The 1990s have witnessed the changing face of cholera—its reemergence in one continent, a new epidemic strain in another, and a tragic reminder of the impact of the disease in an already suffering population.

In January, 1991, cholera was reintroduced into Latin America after an absence of more than 100 years.3 Within two years the disease had spread from Peru to Mexico,2 and in the past six years there have been 1·4 million reported cases of cholera causing more than 10 000 deaths in the Americas (figure). As a result of the Latin American outbreak, more cases of cholera have been reported to WHO every year in the 1990s than in any other year since surveillance began. October, 1992, saw another unprecedented epidemiological event; a new epidemic strain of Vibrio cholerae emerged in India and Bangladesh.3 This cholera toxin (CT)-producing strain was the first non-O1 strain of V cholerae capable of causing epidemics, and it has since been classified as V cholerae O139 Bengal.3 The lack of cross-immunity between the Bengal strain and other O1 cholera strains led to major epidemics of cholera (up to 200 000 cases) in India, Bangladesh, and five other countries of South-East Asia.4 Travel-associated (imported) cases were reported in the USA, Europe, and Japan.4,5

Finally, the massive outbreak of El Tor cholera among Rwandan refugees in Goma, Zaire, resulting in 70 000 cases and 12 000 deaths in July, 1994, showed that during times of crisis cholera can be catastrophic.6,7 The very high cholera death rate (15 per 1000), with a fatality ratio as high as 48% at one camp, were principally due to the rapid waterborne spread of cholera (as well as shigellosis), which quickly overwhelmed the existing medical services and the capacity for oral rehydration therapy (ORT) at diarrhoea treatment centres.7 The morbidity and mortality rates during the Rwandan refugee crisis contrast sharply with Latin America, where mortality rates were consistently below 1%.2,8 Access to health care is an important factor determining outcome. In Peru, for example, most cholera was reported in urban areas where hospitals and rehydration units were readily available but in rural areas mortality rates were 5% and continued to be high for years after the initial outbreak.8,9

**Cholera and international travel**

The resurgence of cholera has led to an increase both in the awareness of cholera and in the number of cases detected in travellers and workers going from the developed world to endemic areas. The most important example occurred in February, 1992, when 75 of 336 passengers from an airliner returning to Los Angeles from South America developed cholera.7 The flight originated in Argentina but the outbreak was traced to a seafood salad prepared by the caterer in Peru that was picked up en route to Los Angeles. Many of the passengers had severe diarrhoea and had to be admitted to hospital; five progressed to renal failure and one died.

In surveys done from the 1960s to 1980s cholera was rare in travellers but this was because the disease was present in areas that few people visited; also microbiology-based surveillance was lacking.10 In Japan, where there is regular microbiological screening for cholera by culture in returning travellers with diarrhoea, the incidence of cholera for all destinations was 5 per 100 000; it was 13 per 100 000 in Japanese travellers returning from Bali (table 1).11 In the USA the 10-fold increase in cholera cases noted by Centers for Disease Control and Prevention investigators was due to the proximity of the Latin American outbreak and increased awareness.8 Routine surveillance of expatriates living in endemic areas suggests a substantial risk exists. In a study of US embassy personnel in Lima, Peru, during the height of the cholera outbreak and among those who presented with diarrhoea,
Microbiology of V cholerae

V cholerae is a motile, curved, gram-negative bacillus, first described in 1854 in Italy by Filippo Pacini. In 1883, Robert Koch demonstrated that cholera was caused by this microorganism (Kommabazillen). It is a well-defined species. Of the 139 serogroups, as determined by the composition of the major surface antigen of the cell wall (O), only two, O1 and O139, have been associated with epidemics; these two serogroups produce cholera toxin, which is responsible for the fluid secretion. Other serogroups have only been associated with sporadic cases and small clusters of non-cholera diarrhoea. Serogroup O1 can be further subdivided into three serotypes, named Ogawa, Inaba, and Hikojima, based on quantitative differences of factors A, B, and C of the O antigen. V cholerae O1 strains are also divided into two biotypes, classical and El Tor. Isolates from the third pandemic (1852–59) to the sixth (1899–1923) were caused by the classical biotype. El Tor gave rise to the seventh pandemic, which originated in Sulawesi, Indonesia, in 1961, and is still ongoing. El Tor is now the predominant biotype but classical is still common in Bangladesh. The Latin American outbreak was exclusively El Tor Inaba at first but was replaced by El Tor Ogawa after the first year. Cholera due to classical or O139 strains has not been detected in Latin America.

V cholerae O139 is genetically related to the seventh pandemic O1 strains (see below) because it possesses all of the virulence determinants typical of O1 biotype El Tor, but there is a mutation in the genes producing the O antigen. O139 strains can produce a polysaccharide capsule and have an increased capacity both for cholera toxin production and for spread and proliferation within the environment.

Epidemiology

Epidemics of cholera arise after the introduction of V cholerae in non-endemic areas where most of the population is non-immune. Under these circumstances the attack rates can be as high as 10% and all age groups are affected. The morbidity and mortality can be considerable—for example, the introduction of cholera into West Africa in 1970 resulted in over 150,000 cases and more than 20,000 deaths in the first year, and in Peru there were over 420,000 cases and 3300 deaths within the first 15 months of the start of the epidemic. Epidemics are often unpredictable but they are usually seasonal: in Bangladesh the main peak is in the winter (September to November) with a summer peak in March to May, in coastal Pacific regions of South America cholera epidemics occur during the summer (January to May), and in the tropical jungle regions epidemics tend to coincide with the cool, dry season (June to September). Chronic carriers are rare and do not play a significant part in maintaining the microorganism between epidemics. Asymptomatic infection is often found at the time that symptomatic cholera is occurring, and is probably important in intrafamilial transmission.

The transition from the epidemic to an endemic phase occurs after a large proportion of the population is immune or semi-immune. Previous immunity decreases in adults so higher attack rates are seen in children and in women of childbearing age, who are exposed to large inocula of V cholerae organisms while caring for the very young. Attack rates tend to be low in adults in this situation (<1% per year). Studies in Bangladesh, where both classical and El Tor biotypes are present, have indicated that infection with classical organisms provides more potent and long-lasting immunity than infection with El Tor. In Peru, where outbreaks were caused exclusively by V cholerae El Tor, numbers of cholera cases fell from 322,000 cases in 1991 to only 4500 cases in 1996 and this decrease was at least in part due to heightened immunity. During the endemic phase secondary transmission of cholera occurs, principally by intrafamilial spread of infection among family contacts in the range 4–22% and sometimes as high as 50%. Contamination of food, at home, shared social functions, in markets, and by street vendors, is common. V cholerae O1 can survive for 2–14 days in food and for many weeks in shellfish and molluscs. Widespread contamination of surface water sources also contributes to the transmission of cholera.

The epidemiology and transmission patterns of O139 seem similar to those of O1 strains. High attack rates of severe cholera due to O139 seen among adults in areas long endemic for O1 indicate that infection by V cholerae O1 strains does not provide cross-protection. The secondary infection rate among family members is about 25% within 10 days of the index case. If outbreaks of cholera due to this new serogroup continue to occur in newly affected countries, they may represent the advent of the eighth cholera pandemic. Bengal strains survive well in environmental water. Household (tubewell) water has often been found to be the principal source of infection in Bangladesh. Thus, the predominant mode of transmission of V cholerae O139 appears to be waterborne.

Ecology of V cholerae

Non-O1 V cholerae have been identified as free-living bacterial flora in estuarine areas. By contrast, V cholerae O1 is very difficult to isolate unless there is cholera in the population. The persistence of V cholerae within the environment, for months and probably years, is facilitated by its ability to enter a viable, nonculturable, dormant state.
2% of El Tor result in severe cholera and in Peru only. In Bangladesh only 11% of classical infections and infected with intervention in controlling cholera. Rugose strains, if size and whether food was the vehicle of transmission exopolysaccharide which promotes cell aggregation. This form, associated with the production of an epidemic.23,25

Clinical features
The incubation period of cholera ranges from several hours up to 5 days and is determined by the inoculum size and whether food was the vehicle of transmission (food protects vibrios from the action of stomach acid). Inoculum sizes as low as 100–1000 organisms may cause disease but doses of around 1 million are needed to reliably induce disease in volunteers.26,27 Few patients infected with V cholerae O1 develop severe cholera (cholera gravis). In Bangladesh only 11% of classical infections and 2% of El Tor result in severe cholera and in Peru only 25% of cholera illnesses among the local population were detected in hospital.11

The most striking feature of severe cholera is the voluminous water stool output, and the dehydration it causes (table 2). Stool output can reach 500–1000 mL per hour, leading rapidly to hypotension, tachycardia, and vascular collapse. The patient becomes lethargic or stuporous with sunken eyes and cheeks and dry mucous membranes. Decreased skin turgor (skin-pinch sign) is found in all such cases. Urine flow is decreased or absent and serum specific gravity is consistently raised. Clinical illness which goes untreated resolves in 4–6 days in most cases (unless circulatory collapse occurs).

Table 2: Guidelines for clinical evaluation of dehydration and recommendations for rehydration and maintenance fluid therapy

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Degree of dehydration</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental status</td>
<td>Alert</td>
<td>Restless or lethargic</td>
<td>Lethargic, stuporous</td>
</tr>
<tr>
<td>Thirst</td>
<td>Present</td>
<td>Rapid</td>
<td>Marked</td>
</tr>
<tr>
<td>Radial pulse</td>
<td>Normal</td>
<td>Tachypnoeic</td>
<td>Rapid and feeble or imperceptible</td>
</tr>
<tr>
<td>Respirations</td>
<td>Normal</td>
<td>Skin retracts slowly (1–2 s)</td>
<td>Tachypnoeic, deep, laboured</td>
</tr>
<tr>
<td>Skin-pinch sign</td>
<td>Skin retracts immediately</td>
<td>Sunken</td>
<td>Skin retracts very slowly (&gt;2 s)</td>
</tr>
<tr>
<td>Eyes</td>
<td>Normal</td>
<td>Scant and dark</td>
<td>Dramatically sunken</td>
</tr>
<tr>
<td>Urine flow</td>
<td>Normal</td>
<td>1·028–1·034</td>
<td>Scant or absent</td>
</tr>
<tr>
<td>Serum specific gravity</td>
<td>&lt;1·027</td>
<td>51–90</td>
<td>&gt;1·034</td>
</tr>
<tr>
<td>Fluid deficit (mL/kg body weight)</td>
<td>20–50</td>
<td>ORT and/or IVRT; depends on presence of vomiting and stool losses</td>
<td>91–120</td>
</tr>
<tr>
<td>Preferred method of rehydration</td>
<td>ORT in 4–6 h</td>
<td>IVRT, 2 L in 30–60 min, remainder in 3–4 h</td>
<td>WHO ORT and/or IVRT</td>
</tr>
<tr>
<td>Preferred type of rehydration</td>
<td>ORT or (all ages), Rehydralyte (adults), Pedialyte (children), Infalyte (infants)</td>
<td>WHO ORT and/or IVRT</td>
<td>Lactate Ringer’s*</td>
</tr>
<tr>
<td>Maintenance</td>
<td>ORT for as long as diarrhoea persists</td>
<td>Normal saline*+</td>
<td>NA</td>
</tr>
</tbody>
</table>

ORS=oral rehydration therapy, IVRT=intravenous rehydration therapy, NA=not applicable. *Dextrose-containing solutions (2–5%) are preferred due to risk of hypoglycaemia in cholera. Addition of potassium chloride (10 mmol/L) is recommended to reduce risk of hypokalaemia.

Table 2: Guidelines for clinical evaluation of dehydration and recommendations for rehydration and maintenance fluid therapy

Public health prevention and control of cholera
Strategies for the prevention and control of cholera are:10,26

- Early detection of epidemics through diarrhoeal disease surveillance and investigation of severe cases and clusters of illness.
- Education to promote good personal hygiene emphasising proper handwashing with soap and food
preparation techniques. Bathing in potentially contaminated open water should also be discouraged.

- Construction and maintenance of sewage disposal facilities.
- Provision and protection of safe and plentiful water and storage (eg, in homes and restaurants). Simple and inexpensive methods of domestic water disinfection and storage have been developed and they reduce the risk of cholera and diarrhoeal diseases. Point-of-use disinfection and the appropriate use of safe water storage containers is important in maintaining the water supply.

**Recent advances in vaccine development**

Parenteral, whole-cell cholera vaccines have been in use since the late 19th century. Controlled trials in the 1960s in cholera-endemic areas demonstrated they were only 60% effective for the first 3 months, declining to 30% during months 4–6 after vaccination. Moreover, this vaccine has to be given in two doses at 7–28 days apart and is associated with significant local reactions in up to 30% of vaccinees. This vaccine does not reduce carriage of *V cholera* O1 and its protective efficacy is below 30% in children. This vaccine is no longer recommended.

Beginning in the early 1980s inactivated oral cholera vaccine candidates have been developed. An oral vaccine, consisting of the B subunit of cholera toxin (1 mg) and 10⁷ whole cells (WC/BS, Cholerax; SBL Vaccin AB, Stockholm, Sweden), was found to protect against diarrhoeal illness caused by *V cholerae* O1 and, to some extent, against enterotoxigenic *Escherichia coli* in Bangladesh. This vaccine provided 85% efficacy against cholera in the first 6 months and a cumulative efficacy of 50% over 3 years when two or three doses were given 6 weeks apart. Protection was also found to be better against classical than El Tor cholera, especially among young children less than 5 years of age.

A cheaper, recombinant formulation (WC/rBS, SBL Vaccin AB), developed in the late 1980s, was also found to be safe and immunogenic in volunteers. Immunity is conferred 7–10 days after the second dose. This oral vaccine, given in two doses 1–2 weeks apart, provided 86% efficacy for 3 months against cholera among Peruvian military personnel immediately before an epidemic of El Tor with high attack rates (2–3%) in the summer of 1994.

A similar inactivated oral cholera vaccine, manufactured in Vietnam, had a protective efficacy of 66% against El Tor cholera. Protection in the Vietnam study was found to be similar in young children (68%) and older people (66%).

The main drawback of the oral, inactivated vaccines is the need for two or three doses, 1–2 weeks apart. If immunity could be obtained more rapidly, a vaccine could be considered as an option for immunisation in the military and/or for travellers and for the control of threatened cholera epidemics or epidemics already in progress. Live attenuated, oral cholera vaccines would be ideal for these needs. The best studied of these vaccines is CVD 103-HgR (Orochol Berna; Swiss Serum and Vaccine Institute, Berne, Switzerland). This vaccine confers an immune response and (protection in challenged volunteers) within 8 days. It is safe and produces after one dose, in the immunologically naive individual, a vibriocidal immune response that approximates natural infection.

In the volunteer challenge model, CVD103-HgR produces higher protection against the homologous classical strain than against El Tor. This vaccine is licensed in Switzerland and in parts of Latin America. A trial in north Jakarta, Indonesia, in 68 000 people given a single dose of CVD 103-HgR is being organised at the US Naval Medical Research Unit No 2. Other single-dose, live attenuated vaccines developed at Harvard Medical School (Mekalanos et al) and at the Center for Vaccine Development, Baltimore (Levine et al), are being tested in volunteers.

Attenuation of El Tor was unsuccessful for many years but new methods of strain selection and attenuation have now led to several new candidate vaccines. For example, two El Tor based, live attenuated, oral vaccines developed from Peruvian strains (Peru-14 and Peru-15) show promise as safe, effective vaccines against El Tor cholera, and Peru-15 has proved to be safe and highly protective against El Tor cholera in human challenge studies.

The rapid spread of *V cholerae* O139 among all age groups in areas where *V cholerae* O1 is endemic indicates that immunity to O1 type is not protective against O139. Epidemiological and laboratory studies suggest that natural immunity to O1 is not protective against O139, and this has been confirmed in challenge studies in rabbits and volunteers. The high rates of severe illness seen with this new strain and its potential to cause large epidemics among non-immune adults mean that attenuated *V cholerae* O139 type vaccines are needed urgently. Such vaccines are being developed and tested in animal models. Initial volunteer studies with live, attenuated O139 vaccine candidates seem promising, and protective efficacies as high as 83% have been recorded with one candidate vaccine, Bengal-15.

The hope is that oral cholera vaccines, killed and live, will become readily available for use in immunisation programmes in developing countries, and for travellers, expatriates, and military personnel. Other possibly important, albeit controversial, applications are during emergencies (such as famines, typhoons, and floods) and for refugees in both primitive and well-established camps where the risk of cholera outbreaks is considered high.
References


Further reading


Microbiology


Mhalu FS, Mmari PW, Ijumba J. Rapid emergence of El Tor Vibrio cholerae resistant to antimicrobial agents during the first six months of the fourth cholera epidemic in Tanzania. Lancet 1979; 1: 345–47.


Epidemiology and ecology


Clinical features and treatment


Prevention and control


Recent advances in vaccine development


