

Cholera

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Cholera

Summary

- Toxin from intraluminal intestinal bacteria, *Vibrio cholerae* O1 and O139
- Acute profuse to catastrophic watery diarrhoea with severe dehydration and ion loss
- Low or no fever and limited abdominal cramps
- Rehydration essential; preferably Ringer's lactate
- Antibiotics are of secondary importance

General

Cholera is an acute infectious disease, characterised by profuse watery diarrhoea. It is caused by a Gram-negative bacterium: *Vibrio cholerae* O1 (the characters O1 indicate the serogroup). It is a very small, motile, curved bacterium (vibrio is the Greek word for comma). Various subtypes exist, with classification according to biological and biochemical behaviour (biotypes) and serological characteristics (serotypes). Until 1992 it was thought that bacteria causing cholera must belong to *V. cholerae*, serogroup O1 and that they must be toxicogenic (must possess and express the genes for toxins). It was known that non-O1 *Vibrio cholerae* could sometimes cause mild gastro-enteritis or even bloodstream infection in immune depressed patients, but not cholera. In October 1992 in Madras (India), a mutated pathogenic bacterium (a new serogroup) was discovered, which also causes cholera. The isolate was given the name *Vibrio cholerae* O139 (nicknamed Bengalen). After a short bloom, the traditional strains (O1) have become more common again. A few years later, *V. cholerae* O139 Calcutta was identified.

Antibiotic resistance genes in *V. cholerae* are often positioned on plasmids and can be transmitted to vibrios from non-pathogenic intestinal flora.

V. cholerae can only cause disease if there are **pili** [Lat.: "hairs"] present. Pili are shorter and thinner than flagella. The pili adhere to the intestinal mucosa.

Sometimes **other Vibrio species** are responsible for diarrhoea, e.g. *Vibrio cholerae* non-O1, *V. parahaemolyticus*, *V. hollisae*, *V. minicus* and *V. fluvialis*. Our knowledge of these latter bacteria is clearly insufficient. *Vibrio vulnificus* is an aggressive species present in seawater and filter-feeding organisms such as oysters. This bacterium may cause bloodstream infection and wound infections, certainly in patients with liver cirrhosis.

Biotypes

There are **2 biotypes**: classic *Vibrio cholerae* and *V. cholerae* biotype El Tor. Biotype El Tor agglutinates chicken erythrocytes and causes lysis of sheep erythrocytes, unlike the classic biotype. The name El Tor originates from the Egyptian town and quarantine camp El Tor in the Southern Sinai desert, where the bacterium was isolated for the first time in 1905 (during the 6th pandemic) from an asymptomatic Hajj pilgrim from Mecca. The importance of this germ was long disputed (until 1961). At present El Tor has replaced the classic variant in most places, except in the Ganges and Brahmaputra delta. El Tor may also survive longer in the environment, is less dependent on transmission via water and produces more asymptomatic infections (symptomatic/asymptomatic infections = 7/100).

Serotypes

V. cholerae O1 of both biotypes can be subdivided into **serotypes according to the structure of the O antigen**. If only O antigen A and C are present, the bacterium is known as serotype Inaba. If only A and B are present, the bacterium is known as serotype Ogawa. If A, B and C are present, the name Hikojima is given. Serotype shift seldom occurs (from Ogawa to Inaba and vice versa).

The difference between these serotypes is only of importance for epidemiological studies. For example: in 1991 all cases of cholera in South America were caused by toxin-producing *Vibrio cholerae*, serogroup O1, biotype El Tor, serotype Inaba. The cholera epidemic in the Rwandan refugees in DRC (July and August 1994) was caused by El Tor, serotype Ogawa. These bacteria were resistant to tetracyclines, cotrimoxazole, chloramphenicol and ampicillin. The outbreak in Haiti in October 2010, 9 months after an earthquake was due to *V. cholerae* O1, Biotype El Tor.

Flagella, pili and fimbriae

Most motile bacteria move about with structures called flagella (spirochaetes move with the help of axial filaments). Do not confuse active bacterial movement with random Brownian movement. Do not confuse a bacterial flagellum with the flagellum of a eukaryote such as *Giardia* (cf. also the remark concerning cilia in *Balantidium coli*). The flagella are too thin (0.2 μm) to be observed with a standard light microscope. The bacterial flagellum carries out a rotating movement. Some bacteria have several flagella. When the flagella rotate anti-clockwise, they form a coherent bundle, so that the bacterium moves in a straight line. On the other hand when the flagella turn clockwise, there is no longer any co-ordination and the bacteria move randomly. By timing the

duration of clockwise and anti-clockwise spinning, this mechanism can be used in chemotaxis. The motor is in the membrane and the immediate driving power is not ATP, but a proton gradient. The bacterial flagella must not be confused with fimbriae, thread-like appendages which have no function in movement, but play a part in adherence to cells or tissues (important for virulence). Flagella rotate, fimbriae do not. Pili (singular pilum) are important in conjugation, the bacterial equivalent of sex. These hollow rigid tubes permit DNA transfer between bacteria. F-pili [Fertility] are important in the spread of resistance to antibiotics. Pili may also act as receptors for bacteriophages.

Epidemiology

Epidemics, Pandemics

Cholera has always been endemic in India and Bangladesh, in the huge delta formed by the confluence of the Ganges, Brahmaputra, Jamuna and Meghna rivers. Probably there was no cholera in Europe or America before the 19th century.

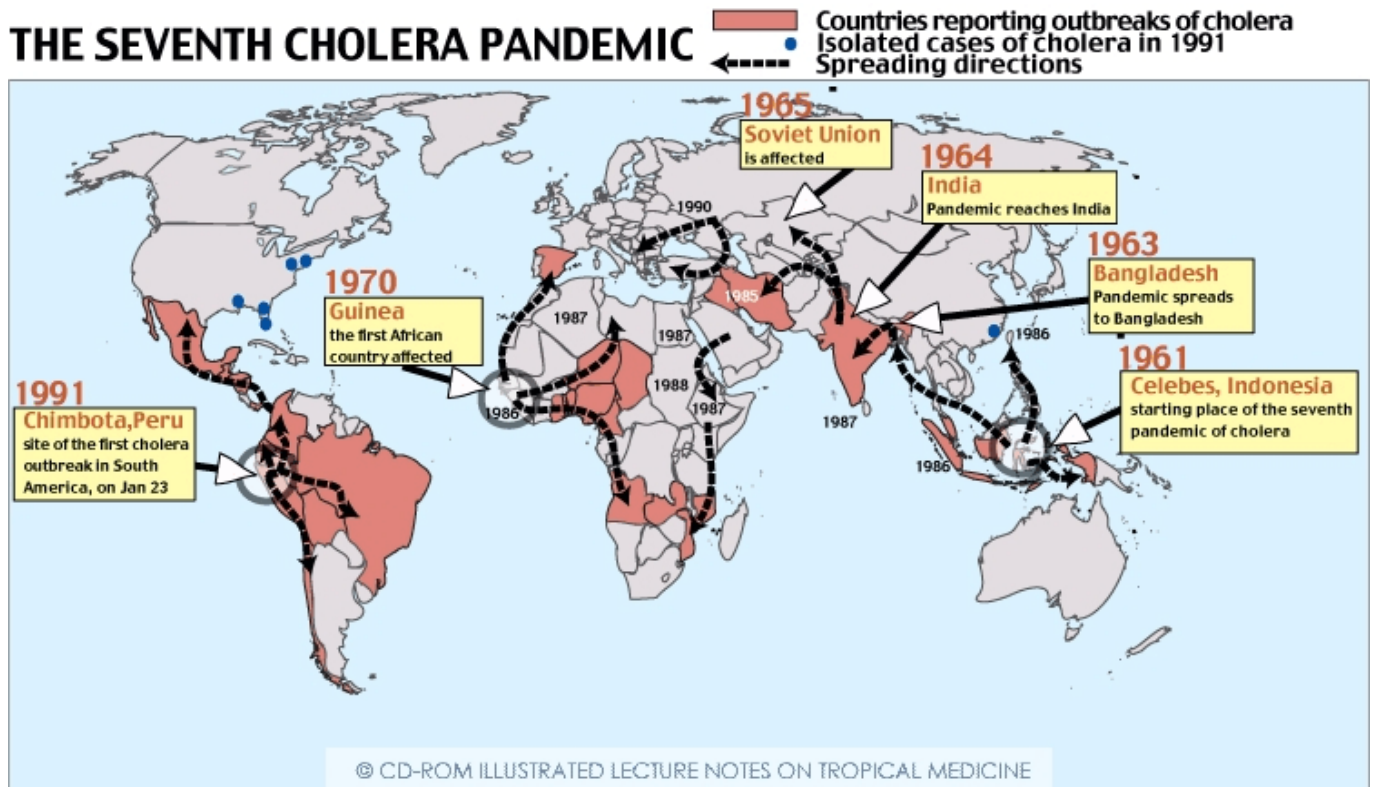
Between 1817 and 1923 there were various great pandemics, probably caused by the classic *V. cholerae* (there is no certainty as to the exact strain). The first pandemic which started in 1817 did not reach Western Europe. In 1829 the bacterium was introduced into the countries around the Persian Gulf via a British army unit stationed in India. From Iran the infection spread to Iraq, Syria, Georgia and Astrakhan (north of the Black Sea). It then travelled towards Odessa, Moscow, Vienna, Warsaw and Hamburg reaching England via the port of Sunderland. The first cases in London were seen in February 1832. The third pandemic merged with the second and was amplified by the miserable conditions during the Crimean war.

When each pandemic began and ended is rather unclear. There was cholera in Belgium in 1832, 1848, 1854, 1859, 1866 and 1892. In 1866, 1 in 100 Belgians died of cholera.

The pathogen was discovered in 1884 by Robert Koch during the fifth pandemic (first work in 1883 in Alexandria, Egypt, confirmation followed by research in India in 1884, with isolation of the bacterium in culture). In fact the bacterium had already been described in 1849 by Pouchet and in 1854 by Filippo Pacini, an Italian physician. However the latter's work on this was not known outside Italy. The germ theory and in particular the work of Koch were attacked by Pettenkofer. Pettenkofer was a proponent of the "ground water theory" believing that the fermentation of organic matter in the subsoil ("miasma") released cholera into the air (no transmission from person-to-person) which then infected the most susceptible e.g. those with poor diet, constitution,

etc. Both Pettenkorfer and his loyal student Emmerich drank a vial filled with cholera bacteria as proof against Koch's type transmission of *V. cholerae*. Amazingly, Pettenkofer did not then get cholera, but Emmerich suffered severe diarrhoea for 48 hours.

After the sixth pandemic there was a strange silence for about 40 years, for which no good explanation exists. The seventh pandemic was caused by El Tor. It started in 1961 in Celebes (Sulawesi), Indonesia, reached India in 1964 and Africa in 1970. In 2 years the infection passed through 29 African countries. In 1973 it arrived in the Gulf of Mexico. Early in 1991 the infection spread rapidly in Peru. In 3 weeks there were 30,000 cases. The bacterium then spread further into South America, causing 360,000 cases within the year. In the summer of 1992 a second, less severe outbreak occurred. Nevertheless by August 1992 "only" 5,000 deaths had been reported (from an estimated total of 600,000 cases), thanks to the wide-spread use of rehydration therapies. The case-fatality ratio varied depending on the region. After 1993 the disease assumed an endemic character in several countries, sometimes with local outbreaks. At the end of 1993 the cumulative total amounted to 900,000 cases in three years (1991-1993), with a cumulative mortality of 8,000. According to one hypothesis cholera bacteria infected the marine plankton off the Peruvian coast via the ballast water from a Chinese freighter. The possible role of changes in the nutrient-rich von Humboldt current is still unclear.



Spread of the seventh cholera pandemic

About 80% of the cholera in 1997 occurred in Africa, chiefly in the horn of Africa (118,000 cases were reported officially). The increase in cholera in this region followed heavy rains and flooding (possibly associated with the El Niño weather phenomenon).

Since 1992 *V. cholerae* O139 is recognised as a cause of a disease which is clinically identical to classic cholera, but which also occurs frequently in adults. Classic cholera in India, on the other hand, is common in children. There is no cross immunity with *V. cholerae* O1. Bacteria of the O139 serogroup have a polysaccharide capsule (unlike *V. cholerae* O1), which may explain the increased risk of bloodstream infection.

Cholera O139

After 1992 this new serogroup spread across Bangladesh, India, Pakistan and Southeast Asia. By the end of March 1993 more than 100,000 cases had been reported in Bangladesh. Further spread continued, but somehow diminished again, as the classic form and El Tor took over, reducing the

incidence of the new serogroup. The reason is unknown. Therefore it is difficult to make then new Bengalen serogroup responsible for an 8th pandemic. It was observed in India that, after the first spread of *V. cholerae* O139, new variants (clones) of *V. cholerae* O1 El Tor once more gained the upper hand.

Cholera also surfaces regularly in Madagascar. From the beginning of December 1999 until the end of February 2000 more than 12,400 cases were reported. **The disease can thus certainly not be regarded as an entity which only existed “in the past”.**

Recent Epidemics

At the end of 2008 a large cholera outbreak appeared in Zimbabwe. By February 2009, this led to more than 60,000 cases with a mortality of more than 5%, reflecting the general degradation of the nation’s basic infrastructure and the crumbling Zimbabwean health care system. By mid-April 2009 the official count was 96,591 cases with 4,201 deaths.

In 2010, more than 38,000 cases of cholera were identified in Nigeria.

In January 2010, a devastating earthquake hit **Haiti**, with its epicentre 25 km from Port-au-Prince, the capital. A couple of weeks before Nepalese United Nations peacekeepers arrived in Haiti, a cholera outbreak occurred in Kathmandu, the Nepalese capital. The forces were stationed in Mirebalais, 60 km north-east of Port-au-Prince. Late October 2010, patients with cholera were recognized in some Haitian rural areas. **In less than 6 weeks, more than 10,000 cholera cases** were identified. The disease quickly spread to the capital, where many people were still living in temporary shelters and tents, without access to safe drinking water or proper sanitary facilities. By January 1, 2011, the Ministry reported **171,304**, with a cumulative mortality of **3651**. The hospital case fatality rate was too high, and a target of hospital CFR of < 1% should be achievable. A possible epidemiological connection with the Nepalese forces was suspected and created tension between the local population and the UN forces. The current Haitian strain of cholera was identified as a **virulent hybrid of the El Tor O1 biotype and the classic type, serotype Ogawa**.

Transmission

Cholera is spread by **the faecal-oral route**, via contaminated water and food. The infectious dose of bacteria required to cause clinical disease varies according to the mode of transmission and varies

according to bacterial strain, with hyper-infective strains occurring immediately after gut passage. In people with normal gastric function and if ingested with water, **the infectious dose is one thousand to one million vibrios**. When ingested with food, it is lower about one hundred to ten thousand vibrios. The low pH of stomach acid kills most vibrios. When a person uses antacids, proton pump inhibitors or ranitidine, a lower infectious dose is required to trigger infection. The same applies to chronic atrophic gastritis and status post-gastrectomy. Asymptomatic infections are common, especially in case of El Tor. People excrete bacteria for about 10 days. This is sufficient time to ensure continued contamination of the environment. Chronic carriers are very rare, but occur, sometimes with vibrios lodging in in the biliary tract.

In third world countries many people have no chlorinated, filtered, treated, pure **drinking water**. The **lack of good toilets and sewers** leads to contamination of the surface or ground water. Too often untreated sewage water is still poured into surface water. Sometimes sewage pipes and drinking water pipes are laid in the same trench, which may result in contamination if there are leaks or greatly varying water pressures in the pipes. If drinking water is contaminated in this way, bacteriological checks of the drinking water when it leaves the pumping station will not show anything amiss. In houses, drinking water containers with a wide openings often become contaminated, because people are inclined to scoop up water in their (dirty) hands. Containers with a small spout, from which water must be poured are safer.

There is also direct **transmission from person to person, but it is rare**. The number of bacteria on dirty hands is usually lower than the minimum infectious dose necessary for direct transmission. Health workers who respect basic hygiene are at extremely low risk. **Filter feeders** such as mussels or oysters (especially in estuaria) concentrate the bacteria in their bodies. When the organisms adhere to food particles (e.g. the chitin of crustaceans) and in the case of hypochlorhydria, lack of gastric acid due to gastric surgery, antacids, anti-ulcer drugs or atrophic gastritis, the number of organisms needed to trigger infection is much smaller. Food may be infected by **dirty hands** during or after preparation. The **bacteria can survive and reproduce in food** such as cereals, rice or lentils and crustaceans. This intermediate replication step is very important. If someone dies of cholera and a meal is made for the mourners at the funeral by the persons who have washed the corpse, the risk of further transmission is very real. The bacteria are very sensitive to drying out, sunlight and acid. Meals which contain acid e.g. tomatoes and/or lemon, are much less dangerous than neutral or alkaline meals. Vegetables and fruit on the market are often sprayed with water to make them appear fresher and more attractive. If this is done with contaminated water, transmission may occur.

Historical note: John Snow and contaminated water

In the first half of the nineteenth century a cholera epidemic occurred in London. In 1848, a cholera outbreak started which would kill more than 14,000 people in London. Another outbreak in 1853 killed more than 10,000 people. The physician John Snow, already well known in 1853 as anaesthetist to Queen Victoria. Dr Snow had also a special interest in cholera. In 1854 he examined the various families presenting cases and calculated that the mortality in the houses that were supplied with water by the Southwark and Vauxhall Water Company was 31/1000 houses. This was 8.5 times higher than in houses supplied by the rival Lambeth Company. Although neither of the two companies offered purified water, the first company took its water from the Thames near London Bridge, downstream from the city sewage outlets while the second company pumped its water upstream from the city at Thames Ditton. In 1849 there had been no difference in mortality between the families that received water from Lambeth or Southwark. Before 1851 the Lambeth Company drew its water from a highly contaminated stretch of the Thames near Hungerford Market. It was this spectacular change (1849 compared to 1854) which made Snow conclude that contaminated water had a causal connection with cholera. Although both companies delivered water in the same streets, the water used in any particular house could be identified by its salt content (London Bridge is closer to the sea and its water is saltier than that at Thames Ditton). Adding silver nitrate leads to precipitation of silver chloride, proportionate to the amount of salt in the water. This was the basis of a simple test that could be carried out in every house.

Similar findings were made in Hamburg in 1890. The incidence of cholera was 34/1000 in Hamburg, where the drinking water was drawn from the river Elbe, and 3.9/1000 for the surrounding areas where other sources were used. In Altona, to the west and downstream from Hamburg, contaminated water was also taken from the Elbe, but there was less cholera. How could this be explained? If anything, more cholera would be expected in Altona. The difference was that in Altona the water was first filtered slowly through sand before being supplied for consumption. These observations led to attempts to provide cities with clean drinking water and to construct adequate sewers. Cholera was the first disease for which surveillance was set up and because of this the disease still has code number 001 in the international classification list of diseases.

Reservoir

Humans are the only vertebrate hosts. *Vibrio cholerae* can survive long-term and probably

permanently in brackish water, especially if there is a neutral or slightly alkaline pH and the water contains minerals and organic material. The bacteria are concentrated in phytoplankton (certain algae) and zooplankton which live in this water. Among the latter, **copepods**, a group of crustaceans, are important.

Cholera is clearly seasonal. A chronic aquatic reservoir is likely and this might be independent of continuous human faecal pollution. *V. cholerae* excreted by humans can be cultured in the laboratory. These bacteria however may assume a living form which cannot be cultured in vitro and which do not multiply in the environment. However, those bacteria are not dead as they are known to multiply when instilled in a rabbit's ileum. They are called 'viable but not culturable'. It may revert to a replicating form in its natural environment when there are favourable environmental factors and this has important epidemiological implications. The living, but non-reproducing form of *V. cholerae* can probably cause disease. Traditional culture methods for tracing *V. cholerae* in water miss these "dormant" bacteria. Tests based on fluorescent antibodies may offer a practical solution, as they stain both dormant and active bacteria.

Physiopathology

The bacterium **multiplies in the small intestine**, where it adheres to the **mucosal brush border**. The bacterium is **not invasive**, in other words it does not penetrate the intestinal wall or pass into the blood. It excretes a **very powerful toxin** which causes active fluid secretion towards the lumen. This fluid is isotonic, ion-rich and protein free. There are no intestinal ulcerations and the faeces do not contain blood. There is little if any fever. There is no tenesmus. **The faeces contain significant amounts of sodium, potassium and bicarbonate.** Because of this the intestinal content is slightly alkaline (*V. cholerae* thrives best in a slightly alkaline environment and the bacteria are therefore producing the conditions which are optimal for their own survival). The loss of large amounts of alkaline faeces results in metabolic acidosis. People with blood group O have an equal risk of infection but are at a significantly higher risk of clinically severe cholera if they become infected. The reason is unknown.

Hypervirulent and hyperinfective strains play an important role in epidemics. Passage of *Vibrio cholerae* through the gastrointestinal tract results in a short-lived, hyperinfectious state of the organism that decays in a matter of hours into a state of lower infectiousness. Such strains have a much lower ID₅₀ (the number of micro-organisms that will disease 50% of a population in normal conditions = measurement for virulence) than strains occurring in natural water reservoirs. The classic strain is associated with more severe illness. Faecal excretion of *V. cholerae* for up to two weeks has been documented and occasional asymptomatic carriers occur. Asymptomatic patients

typically shed bacteria in their stools at about 1000 *V. cholerae* bacteria per gram of stool, which is a low level of shedding, compared with the 100 million bacteria per gram in case of ricewater stools.

Toxins

Vibrio cholerae produces several toxins: **cholera toxin (Ctx)**, the zona occludens toxin (*Zot*) and the accessory cholera enterotoxin (*Ace*). The role of the two latter toxins is not entirely clear. The *Ctx* enterotoxin of *V. cholerae* consists of 2 parts: **A and B, where A stands for active and B for binding.**

They stimulate adenylate cyclase. Adenylate cyclase **increases intracellular cyclic-AMP, which inhibits salt absorption by the microvilli and promotes active chloride excretion by the crypt cells.**

Water and potassium bicarbonate passively follow the chloride. In the end there is an overall water loss to the intestinal lumen. Fluid loss originates in the duodenum and upper jejunum, the ileum is less affected. The colon is insensitive to the toxin and cannot absorb the large amount of fluid quickly enough. Catastrophic diarrhoea follows.

Cholera toxin

Part A is a monomer, while part B consist of 5 identical subunits (a pentamer). The polypeptides of part B bind to a receptor (Gm1 ganglioside, a glycolipid) on the epithelium of the small intestine, after which part A can penetrate the cell. Part A binds covalently to an intracellular protein (Gs-protein; s for stimulatory) which irreversibly activates it, leading to the persistent stimulation of another intracellular enzyme, adenylate cyclase.

The toxic A-subunit also has other effects such as disturbing the expression of some genes, increasing inflammatory cytokines and inhibiting antigen presentation by macrophages. On the other hand, the B-subunits of cholera toxin have anti-inflammatory properties. These are under intense study at present for possible therapeutic use in immune abnormalities. While cholera toxin adheres to the intestinal villus cells and disables the cellular saltwater pumps, the *Zot* toxin loosens the junctions that binds intestinal epithelial cells together. This contributes to the loss of water to the intestinal lumen.

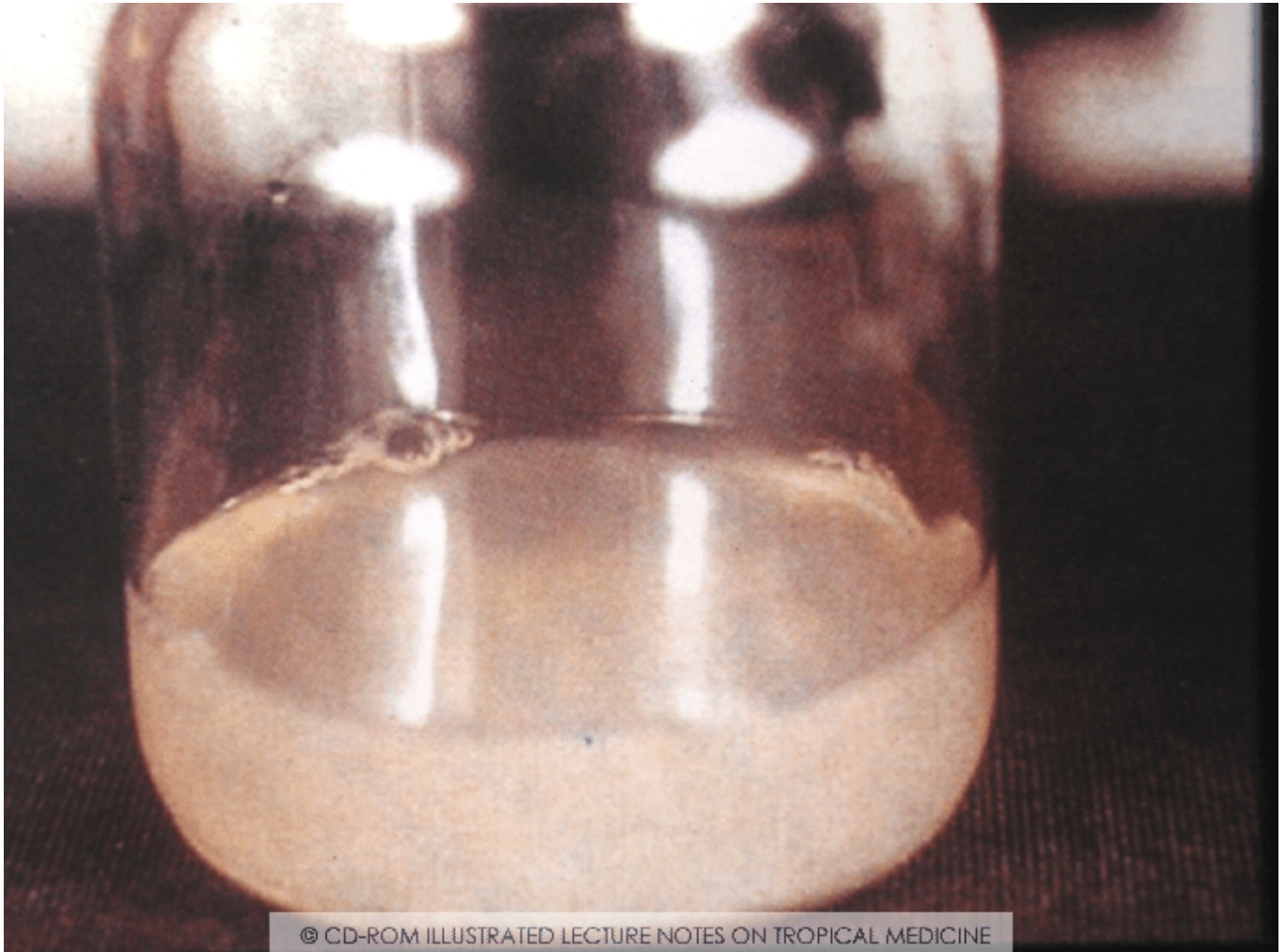
The in-vivo detailed mechanism is probably more complicated. Cholera toxin also stimulates the

nervous system in the intestinal wall, the myenteric plexus. This results in the release of 5-hydroxytryptamine (serotonin) from the enterochromaffin cells, leading to an additional fluid loss to the lumen. Granisetron, a 5-HT₃ receptor blocker, partially reverses this effect. More research is needed to determine the role of this mechanism in the pathophysiology.

Clinical aspects

The **incubation period is brief**: sometimes only hours, more commonly 1 to 5 days (average 2 days). It is one of the few infectious diseases where -in case of a very severe infection- you can be well in the morning and dead by sunset the same day. Asymptomatic infections are common (about 93%), but chronic carriers are very rare. Sometimes there is an initial transient fever (more seen in children).

Massive watery diarrhoea starts suddenly. The faeces very rapidly look like water in which rice has been boiled: watery with flakes of mucus. The faeces have a fish-like smell. The volume of faeces may rise to 500 ml per hour. Vomiting is common, but abdominal cramps are unusual. The onset of thirst, oliguria or anuria and weakness is rapid. In a short time the patient develops severe dehydration and can die within 24 hours. In other cases the diarrhoea is less severe, especially with infections with El Tor. As the patient's condition deteriorates, hoarseness of the voice and temporary deafness are often observed. Children with severe cholera may present with drowsiness or coma.



'Cholera feces which look like "rice water". Copyright Alexander von Humboldt Institute, Peru'

The signs of dehydration are thirst, dry mouth and lips (if the patient has not vomited recently), hollow eyes and sunken fontanel in children. The skin turgor diminishes. The skin becomes wrinkled (washerwoman's hands). Often, the voice becomes weak and hoarse, the pulse quickens and is difficult to feel. The radial pulse might be impossible to detect. Blood pressure falls. There is little or no urine production (prerenal failure). Respiration becomes faster due to metabolic acidosis secondary to loss of bicarbonate in the faeces (bicarbonate is alkaline). This acidosis causes vomiting and muscle cramps.

There is also significant potassium loss in the faeces. If rehydration is carried out using fluid without potassium, severe hypokalaemia may result. Nevertheless, quite often normokalaemia is found, together with an increased anion gap. The increase in anions (= negative ions) is multifactorial due to

the hyperproteinaemia (hemoconcentration), hyperphosphataemia (internal shifts and renal failure) and lactate acidosis (shock). Ketones play little if any role. An elevated hematocrit (hemoconcentration) can be found in nonanemic patients, as can neutrophil leukocytosis in severe cases.

The mortality from classic cholera may reach 50 %, but can be brought down to < 1 % with correct therapy. Mortality is chiefly due to dehydration with kidney failure, hypokalaemia, hypoglycaemia and aspiration pneumonia during vomiting.

Diagnosis

Cholera should be suspected in acute massive rice-water diarrhoea, certainly if there have been several cases in a short time (epidemic). The clinical picture of severe cholera is so spectacular that differential diagnosis does not present many difficulties. Milder cholera may be similar to other forms of gastro-enteritis (but not to dysentery). A child above the age of five years who develops acute dehydration, or dies as the result of acute diarrhoea, is always suggestive for cholera.

The vibrios are very small and can best be seen in a fresh faecal specimen with the help of dark field microscopy. There is characteristic motility (“star shooting”) which stops immediately after adding anti-O1 antiserum. This does not give any information on possible toxin production.

Confirmation is best made via a bacteriological culture.

Culture of *Vibrio cholerae*

Culturing should preferably be on a special medium in a bacteriology lab, e.g. TCBS-agar [=Thiosulphate-Citrate-Bile salts-Sucrose], polymyxin mannose tellurite agar (PMT) or another selective medium. TCBS agar is green before inoculation; sucrose-fermenting organisms such as *V. cholerae* turn it yellow. TCBS agar is important for rapid isolation and identification, but *V. cholerae* also grows on routine agar media. For routine media, large numbers of bacteria per gram of stool should be present to allow detection. Patients or carriers with low burden of bacteria will be missed with routine culture media. Overgrowth by normal faecal flora limits recovery of colonies. In order to identify the serogroup and the serotype one subsequently finds out to which antibodies (antiserum) the colonies obtained exhibit an agglutination reaction. It is also possible to find out whether the vibrios are toxicogenic (produce toxin), e.g. by a PCR variant called a loop-mediated isothermal amplification (LAMP) assay. Definitive identification is made in a reference laboratory.

Specimens may be transported in a transport medium, e.g. Cary-Blair. This is a kind of mild alkaline buffered gelatine in seawater with low redox potential in which the bacteria will survive for 4 weeks. If it is not available, a filter paper can be soaked with faeces and transported in an airtight bag to a well-equipped laboratory. A sample treated in this way remains usable for 1 week, but the recommendation is “the faster the analysis, the more reliable”. Blotting paper, soaked with liquid faeces and if possible placed in a 1% saline solution, can be kept for several weeks at 37°C (not in the freezer). This is useful if there are initial transport problems. Nevertheless it is better to have a fresh faecal specimen. For specimens from the environment or from food, in which the number of bacteria is much lower than in faeces, enrichment is necessary. The specimen can be incubated for 8 hours in alkaline peptone water, after which a TCBS agar is used.

About 10 days after infection with *V. cholerae* O1 the patient produces vibriocidal antibodies. They start diminishing after only one month and disappear within the year. Antibodies against cholera toxin are produced more slowly and remain for years. However these cross-react with enterotoxin produced by ETEC bacteria [enterotoxigenic *Escherichia coli*]. The immune response to *V. cholerae* O139 is not well understood. **The detection of antibodies is not important for the urgent care of the individual patient** but does permit retrospective diagnosis.

Other Vibrios

Sometimes other *Vibrio* species are responsible for diarrhea, e.g. *Vibrio cholerae* non-O1, *V. parahaemolyticus*, *V. hollisae*, *V. minicus* and *V. fluvialis*. Our knowledge of these latter bacteria is clearly insufficient. *Vibrio vulnificus* is an aggressive species present in seawater and filter-feeding organisms such as oysters. This bacterium may cause bloodstream infection and wound infections, certainly in patients with liver cirrhosis or otherwise immune depressed.

Treatment

Rehydration is essential and must be instituted as soon as possible. **Two phases** are distinguished. First it is important to replenish what has been lost in the previous hours or days. Then one must compensate the persistent fluid loss (e.g. the amount of fluid that is lost every hour). In mild cholera without vomiting oral rehydration may suffice. In severe forms IV fluids should be administered.

There are several possible compositions of rehydration fluids. **Solutions containing salt, sugar, potassium and bicarbonate are recommended.** Lactate is also good because it is converted in the body to bicarbonate. In cholera it is preferable to use Ringer's lactate (= Hartmann's solution). Normal physiological saline is second choice because it does not correct the acidosis nor does it

contain potassium. Severe hypokalaemia may occur, with cardiac arrhythmias, kidney damage, paralytic ileus and significant muscle weakness with reduced or absent tendon reflexes. Dextrose (= glucose) 5 % without electrolytes is not advised as a rehydration fluid. A reminder: 1 gr KCl = 13 mEq KCl. **So: Hartmann = Ringer's lactate >Ringer> physiological saline>>> not glucose infusion if there is an alternative.**

In severe cholera (fluid loss > 10 % of weight) the missing fluids should be administered quickly, e.g. 6 litres over 4 hours for a patient weighing 60 kg. The first 3 litres may each be administered in 10 minute boluses (total therefore 30 minutes). After administration of the lost volume, losses are compensated with further IV and/or PO fluids (faeces volume + urine volume + 500 ml). Vomiting may make oral administration of fluids difficult. **Generally a total of 6 to 10 litres per patient is necessary.** When patients start to drink and stop vomiting, it is advised to leave IV lines in place for a while until you are sure rehydration will not pose any more problems.



Cholera epidemic in Congo. Refugees live in very poor conditions. Photo courtesy Els De Temmerman



Cholera bed used by AIDS patients with chronic diarrhoea. With special thanks to Prof. Dr R. Colebunders. Copyright ITM

Special cholera beds are useful: they have a central opening to allow the liquid faeces to pass through, and they can be collected in a bucket. This makes it possible to quickly determine the amount of fluid loss. During an epidemic people who can still hold themselves upright can sit on a bucket and try to drink as much ORS [oral rehydration solution] as possible. Children quickly develop convulsions and coma. It is important that hypoglycaemia should be considered. For an adult 50 ml of a 50% glucose solution is given IV, for a child 2-4 ml/kg 25% glucose or 10 ml/kg of a 10% glucose solution.

Antibiotics may be useful because they reduce the duration and thus the total volume of the diarrhoea and may therefore reduce the need for rehydration fluids. On the other hand, they are not

essential (given the non-invasive character of the infection) and resistance often occurs. At present a single dose of azithomycin 1 gram (child 20 mg/kg) or a single dose of doxycycline 300 mg (child 4 mg/kg) are possible treatments. Doxycycline is usually contraindicated in pregnant women and children under 8 years. However, the administration of a single dose should not provoke major adverse effects. Single-dose ciprofloxacin may also be effective. *V. cholerae* O139 is often resistant to cotrimoxazole (sulphamethoxazole-trimethoprim). There is insufficient data examining the effect of antibiotics on secondary transmission of cholera. However in published studies to date antibiotics have not been shown to decrease secondary transmission of cholera within households. Anti-peristaltic drugs such as loperamide may cause accumulation of fluid in the intestinal lumen with unfavourable consequences and should be avoided.

Prevention

In the industrialised world a patient with cholera will remain a sporadic case. In developing countries one case can lead to several secondary cases. Therefore **'enteric contact precautions'** are essential in health care settings, focusing on very strict hand hygiene and thorough environmental cleaning and disinfection. The contamination of clothing and bedding is unavoidable. Boiling in water for five minutes is sufficient for disinfection. Mattresses and blankets can be dried in the sun. It is better to do this before washing them, to prevent infection of the washing area.

After surviving cholera a patient is probably immune for homologous biotypes for more than 3 years. There is some controversy: infection with the classic biotype seems to protect against recurrent infection by either biotype, but El Tor does not. No cross-immunity between *V. cholerae* O1 and *V. cholerae* O139 is seen, although they produce the same toxin. Immunity relies on antibodies in the intestinal lumen (the bacteria are not invasive). Systemic vibriocidal as well as anti-toxin antibodies develop during illness. Babies which are being breast-fed receive protective antibodies in their mother's milk.

Vaccination

Former parenteral vaccination with dead *V. cholerae* bacteria (IM administration) did not lead to sufficient formation of protective antibodies in the intestinal lumen. Only about 50-65% of people living in endemic areas were protected for 3-6 months. The IM vaccine was associated with local reactions in 50% and systemic reactions (fever, malaise) in 10-30%. Advice to vaccinate with this type of vaccine was discontinued in 1972 by the WHO [World Health Organisation]. Parenteral vaccination, mass chemoprophylaxis and "cordon sanitaire" (= restrictions on travel and trade) are not effective in preventing or limiting outbreaks. A **newly developed oral cholera vaccine** is based on a killed

whole cell cholera vaccine combined with the recombinant B subunit of cholera toxin (**Dukoral[®]**). The vaccine contains 1 mg of recombinant B subunit, as well as 25×10^9 bacteria each of *V. cholerae* O1 classic Inaba, *V. cholerae* O1 classic Ogawa, *V. cholerae* O1 El Tor Inaba (heat-inactivated), *V. cholerae* O1 El Tor Inaba (formaline inactivated). Dukoral does not contain the A subunit of cholera toxin and therefore, no pathogenic toxin is present. Two to three doses need to be given. Two recent studies showed an effectiveness of 86% and 40% respectively; the latter study indicating a 63% protection against severely dehydrating cholera episodes. Lower levels of protection continue for 3 years. Protection wanes rapidly in young children. **A herd immunity effect is expected in areas where vaccine coverage is more than 50%**. Because the risk of cholera for most travellers is extremely low, vaccination should be considered only for those working in relief or refugee settings or for those who will be travelling in cholera-epidemic areas and who will be unable to obtain prompt medical care. WHO recommends that current available cholera vaccines be used as complements to traditional control and preventive measures in areas where the disease is endemic and should be considered in areas at risk for outbreaks.

Mass (antibiotic) chemoprophylaxis is not effective because (1) the infection spreads faster than the organisation of drug distribution, (2) the effect of a drug only lasts 2 days, after which re-infection may occur, (3) the whole population needs to be treated simultaneously and people should then be isolated and (4) it is difficult to convince asymptomatic people to take a drug. In addition, the selection of highly resistant strains has been observed in settings using mass-administration of antibiotics.

Correct eating and drinking habits, safe stool habits and personal hygiene are the most effective means for individuals to limit their risk of cholera. Improved sanitation is the pre-eminent method of eliminating cholera and many other faecal-orally transmitted infections. This is directly linked to the degree of poverty in a region. Boiling drinking water is often difficult since fuel may be scarce and expensive. Since a significant proportion of *Vibrio cholerae* can adhere to plankton, the drinking water can be filtered through a fine cloth, which removes both plankton and a lot of bacteria in a single operation. This is of course less effective than obtaining water from a clean pipe or pump but it is cheaper. Chlorination of drinking water may be important (piped water or via water trucks). This is difficult to accomplish in rural areas. Chlorination is much less effective if the water is turbid due to organic debris.

Eating raw fish, shellfish (e.g. oysters, mussels) and crustaceans (such as crabs, shrimps) should be avoided. Washing hands is important for transmission control within a household. Infected faeces should not be disposed of in a poorly functioning drain (hospital: e.g. in pit with unslaked lime = CaO).

When large groups of people come together (funerals, festivals, etc.) there should be latrines with facilities for washing hands and plenty of soap.

An attempt must be made to trace the source of small, local outbreaks (see Historical note on John Snow). Contaminated water is the chief suspect in a sudden local epidemic, while in isolated cases the cause should be sought in contaminated food. This is of course not an absolute rule. Food cooked by street vendors and in restaurants poses specific problems. Flies probably play an underestimated part in transmission, but their numbers also reflect the sanitary conditions in a region.

The following points should be emphasised during information campaigns:

1. Drink only clean water (boiled or chlorinated)
2. Cook food completely and eat it while it is hot
3. Avoid uncooked food, unless it can be peeled
4. Wash hands after a bowel movement
5. Wash hands before preparing food
6. Wash hands before eating
7. Correct use of a good latrine (also for children)
8. With correct treatment cholera is rarely fatal
9. If cholera is suspected medical help should be sought immediately
10. In diarrhoea, give plenty of fluids (e.g. ORS)
11. Cholera vaccines should be used as complements to traditional control and preventive measures in areas where the disease is endemic and should be considered in areas at risk for outbreaks

In case of an epidemic, it is important to have a **large stock of IV rehydration fluid available** as well as the means of **preparing large amounts of oral rehydration fluid**. Normally such buffer stocks should be stored at various strategic points. The stocks for cholera treatment should not be segregated in storage, but should be rotated during normal use to avoid stock expiring. As soon as an epidemic is suspected, use as much oral rehydration as possible first so that stocks of the IV solutions last as long as possible. Cholera beds should be made ready. In a normal epidemic an attack rate of 0.2% can be taken as a rule of thumb (i.e. 200 cases can be expected in a population of 100,000). This is useful for estimating the size of stocks that will be needed. Sometimes the attack ratios are higher (e.g. the Rwanda-Zaire border in 1994). All this requires a solid epidemic preparedness.