1307, Inaba-16, and 12Rx-1 showed negative responses. Mutants derived from 569B were isolated on the basis of producing less or no detectable enterotoxin. Quantitation with Ouchterlony and modified Oakley Fulthorpe techniques indicated that mutant colonies that failed to produce halos in antitoxin agar produced detectable amounts of toxin antigen. One of the mutants completely lacked the capacity to produce measurable amounts of toxin was avirulent for infant rabbits although it was prototrophic and colonized in the intestine of rabbits. In addition, the study revealed that an

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El Tor vibrio strain 3083 Ogawa produced an enterotoxin that lacked an antigenic determinant and was found similar to choleragen elaborated by strain 569B Inaba. Thus the study concluded that some antigenic variation exists among cholera entero- toxins derived from the strains of *V. cholerae* from different sources.

Finkelstein RA see Holmes RK

Finkelstein RA see Vasil ML

Forbes GI, Lockhart JDF, Bowman RK. Cholera and nightsoil infection in Hong Kong, 1966. Bull WHO 1967;36(2):367-73

Forbes GI, Lockhart JDF, Robertson MJ, Allan WGL. Cholera case investigation and the detection and treatment of cholera carriers in Hong Kong. Bull WHO 1968;39(3): 381-8

Forbes GI see Van de Linde PAM

Formal SB, Bohner H, Schneider H, Baron LS. Virulence of a nutritional mutant of *Vibrio comma*. Proc Soc Exp Biol Med 1960 Feb;103(2):359-61

Formal SB see LaBrec EH

Foster JW, Brestel C. NAD metabolism in *Vibrio cholerae*. J Bacteriol 1982 Jan;149 (1):368-71

Crude cholera toxin obtained from various mutants of *Vibrio cholerae* were assayed for several pyridine nucleotide cyclic components at different pH values. Toxins were assayed for anzymatic activities associated with the pyridine nucleotide cycle metabolism which included NAD glycohydrolase, nicotinamide (NA) deamidase, nicoti- namide mononucleotide (NMN) deamidase and nicotinic acid phosphoribosyltransferase. Crude extracts of *V. cholerae* 569B and CA401 showed similar results. *V. cholerae* 569B grown in the presence of nicotinic acid showed complete recycling of nicotinic acid to NAD. NMN deamidase activity was readily observed in crude extracts of strain 569B and CA401. Molecular seive chromatography with sephadex G-150 revealed that both NMN deamidase and NA deamidase are two separate enzymes having molecular weights of 43,000 and 35,000 respectively. The existence of 5-membered pyridine nucleotide cycle and 4-membered pyridine nucleotide cycle in *V. cholerae* were demons- trated. Toxin producing strain, FA86, showed twice the parental glycohydrolase activity. Extracts of strain FA64 showed that NMN deamidase activity was 2.5 times higher than that of the parent. However, none of the mutant strains exhibited NAD pyrophosphatase activity. The results suggest that most of the NAD-glycohydrolase in *V. cholerae* extracts were not directly related to cholera toxin.

Francis DW see Twedt RM

Freter R, Jones GW. Adhesive properties of *Vibrio cholerae:* nature of the inter- action with intact mucosal surfaces. Infect Immun 1976 Jul;14(1):246-56

Adhesion of *Vibrio cholerae* and *Salmonella enteritidis* on the intestinal tissue slices were studied. Tissue slices were incubated for 1 h at 37°C with *V. cholerae* and *S. enteritidis* suspensions. By fluorescent antibody techniques large numbers of *V. cholerae* were observed in association with the mucosal surface in contrast with serosal surface of the sliced sections. Two receptors were found for the adhesion of vibrios on the mucosal surface of rabbit small intestine. The receptor concerned

with the adhesion of *Salmonella* differed from that of the vibrios. Non-motile *Vibrio* mutants failed to adhere to intact mucosa due to its lack of adhesion that enabled the strain to bend to the L-fucose-resistant mucosal receptor. However, it was suggested that motility might play a vital role in the transport of vibrios to the location of the L-fucose-resistant receptor. Considerable differences in the *Vibrio* adhesion to brush borders and intact mucosa was observed. L-fucose, a specific inhibitor of *Vibrio* adhesion to brush border membrane, surprisingly failed to reduce the adhesion of vibrios and *Salmonella* to the mucosa of intestinal tissue slice. The relation between agglutination and inhibition of adhesion was also investigated. The inhibitory effect of antibody on adhesion of vibrios to the intestinal tissue slice occurred in the absence of bacterial agglutination. The bacterial agglutination occurred at high concentration of vibrios. It was speculated that during the infection high concentration of vibrios were not present in human. Agglutination in the lumen of intestine seems to play a minor role in prophylactic immunity against cholera and other enteric diseases.

Freter R see Jones GW

Furniss AL see Bashford DJ

Gallut J, Quiniou J. Interactions between classical *Vibrio cholerae*, the El Tor biotype and non-agglutinable vibrios. Bull WHO 1970;42(3):464-6

Gangarosa EJ, Mosley WH. Asiatic cholera. *In:* Tice's Practice of medicine, ch 28, vol. 3. Maryland: Harper and Row, 1970:1-13

Gangarosa EJ, DeWitt WE, Huq I, Zarifi A. Laboratory methods in cholera: isolation of *Vibrio cholerae* (El Tor and classical) on TCBS medium in minimally equipped laboratories. Trans R Soc Trop Med Hyg 1968 Oct 9;62(5):693-9

The efficiency of thiosulphate citrate bile salt sucrose agar (TCBS) medium for *Vibrio cholerae* isolation was compared to two other well-established cholera media during an El Tor epidemic in Iran in 1965 and a classical cholera outbreak in the erstwhile East Pakistan (now Bangladesh) in 1966. Three plating media were used: (1) gelatin agar (GA); (2) Tellurite taurocholate gelatin agar (TTGA); and (3) TCBS. TCBS was compared with TTGA media during a clinical investigation of the duration of *Vibrio* excretion among Iranian cholera patients. Recently passed stool specimens were streaked directly on these media. Two comparative studies were conducted in parallel in the then East Pakistan, and specimens from acute and convalescent patients were promptly streaked directly on to all three media at the bedside, with separate rectal swabs. Six separate studies were conducted to compare the efficiency of 3 commercially available TCBS media. All 3 commercially available TCBS media were found equally efficient. TCBS medium was as effective as the other two widely-used media for *V. cholerae* isolation. The simplified isolation method, using TCBS, as highlighted in this study, has important implications for cholera surveillance.

Gangarosa EJ see DeWitt WE

Ganguly U, Greenough WB, III. Adenosine 3',5'-cyclic monophosphate in *Vibrio cholerae*. Infect Immun 1975 Feb;11(2):343-9

This study evaluates the correlation between toxin production and adenylate cyclase activity in 3 different *Vibrio cholerae* strains (Inaba 569 B, Ogawa 395 and Inaba 569 B M-13 mutant). The strains were grown in syncase medium and their extracellular concentration of cyclic adenosine 3',5'-monophosphate (AMP) was measured.

Both the cultures of wild type Inaba 569 B and Ogawa 395 accumulated comparable concentration of cyclic AMP and there were no significant differences in cyclic AMP levels. However, cyclic AMP produced by mutant M-13 Inaba was approximately half of that formed by the wild type Inaba 569 B. This may be due to the lower activity of adenylate cyclase observed in the mutant. The effect of low and high glucose concentration on cyclic AMP were also studied. In presence of 3% glucose, cyclic AMP levels were reduced without any changes of adenylate cyclase activity. On the other hand, in presence of 2.5% glucose a marked rise of cyclic AMP was observed. Enterotoxin production was same at both concentration of glucose when pH of the medium was maintained between 7.6 and 8.0. Hence the toxin production was not directly dependent on the cyclic AMP level of *V. cholerae* and the maximum concentration of toxin formed extracellularly varied with the carbon sources. In wild-type *V. cholerae* Inaba 569 B neither the adenylate cyclase activity nor the toxin production was reduced by an increased glucose concentration, whereas cyclic AMP levels were reduced by six folds. Mutant M-13, which did not produce any toxin, showed one-third of activity of adenylate cyclase found in wild-type 569 B. Thus a correlation was found between the toxin production of *V. cholerae* and adenylate cyclase activity.

Ganguly U see Chakrabarti MK

Ganguly U see Chakraborty MK

Gardner EW, Lyles ST, Lankford CE. A comparison of virulence of *Vibrio cholerae* strains for the embryonated egg. J Infect Dis 1964 Dec;114:412-6

The LD_{50} values of *Vibrio cholerae* in the 13-day embryonated egg were used to determine whether strains from patients of the same epidemic locality were identical or different, whether the LD_{50} values of antigenically smooth cultures isolated from the same patient at different times in the course of an infection were constant, and whether strains from different epidemic sources might show trends toward higher or lower LD_{50} values. Chick embryo virulence of certain *V. cholerae* strains from the same source were compared with their virulence for the mouse. The LD_{50} tests of daily isolates obtained from individual patients indicated that the infecting strain may remain stable in some cases, but in others it appears to effect temporary or progressive selection of mutants which arise during the course of infection. The R mutant strain was avirulent for the 13-day embryo. The result suggested that the embryo and mouse virulence of *V. cholerae* were associated with humans virulence as the strains were found virulent in each host. Thus the avirulence of R strains for the embryonated egg and certain data obtained with S strains indicated that embryo virulence of *V. cholerae* were associated to an uncertain degree with its virulence in humans.

Gardner EW, Lyles ST, Lankford CE, Hagens SJ. *Vibrio cholerae* infection in the embryonated egg. J Infect Dis 1963 May-Jun;112(3):264-72

Virulence titrations were made in the 13-day chick embryo with certain cultures to test the reproducibility of LD_{50} determinations for 24 h deaths. Possible causes of virulence variation and the nature of the virulence mechanism of *V. cholerae* in the chick embryo are discussed. Preliminary results indicated that comparisons of strain virulence could be made on the basis of LD_{50} values obtained when deaths were scored at 24 h after inoculation. Significant changes in the embryo LD_{50} were also noted with colonical variation of *V. cholerae* strains. There is evidence of the decisive role of the O antigen, and the inherent differences in the capacity of vibrios to invade the circulatory system, which may be determining factors of virulence.

Classical *Vibrio cholerae*

Garges S see Collwell RR

Gauthier D see Parker C

Ghoda A see Yamamoto A

Ghosh BN see Niyogi SG

Ghosh RK see Roy NK·

Ghosh S see Chatterjee SN

Ghosh SK see Panja G

Gildemeister E, Baerthlein K. [Asiatic cholera]. MMW 1915;62:705

Glass RI, Becker S, Huq MI, Stoll BJ, Khan MU, Merson MH, Lee JV, Black RE. Endemic cholera in rural Bangladesh, 1966-1980. Am J Epidemiol 1982 Dec;116(6):959-70

Cholera in Bangladesh was due to the classical biotype of *Vibrio cholerae* until 1972 and this was replaced by the El Tor biotype in 1973. Earlier studies of El Tor cholera in rural and urban areas of the country -- the same communities where classical cholera was existing -- demonstrated that there were many more unreported infections for every clinical case of El Tor as compared to classical cholera, where both infections were present simultaneously. The present family study of El Tor cholera cases with a 10-day follow-up period delineates the characteristics of this disease in a congested urban setting, the same community of Dhaka, where many studies on cholera had been done. Cases were selected from the hospital of International Centre for Diarrhoeal Disease Research, Bangladesh. Results showed that the rate (31.3%) was higher than previously recorded both for classical (13%-24.6%) and El Tor (8.7%-20.6%) strains. The infection to case ratio of 4:3 in this study differed markedly from the ratio of 100:1 and 4-36:1 reported earlier. Thus, under certain socio-environmental conditions the virulence of *V. cholerae* El Tor equals that of the classical biotype.

Glass RI see Huq MI

Gordon RS see Lindenbaum J

Gormly AA see Horsfall DJ

Greenough WB, III see Benenson AS

Greenough WB, III see Ganguly U

Greenough WB, III see Lindenbaum J

Guentzel MN, Berry LJ. Motility as a virulence factor for *Vibrio cholerae*. Infect Immun 1975 May;11(5):890-7

Guha DK see Mukerjee S

Guhathakurta B, Sasmal D, Datta GC, Datta A. Immunological studies on the ammonium sulphate fractions of *Vibrio cholerae* cell lysate. Indian J Exp Biol 1978 May;16(5):569-72

Vibrio cholerae cell lysate was fractionated with ammonium sulphate and their immu-

nogenicity of the fractions was compared with that of conventional vaccine both with and without supplementation of pure lipopolysaccharide (LPS) and aluminium hydroxide adjuvant. Comparison of different ammonium sulphate fractions indicated that the 60% aluminium sulphate fraction (with LPS and adjuvant), irrespective of the serotype, was more efficient in eliciting antibody production. The 60% fraction contained more protein and less hexose and showed more precipitin lines than the corresponding 30% fraction. Conventional vaccine alone gave insignificant protection. However, the vaccines when supplemented with adjuvant showed appreciable improvement in immunogenicity.

Guhathakurta B see Sen AK

Gyr K, Felsenfeld O. The effect of oral pancreatic extract on jejunal bactericidal activity in protein-deficient vervet monkeys challenged with *Vibrio cholerae*. Acta Trop (Basel) 1979 Jul;36(2):147-50

Hagens SJ see Gardner EW

Han GK, Khie TS. A new method for the differentiation of *Vibrio comma* and *Vibrio* El Tor. Am J Hyg 1963 Mar;77(2):184-6

This report describes a new method for the differentiation of the *Vibrio comma* and El Tor vibrios based on studies conducted at the Department of Microbiology, School of Medicine, University of Indonesia. Twenty-seven strains of "true" (classical) *V. cholerae*, 258 strains of El Tor vibrios and 12 strains of nonagglutinable vibrios were examined. It was found that the growth of agglutinable vibrios in 0.9% trypsin broth of pH 7 and the sensitivity test with Bacto sensitivity disks containing 50 units of polymyxin B were useful for classifying classical and El Tor biotypes. A definite distinction was observed between the two biotypes. The phenomemon being highly speci·fic could serve as a method of differentiation of classical *V. cholerae* from El Tor biotype. All classical *V. cholerae* showed clear growth in 0.9% trypsin broth, and were sensitive to 50 units of polymyxin B. Of the 258 El Tor strains, 256 produced either turbid growth in the broth or a distinct surface pellicle, and all were resistant to polymyxin B. Only 2 El Tor strains showed a clear growth in the Trypsin medium, but they were resistant to polymyxin B. Although the growth of the nonagglutinable vibrios in 0.9% trypsin was not different from that of the El Tor vibrios, they could be differentiated easily from other vibrios by means of the serological test. This simple and inexpensive method was also valid for both freshly isolated strains and old laboratory strains.

Harada K see Hayashi F

Hart LT see Hranitzky KW

Hayashi F, Nakajima T, Suzuki M, Harada K, Inoue M, Mitsuhashi S. Stability of *Vibrio anguillarum* R plasmids in *Vibrio parahaemolyticus* and *Vibrio cholerae*. *In:* Mitsuhashi S, ed. Drug resistance in bacteria: genetics, biochemistry, and molecular biology. Tokyo: Japan Scientific Society, 1982:381-5

Hebert WO see Twedt RM

Heddle RJ see Horsfall DJ

Heiberg B. The biochemical reactions of vibrios. J Hyg (Lond) 1936;36:114-7

Hirschhorn N see Lindenbaum J

Hisatsune K, Kondo S, Kawata T, Kishimoto Y. Fatty acid composition of lipopoly-saccharides of *Vibrio cholerae* 35A3 (Inaba), NIH 90 (Ogawa), and 4715 (Nag). J Bacteriol 1979 Apr;138(1):288-90

The fatty acid composition of lipopolysaccharides (LPS) of *Vibrio cholerae* has been investigated. *V. cholerae* 35A3 (Inaba), NIH 90 (Ogawa) and 4715 (Nag) types were cultured in 1% glucose-peptone medium of pH 8.0 at 30°C for 16 h. The cells were then prepared, and a comparative study of the fatty acid composition of LPS isolated from these strains was done by gas-liquid chromatography. The total lipid content of LPS from the cell walls of the three strains were between 25 and 34%. Total fatty acid content recovered as methyl ester was unexpectedly low (7%) in the LPS of NIH 90 (Ogawa). The major nonhydroxy fatty acid components of all three strains were found to be $C_{14:0}$ and $C_{16:0}$. In addition, small but significant amounts of $C_{18:0}$, $C_{16:1}$ and $C_{18:1}$ were observed in the LPS of all three strains. Considerable amounts of odd-numbered fatty acids, such as nonhydroxy $C_{15:0}$ and $C_{17:0}$ were found in the LPS of only strain 35A3 (Inaba). 3-hydroxy fatty acid composition $C_{12h:0}$ and $C_{14h:0}$ were found to be present in all three strains. Further more substantial amounts of odd-numbered fatty acid such as 3-hydroxy $C_{11h:0}$ and $C_{13h:0}$ were also observed particularly in the LPS of strain 35A3 (Inaba).

Hisatsune K, Kondo S. Lipopolysaccharide of R mutants isolated from *Vibrio cholerae*. Biochem J 1980 Jan 1;185(1):77-81

Hisatsune K, Kondo S. Lipopolysaccharide of *Vibrio cholerae*. (III)-chemical and serological study on Rantigen. Jpn J Med Sci Biol 1979 Apr;32(2):106-9

Holmes RK, Baine WB, Vasil ML. Quantitative measurements of cholera enterotoxin in cultures of toxinogenic wild-type and nontoxinogenic mutant strains of *Vibrio cholerae* by using a sensitive and specific reversed passive hemagglutination assay for cholera enterotoxin. Infect Immun 1978 Jan;19(1):101-6

A reversed passive hemagglutination (RPHA) assay for cholera enterotoxin has been developed. The RPHA assay is very sensitive and specific and antibodies against enterotoxin are assayed by inhibition of RPHA (RPHA-I). Equine antichloregenoid antibodies were purified by immunoadsorption and was covalently coupled to formali-nized sheep erythrocytes suing bis-diazotized benzidine. The antitoxin-sensitized erythrocytes were shown to agglutinate specifically in the presence of cholera enterotoxin. By using standard microtiter techniques a quantitative RPHA assay for cholera enterotoxin and a corresponding RPHA-I assay for antibody to enterotoxin was developed. In this RPHA assay system, the smallest quantity of enterotoxin that caused hemagglutination was found to be approximately 20 kg. By using this assay it was demonstrated that several nontoxinogenic (tox-) strains of *Vibrio cholerae* produced small but detectable yields of enterotoxin, 4 to 16 μg/ml whereas the highly toxinogenic strain 569B Inaba produces approximately 16 μg of enterotoxin per ml. The enterotoxin produced by (tox-1) strains was found to be identical to the entero-toxin from *V. cholerae* 569B Inaba in its immunological and biological activities. Strains of *V. cholerae* producing intermediate yields of enterotoxin were obtained by two techniques (i) as less toxinogenic mutants derived from highly toxinogenic strains and (ii) as more toxinogenic mutants derived from tox- strains. It was con-cluded that the yield of enterotoxin in cultures of *V. cholerae* grown under stan-dardized conditions is genetically controlled which can be altered by mutation.

Holmes RK, Vasil ML, Finkelstein RA. Studies on toxinogenesis in *Vibrio cholerae*.
III. Characterization of nontoxinogenic mutants *in vitro* and in experimental animals
J Clin Invest 1975 Mar;55(3):551-60

Mutants that are altered in their ability to synthesize or release choleragen have
been isolated from *Vibrio cholerae* strains 569B Inaba, a classical cholera vibrio
and 3083-2 Ogawa biotype El Tor. The independently isolated mutants of *V. cholerae*
with altered toxinogenicity could be separated by qualitative and quantitative immu-
nological assay into groups with different phenotypic properties. The mutants that
make no choleragen detectable *in vitro* by precipitin test or by electrophoresis in
polyacrylamide gels were designated tox⁻. Analysis of proteins in concentrated
culture supernates by electrophoresis in polyacrylamide gels showed that cultures
from tox⁻ strains lacked proteins with electrophoretic mobility corresponding with
choleragen or the spontaneously formed toxoid (choleragenoid). Eight of the tox-
mutants were examined for choleragenicity in experimental animals. Data derived from
intraintestinal infection of infant rabbits showed that rabbits infected with tox⁻
strains remained disease-free or developed milder disease than rabbits infected with
tox⁺ parental strains. When signs of cholera developed after inoculation with tox⁻
mutants, detectable numbers of tox⁺ revertants could be isolated from the intestines
of the infected animals. Two tox⁻ strains M13 and M27 were totally avirulent and
produced no signs of diarrhoeal illness in any of the infected animals, and mutant
M13 also remained avirulent and stably tox⁻ during six cycles of serial passage in
infant rabbits. The two strains were noncholerogenic in adult rabbit ileal loops.
Several experiments were performed to compare colonization of infant rabbits by the
virulent parental strain 569B and by the stably tox⁻ avirulent mutant M13. It was
clear that strain M13 could multiply *in vivo* and colonize the intestinal tract of
infant rabbits for upto 48 h periods.

Holmes RK see Baine WB

Holmes RK see Finkelstein RA

Holmes RK see Ivins BE

Holmes RK see Vasil ML

Holmgren J, Svennerholm AM, Lindblad M. Receptor-like glycocompounds in human milk
that inhibit classical and El Tor *Vibrio cholerae* cell adherence (hemagglutination).
Infect Immun 1983 Jan;39(1):147-54

Holmgren J see Islam MR

Homma JY see Yamamoto A

Honda T, Takeda Y, Miwatani T. Isolation of special antibodies which react only
with homologous enterotoxins from *Vibrio cholerae* and enterotoxigenic *Escherichia
coli*. Infect Immun 1981;34(2):333-6

Purified cholera enterotoxin (CT) of *Vibrio cholerae* 569B and heat-labile entero-
toxin (LT) obtained from culture filtrates of enterotoxigenic *Escherichia coli*
536-5 showed a common antigenic determinant. Antibodies against these unique anti-
genic determinants was prepared by immunoaffinity column chromatographies -- that
is, antiserum that reacted with CT but not with LT, and antiserum that reacted with
LT but not with CT. The specificities of these antisera were demonstrated by
Ouchterlony double-gel diffusion tests and by neutralization of the activities for

CT and LT to cause morphological changes in Chinese hamster ovary (CHO) cells. The data confirmed the findings of other investigations, where the existence of common and unique antigens in CT and LT were seen.

Hood MA, Ness GE. Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. Appl Environ Microbiol 1982 Mar;43(3):578-84

The comparative survival of *Vibrio cholerae* and *Escherichia coli* in estuarine water and sediment chambers was studied using plate counting and direct counting techniques. *V. cholerae* strains including environmental, clinical, and serotype 01 and non-01 isolates and *E. coli* strains of ATCC 25922 and a freshly cultured human isolate were studied. The environmental *V. cholerae* serotype 01 strain survived well in estuarine waters and sediments and the recovery varied significantly with incubation temperature. After 7 days the organism grew abundantly in the sterile sediment but viable cells declined in nonsterile sediments as well as in nonsterile water. On the other hand, strains of *E. coli* did not survive in both sterile and nonsterile estuarine waters and could not be recovered by plating. *E. coli* did not survive well in sterile sediment but in nonsterile sediment its growth was observed to be better than the *V. cholerae* strains. Freshly cultured human isolates of *E. coli* did not survive as well as the *V. cholerae* strains in sterile estuarine water. The differences between direct counts and viable counts of *V. cholerae* strains in sterile estuarine water were notably less than with *E. coli* strains. Significant differences between viable and direct counts of *V. cholerae* were observed with the 01 strain. These findings suggest that *V. cholerae* can survive better in estuarine water than *E. coli*. This in time, has implications for the validity of using fecal coliform *E. coli* levels to indicate the quantity of water and shellfish. Other environmental studies also showed that fecal coliform levels in estuarine waters do not correlate well with *V. cholerae* levels.

Hornick RB, Music SI, Wenzel R, Cash R, Libonati JP, Snyder MJ, Woodward TE. The broad street pump revisited: response of volunteers to ingested cholera vibrios. Bull N Y Acad Med 1971 Oct;47(10):1181-91

Hornick RB see Cash RA

Horsfall DJ, Rowley D. A comparison of methods for measuring intestinal antibodies against *Vibrio cholerae* in mice. Aust J Exp Biol Med Sci 1979 Feb;57(1):61-73

To enable quantitative comparisons to be made, the sensitivities of the various techniques for detection of purified antibodies of the major mouse immunoglobulin classes were estimated. A baby mouse cholera model was included in these studies. This study indicates that the most sensitive assays for detecting intestinal antibodies are the baby mouse protection test and the radioimmunoassay. Of the two most successful assays for the determination of mouse intestinal antibody, the use of the baby mouse protection assay was confined to *Vibrio cholerae* infection. Radioimmunoassay, on the other hand could be used to determine intestinal levels of antibody induced by any enteric infection. The method was reproducible and sensitive. The baby mouse protection test had the advantage of being equally efficient in the detection of antibody of all classes, and highly efficient for detection of antibody fragments.

Horsfall DJ, Rowley D. Intestinal antibody to *Vibrio cholerae* in immunised mice. Aust J Exp Biol Med Sci 1979 Feb;57(1):75-85

Horsfall DJ, Heddle RJ, Gormly AA, Rowley D. *In vitro* degradation of mouse, rabbit and dog antibodies to *Vibrio cholerae* by succus entericus. Aust J Exp Biol Med Sci 1978 Dec;56(Pt.6):681-93

This study examines the rates of degradation of the major immunoglobulin classes, as measured by two functional abilities. One, the ability to kill vibrios in conjunction with complement (vibriocidal reaction) is dependent on intact Fc function. The other, the *in vivo* protection of infant mice from orally-induced choleraic diarrhoeal-death syndrome, is dependent on the antibody-combining sites. Purified antibodies to *V. cholerae* from mouse, rabbit and dog were digested *in vitro* by homologous intestinal secretions. When assessed with regard to their complement-dependent vibriocidal activity, IgG antibodies generally were more susceptible to degradation than were IgM antibodies. High levels of tryptic inhibitors were required to inhibit this digestion. Rabbit IgG was unusual in being resistant to digestion. Gel filtration studies demonstrated that secretory IgA, isolated from mouse intestinal secretions, was resistant to proteolysis. Similar studies on dog IgG and mouse IgM demonstrated production of F (ab')$_2$-like fragments. Digests of these antibodies, while devoid of Fc-mediated vibriocidal activity, retained significant protective activity for baby mice.

Horsfall DJ, Rowley D. Modification of the local immune response to *Vibrio cholerae* attributed to the intestinal microbial flora of the mouse. Aust J Exp Biol Med Sci 1978 Oct;56(5):579-86

Horsfall DJ, Brown MA, Rowley D. The suppressive effect of circulating specific antibody on the response to oral immunisation with *Vibrio cholerae*. Aust J Exp Biol Med Sci 1979 Jun;57(3):271-8

The effect of the presence of very small amounts of circulating antibody on the response to orally administered *Vibrio cholerae* was studied. Infant mouse-passaged *V. cholerae* Inaba 569B was used as bacterial strain, and the animals were two-month-old specific pathogen-free outbred LAC strain mice of either sex. Specific IgG antibody given intravenously 3-4 h prior to oral immunization with *V. cholerae* led to a specific depression of immune response to oral vaccination. The suppression extended not only to antibody found locally in the gut, but also to that circulating in the serum. One vibriocidal unit of passive antibody was sufficient to produce marked suppression of the response following the shorter immunization regimes. The suppression was considered to be due to central repression of the antigen-reactive lymphocyte, rather than to antigen exclusion at the gut mucosal surface. Investigation of the suppressed responses by the Coombs antiglobulin-enhancement of hemagglutination suggested that the only classes of antibody repressed were IgA and IgM. It was suggested that, if these results are applicable to other species, oral immunization of individuals possessing circulating antibody should be avoided.

Howard-Jones N. Cholera nomenclature and nosology: a historical note. Bull WHO 1974; 51(3):317-23

Hranitzky KW, Mulholland A, Larson AD, Eubanks ER, Hart LT. Characterization of a flagellar sheath protein of *Vibrio cholerae*. Infect Immun 1980 Feb;27(2):597-603

Hugh R. A comparison of the proposed neotype strain and 258 isolates of *Vibrio cholerae* pacini. Int Bull Bact Nomencl Taxon 1965;15:13

Hunt JM see Twedt RM

Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl Environ Microbiol 1983 Jan;45(1):275-83

The study examines the association between *Vibrio cholerae* and zooplankton and determines whether the presence of copepods influence the survival of *V. cholerae*

in the aquatic environment. *V. cholerae* 01 (classical Inaba and El Tor Ogawa), *V. cholerae* non-01, *V. parahaemolyticus*, *Escherichia coli* and *Pseudomonas* sp were used. Strains of *V. cholerae*, both 01 and non-01 serovars, were found to attach to the surfaces of live copepods maintained in natural water samples collected from the Chesapeake Bay and Bangladesh environments. Scanning electron microscopy (SEM) confirmed the specificity of attachment of *V. cholerae* to live copepods. Attachment to live copepods appeared to be selective, since the heaviest concentrations of bacterial cells were observed in the oral region and on the egg sac of the copepods. In addition, survival of *V. cholerae* in water was extended in the presence of live copepods. SEM revealed that there was no attachment when cold-killed (by exposure to -60°C) copepods were employed. *V. cholerae* survival was not as long in the presence of dead copepods as in the live copepod system. *V. parahaemolyticus* also adhered to live copepods, but without selectivity, i.e., the cells covered the whole copepod. The attachment had no effect on survival of the organism in water. Strains of *Pseudomonas* sp and *E. coli* did not attach to live or dead copepods. The attachment of vibrios to copepods is significant since strains of other bacteria used in the study did not show any adherence. The attachment between *V. cholerae* and live copepods is suggested to have ecological as well as epidemiological significance. Since *V. cholerae* serovar 01 is the causative organism for cholera, the results also have epidemiological implications.

Huq A see Colwell RR

Huq I see Colwell RR

Huq I see Cook WL

Huq I see DeWitt WE

Huq I see Gangarosa EJ

Huq I see Martin AR

Huq MI, Rahaman MM. Blotting paper strip for the transport of stool specimens for laboratory diagnosis of cholera. Indian J Med Res 1983 Dec;78:765-8

The use of blotting paper strips, compared to Cary-Blair transport medium, culture plates and enrichment broth, for transporting fecal materials from suspected cholera patients was evaluated. 4 x 2.5 cm pieces of white blotting paper soaked in patient's stool, and inserted in polythene bags were mailed to a central laboratory, while rectal swabs also were collected in Cary-Blair transport medium, culture plates and enrichment broth for laboratory analysis. Taking the direct culture as 100 percent, the recovery rate from the paper strips was 93.7 percent, in contrast to 83.4 percent for Cary-Blair transport medium. The higher rate was statistically significant (p<0.001). Compared to Cary-Blair medium, blotting paper strips were able to keep *Vibrio cholerae* viable for 35 days, and allowed a higher percentage of isolation upto the third week. A recovery rate of 93.4 percent by the 14th day was considered highly satisfactory. The method eliminates the need for culture tubes and transport medium, and drastically reduces the cost of transporting specimens, thus facilitating laboratory diagnosis of cholera in areas where the disease is endemic.

Huq MI, Sanyal SC, Samadi AR, Monsur KA. Comparative behaviour of classical and El Tor biotypes of *Vibrio cholerae* 01 isolated in Bangladesh during 1982. J Diar Dis Res 1983 Mar;1(1):5-9

The change from classical to the El Tor biotype of *Vibrio cholerae* during the early seventies in Bangladesh remains to be elucidated. The shift in 1982 from El Tor back to the classical was not predicted, but provided an opportunity to study both the biotypes occurring simultaneously in nature, compared with earlier isolates. Comparative studies showed TCBS to be as good as TTGA for isolation of the two biotypes. Replacement of the El Tor by the classical biotype as the dominant epidemic strain, occurred over four months. Isolates of both biotypes from 1982 were found to be slow in mannitol fermentation. The isolates of El Tor were frankly hemolytic and belonged to phage type 4. The classical strains were of phage type 3. *V. cholerae* strains isolated in the late sixties and early seventies were similar in these markers. This suggested that the epidemic was caused by strains indigenous to Bangladesh. A classical strain of *V. cholerae* isolated in 1969 was overgrown by an El Tor strain of the same year when grown together in peptone water. Classical strains of 1982 however grew competitively with 1969 and 1982 strains of El Tor. One classical isolate of 1982 survived for 50 days when grown with an El Tor strain of 1969. These findings suggest that the classical *V. cholerae* strains of 1982 successfully compete with the El Tor strain. They are more toxigenic than the prevailing El Tor biotype.

Huq MI, Glass RI, Stoll BJ. Epidemiology of cholera (letter). N Engl J Med 1980 Sep 11;303(11):643-4

This report observes a simultaneous occurrence of sporadic cases of El Tor cholera and cases of classical cholera in Louisiana and Texas, USA, and in India, Thailand and Bangladesh respectively. The classical biotype of *Vibrio cholerae* O Group 1 which were known to have disappeared from Asia in 1960s and again in early 1970s was replaced by the El Tor biotype, but the National Institute of Cholera & Enteric Diseases, Calcutta (W.H.O. reference laboratory of Southeast Asia) report of having isolated several classical *V. cholerae* strains from the several *V. cholerae* O1 specimens received at their laboratory. Similar isolation was done at the International Centre for Diarrhoeal Disease Research, Bangladesh, where the biotypes of over 5,000 *V. cholerae* O1 were examined since the transition from classical to El Tor biotype during 1973. They have recorded only five classical strains since that time. The reappearance of this sporadic case of classical cholera could be due to the mutations from the El Tor biotype or due to the chronic carriage by persons who had previously been exposed to this organism. The occurrence of sporadic cases was due to the presence of *V. cholerae* O1 in the environment in association with copepods or shellfish. Investigation of sporadic cases of classical cholera during an El Tor outbreak may provide leads to further findings in the epidemiology of cholera, similar to those discovered in earlier studies.

Huq MI. A simple laboratory method for the diagnosis of *V. cholerae*. Trans R Soc Trop Med Hyg 1979 Oct 13;73(5):553-6

A simple, practical and most reliable method for the diagnosis of *Vibrio cholerae* in small hospitals, health centers and in places where inadequate bacteriological facilities are available has been evaluated in the Cholera Research Laboratory, Dhaka (now the International Centre for Diarrhoeal Disease Research, Bangladesh). Swabs collected from 309 suspected cholera patients were streaked on gelatin agar (GA), taurocholate gelatin agar (TTGA) and thiosulphate citrate bile-salt sucrose (TCBS) media. The plates, after overnight incubation at 35°C, were observed for growth. A total of 309 positive cultures with colonies morphologically resembling *V. cholerae* were confirmed. All 309 samples showed positive growth on TTGA medium whereas 96.7% of the samples showed positive growth on TCBS. Inoculation on TTGA for 24 h showed flat, smooth grey colonies of *V. cholerae* with blackish centers

Classical *Vibrio cholerae*

and translucent peripheries, with a definite surrounding halo due to gelatin liqui-
fication. Non-cholera vibrios also showed larger colonies having a marked black
center, with translucent to opaque peripheries. Proteus colonies were identified
as larger colonies with a black center and with opaque, crenated peripheries. The
Pseudomonas colonies were recognized as crenated spreading colonies with pigments.
After incubation for 24 h on TCBS medium, *V. cholerae* showed distinct yellow colo-
nies surrounded by smooth edges with transparent peripheries. Of the samples, 87.7%
produced 30 or more vibrio colonies and only 4.9% required enrichment for detection.
V. cholerae grown in petri dishes and in four-ounce flat prescription bottles did
not show any significant differences in colony size; the appearance of the colonies
of either *V. cholerae* or the common contaminants did not change on either TTGA or
TCBS medium. TTGA medium is preferred to TCBS on account of its slightly higher
percentage of positive response, and slide agglutination with 0 group I sera can be
carried out reliably with colonies from the primary plates.

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Huq MI see Huq A

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of *Vibrio cholerae* for the mouse. J Infect Dis 1956;99:90-102

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Islam MR, Sack DA, Holmgren J, Bardhan PK, Rabbani GH. Use of chlorpromazine in
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Ivins BE, Holmes RK. Isolation and characterization of melanin-producing (mel) mutants of *Vibrio cholerae*. Infect Immun 1980 Mar;27(3):721-9

The biochemical basis for pigment production in *Vibrio cholerae* was investigated. Since the ability to produce pigment is not a property of wild-type *V. cholerae* 569B, pigment-producing mutant strains were isolated and characterized. Because pigment production and hypertoxinogenicity appeared simultaneously during the derivation of strain Htx-3, all newly isolated pigment-producing strains were also tested for associated phenotypic changes in toxinogenicity. After mutagenesis of *V. cholerae* 569B Inaba, a prototrophic highly toxinogenic strain of the classical biotype, with N-methyl-nitro-N-nitrosoguanidine, 28 independently derived pigment-forming (mel) mutants were isolated and characterized. Some of the newly isolated mel mutants differed from parental strain 569B in other characteristics besides pigmentation, including failure to grow on minimal medium, decreased or increased motility, increased resistance to streptomycin or novobiocin, and hypotoxinogenicity. It has not yet been established whether multiple phenotypic changes in individual mel mutants represents pleiotropic effects of single mutations or induction of multiple mutations by N-methyl-N'-nitro-N-nitrosoguanidine or both. Production of pigment by mel mutants occurred at temperatures from 22°C, and was inhibited by anaerobiosis. Specific nutritional requirements for pigment formation were investigated which revealed that supplementation of growth media with amino acid precursors of melanin (L-phenylalanine, L-tyrosine, or L-tyrosine plus L-cysteine) stimulated the production of pigment. Further studies performed to demonstrate similarity of the pigment to other microbial melanins provided evidences that the pigment produced by mel mutants of *V. cholerae* was related to the melanins. The experiments reported here established that a biochemical pathway leading to formation of melanin exists in *V. cholerae*. It was concluded that this pathway cannot be fully expressed in wild-type strain 569B, and that mutations in the gene(s) which were designated mel can permit hyperproduction of melanin under appropriate conditions.

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Iwanaga M, Morik, Kaviti JN. The routes of cholera spreading in Kenya. Trop Med 1981 Dec;23(4):217-22

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Johnson SR, Romig WR. *Vibrio cholerae* hybrid sex factor that contains ampicillin transposon Tn 1. J Bacteriol 1979 Jan;137(1):531-6

Johnson SR see Cook WL

Jones GW, Abrams GD, Freter R. Adhesive properties of *Vibrio cholerae*: adhesion to isolated rabbit brush border membranes and hemagglutinating activity. Infect Immun 1976 Jul;14(1):232-9

This report observes that vibrios readily adhere to isolated rabbit brush border membrane obtained from intestinal epithelial cells. Attachment of vibrios to mucus and agglutination of vibrios by mucus was not observed. Vibrios were seen to adhere

to the microvillus surface of the brush border. The adhesion was temperature-dependent and required calcium ions (optimal concentration range: 1 to 10 mmol CaCl₂). Adhesion of vibrios occurred rapidly after incubation at 37°C and reached a maximum after approximately 15 min. The number of vibrios attached to the brush border was reduced after 15 min incubation. The agglutination of human O erythrocytes by *Vibrio cholerae* was also observed and the hemagglutination test appeared to show the same mechanism that was involved in the adhesion of vibrios to brush border. Vibrios grown in TSB culture medium were adhesive and hemagglutinating but vibrios grown on TSA plates or suspended in buffer for 15 min at 37°C lacked these abilities. Strontium ions did not promote hemagglutination. Vibrios penetrated the intestinal mucus gel and occasionally became entrapped within. They did not attach to the mucus gel, but were moving along numerous tracks within the mucus gel.

Jones GW, Freter R. Adhesive properties of *Vibrio cholerae:* nature of the interaction with isolated rabbit brush border membranes and human erythrocytes. Infect Immun 1976 Jul;14(1):240-5

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Kabir S. Characterization of the lipopolysaccharide from *Vibrio cholerae* 395 (Ogawa). Infect Immun 1982 Dec;38(3):1263-72

Lipopolysaccharide (LPS) isolated from *Vibrio cholerae* 395 (Ogawa) by phenol-water techniques were studied in order to determine their chemical composition and biological properties. The LPS preparation did not contain any detectable amount of protein or nucleic acid, or other cellular contaminants. Upon acid hydrolysis the LPS was split into its polysaccharide (PS) and lipid A moieties. Thin-layer chromatography revealed that the PS contained both neutral (glucose, heptose, fructose) and amino (glucosamine, quinosamine) sugars. The lipid A part contained only the amino sugar glucosamine and its phosphate derivative, glucosamine phosphate. An important finding was that the LPS of *V. cholerae* 395 (Ogawa) contained an acid-labile amino sugar, 4-amino-arabinose, which however, was not present in the Inaba serotype of *V. cholerae*. Gas-liquid chromatography and mass spectrometry showed that the polysaccharide had a branched structure with glucose and heptose residues, primarily at the non-reducing-end groups. The LPS of *V. cholerae* 395 (Ogawa) differed from the LPS of *Enterobacteriaceae* by the absence of 2-keto-3-deoxyoctonate and presence of fructose. The lipid portion of the LPS consisted of both hydroxy and non-hydroxy fatty acids. The main fatty acid components were 3-hydroxylauric (3-OH $C_{12:0}$), 3-hydroxymyristic (3-OH $C_{14:0}$) and myristic ($C$14:0) acids. Minor amounts of palmitic($C$16:0), stearic ($C$18:0) and oleic ($C$18:1) acids were detected. The relative proportion of ester - and amide-bound fatty acids of the LPS was also determined. Interaction of LPS with lectins, concanavalin A and wheat germ agglutinin indicated that terminal glucose residues were α-linked and the terminal glucosamine residues were connected by -1, 3 linkages. The LPS of *V. cholerae* 395 (Ogawa) showed several biological properties in common with the LPS from other Gram-negative bacteria, i.e. it is pyrogenic, causes gelation of limulus lysate and induces lethal toxicity in mice. The LPS also induced non-specific resistance to infection when injected in mice in a sublethal dose. Biological reactions are mediated by the lipid A moiety of the LPS from Gram-negative bacteria. The general structural similarity between

the lipid A part of the *V. cholerae* 395 (Ogawa) LPS and the lipid A of *Enterobacteriaceae* accounts for their similar biological properties.

Kabir S, Ali S. Characterization of the surface to *Vibrio cholerae*. Infect Immun 1983 Mar;39(3):1048-58

In the bacterial disease process, host-parasite recognition is the prime criterion. It has been suggested that surface properties, such as hydrophobicity, electrostatic forces and the hemagglutinating activity of bacteria, might play an important role in mediating the interaction between bacteria and host cell surfaces. In the present study, a large number of freshly isolated *V. cholerae* strains of both classical and El Tor biotypes and serotypes Ogawa and Inaba were examined for these surface properties. The strains' hydrophobicity, surface charge density and hemagglutinating activity were found to depend on the medium and growth conditions. Outer membrane proteins were conspicuously hydrophobic amongst all the cell components examined. The *V. cholerae* strains adhered strongly to the anio-exchange matrix DEAE-cellulose. All El Tor strains possessed cell-bound hemagglutinin. Eighty percent of the El Tor strains were D-mannose sensitive, but all were L-fucose resistant. The results indicated that the *V. cholerae* surface contains both specific hemagglutinating and non-specific hydrophobic and ionic factors, which may influence the bacteria's eventual adherence to the host cell surface.

Kabir S. Immunochemical properties of the major outer membrane protein of *Vibrio cholerae*. Infect Immun 1983 Jan;39(1):452-5

The author of an earlier communication suggested that the outer membrane of *Vibrio cholerae* 01 strains contains a protein of molecular weight 48K, that is common to both the biotypes classical and El Tor and to the serotypes Ogawa and Inaba. In the present study, this protein was characterized after isolation from a *V. cholerae* strain of classical biotype and Inaba serotype, by polyacrylamide gel electrophoresis. The antiserum raised in rabbits against this protein agglutinated several strains of *V. cholerae*. An immunoprecipitation line of identity was observed, when the outer membrane proteins prepared from *V. cholerae* 01 strains of both the biotypes and serotypes were allowed to diffuse against this antiserum. Using enzyme-linked immunosorbent assay antibodies of all isotypes to the 48K outer membrane, protein was detected in the serum of humans immunized with a bivalent cholera vaccine, containing equal amounts of Ogawa and Inaba organisms. These results conclusively established that the 48K protein is immunogenic for man and is the common outer membrane antigen of *V. cholerae*.

Kabir S, Ahmad N, Ali S. Neuraminidase production by *Vibrio cholerae* 01 and other diarrheagenic bacteria. Infect Immun 1984 Jun;44(3):747-9

The study investigates neuraminidase production by a range of diarrhoea-causing enteric bacteria, using mostly the clinical isolates obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh. *Vibrio cholerae* 01 strains belonging to both biotypes (classical and El Tor) and both serotypes (Ogawa and Inaba) produced neuraminidase which was released rather than cell bound classical strains produced significantly more neuraminidase than did El Tor (p<0.001). Besides, classical strains produced neuraminidase with a higher specific activity than that secreted by El Tor and non-01 strains (p<0.001). Only one-third of *V. cholerae* non-01 and one-fourth of *Aeromonas hydrophila* strains were neuraminidase positive. There is, however, a similarity between the neuraminidase produced by *V. cholerae* 01 (classical and El Tor) and non-01 strains, as the neuraminidase produced by them cleaved ($\alpha \rightarrow 2 \rightarrow 3$) linkage of neuraminosyllactose. The enzyme's role in *V. cholerae*

pathogenicity is discussed. It is most likely that neuraminidase, after being relea-
sed by the bacteria, attacks intestinal glycoproteins and gangliosides, thus unmas-
king receptor sites for cholera toxin. Strains of enterotoxigenic *Escherichia coli*,
Vibrio parahaemolyticus, and *Shigella* spp. did not produce detectable neuraminidase.

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duction of *Vibrio cholerae* in Chesapeake Bay. Appl Environ Microbiol 1979 Jan;37(1):
91-103

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Kaper JB, Moseley SL, Falkow S. Molecular characterization of environmental and
nontoxigenic strains of *Vibrio cholerae*. Infect Immun 1981 May;32(1):661-7

Kauffman PE see Shah DB

Kaur J, Shrivastav JB. Immunochemical studies on *Vibrio polysaccharides*. Indian
J Med Res 1964 Aug;52(8):809-15

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Khan M, Shahidullah M. Cholera due to the El Tor biotype equals the classical
biotype in severity and attack rates. J Trop Med Hyg 1980 Feb;83(1):35-9

The Matlab Treatment Centre of the ICDDR,B has been treating patients of cholera
and other diarrhoeal diseases since 1963. In 1966, the first census was performed
to define the surveillance area population for further studies. This study analyzes
7,141 surveillance-area patients culture positive for *Vibrio cholerae* 01 in the
15-year period (1966-1980) to examine the epidemiology of endemic cholera and to
consider issues which relate to possible future prevention and control measures.
Of this 15-year period, the classical period ranged for 7 years (1966-72) and the
following 8 years (1973-1980) constituted the El Tor period. Children aged 2-9
years and adult women were most commonly hospitalized for cholera. Three patients
were hospitalized with cholera for the second time while 29 were expected on the
basis of life-table analysis. The results suggest that immunity to severe disease
conferred by previous illness might be stable and long-lasting. Time of cholera
onset or the peak of the epidemic showed no relationship with peaks of the monsoon
rain or river water level.

Khan M see Bart KJ

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a semi-urban community. J Pak Med Assoc 1969 Aug;19(8):380-5

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Pak Med J 1967 Apr;11(2):61-5

Khan MU, Samadi AR, Huq MI, Yunus M, Eusof A. Simultaneous classical and El Tor cholera in Bangladesh. J Diar Dis Res 1984 Mar;2(1):13-8

Cholera appears annually in Bangladesh, with peak incidence from September through December and April through June. After being absent for a decade the classical biotype of *Vibrio cholerae* reappeared in Comilla and Dhaka on September 1 and 3, 1982, and soon thereafter appeared in many other districts. Cases due to the newly emerged classical biotype of *V. cholerae* rapidly surpassed those due to the El Tor biotype. Before 1970 classical *V. cholerae* had been responsible globally for both endemic and epidemic cholera. In Bangladesh, El Tor first was isolated in 1964. By 1973, it had replaced the classical strain. Subsequently until 1981, only six classical isolates were noted. The new classical strains were almost identical to those isolated a decade earlier, except for their ability to successfully displace El Tor in an epidemic setting. Investigation of the first 10 cholera cases suggests a multiple source outbreak. The age distribution of the classical and El Tor was similar, suggesting a lack of important new antigenic determinants, which could circumvent existing immunity in the population. Both strains followed known seasonal patterns.

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Khan S, Agarwal MN. On the duration of the life of vibrios in the Ganges and Jumna river water. Indian J Med Res 1929;16:993-1008

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Kolvin JL, Roberts D. Studies on the growth of *Vibrio cholerae* biotype El Tor and biotype classical in foods. J Hyg 1982 Oct;89(2):243-52

The growth of the El Tor and classical biotypes of *Vibrio cholerae* was investigated in a range of foods commonly incriminated in cholera outbreaks. Both biotypes grew well in all the cooked foods but not in raw shellfish. The growth rate was fastest on cooked mussels, prawns and eggs with an alkaline pH. At 37°C, levels of 10^{10}/g were reached within 12 h on mussels and prawns and within 14 h on eggs. At 22°C both biotypes grew in all cooked foods except in pate, where there was no growth even after 48 h. The classical biotype had a lag phase of 15 h and the El Tor biotype 8-10 h when held in rice at 22°C. Similarly, in mussels the classical biotype had a lag phase of 8 h, compared with only 4 h for the El Tor biotype at the same temperature. *V. cholerae* failed to grow on either bean shoots or mushrooms at the tested

temperatures. Growth of El Tor biotypes were examined at 22°C, 30° and 37°C on both courgettesand fennel but classical biotypes grew only at 30° and 37°C on cour- gettes and only at 37°C on fennel. On some vegetables the vibrios multiplied to levels of 10^6/g. The El Tor biotypes reached a higher level in the stationary phase than did the classical biotypes. Thus, multiplication of *V. cholerae* on cooked foods and on some raw foods is of an order that constitutes an infectious dose which in endemic areas could be a mode of disease transmission within homes and small communities with an unhealthy environment.

Konchady D, Prescott LM, Datta A, De SP. Survival of *Vibrio cholerae* in some of the waters of Calcutta. Indian J Med Res 1969 Jan;57(1):60-5

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Kusama H, Craig JP. Production of biologically active substances by two strains of *Vibrio cholerae*. Infect Immun 1970 Jan;1(1):80-7

Two strains of *Vibrio cholerae* Inaba 569B and Ogawa B1307 were cultured in Casamino Acids-yeast extract-glucose medium incubated at 30 to 37°C with continuous shaking for four days after which biologically active substances produced by these strains were studied. Biological enzymes such as neuraminidase (NM), mucinase (MC), protei- nase (PR) as well as lytic factor (LF) and vascular permeability factor (PF) produced by the strains were compared by taking culture filtrates at various time intervals. Both the culture filtrates showed higher turbidities at 30°C than at 37°C while pH changes remained the same among the filtrates. Between 4 and 10 h the pH rose rather sharply, reaching a peak of 8.5 and 8.2, for 569B and B1307 respectively, at 96 h. Both strains showed bacterial lysis when maximal growth was obtained at 48 h at both temperatures. NM production reached the peak at about 10 h of incubation at 37°C but disappeared quickly. Strain of 569B showed higher yield of NM. With 569B maxi- mum MC production was achieved at 24 h. The filtrates of strain 569B did not show any PR activity but in filtrates of B1307, PR was detectable at 10 h incubation at 37°C and similarly at 24 h at 30°C followed by a gradual increase in the activity. LF was not detected from the filtrate of 569B whereas strain B1307 showed greater LF production at 37°C. The strains 569B and B1307 yielded PF at 30°C. Strain 569B produced more PF than B1307. At 30°C, PF was detectable during the exponential growth phase of 569B which peaked at 24 h. This was well maintained for 96 h, but fell moderately at 37°C. On the other hand, with B1307, PF fell sharply and virtu- ally disappeared after 24 h at both temperatures, with concomitant rise in late products. PR and LF were late products; neither was detected in 569B filtrates, whereas older cultures of B1307 showed high levels of both enzymes. MC was an early product with 569B, resembling PF, whereas it was a late product with B1307.

LaBrec EH, Sprinz H, Schneider H, Formal SB. Localization of vibrios in experi- mental cholera: a fluorescent antibody study in guinea pigs. *In:* Proceedings of the Cholera Research Symposium, Honolulu, Hawaii, 24-29 Jan 1965. Washington, D.C.: U S Government Printing Office, 1965:272-6

This study examines whether, in the course of infection in the experimental animal, the cholera vibrio penetrates the intact intestinal epithelial barrier as a neces- sary feature in cholera pathogenesis. Hartley strain guinea pigs were fed with *Vibrio comma* suspension along with tincture opium, injected intraperitoneally after challenge. The animals were sacrificed at various time intervals ($\frac{1}{2}$, 1, 2, 4, 8, 12, 16, 24 h until death). The intestinal tract was removed and a portion of the ileum was used to determine cholera vibrio counts, while the remaining intestine

was utilized for fluorescent antibody study and light microscopy. Fluorescent antibody studies and conventional histologic examinations indicated that while large numbers of cholera vibrios can be found covering the intact mucosal epithelium, they were not found in the lamina propria of the villi. In the small intestine, at the height of *Vibrio* proliferation, an emptying of crypts glands and individual goblet cells of mucus occurred, as part of the inflammatory response. These findings contrast with what was observed by others, when similarly-prepared animals were infected with a strain of *Shigella flexneri*, resulting in ulceration. Cells of *S. flexneri* did not pack the crypts of the villi as did the vibrios.

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Lalithamma BP, Sagar IK, Rao SN, Nagesha CN. Role of magnesium and iron in the reversal of nickel toxicity in *Ogawa* serotypes of *Vibrio cholerae* and *Vibrio El Tor*. Curr Sci 1976 Aug 20;45(16):578-80

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Lam SYS. A rapid test for the identification of *Vibrio cholerae* in stools. J Diar Dis Res 1983 Jun;1(2):87-9

A rapid test to identify *Vibrio cholerae* in stools has been developed. The test depends on the ability of the vibrios to multiply in a specially designed medium in the presence of other intestinal bacteria and to agglutinate against specific antisera directly. Results are available within 4 hours. In view of the simple technique, low cost and easy preparation, the test can be performed in a laboratory with minimal facilities. Results obtained for the cholera and halophilic vibrios indicated a close correlation between the present method and the slide agglutination test.

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Levine RJ, Khan MR, D'Souza S, Nalin DR. Cholera transmission near a cholera hospital. Lancet 1976 Jul 10;2(7967):84-6

This is the first report of cholera ascribed to contamination of a canal water from a hospital outlet. A review of cholera incidence from 1964 to 1974 in Matlab, a rural area of Bangladesh, revealed very high incidence rates in several villages. Higher cholera rates (8.6 and 10.9 per 1,000 annually) in two of the villages were probably due to the heavy contamination of canal water from a nearby cholera hospital that was established in 1963. The high-incidence and average-incidence villages were almost within the same distance from the hospital. The ratio of mild to severely affected cases seeking medical attention was 1:3 in both areas suggesting that admission rates did not vary with distance from the hospital. During five epidemics studied from November 1968 through February 1971, the hospitalization rate was higher in those two villages near the cholera hospital. A high cholera reinfection rate, 13 times higher than that of the other villages, was observed

between 1963 and 1969 in these villages. An analysis of water samples from the canal confirmed the presence of cholera vibrios. The higher incidence rate of cholera among the population of these two villages and a decline in rate with increased distance from the canal site indicated that canal water was the vehicle of cholera transmission.

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McCormack WM, Islam MS, Fahimuddin M, Mosley WH. A community study of inapparent cholera infections. Am J Epidemiol 1969 Jun;89(6):658-64

A community study during the 1966-67 and 1967-68 cholera epidemic evaluates the relative frequency of mild and inapparent *Vibrio cholerae* infections. A village located in Matlab, Bangladesh, an endemic cholera area of rural Bangladesh with a population of 1,609, was studied for 10 weeks. Almost one-half of the village population was aged below 15. Blood specimen was collected from each available village resident for serological study at the beginning and at the end of the study period. Serum specimens collected from the village residents were examined for vibriocidal antibodies. During the intensive surveillance period between November and February, five cases of mild diarrhoea associated with *V. cholerae* were observed. Four of the five cases were young children. In all cases, illness was associated with a few loose stools over a period of one or two days but was not severe enough to require hospitalization. Two serum specimens obtained from the young children showed a four-fold or greater rise in vibriocidal titers. Evidence of an additional 22 inapparent *V. cholerae* infections was found from the vibriocidal antibody titrations of 948 paired sera. The cholera infection rate in the communities during 1966-67 was estimated to be 27.4 per 1000 without any single clinically recognized cholera case. During 1967-68, rectal swab cultures

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of 120 persons with diarrhoea showed negative responses to *V. cholerae*. Findings suggest that cholera cases have a high infection to case ratio.

McCormack WM, Mosley WH, Fahimuddin M, Beneson AS. Endemic cholera in rural East Pakistan. Am J Epidemiol 1969 Apr;89(4):393-404

Madden JM see Twedt RM

Maiti M. Mode of action of bacteriophage Ø149 on cholera and El Tor vibrios. Can J Microbiol 1978 Dec;24(12):1583-9

The mode of action of phage Ø149 on cholera El Tor vibrios were investigated in India. Bacteriophage Ø149 which was propagated in *Vibrio cholerae* (classical) strain 154, killed *V. cholerae* (El Tor) strain MAK 757 without phage propagation. This investigation showed that Mukerjee's group IV cholera phage Ø149 was adsorbed to El Tor strain MAK 757 and also to classical strain Ogawa 154 cells. Infection of El Tor strain MAK 757 with phage Ø149 resulted in an immediate blockage of bacterial growth. About 10 minutes later this was followed by slow lysis of bacteria, though no propagation of phage Ø149 took place. After infection with the phage Ø149, the El Tor strain MAK 757 under-went significant loss of viability. To study the kinetics of phage adsorption, ^{32}P-labelled phage Ø149 were used. To test whether the phage DNA penetrated into both vibrios, the cells, after infection with ^{32}P-labelled phage Ø149 were lysed by shaking with chloroform. Evidence was obtained by this test for penetration of phage DNA into both bacterial strains. In the host strain (classical strain 154) phage particle synthesis occurred normally. The phage DNA was not degraded in El Tor strain MAK 757 while the expression of the phage DNA was blocked. The lack of phage DNA expression in El Tor strain MAK 757 could occur because the repair of the initial damage of the cytoplasmic membrane, accruing from phage Ø149 infection, did not occur. To support this effect, the influence of bacteriophage Ø149 on membrane activities such as respiration and synthesis of macromolecules (DNA, RNA and protein) were studied in both strains. Cell respiration and RNA and protein synthesis was blocked immediately after infection with strain MAK 757 and the observations are in accord with the conclusion that phage Ø149 was not propagated in El Tor vibrios because of the damage to the cytoplasmic membrane, leading to reduction of energy metabolism, cellular synthesis and phage synthesis. These effects were not observed in host strain classical Ogawa 154.

Maiti M, Sur P, Chatterjee SN. Polyacrylamide gel electrophoresis and infrared spectroscopy of *Vibrio* biotypes. Can J Microbiol 1981 Oct;27(10):1048-52

The techniques of polyacrylamide gel electrophoresis (PAGE) and infrared spectroscopy which were proved to be successful in the classification of bacteria were used in this study to classify *Vibrio* biotypes. The methods were employed to study the relationship between *Vibrio cholerae* classical, *V. cholerae* El Tor and nonagglutinable (NAG) vibrios. Phenol-acetic acid-water (PAW) extracts of the different strains produced type-specific electrophoretic patterns, and the infrared spectra revealed broad absorption maxima which largely corresponded to those found in other organisms. Thus the present study showed that *V. cholerae* classical, *V. cholerae* El Tor and NAG vibrios may be differentiated on the basis of their protein spectra when subjected to PAGE by the PAW method. Classical and El Tor biotypes, with the exception of NAG vibrios, presented identical patterns in the spectral range. The individual strains of any biotype could not be differentiated by their infrared spectra. The study further recorded strain-specific differences in the exoprotein spectra of the vibrios obtained by the sodium-dodecyl sulphate-PAGE technique.

Maiti M, Chaudhuri K. Some properties of a *Vibrio cholerae* bacteriophage phi 2.
Indian J Exp Biol 1979 Jul;17(7):628-31

Vibrio cholerae phage Ø2 belonging to Mukerjee's group II, gave clear circular
plaques with a diameter 0.5 to 1.5 mm, after 20 h incubation at 37°C when plated
on a lawn of host *V. cholerae* Ogawa 154. Adsorption kinetics showed that the ad-
sorption of phage Ø2 to host was biphasic with an adsorption rate constant of
1.09×10^{-9} ml/min up to 90% adsorption and 2.085×10^{-10} ml/min thereafter.
Adsorption of this phage to isolated cell envelope and isolated lipopolysaccharide
followed first order reaction kinetics resulting in 80% inactivation by cell
envelope and 50% by lipopolysaccharide in 30 min at 37°C. Intracellular growth
characteristics of phage Ø2 within cholera strain Ogawa 154 was studied. The
intracellular phage multiplication was characterized by an eclipse period of 22 min,
latent period of 38 min and the rise period of 17 min. The average burst size was
120 PFU/cell. The single burst technique was used to study the number of phage
particles produced by individual bacteria. The average burst size calculated from
the Poisson's distribution formula was 124 PFU/cell. Phage Ø2 was fairly stable in
pH 6 to 11. Its thermal inactivation was characterized by half-lives of 42.5,
8.4 and 1.5 min at 55°, 60° and 70°C respectively. The thermodynamic parameters
H, F and S were determined at these temperatures. The phase was resistant *in vitro*
to chloroform, ribonuclease, deoxyribonuclease, sodium deoxycholate, polymyxin B
and mitomycin C. The diphenylamine test (for DNA) was positive and orcinol (for
RNA) was negative, confirming that the phage contained the genetic material DNA.

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Manning PA see Imbesi F

Martin AR, Mosley WH, Sau BB, Ahmed S, Huq I. Epidemiologic analysis of endemic
cholera in urban East Pakistan, 1964-66. Am J Epidemiol 1969 May;89(5):572-82

This is a combined retrospective and prospective analysis of the epidemiological
characteristics of 983 cholera patients admitted to the Pakistan-SEATO Cholera
Research Laboratory (now the International Centre for Diarrhoeal Disease Research,
Bangladesh), during the cholera seasons of 1964-65 and 1965-66. In both seasons,
less than half those studied were found to be infected with *V. cholerae* 01. A
striking preponderance of the Inaba serovar was seen. The disease was seasonal,
with a peak during the dry winter months and virtual disappearance during the
summer monsoon. In 1965-66, a second, smaller cluster of cholera cases occurred
in April and May. The outbreaks tended to be localized in small communities using
common facilities for water, food and sanitation. The infection rate was higher
in children than in adults, and was almost equal in distribution in both sexes.
Multiple family members were infected where there were known prior cholera cases,
or due to exposure resulting from ingestion of contaminated water or food, in an
area where other cholera cases were present, due to family outbreaks.

Martinez B see Chaicumpa W

Medina RA see Baselski VS

Mekalanos JJ, Sublett RD, Romig WR. Genetic mapping of toxin regulatory mutations
in *Vibrio cholerae*. J Bacteriol 1979 Sep;139(3):859-65

Procedures were developed for analyzing the properties of *Vibrio cholerae* mutants
genetically. Transposon-facilitated recombination was used for mapping the regu--

latory site mediating the hyper-production of toxin in mutant of *V. cholerae* strain 569B Inaba. Mutation in this locus, called htx occurred in the hypertoxinogenic phenotype and was measured by the ganglioside filter assay and immunoradial diffusion. Subsequent mapping by conjugation demonstrated that the htx locus was closely linked to the gene rif, str and ilv of *V. cholerae*. Analysis of recombinants suggested the following gene order: thy str htx rif ilv arg. The high comutation frequency between the rif and htx (as high as 98%) was observed after nitrosoguanidine mutagenesis due to the close genetic linkage. Elevation of toxin production in the recipients occurred due to the transfer of htx mutant locus from a hypertoxinogenic donor to several unrelated Tox$^+$ *V. cholerae* strains. Thus, the htx gene product played a significant role in the regulatory mechanism of toxin production in strains of *V. cholerae*.

Mekalanos JJ, Murphy JR. Regulation of cholera toxin production in *Vibrio cholerae*: genetic analysis of phenotypic instability in hypertoxinogenic mutants. J Bacteriol 1980 Feb;141(2):570-6

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Misra SB, Shrivastava DL. Serological diagnosis of cholera: preliminary report. Indian J Med Res 1964 Aug;52(8):824-7

Mitra PP see Wallace CK

Mitsuhashi S see Hayashi F

Miwatani T see Honda T

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Mosley WH, Aziz KMS, Ahmed A. Serological evidence for the identity of the vascular permeability factor and ileal loop toxin of *Vibrio cholerae*. J Infect Dis 1970 Mar;121(3):243-50

Mosley WH see Bart KJ

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Mukerjee S. The bacteriophage. *In:* Frankel S, Reitman S, Sonnenwirth AC, eds. Gradwohl's clinical laboratory methods and diagnosis. St Louis: Mosby, 1963:697-705

Mukerjee S. The bacteriophage-susceptibility test in differentiating *Vibrio cholerae* and *Vibrio el tor*. Bull WHO 1963;28(3):333-6

This study differentiates between *Vibrio cholerae* and El Tor vibrios by a bacterio-phage susceptibility test. Usually, El Tor vibrios are hemolytic for sheep or goat erythrocytes in the Greig test, while the classical cholera vibrios are nonhemolytic in nature. In some fresh isolations of El Tor strains, the hemolytic properties remain ill-developed and the strains sometimes show both negative and positive results in the Greig test. Thus, a differentiation between cholera and El Tor vibrios was done by the bacteriophage susceptibility patterns of the vibrios. Four groups of cholera bacteriophages used in this study were isolated from cholera epidemics in Calcutta. A total of 4446 classical *V. cholerae* strains and a number of El Tor strains were tested. A loopful of cholera bacteriophage was added on a spot culture of test *Vibrio,* and after overnight incubation at 37°C, it was observed that group IV cholera bacteriophage was universally lytic for all the strains of classical *V. cholerae,* though none of the El Tor strains were lysed by it. It was suggested that this phage-sensitivity test is suitable for routine diagnostic work and is simple and rapid for bacteriological characterization of vibrios.

Mukerjee S. Diagnostic uses of cholera bacteriophages. J Hyg 1961 Mar;59(1):109-15

Mukerjee S. Recent incidence of cholera outside India. Indian J Med Res 1964 Aug; 52(8):771-6

Cholera incidence outside India during 1954-64 has been reported. Countries affected by classical cholera outside the Indo-Pakistan subcontinent were Afghanistan, Thailand, Nepal and Burma. In 1962, cholera cases were reported from India and Pakistan (inclu-ding Bangladesh) only. El Tor cholera was restricted only to a small geographical area in South Celebes island in Indonesia. From 1961 this spread in a pandemic form to regions in South and South East Asia. El Tor cholera invaded Burma in 1963 and came close to the Indian subcontinent. The study, being carried out during El Tor's outbreak, prompted the author to recommend strict vigilance and quarantine measures to prevent the entry of El Tor cholera into the subcontinent.

Mukerjee S, Guha DK, Roy UKG. Studies on typing of cholera by bacteriophage I. Phage typing of *Vibrio cholerae* from Calcutta epidemics. Ann Biochem Exp Med 1957; 17:161-76

Mukerjee S see Biswas K

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Mukherjee AK see Sen AK

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Murphy JR see Mekalanos JJ

Classical *Vibrio cholerae*

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Nagesha CN, Lalithamma BP, Rao SN, Ananthakrishna NC, Sagar IK. Cobalt toxicity and its reversal by iron and magnesium in Ogawa serotypes of *Vibrio cholerae* and *Vibrio El Tor*. Curr Sci 1977 Nov 20;46(22):771-3

Nagesha CN see Lalithamma BP

Nagesha CN see Sagar IK

Naito T see Iwanaga M

Nakajima T see Hayashi F

Nalin DR. Cholera, copepods and chitinase. Lancet 1976 Oct 30;2(7992):958

Though little is known about *Vibrio cholerae* bioecology, much more is known about the bioecology of *Vibrio parahaemolyticus* -- which can adsorb onto the chitin of copepods and which, with related vibrios, constitutes nearly the total viable bacterial flora of these plankton. These vibrios produce chitinase, and can use chitin as a nutrient. It is suggested that *V: cholerae* likewise may survive unfavorable environmental conditions by adhering to or by colonizing copepods or related species, in the Ganges delta area. This would explain why *V. cholerae* too produces chitinase, and also indicate why surface water vibrios in endemic areas are far lower than the counts needed to pass the stomach acid barrier and causing infection. Low *Vibrio* counts probably could cause small bowel infection, by traversing stomach acid inside the indigestible chitin of copepods and then reaching human intestines via drinking water, etc. As with *V. parahaemolyticus*, a seasonal persistence cycle in sediments, bacterial increase during plankton bloom, and vibrios appearance in the water column leading to infection, could account for the seasonality of endemic cholera.

Nalin DR see Levine RJ

Neogi PKB see Sanyal SC

Neogy KN. Viability of *V. cholerae* and *V. El Tor* in food and water. Bull Calcutta Sch Trop Med 1965;13(1):10-1

This study evaluates the viability of *Vibrio cholerae* (classical) and *V. cholerae* El Tor, in some commonly used foodstuffs, cutfruit, and in tank water under laboratory conditions. Organisms, obtained from 24 h nutrient agar cultures were inoculated in samples of tank water, curd and "panta rice" (boiled rice soaked overnight in water), and suspension of agar cultures in 0.1% peptone saline was smeared on the surface of the cut fruit and sweets. The specimens were then cultured at intervals after primary enrichment in alkaline peptone water. El Tor vibrios survived one week longer than *V. cholerae* in tank water having pH 6.4-6.6. Both organisms survived well in other foodstuffs and their viability appeared to be similar. In the cucumber (cut fruit, at pH 6.6), organisms survived for more

than 24 h. The viability of *V. cholerae* El Tor in water and foodstuff is a valuable finding, from the public health point of view.

Ness GE see Hood MA

Niyogi SG, Deb BC, Sircar BK, Sengupta PG, Sen D, Ghosh BN. Studies on cholera carriers and their role in transmission of the infection: a preliminary report. Indian J Med Res 1979 Dec;70:892-7

Northrup RS. Antibiotics in cholera therapy. J Pak Med Assoc 1969 Aug;19:363-5

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Oleinick A see Wallace CK

Oseasohn R, Ahmed S, Islam MA, Rahman ASMM. Clinical and bacteriological findings among families of cholera patients. Lancet 1966 Feb 12;1(7433):340-2

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Panja G, Ghosh SK. Isolation of cholera vibrios from Hooghly river water at Calcutta. Indian J Med Res 1947 Jan;35(1):1-2

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Park RWA see Davies GHG

Parker C, Gauthier D, Tate A, Richardson K, Romig WR. Expanded linkage map of *Vibrio cholerae*. Genetics 1979 Feb;91:191-214

This paper summarizes the current *Vibrio cholerae* map, and also proposes a numbering system for mutant identification in *V. cholerae*. Using a variety of new auxotropic, mutants, an expanded linkage map of the chromosome from *V. cholerae* classical strain 162 was prepared. The chromosome consists of a single, linear linkage group. The organism was shown to be amenable to genetic mapping by two independent methods: linkage analysis and crossover class analysis. Map orders derived in these two ways were similar. A proposal to standardize genetic nomenclature in *V. cholerae* genetic studies is made. The map and the data given can be of value in future genetic studies of *V. cholerae*.

Classical *Vibrio cholerae*

Parker C, Romig WR. Self transfer and genetic recombination mediated by P, the sex factor of *Vibrio cholerae*. J Bacteriol 1972 Nov;112(2):707-14

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Peeler JT see Twedt RM

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Rabbani GH see Islam MR

Rahaman MM see Huq MI

Rahman ASMM see Akef QMA

Rahman ASMM see Oseasohn R

Rahman ASMM see Samadi AR

Rahman R see Huq A

Rammers E see Colwell RR

Rao SN see Lalithamma BP

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Rao SN see Sagar IK

Raziuddin S. Immunochemical studies of the lipopolysaccharides of *Vibrio cholerae*: constitution of O specific side chain and core polysaccharide. Infect Immun 1980 Jan;27(1):211-5

Classical *Vibrio cholerae*

Read WDB, Pandit SR. Distribution of *V. cholerae* and El Tor type strains in certain rural areas in India. Indian J Med Res 1941 Jul;29:403-18

Read WDB, Singh G, Seal SC, Bose S. Growth and survival of *V. cholerae* with special reference to growth and survival in water. Indian J Med Res 1939 Jul;27(1):1-40

Richardson K see Parker C

Richardson SH. Factors influencing in vitro skin permeability factor production by *Vibrio cholerae*. J Bacteriol 1969 Oct;100(1):27-34

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Roberts N see Colwell RR

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Roderick CN see Twedt RM

Romig WR see Johnson SR

Romig WR see Mekalanos JJ

Romig WR see Parker C

Romig WR see Sublett RD

Rowley D see Attridge SR

Rowley D see Bloom L

Rowley D see Bloom LD

Rowley D see Horsfall DJ

Roy NK, Das G, Balganesh TS, Dey SN, Ghosh RK, Das J. Enterotoxin production, DNA repair and alkaline phosphatase of *Vibrio cholerae* before and after animal passage. J Gen Microbiol 1982;128(Pt.9):1927-32

The present report compared enterotoxin production, the capability for dark repair of UV-induced damage to DNA and the stability of alkaline phosphatase in three laboratory strains of *Vibrio cholerae* before and after animal passage. Three strains of *V. cholerae*, differing in biotype, serotype and/or toxinogenicity used in this study, were the highly toxigenic strain 569B, the mildly toxigenic strain 154 and the non-toxigenic strain EW6. The study was done at the Indian Institute of Chemical Biology, Calcutta, India. The results showed that during laboratory maintenance, DNA repair capabilities, and the stability of alkaline phosphatase were reduced concomitantly with a reduction in the toxigenicity of strain 569B. Moreover, strain 569B showed decreased viability in each of the broth media after five to seven subcultures, but all three properties could be restored by passaging strain 569B once through a guinea pig. In contrast, laboratory maintenance had little effect on these three properties of strains EW6 & 154. Significantly, none of the strain 569B isolates had only one of the three properties restored, making it appear that the three processes might be interdependent. While conclusions could not be drawn as to any direct correlation between toxinogenicity and the

phenotypic changes observed, this study provides the first systematic examination of the possibility that enterotoxin production might play an important role in the physiology of classical vibrios.

Roy UKG see Mukerjee S.

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Sack DA see Islam MR

Sack RB see Wallace CK

Sagar IK, Rao SN, Nagesha CN. Effect of copper on *Inaba* serotypes of *Vibrio cholerae* and *Vibrio el tor*. J Health Sci 1975;1:60-4

The effect of different concentrations of copper (Cu^{2+}) on Inaba serotypes of *Vibrio cholerae* classical El Tor was studied in India, using nutrient broth and synthetic medium. Cu^{2+} was toxic for both the biotypes in nutrient broth. At the 8 µg/ml concentration of copper 24% and 8% fall in growth was observed, respectively, for El Tor and classical biotypes. At about 80 µg/ml level the growth was at par in both, but at 100 µg/ml the growth reached almost extinction level for the classical biotype. With synthetic medium, lower concentration (12 µg/ml) of Cu^{2+} stimulated the growth of both biotypes while higher concentration (20 µg/ml) resulted in a sudden fall in growth to 25%. At 28 µg/ml Cu^{2+} no growth was observed. In presence of high concentration of Cu^{2+}, acid production was impaired.

Sagar IK, Rao SN, Nagesha CN. Interrelationship of magnesium and manganese in the metabolism of Inaba serotypes of *Vibrio cholerae* and *Vibrio el tor*. J Health Sci 1975;1:146-8

Sagar IK, Rao SN, Nagesha CN, Bhat JV. Reversal of zinc toxicity by iron and magnesium in Inaba serotypes of *Vibrio cholerae* and *Vibrio eltor*. J Health Sci 1976; 2:50-4

The metabolic behavior of zinc towards Inaba serotypes of *Vibrio cholerae* classical and El Tor and the role of magnesium and iron in reversing this toxicity was studied. Zn^{2+} was toxic to both biotypes, particularly to classical *V. cholerae*. Complete inhibition of growth was attained at continuous concentration of 900 µg in El Tor and 1000 µg in classical, while 50% growth inhibition was noted in the classical biotype at a much lower concentration (400 µg) than in the El Tor (700 µg). Fe^{3+} and Mg^{2+} supplementation revealed that Fe^{3+} is partially effective in both biotypes to counteract zinc toxicity. Mg^{2+} failed to counteract zinc toxicity except for a marginal increase in the growth of the El Tor biotype.

Sagar IK see Lalithamma BP

Sagar IK see Nagesha CN

Samadi AR, Huq MI, Shahid N, Khan MU, Eusof A, Rahman ASMM, Yunus M, Faruque ASG. Classical *Vibrio cholerae* biotype displaces El Tor in Bangladesh. Lancet 1983 Apr 9;1(8328):805-7

The El Tor biotype of *Vibrio cholerae* caused all endemic and epidemic cholera in

Bangladesh from 1973 until Sept. 3, 1982, when the first classical strain was isolated from a patient in Matlab. Since then the number of isolations of the classical biotype has increased very rapidly and spread to other districts, replacing the El Tor biotype as the main epidemic strain. The classical strains isolated in the 1982 outbreak were indistinguishable by the standard tests from those isolated a decade ago and the very few isolates in 1979, 1980, and 1981. This suggests that beyond the taxonomic traits used to identify the classical and El Tor strains, there may be other more crucial biological characteristics that have given this new strain an advantage over the existing strains. The mechanism by which a new biotype of *V. cholerae* 01 achieves such a crucial biological advantage to displace the existing strains may be a key point in control of the global spread of cholera.

Samadi AR, Chowdhury MK, Huq MI, Khan MU. Seasonality of classical and El Tor cholera in Dhaka, Bangladesh: 17-year trends. Trans R Soc Trop Med Hyg 1983;77(6):853-6

Data on cholera patients admitted monthly to the ICDDR,B's Dhaka hospital from 1964 through 1980 were studied and analyzed. Although *Vibrio cholerae* biotype El Tor did not enter Bangladesh until 1963, it has completely displaced the classical biotype since 1973. There was a significant seasonal pattern (p<0.05) for both El Tor and classical cholera, with the estimated peaks on October 19 for El Tor and on December 5 for classical. The estimated 47 days difference in which the El Tor peak precedes the classical one also is statistically significant (Z=1.76, p<0.05). The findings may suggest that the change in seasonality of cholera in Bangladesh could be due to a change in *V. cholerae* biotype from classical to El Tor.

Samadi AR see Cook WL

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Samadi AR see Khan MU

Samadi AR see Shahid NS

Sanyal SC, Neogi PKB, Alam K, Huq MI, Al-Mahmud KA. A new enterotoxin produced by *Vibrio cholerae* 01. J Diar Dis Res 1984 May;2(1):3-12

Isolation from diverse environmental sources of *Vibrio cholerae* 01 that lack the cholera toxin (CT) gene has encouraged researchers to use them, or CT gene-deletion mutant strains, as potential candidates for a live oral cholera vaccine. Thirteen such strains were examined in greater detail, using various enterotoxin assay systems, to determine whether they do completely lack the capability to produce any toxin(s). Live cells of all these strains caused significant accumulation of fluid in ligated adult rabbit ileal loops and diarrhoea in infant rabbits. Similar results were obtained using their culture filtrates. The filtrates also increased capillary permeability of rabbits' skin, and blueing was accompanied by blanching or necrosis. Suckling mice assays were negative for all test materials. The culture filtrates lost their loop and skin toxic activities when held at 56°C for 30 min. However, the toxin could not be neutralized by antisera against CT or its A and subunits in rabbits' skin and ileal loop assays. The culture supernates did not cause cytotonic effects on Chinese hamster ovarian and mouse adrenal cells; and did not bind to GM_1 ganglioside, in enzyme-linked immunosorbent assay. When tested against anti-CT, no precipitin band was observed in Ouchterlony's gel-diffusion technique with any of the concentrated culture filtrates. The strains did not show any homology when retested with CT or LT probes. Thus, this study indicates that these *V. cholerae* 01 strains produce a toxin not previously recognized. This new toxin seems to differ

from the knwon CT in antigenic nature, receptor site, mode of action and genetic homology. Before embarking upon a direct vaccine development program, this toxin requires further immuno-biologic and genetic studies. The general belief about the non-toxic nature of environmental *V. cholerae* isolates also may be a myth.

Sanyal SC, Sil J, Dutta NK, Mukerjee S. Present status of classical cholera in India. Indian J Med Res 1972 Nov;60(11):1564-7

The progressive changes observed in the prevalent biotypes of *Vibrio cholerae* in India from 1967 to 1969 is reported, their epidemiological significance is also discussed. *V. cholerae* strains isolated from different outbreaks and sporadic cases were examined at the WHO International Reference Centre for Vibrios, in Calcutta, India. It was found that since the entry of *V. cholerae* biotype El Tor in 1964, the classical biotype disappeared from all parts of India and in 1966 no classical *V. cholerae* strain could be isolated from cholera patients. During the 3-year period between 1967 and 1969, nearly 5,000 strains of cholera vibrios isolated from most states in India were received. Of the 5,000 strains, 92 were classical *V. cholerae*; 9 received in 1967, 58 in 1968 and 25 in 1969. These classical strains were received from Assam, Calcutta, Orissa, Tripura and Maharashtra. The number of classical strains were insignificantly low in proportion to El Tor strains. Seven of the strains in 1967 and 21 of the 37 strains in 1968 isolated in Calcutta were from carriers. This indicates that the classical *V. cholerae* strains are being maintained in the community through carriers. It was considered likely that other places in India from where *V. cholerae* classical had not been isolated during the study period, continued to harbor the classical *V. cholerae* in the carrier state among a small fraction of the population. In contrast to the overall trend of dominance of the El Tor biotype over the classical *V. cholerae*, classical strains re-emerged in Assam in 1968. All the 12 strains referred from Assam in 1969 were *V. cholerae* classical and no El Tor strain was received. The possibility of reversal of dominant biotypes in cholera epidemiology requires close observation.

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Sasmal D see Guhathakurta B

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Sau BB see Martin AR

Saxena KC, Murti CRK. Intracellular peptidases of *Vibrio cholerae* and their possible role in pathogenicity. Indian J Med Res 1964 Aug;52(8):795-800

Schneider DR, Parker C. Isolation and characterization of protease-deficient mutants of *Vibrio cholerae*. J Infect Dis 1978 Aug;138(2):143-51

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Seal SC. Centenary of discovery of cholera vibrio (editorial). Indian J Public Health 1983 Jan-Mar;27(1):1-4

Classical *Vibrio cholerae*

On the occasion of the centenary of the discovery of *Vibrio cholerae* by Robert Koch of Germany, the author discusses the reasons why cholera is still menacing Asiatic countries, particularly India, despite 50-year-long efforts to curtail the problem. A number of mistakes committed by serological researchers and health authorities, particularly regarding classification, are discussed. For example, vibrios, including El Tor, not agglutinated by the serum raised against the so-called "true cholera vibrio" were regarded as non-choleragenic. The author concludes that attempts to control cholera should place more importance on human carriers.

Seal SC see Read WDB

Seidler RJ see Colwell RR

Sen AK, Mukherjee AK, Guhathakurta B, Dutta A, Sasmal D. Structural investigations of the lipopolysaccharide isolated from *Vibrio cholerae*, Inaba 569 B. Carbohydr Res 1979 Jul;72:191-9

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Shah DB, Kauffman PE, Boutin BK, Johnson CH. Detection of heat-labile-enterotoxin-producing colonies of *Escherichia coli* and *Vibrio cholerae* by solid-phase sandwich radioimmunoassays. J Clin Microbiol 1982 Sep;16(3):504-8

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Shahidullah M see Khan M

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Shrivastava DL. Enzymes in *Vibrio cholerae*. Indian J Med Res 1964 Aug;52(8):801-8

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Siddiqui KAI, Bhattacharyya FK. Role of temperate phage in determining lytic phage sensitivity and serotype of *Vibrio cholerae*. Infect Immun 1982 Sep;37(3):847-51

The correlation between serotype, phage type and lysogeny in *Vibrio cholerae* was investigated in the present study undertaken in Calcutta, India. The effect of lysogenization with five temperate phages from various sources on serotype and lytic phage sensitivity was investigated in six cultures of both classical and El Tor biotypes of *V. cholerae*. Serological conversion by any of the phages investigated here could not be demonstrated. No change was observed in classical biotype. Four of the five phages showed homology in their immunity profiles and type-converting activities. Typical of them was K-phage 22. Changes in phage type caused by K-phages involved only induction of resistance to lytic phage E3, thereby causing a type change in El Tor strains. The sensitivity to the other phages was not changed. In 14 natural isolates, E3 (group III) phage resistance correlated with the presence of temperate phage. The results showed that E3 resistance was due to post-adsorption exclusion in K-lysogens. These lysogens were able to absorb phage E3, but unproductively. The fifth temperate phage

VcA-1 did not induce a change in lytic phage sensitivity and had a unique immunity profile. It was demonstrated that the El Tor vibrio could act as a host to this phage, without causing changes in the host properties investigated. It was suggested that a derivative of this phage may prove to be a useful addition to the typing series.

Sigel SP, Lanier S, Baselski VS, Parker CD. In vivo evaluation of pathogenicity of clinical and environmental isolates of *Vibrio cholerae*. Infect Immun 1980 Jun; 28(3):681-7

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Singh G see Read WDB

Sinha VB, Srivastava BS. Attenuation of virulence by P and V plasmids in *Vibrio cholerae:* strains suitable for oral immunization. Bull WHO 1979;57(4):643-7

A new procedure for producing attenuated PV strains of *Vibrio cholerae*, by introducing P and V plasmids was demonstrated. In a virulent strain of *V. cholerae*, KB9, P and V plasmids were introduced by bacterial conjugation. Characterization of PV isolates was done. Their systematic screening in animal models of cholera among mice and rabbits revealed that the pathogenicity of a large number of PV isolates of *V. cholerae* was significantly suppressed. This loss of pathogenicity was due to the inability to synthesize biologically active toxin. Results obtained with two such strains designated as KB9:PV and CD24 were described. The plasmid status of these strains appeared to be stable, both *in vitro* and *in vivo*. Some of these strains were maintained and passaged on nutrient agar for several years and loss of plasmid(s) was not observed. Successive passages in rabbit intestine had not effect on the plasmids. These attenuated strains of *V. cholerae* appeared to be immunogenic as shown in the mouse protection test and an ileal loop challenge experiments in adult rabbits. It was suggested that PV-bearing attenuated strains be tried in oral vaccination.

Sinha VB. Effect of carbohydrates on adherence of *Vibrio cholerae* to rabbit intestine and haemagglutination of sheep erythrocytes. Indian J Med Res 1984 Feb;79: 159-62

The effect of some mono- and di-saccharides on the adherence and hemagglutination of some classical and EL Tor *Vibrio cholerae* strains was studied. All *V. cholerae* strains agglutinated sheep erythrocytes and were adhesive to rabbit intestinal mucosa. D glucose, D mannose, sucrose and maltose inhibited adherence and hemagglutination. This finding, plus the fact that D-mannose is a stereoisomer of D glucose, strongly suggests that a certain sterochemical configuration of these carbohydrate residues might be essential for *V. cholerae* hemagglutinins to interact. This hypothesis is supported by the finding that there was no additive effect of the carbohydrates, which competed for the same receptors.

Sinha VB, Srivastava BS. Plasmid associated suppression of pathogenicity of wild-type strains of *Vibrio cholerae* from cholera patients. Indian J Med Res 1983 Jan;77:1-4

Pathogenicity of *Vibrio cholerae* was suppressed by harboring P and V plasmids. Six pathogenic strains of classical *V. cholerae* isolated from human cholera cases were studied. The strains were devoid of P and V plasmids. Plasmids P and V was transferred to these strains by conjugation with KB9:P and KB9:V an isogenic P and

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V donors respectively. Enteropathogenicity of plasmid (P^+V^+) strains and non-plasmid (P^-V^-) strains were tested in rabbit ileal loops. P^-V^- strains induced fluid accumulation whereas P^+V^+ strains were attenuated and exhibited residual pathogenicity. Virulence test in mice showed that LD_{50} of plasmid associated strain was greater than in the non-plasmid strains. Immunogenicity of both types of strains were examined and no difference between them was observed. The presence of P and V plasmids in pathogenic *V. cholerae* strains renders the strains a pathogenic but do not alter their bacterial antigenicity.

Sinha VB, Bhattacharjee JW. A streptomycin-sensitive revertant of a streptomycin-dependent strain of *Vibrio cholerae*. Bull WHO 1976;53(4):482-4

This study investigates whether a streptomycin-independent revertant derived from streptomycin-dependent mutants of *Vibrio cholerae* would show attenuation of virulence. Strain KB611 was cultured in brain heart infusion broth without streptomycin-independent revertants. The revertants obtained from the strain KB611 were rare and sensitive to streptomycin. Revertant KB599 resembled the parent strain KB611 and was avirulent in animal experimental models. The multiplication of strain KB599 in the infant mouse intestine was not observed as it was found in the ileal loop. Multiplication was not detected even in the infant rabbit gut.

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Sinha VB see Srivastava BS

Sinha VB see Srivastava R

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Sridhar P, Polasa H. Effect of temperature on gene transfer in *Vibrio cholerae:* crosses between P^+ & P^- classical strains of *V. cholerae*. Indian J Exp Biol 1981 Jan;19(1):40-1

Effect of temperature on gene transfer in *Vibrio cholerae* was studied. *V. cholerae* strains used were KB16 (classical, P^+, his^+, ilv^+, str-s [streptomycin sensitive]); KB12 (classical, P^-, arg^+, his^+, ilv^+, str-s) and KB9 (classical P^-, arg^-, his^-, ilv^-, str-r). It was observed that the frequence of arg^+, his^+, ilv^+ recombinants increased when the donor cells were exposed to elevated temperatures prior to mating. The donor cells were pre-incubated at 37°C, 43°, 44°, 46°, 47°, 48°C with three incubation periods of 10, 20 and 30 minutes at each temperature. It was observed that though the frequency of recombinants for all three genes (arg^+, his^+, ilv^+) was enhanced at elevated temperatures, the frequency of arg^+ and his^+ recombinants was always greater than ilv^+ recombinants. This might reflect the order of transfer of genes. It appeared that the efficiency of conjugation, i.e., the frequency of formation of recombinants, varied with respect to temperature and duration of pre-incubation of classical donor cells. The optimum treatment for recovering maximum number of recombinants was exposure to 46°C for 20 min.

Srivastava BS, Sinha VB, Srivastava R. Attenuated recombinant strains of *Vibrio cholerae* for oral immunization. Bull WHO 1979;57(4):649-54

Isolation and properties of the attenuated strains of *Vibrio cholerae* that offer promise of a live oral vaccine were described. In the Cholera Immunology Laboratory of Central Drug Research Institute, Lucknow, India, 2 attenuated *V. cholerae* strains -- CD1 and CD3 -- that remained stable in 1976 were isolated. CD1 and CD3 were motile, adhering to rabbit intestine and were capable of multiplying and surviving in the gut of orally fed infant mice for a week. Both strains were antigenic. They gave rise to good protection to challenge particularly against identical serotypes in the mouse protection test and in the rabbit ileal loop model. Thus, it was clear that CD1 and CD3 meet the requirements expected of a live oral vaccine.

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Srivastava R, Sinha VB, Srivastava BS. Events in the pathogenesis of experimental cholera: role of bacterial adherence and multiplication. J Med Microbiol 1980 Feb; 13(1):1-9

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Takeya K, Otohuji T, Tokiwa H. FK phage for differentiating the classical and El Tor groups of *Vibrio cholerae*. J Clin Microbiol 1981 Aug;14(2):222-4

A new vibrio-infecting phage (FK phage), similar to Mukerjee group IV phage in its lytic spectrum but different in morphology and antigenicity, was isolated from sewage in Fukuoka City, Japan. This FK phage lysed all 25 strains of *Vibrio cholerae* biovar El Tor and 37 strains of *V. cholerae* non 0:1 were resistant to it. Other species of bacteria, such as *Vibrio parahaemolyticus* (35 strains), *Salmonella* (24 strains), *Shigella* (21 strains), *Escherichia* (8 strains), *Proteus* (12 strains), *Pseudomonas* (3 strains), *Bacillus* (4 strains), *Micrococcus* (1 strain), *Bordetella* (1 strain) and *Staphylococcus* (21 strains) were insensitive to FK phage. Mutants resistant to FK, group IV, and kappa-type phages, were selected to study the relationship among these 3 phages. The results of cross sensitivity tests of each mutant to each phage revealed that no relation exists among the three phages. Since there are a few discrepancies in their lytic patterns, it was suggested that the use of FK phage in addition to group IV phage will be useful in the examination and typing of *V. cholerae* strains with atypical biological properties.

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From June 1979 through May 1980, 790 samples of freshly harvested shell-shock oysters were collected (15 to 30 each week). Each sample was examined for *Vibrio cholerae* according to Food and Drug Administration procedures. Of these samples 111 were found to contain organisms identified biochemically and serologically as *V. cholerae* non-01 (611 strains). Seven samples contained *V. cholerae* 01 Inaba (11 strains); in five of these samples both 01 and non-01 organisms were found. Of the 111 samples that were positive for non-01 organisms, 82 were harvested during June-August. In July alone, more than 38% of the samples were positive, and of the 611 non-01 isolates, nearly half were recovered from the July samples. All the 7 samples yielding 11 *V. cholerae* 01 Inaba strains were harvested from May through August. Immunological and biological tests showed that none of the 611 *V. cholerae* non-01 or the 11 *V. cholerae* 01 Inaba isolates were enterotoxigenic.

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This finding casts doubt on their supposed origin from patients and carriers.

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Van de Linde PAM, Forbes GI. Observations on the spread of cholera in Hong Kong, 1961-63. Bull WHO 1965;32(4):515-30

Vasil ML, Holmes RK, Finkelstein RA. Conjugal transfer of a gene determining production of enterotoxin in *Vibrio cholerae*. Science 1975 Mar 7;187(4179):849-50

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A comparative analysis of the clinical manifestations of cholera caused by the classical and El Tor biotypes of *Vibrio cholerae* was done during a study designed to evaluate, under controlled conditions, the effect of small doses of tetracycline in the treatment of the disease. Only male patients aged over 10, admitted to the Infectious Disease Hospital, Calcutta, India, during April through June 1964, having a history of watery diarrhoea and in a hypotensive state were included in the study. Only 63 of the patients were bacteriologically positive for *V. cholerae* out of the 195 admitted and followed up during these observations. Seventeen of the *V. cholerae* posi- tive cases were infected with classical and 34 with El Tor biotype. All the 34 El Tor strains were of Ogawa serovar while 15 of the classical were Inaba and 2 were Ogawa. Measured loss of fluid through diarrhoea and vomiting, values of stool electrolytes, evidence of dehydration and symptoms like abdominal pain and cramps in extremities were compound for the two groups. The data obtained indicated absence of any significant diffe- rence in the clinical and biochemical characters between cholera caused by the biotype classical or El Tor occurring in the same population at the same time. The pattern of the diseases caused by the two types of *V. cholerae* being similar, it was concluded that the disease caused by either organism should be called cholera.

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Woodward HE. Cholera reinfection in man. J Infect Dis 1971 Jan;123(1):61-6

Fourteen individuals with documented reinfection of *Vibrio cholerae* were reported.
Thirteen of the patients were from Matlab, a field study area of Bangladesh under
surveillance since 1962; and one came from a congested area of Dhaka city. All the
patients had two cholera infections during December 1963 to March 1970. *V. cholerae*
classical Inaba was isolated from eight individuals during both the episodes. From
the remaining six, classical Inaba vibrios were isolated during one episode and
either classical Ogawa or El Tor Ogawa during the other. The mean interval between
infections was 19.3 months (varying from 1½ to 60 months). The average interval
for individuals from whom the same type of organism was isolated on both the occa-
sion was 27.3 months whereas it was 8.8 months in those from whom different types
of *V. cholerae* were recovered. Ten of the fourteen patients were hospitalized.
Five were hospitalized during both the infections and classical Inaba vibrios were
isolated from them, one hospitalized during the first infection and four during the
second. The incidence of reinfection with classical Inaba vibrios was much greater
in individuals whose first infection had been due to classical Ogawa than in those
who had been infected initially with the same biotype. The high frequency of rein-
fection suggests that an effective cholera vaccine needs to be developed to stimu-
late greater immunity.

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Woodward WE, Mosley WH. The spectrum of cholera in rural Bangladesh. II. Compari-
son of El Tor Ogawa and classical Inaba infection. Am J Epidemiol 1972 Nov;96(5):
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The epidemiological, serological and bacteriological observations of 197 children
studied during the 1969-1970 El Tor cholera epidemic was compared with a similar
study of 91 children of 1968-1969 classical cholera epidemic. In a village of rural
Bangladesh, epidemics of classical Inaba and El Tor Ogawa occurred in successive
years. Rectal swabs collected from the selected group of infected children were
cultured daily throughout the epidemics. Serial blood specimens were examined for
serological investigations. Infection rates, and infection-to-case ratios were
higher with the El Tor Ogawa than with classical Inaba. Similarly homologous vibrio-
cidal antibody responses were higher in the El Tor Ogawa. Duration of El Tor vibrio
excretion was three times longer than that of the classical Inaba vibrio excretion.
It was found that 86.6% of El Tor Ogawa infection lasted for more than one day while
only 50% of classical Inaba lasted for a longer period. This comparative analysis
indicates that bacteriological and serological investigations into cholera epide-
miology will help biotype characterization.

Woodward WE see Sommer A

Yamamoto A, Homma JY, Choda A, Ishihara T, Takeuchi S. Common protective antigen
between *Pseudomonas aeruginosa* and *Vibrio cholerae*. Jpn J Exp Med 1979 Dec;49(6):
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Crude flagellar (CF) vaccine produced from the classical, Inaba strain CA401 of *Vibrio cholerae* was evaluated for its prophylactic potential in the rabbit ligated ileal loop model. Rabbits immunized with CF showed a high degree of protection against CA401 (classical, Inaba), CA411 (classical, Ogawa) and hypertoxinogenic strains of 569B (classical, Inaba) when challenged. Immunized rabbits require a greater than 1,000 fold increase of the challenge inoculum to induce an intestinal fluid response equivalent to that produced by unimmunized animals. Thus the fluid accumulation (FA) ratios remained significantly depressed in immune animals. The intestinal mucosa of CF immunized rabbits inhibited the association of ^{35}X-labeled vibrios. The effectiveness of CF as an immunogen was compared to those of commonly employed cholera vaccine (CV) and cholera toxoid. The protection conferred by CF immunization was 10 times as effective as CV. CF immunization was also superior to that of glutaraldehyde-treated cholera toxoid. The immunogenicity of CF was destroyed by heat treatment (100°C for 15 min). The immunogenic components of CF appeared to be a flagella-derived protein. Immunogenicity of CF was not diminished by the absorption of CF-immune serum with a flagellated mutant vibrios. By introducing both goat anti-rabbit immunoglobulins A and G intestinal protection of CF-immunized rabbits was completely reversed. The results demonstrated that a level of protection was attained by immunization with the subcellular CF vaccine but a combined CF-toxoid vaccine might be optimal for human prophylaxis against cholera.

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