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Cholera caused by *Vibrio cholerae* O139 - A Case Report

Introduction

Cholera is a clinical-epidemiologic syndrome, caused by *Vibrio cholerae* (*V.cholerae*) of the serogroup O1, usually manifesting as an acute intestinal disease. In its severe form, the clinical disease is characterized by voluminous stools of rice water character that rapidly lead to dehydration. Hypovolemic shock, metabolic acidosis and death can ensue in adults, as well as in children, if prompt and appropriate treatment is not initiated. History has recorded seven pandemics during the 19th and 20th centuries, the first 6 was caused by *V.cholerae* of the classical biotype. The seventh pandemic has been ongoing since 1961, and was caused by *V. cholerae* O1 of the El Tor biotype Beginning in late 1992, in India and then in Bangladesh, there appeared epidemic cholera due to a new, toxigenic, non-O1 *V.cholerae*, which was named *V.cholerae* O139 Bengal, to refer to its origin in areas surrounding the Bay of Bengal [2-4]. The enhanced ability of *V.cholerae* O139 to survive in environmental niches, and its rapid geographic spread, has alerted epidemiologists to the possibility of an eighth pandemic of cholera.

This is a report of the first indigenous case of cholera in Taiwan, caused by *V.cholerae* O139 Bengal.

Case Report

A 71-year-old man presented to our emergency department (E.D.) on August 26, 1997 with vomiting and diarrhea for a duration of 3 hours. He had received a total gastrectomy 24 years ago due to peptic ulcer bleeding. He was otherwise well in the past. Two days prior to presentation, he went on a sightseeing tour in Likang of Pingtung county, and ingested two raw eggs of soft-shelled turtle and some cooked turtle meat during a luncheon attended by some 300 people. Forty-eight hours later, vomiting and profuse, “rice water”-like diarrhea with a frequency of over 20 times

within 3 hours developed. At our emergency department, physical examination found an acutely ill man, clear and alert, with a blood pressure of 120/79 mm Hg, pulse rate 120 per minute, respiratory rate 20 per minute and a body temperature of 36℃. Hyperactive bowel sounds were heard, and the remainder of his physical examination was unremarkable. Laboratory examinations were as follows: blood routine showed a white blood cell (WBC) count of 13170 per cubic millimeter, with 78 % neutrophils, 3% band form, 10 % lymphocytes, 9% monocytes, a hemoglobin of 12.1 gm %, hematocrit 41.7% and a platelet count of 282000 per cubic millimeter; urinalysis showed 0-2 red blood cells (RBC) per high power field (/HPF), WBC 1+, protein 300mg/dL, glucose 0.5g/dL, and was positive for occult blood; stool routine showed RBC 1-2/HPF, WBC 1-2/HPF; serum biochemistry showed GOT 70 UIL, GPT 75 U/L, ALP 111 U/L, LDH 202 U/L, BUN 26mg/dL, creatinine 2.2mg/dL, sodium (Na) 134 mmol/L, potassium (K) 4 mmo/L, chloride 100 mmol/L, glucose 370 mg/dL, and amylase 127 mg/dL; arterial blood gas analysis showed a pH of 7.278, pCO2 19.6 P¢X2 108.3, HCO3 8.9 mmol/L. During observation at E D., his diarrhea persisted, with a frequency of up to 50 times in one day, with a body weight loss of from 75 to 64 kilograms (kg) (a total of 11 kg). Hypovolemic shock developed with a blood pressure of 78/57 mmHg and a heart rate of 160 per minute. Immediate replacement of fluids and electrolytes was begun, and his blood pressure rapidly normalized. However, acute renal failure (BUN 37 mg/dL, Cr 4.1 mg/dL), with hyponatremia (Na 123 mmol/L), hypokalemia (K 3.2 mmollL) and metabolic acidosis (pH 7.227, HCO1 7.9 mmollL) developed as a result of acute diarrhea, he was then admitted for further therapy. Health authorite was notified of a suspected case of cholera. On the second day of admission, diarrhea persisted with rice-water stools at a frequency of about 30 times/day. A Gram's stain of the stool specimen disclosed curved, gram-negative bacilli. Oral tetracycline at a dose of 500mg every 6 hours was administered in addition to replacement of fluids and electrolytes. Diarrhea continued for 3 days after admission, totalling to about 100 stool passages, with a rise in BUN to 62 mg/dL and serum creatinine to 5.7 mg/dL on the third day. After aggressive supportive therapy, his renal function returned to normal on the 7th day after admission Stool culture isolated *V.cholerae* O139, sensitive to tetracycline and resistant to chloramphenicol and trimethoprim-sulfamethoxazole. After 7 days of tetracycline, stool culture was negative for *V.cholerae* O139 over 3 consecutive days, and he was discharged under stable conditions.

Discussion

V.cholerae have been divided into around 150 serotypes on the basis of its lipopolysaccharide (LPS) somatic antigen. Serotypes O1 and O139 produces cholera enterotoxin which causes the clinical syndrome of cholera and has panendernic potential. The remaining serotypes from O2 to O139 are referred to as "non-Ol V.cholerae", and less than 1 % may produce cholera enterotoxins. These strains are not associated with epidemic diarrhea, and are only occasionally isolated

from cases of diarrhea, and rarely, from a variety of extraintestinal infections, including wounds and the ear [5] *V.cholerae* serogroup O1 can be divided into 2 biotypes, classical and El Tor; and has 3 antigenic determinants, factors A, B, and C. This serogroup can be further subdivided into serotypes of the O1 serogroup called Ogawa (contains factor A and B), Inaba (contains factors A and C), and Hikojima (contains factors A, B and C). Ogawa is the most common serotype.

Since 1911, there has been 4 major epidemics in Taiwan, occurring in 1912, 1919-1920, 1946 and 1962, totaling 11,036 patients. Although there has been no case reports of indigenous cases since 1962, there have been occasional imported cases [6]. This article reports the first indigenous case of cholera caused by *V.cholerae* O139Bengal in Taiwan.

Historically, in October 1992, 48 cases of severe, cholera-like diarrheal disease were reported in Madras and Tamilnadu of southern India [3]. In January 1993, and additional 28 cases and 48 cases were reported in Madurai and Vellore. Serological characterization of the 124 strains revealed that they did not agglutinate with O1 antiserum nor with any of the monoclonal antibodies specific for antigenic factors A, B and C. Production of cholera toxins was confirmed by hybridization of DNA probes specific for the cholera toxin gene, and the highly sensitive enzyme-linked immunosorbent assay (ELISA). This strain was identified as *V.cholerae* non-O1. At the same time, in Calcutta of eastern India, there was an unusual increase in the rate of isolation of *V.cholerae* non-O1 compared with that of O1. By the end of December, non-O1s predominated (>95%) in isolates from choleric diarrheal patients. A total of 13,275 patients with diarrhea were admitted to local infectious diseases hospital between January and April, 1993, of which 434 (3.2%) died. Of 115 bacteriologically confirmed *V.cholerae* non-O1 cases, 93% had vomiting and 77.4% had severe dehydration. It is of interest that 17 of 20 cases had leucocytosis ($> 11 \times 10^9$/L), which is not seen in cholera caused by *V.cholerae* O1 strains. The first O139-positive samples were collected in Bagerhat and Perojpur on Dec 22, 1992. By the end of March 1993, the Government of Bangladesh Epidemic Surveillance had reported 107,297 cases of diarrhea and 1473 (1.4%) deaths [4]. Most cases occurred in adults who have been exposed to *V.cholerae* O1 previously. This suggest that the Bangladesh population was immunologically naive and that previous exposure does not provide immunological protection. The case distribution differs from *V.cholerae*O1 during seasonal peaks of disease, in which 60% of the patients are younger than 15 years. The disease is indistinguishable from cholera in clinical features and responses to treatment. However, the causative organism did not agglutinate with the O1 antiserum, nor with antiserum of O2 to O139. The strain has now been assigned to serogroup O139 with the suggested name of “Bengal” to refer to its origin in areas surrounding Bay of Bengal. They resembled El Tor vibrios in being resistant to polymyxin B and positive for agglutination of chicken erythrocytes. All the isolates studied produced large amounts of an enterotoxin apparently identical to cholera toxin. Important virulent characteristics, specifically, cholera enterotoxin and toxin-coregulated pilus (TCP), of *V.cholerae* O139 is indistinguishable from typical El Tor *V.cholerae*. O139 was

therefore considered to be a new hybrid of O1 and non-O1. Characteristic features of the O139 *V.cholerae* are summarized in Table 1.

The O139 strain of *V.cholerae* disseminated more rapidly than the O1 strain establishing the pandemic potential of this new strain.. Within a year since the first recorded outbreak in Madras, Southern India, it appeared in Bangkok, Thailand. Between April and June, 1993, 9 of 17 strains of *V.cholerae* non-O1 isolated from patients with acute watery diarrhea in Bangkok, Thailand were of the O139 serotype. Subsequently, additional cases have been reported in Pakistan, Nepal, China, Kazahcstan, Afghanistan and Malaysia, and several imported cases from the United States and United Kingdom. In contrast, the seventh pandemic strain of cholera, the El Tor *V.cholerae* O1, took over 3 years to spread from Celebes island in Indonesia through Bangkok to India [(7)] Epidemiologists are worried that these epidemics may mark the beginning of the eighth cholera pandemic [(8)] The World Health Organization has requested its members to immediately report all cholera cases due to *V. cholerae* O139.

In June 1993, cultures of 11 of 92 (12%) water samples from ponds, lakes, rivers and canals in Bangladesh yielded *V.cholerae* O139, and 1 yielded *V.cholerae* O1 biotype El Tor and serotype Ogawa. All the strains generated the expected 302 bp fragment of the ctx A gene of the cholera toxin operon of *V.cholerae* by polymerase chain reaction. *V.cholerae* O1 is usually isolated from less than 1 % of water samples during epidemics, thus, such a high isolation rate (12%) indicates that *V.cholerae* O139 may be hardier than and probably has a survival advantage over *V.cholerae* O1; these characteristics increases its potential to cause pandemics. The isolation of *V.cholerae* O139 in the fish ponds in Taiwan should be a warning to our health authorities, and is a challenge to the disease control system in Taiwan. This should also alert the general public to increase vigilance in personal hygiene.

Cholera is transmitted by the fecal-oral route. Therefore, personal hygiene is the key to the prevention of cholera. The avoidance of eating uncooked food or drinking unboiled water cannot be overemphasized. *V.cholerae* can be killed by heating water to a temperature of 55 ¢XC for 10 minutes and pasteurization of milk, water and soft drinks are effective sterilization methods. Gastric acid will kill most of the *V.cholerae* incidentally ingested. Inhibition of growth and multiplication by intestinal bacterial flora, intestinal motility, intestinal mucus and enzyme secretion and bile salts will further hinder establishment of infection. In healthy volunteers, doses of $1O1^{1}FU$ of *V.cholerae* were required to consistently cause diarrhea. When stomach acidity was neutralized with 2g of sodium bicarbonate immediately prior to administration of the inoculum, attack rates of 90% were seen with an inoculum of $10^{6(9)}$. Therefore, gastrectomized patients or those who are achlorhydric should take extra precautions. Our patient had several predisposing factors including having received gastrectomy and ingestion of raw eggs of soft-shelled turtles.

The most important therapy is replacement of fluids and electrolytes Severe cases may die within a few hours without appropriate therapy. The high mortality rate of over 50% may be reduced to less than I % with appropriate therapy

Antimicrobials will shorten the duration of diarrhea and thereby reduce fluid losses in cholera. Tetracycline and its congeners are the drugs of choice in most instances. A dosage of 250mg every 6 hours for 3 to 5 days is adequate. In Bangladesh, 70% of *V.cholerae* O1 is resistant to tetracycline, and alternative drugs include trimethoprim-sulfamethoxazole, furazolidone, erythromycin and quinolones. On the other hand, *V.cholerae* O139 is sensitive to tetracycline, but resistant to trimethoprim-sulfamethoxazole (98%), streptomycin (92%) and furazolidone (86%).

The reporting of the first indigenous case of *V.cholerae* O139 Bengal in Taiwan is a great challenge to our disease control system. Health authorities should closely monitor for a possible outbreak, locate source of infection and educate the public against ingestion of uncooked food or unboiled water. As for physicians, cholera should be listed as one of the differential diagnoses in any patient presenting with acute diarrheal disease.

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Table 1. Characteristics of *Vibrio cholerae* O139

Gram negative
Curved bacilli measuring 2-3 X 0.5 urn
Single polar flagellum
Darting motility not stopped by antisera to *V cholerae* O1
Yellow colonies on TCBS agar
Grayish opaque colonies with dark centers on TTG agar
Positive for oxidase and gelatinase
Fermented glucose, maltose, sucrose and mannose without producing gas
Did not ferment in ostial or arabinose
Decarboxylated lysine and ornithine
Did not dihydrolate arginine
Produced indole
Grew in 0% and 3% salt but not in 8% salt
Variable haemolysis of sheep RBC
Resistant to polymyxin B (50 IU), co-trimoxazole and DADP (10 and 150 ug)
Agglutinated chicken RBS
Resistant to Mukherjee's IV and V phages for O1
Susceptible to tetracycline, ampicillin, chloramphenicol, erythromycin, ciprofloxacin

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TCB S=thiosulfate-citrate-bile salts-sucrose agar
TTG=taurocholate, tellurate, gelatin
RBC=red blood cells
DADP=vibriostatic compound 2, 4-diamino-6, 7-di-isoprophylpteridine (0/129) (see Reference No 4)

Editor's note:

Though this was the first indigenous case of *Vibrio cholera* O139 Bengal infection identified in Taiwan, through the joint effort of the health and medical care units and the disease surveillance system, the infection was soon brought under control. The index case, surnamed Yang, was admitted to the Veterans General Hospital on 26 August for vomiting and diarrhea. VGH soon reported the case to the Kaohsiung City Health Department on 27 August. The case was then confirmed by the Southern Branch Laboratory of the National Institute of Preventive Medicine, DOH, as toxigenic O139 cholera infection on 28 August. 244 contacts were subsequently followed up and specimens collected for laboratory testings to be all negative. The National Quarantine Service of DOH also identified *V cholerae* O139 in the fish ponds where the turtles came from. These ponds were soon disinfected in September by authorities concerned of the Kaohsiung County Government. Soft-shelled turtle farming has been suspended since then.

Following the models of the World Health Organization, the Department, when no further cases were identified during the period starting 26 August, the day of isolation, till two incubation periods of 10 days, announced the end of the disease outbreak.