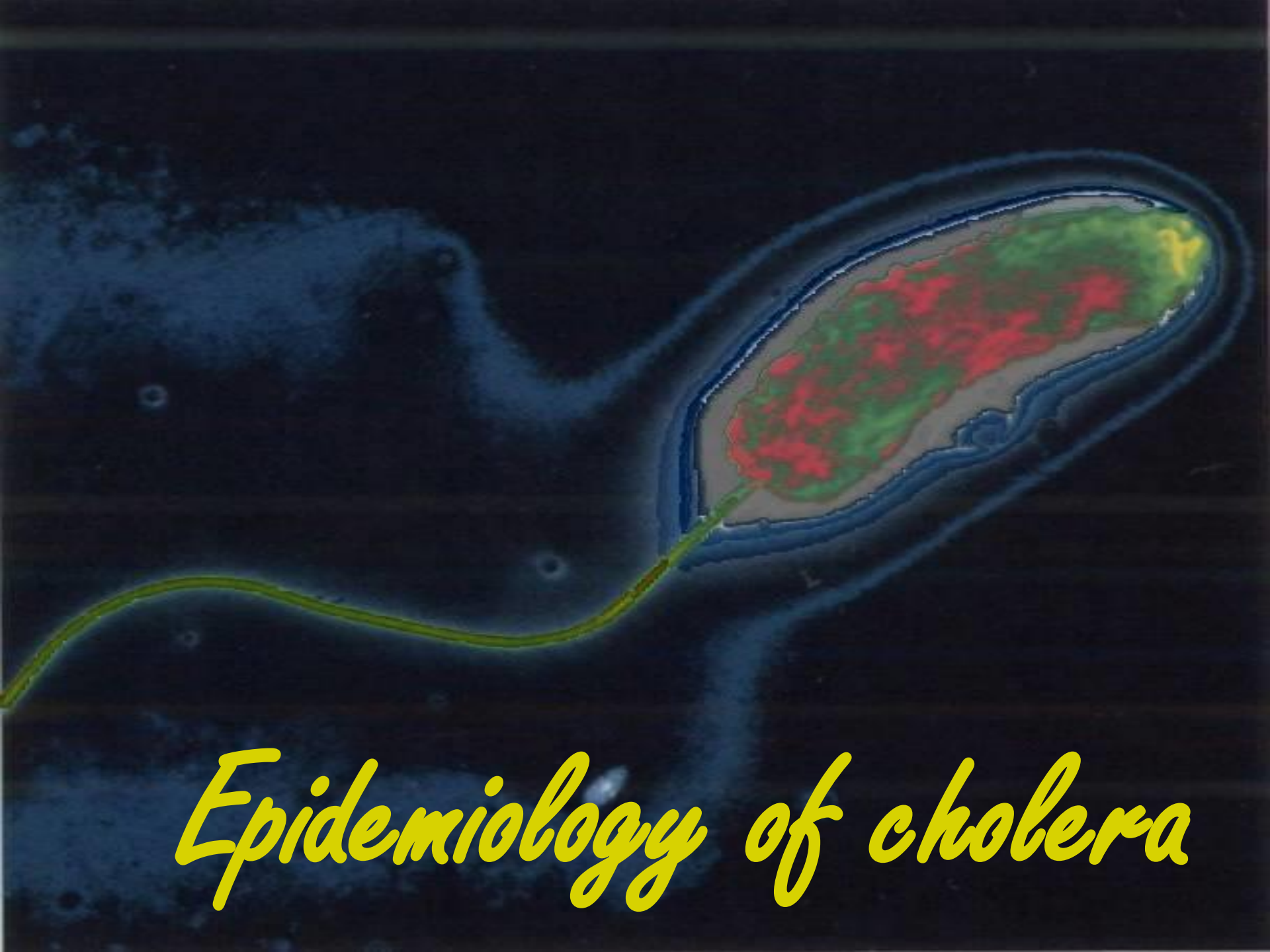
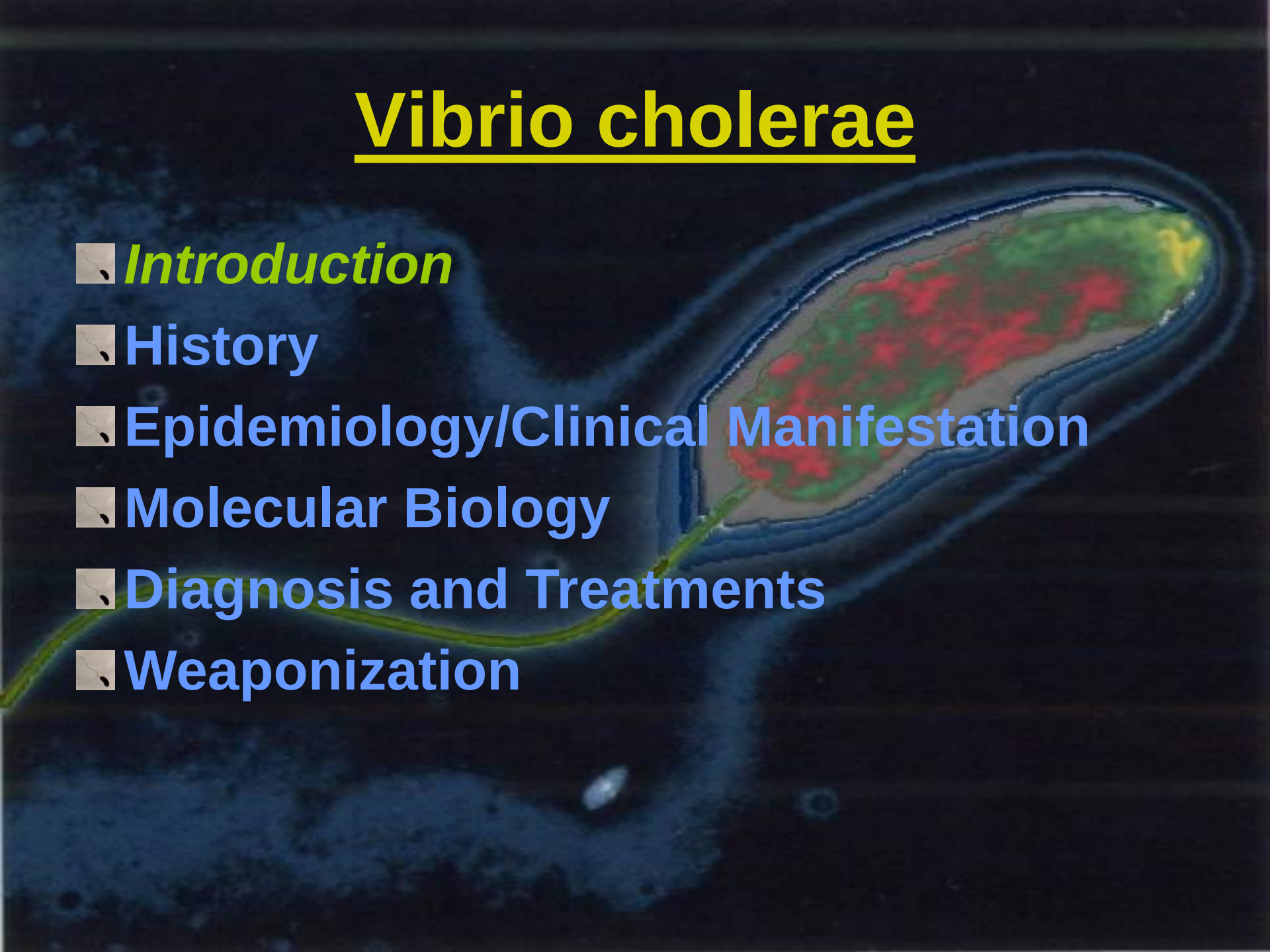
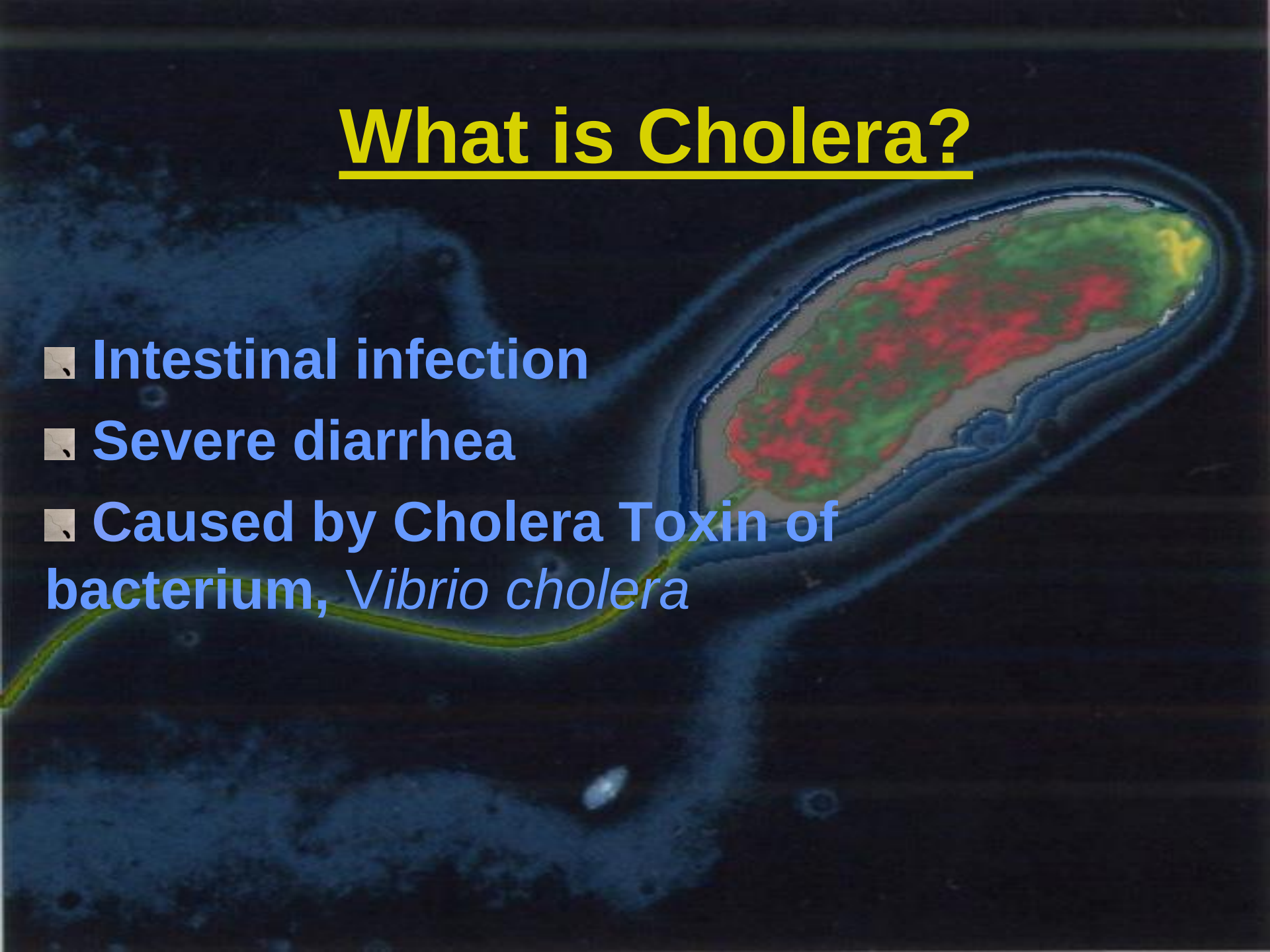

Epidemiology of cholera

Vibrio cholerae

- *Introduction*
- History
- Epidemiology/Clinical Manifestation
- Molecular Biology
- Diagnosis and Treatments
- Weaponization



What is Cholera?



- **Intestinal infection**
- **Severe diarrhea**
- **Caused by Cholera Toxin of bacterium,** *Vibrio cholera*

V. cholerae

- Grows in salt and fresh water
- Can survive and multiply in brackish water by infecting copepods
- Has over 150 identified serotypes based on O-antigen
- Only O1 and O139 are toxigenic and cause Cholera disease
- 2 categories of O1 serotypes – Classical and El Tor

Cholera

- A life-threatening secretory diarrhea induced by enterotoxin secreted by V. cholerae
- Water-borne illness caused by ingesting water/food contaminated by copepods infected by V. cholerae
- An enterotoxic enteropathy (a non-invasive diarrheal disease)
- A major epidemic disease

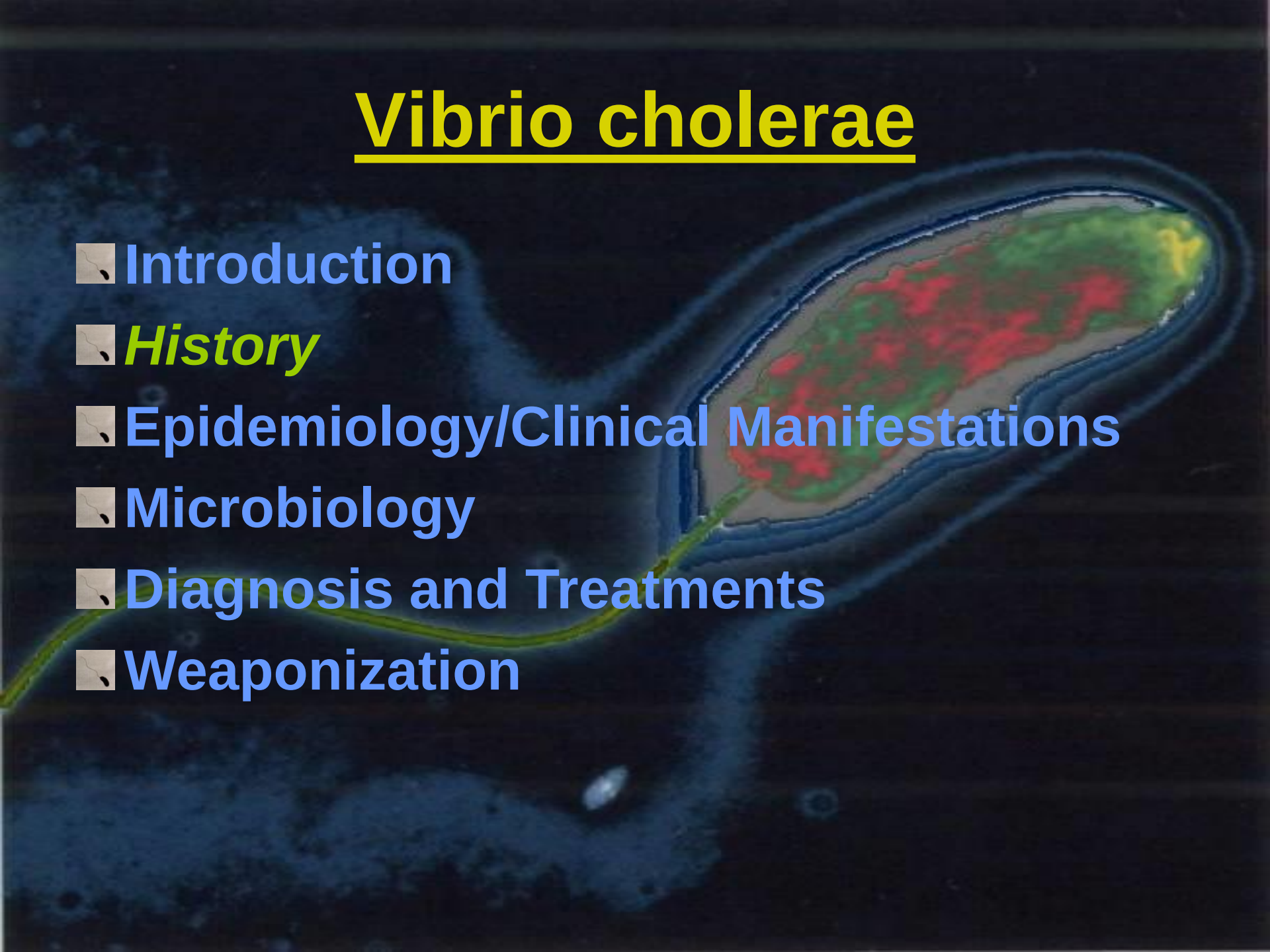
V. cholerae

- Transmitted by fecal-oral route
- Endemic in areas of poor sanitation (India and Bangladesh)
- May persist in shellfish or plankton
- 7 pandemics since 1817 – first 6 from Classical strains, 7th from El Tor
- 1993: Cholera in Bengal caused by O139 – may be cause of 8th pandemic

4. When does cholera become epidemic?

- After heavy period of rainfall
- When water temperatures rise
- When normal diarrhoeal incidence increases
- Endemic cholera with good sanitation needs permanent source of vibrio, but with poor sanitation higher secondary transmission can maintain endemic status

Vibrio cholerae



- Introduction
- *History*
- Epidemiology/Clinical Manifestations
- Microbiology
- Diagnosis and Treatments
- Weaponization

Ancient Texts Describe Cholera

- 500-400 BC: Sanskrit writings
- 500 BC: Hippocrates
- 200 AD: Galen
- 900 AD: Rhazes, Islamic physician
- Sanskrit, Arabic, and Chinese writings dating back 2,000 years

1st Pandemic: 1817-1823

- Started in by Ganges in Calcutta - Kumbh festival
- Polluted water, crowded camps
- 10,000 in British army and hundreds of thousands of natives dead
- Spread by trade routes – Iran, Baku, Astrakhan, Russia
- Cold winter kept it from reaching western Europe

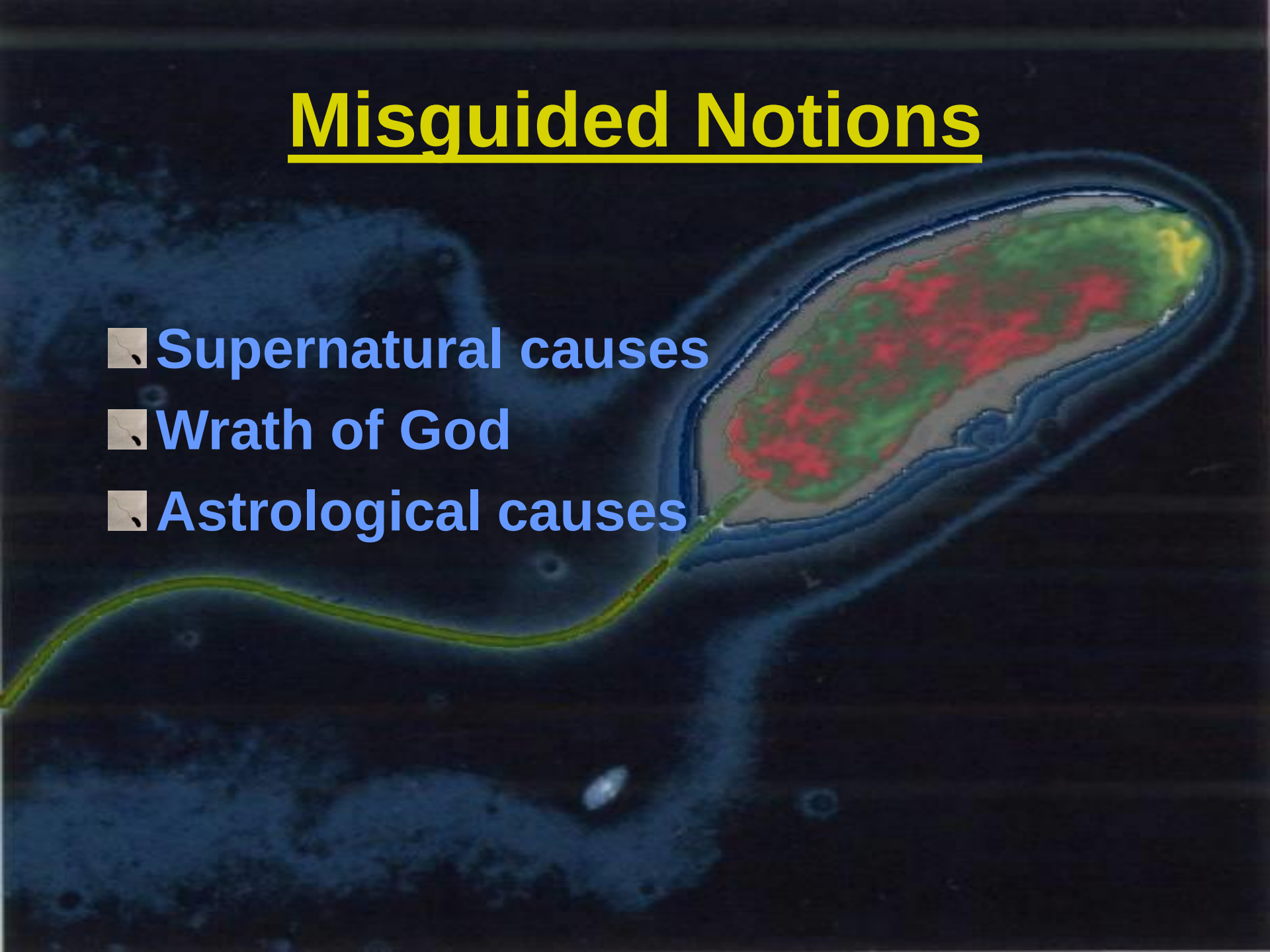
Quarantine Act of 1825

- England's attempt to control spread of infectious disease
- Tried to prevent international movement
- Eventually repealed (based on flawed scientific understanding)

2nd Pandemic: 1829-1852

- Bengal, Afghanistan, Asia, Moscow, England, US
- William Brooke O'Shaughenessy
- Industrial Revolution
- England's Cholera Prevention Act of 1832
- Entered US through NY and New Orleans ports – spread by railway and troop movement after Civil War

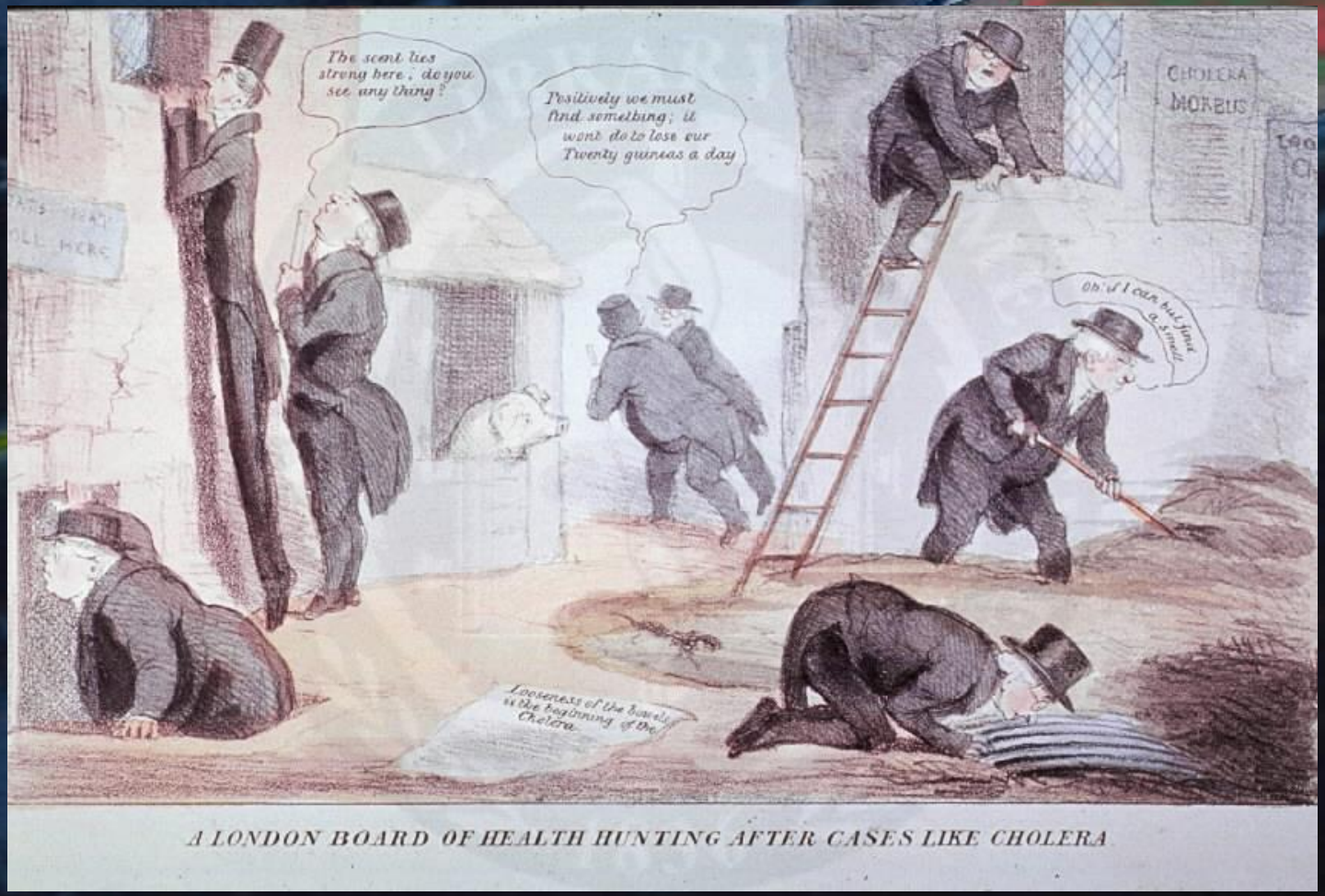
Misguided Notions



- Supernatural causes
- Wrath of God
- Astrological causes

Misguided Notions

- Caused by miasma

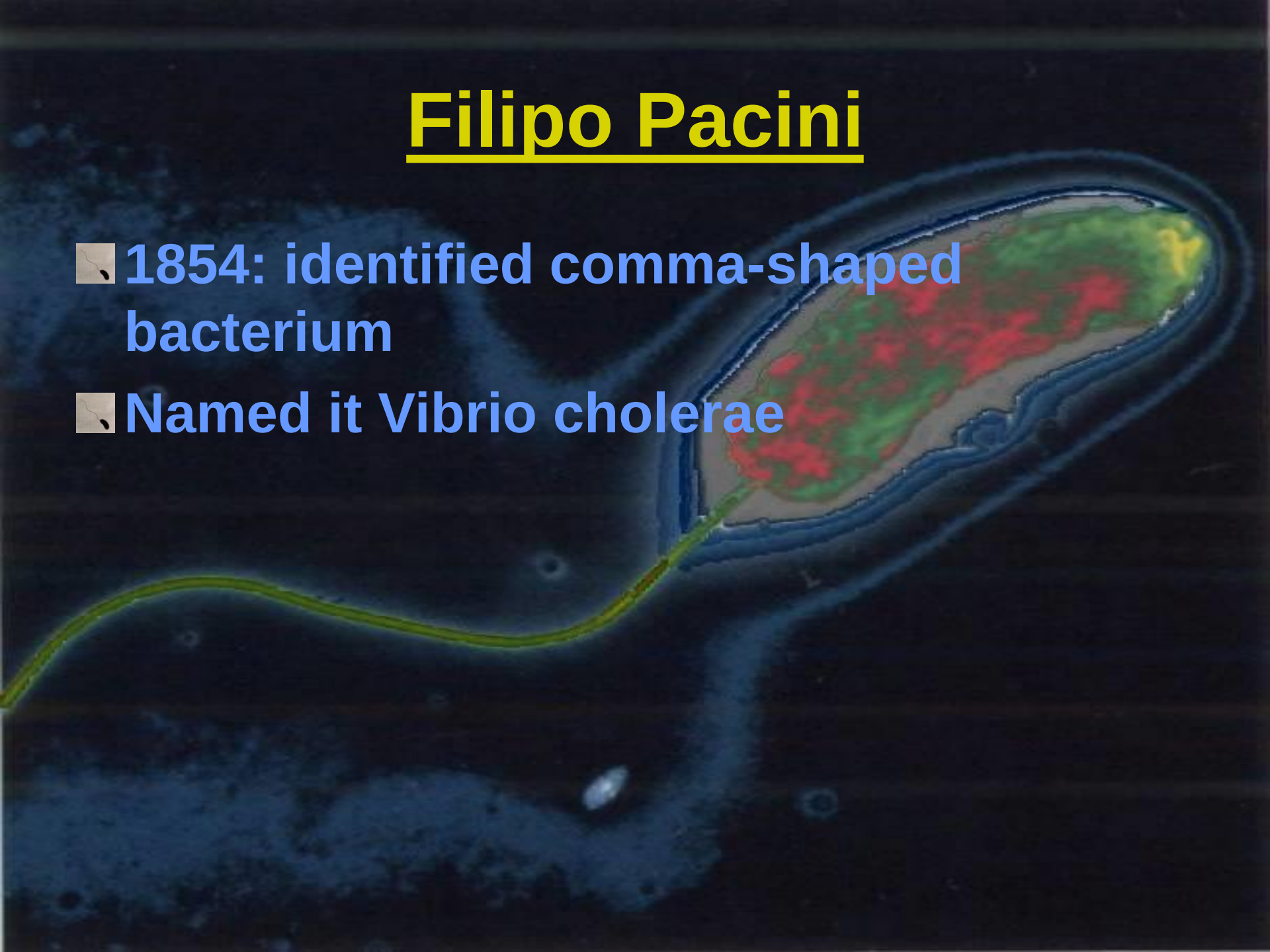


Misguided Notions

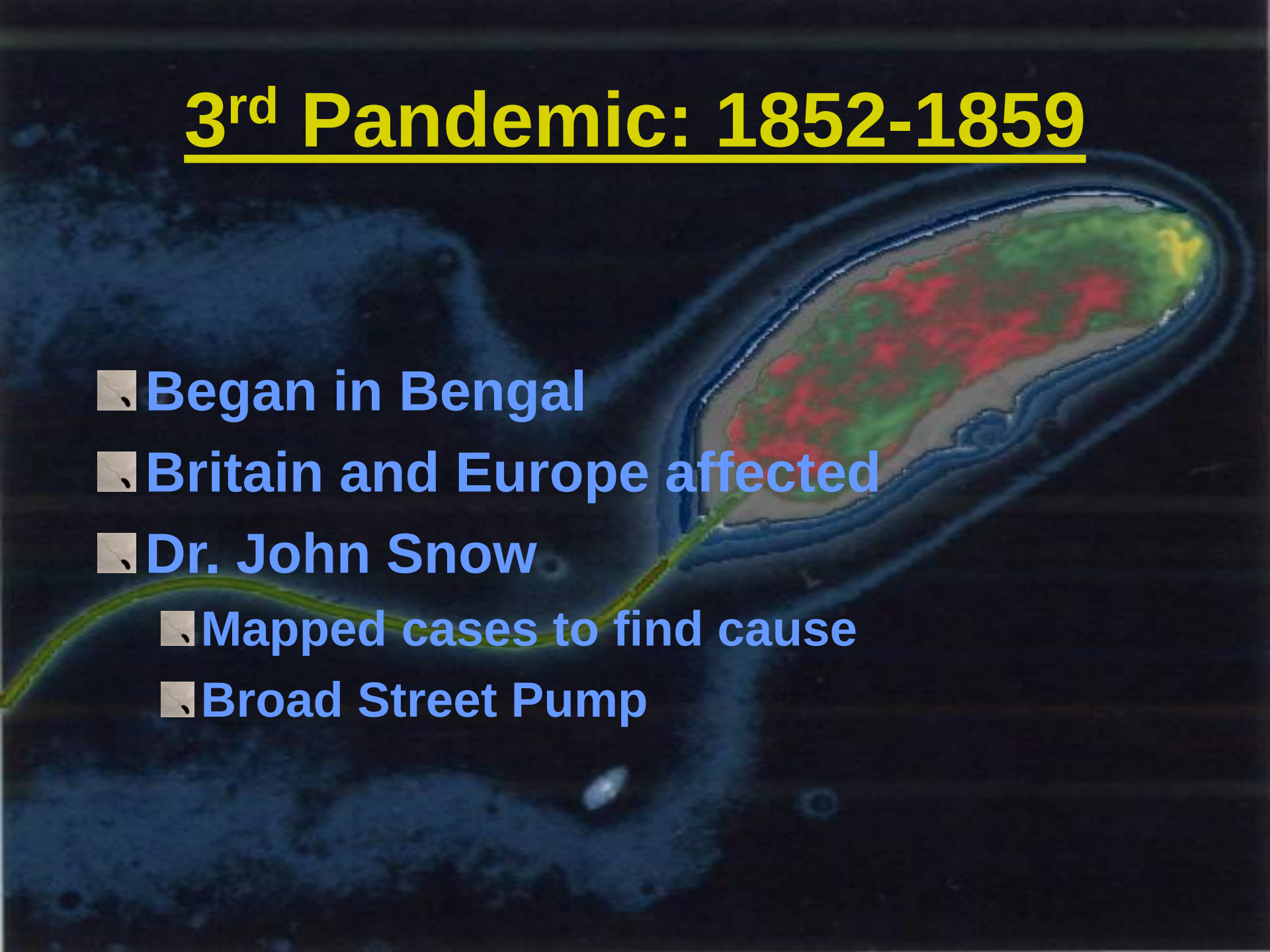
- Prevented by alcohol
- Could be spread by contact with patient or patient's clothes ????

Filipo Pacini

- 1854: identified comma-shaped bacterium
- Named it Vibrio cholerae



3rd Pandemic: 1852-1859



- Began in Bengal
- Britain and Europe affected
- Dr. John Snow
  - Mapped cases to find cause
  - Broad Street Pump

John Snow – Record of Locations of Cholera Cases in London, 1854

Pumps

Deaths from Cholera

Broad Street Pump

- Map led Snow to believe that Broad Street pump was cause of outbreak
- Those affected drank from pump
- Sewage probably contaminated well
- Removal of pump handle - end of outbreak
- Skepticism about Snow's findings

The “Grand Experiment”

- Compared deaths from Cholera between 2 groups
- Group A: Southwark and Vauxhall Water Co. – 70 deaths per 10, 000 (London source of Thames)
- Group B: Lambeth Water Co. – 5 deaths per 10,000 (source upstream from London

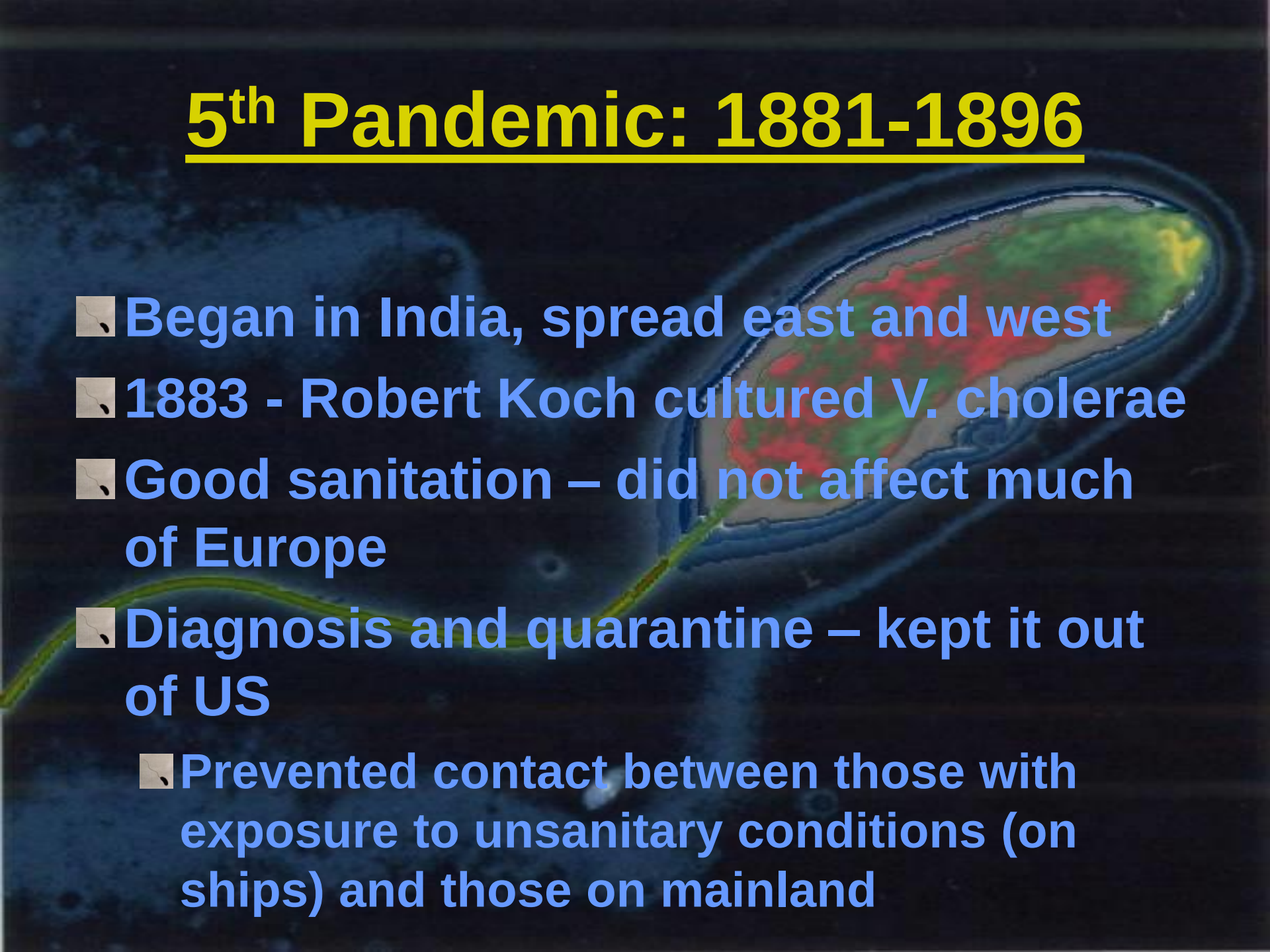
Results

- Massive public health reforms
- Much smaller outbreak in 1866

4th Pandemic: 1863-1879

- From Egypt to Europe by returning pilgrims from the Haj at Mecca
- Imported into NY by ship
- Last time cholera in England
- Third and Fourth International Sanitary Conferences (Paris and Vienna)
  - International Health Regulations
  - International Sanitary Commission – precursor of PAHO (Pan American Health Organization)

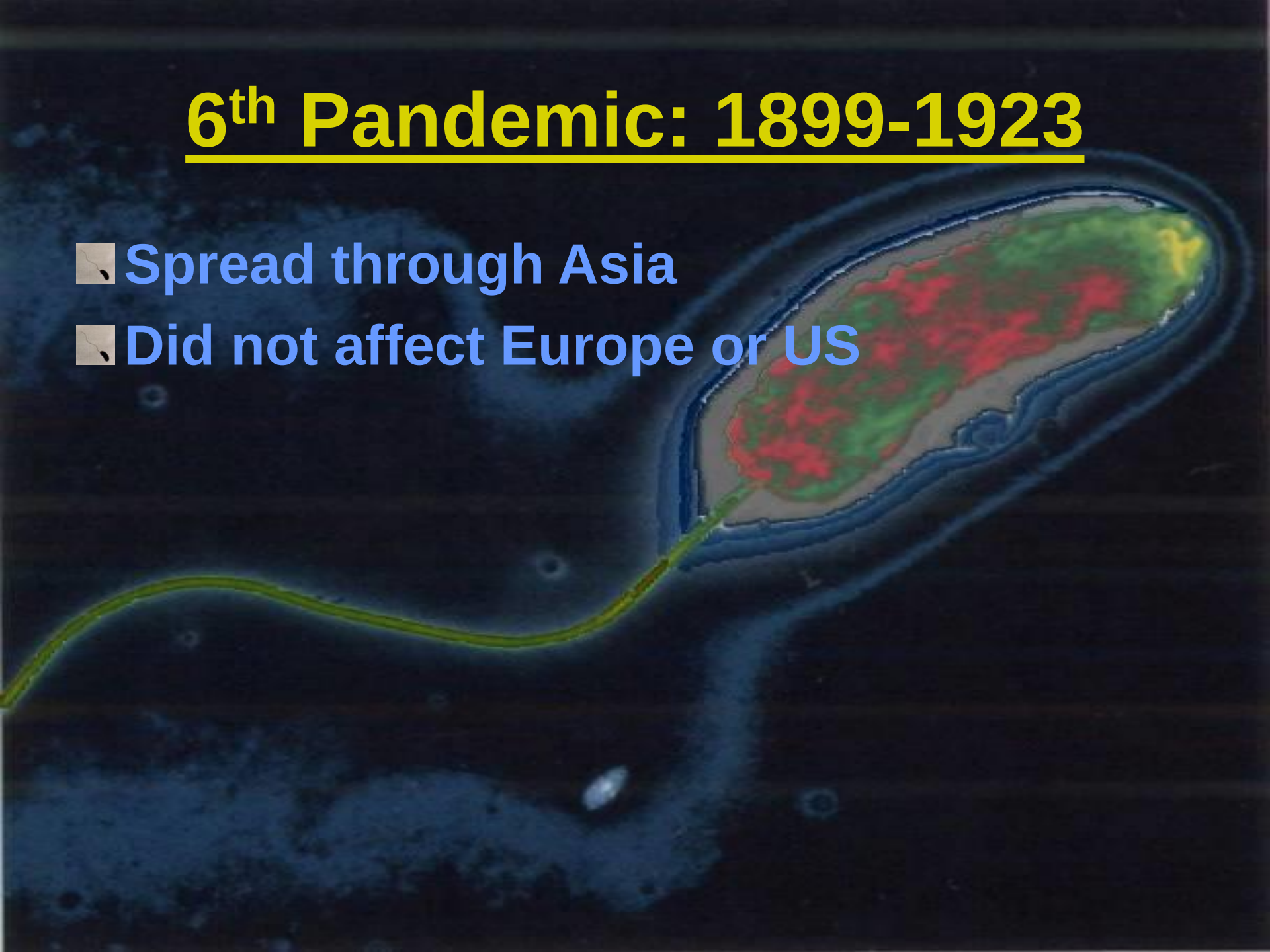
5th Pandemic: 1881-1896



- Began in India, spread east and west
- 1883 - Robert Koch cultured V. cholerae
- Good sanitation – did not affect much of Europe
- Diagnosis and quarantine – kept it out of US
  - Prevented contact between those with exposure to unsanitary conditions (on ships) and those on mainland

6th Pandemic: 1899-1923

- Spread through Asia
- Did not affect Europe or US



Discoveries

- 1959: cholera enterotoxin by S.N. De in Calcutta
  - Cholera bacillus is not harmful – toxin is what induces outpouring of fluid and inhibits sodium transport
- Treatment by rehydration (oral or intravenously) of fluid and electrolytes
- How to measure rapid fluid loss

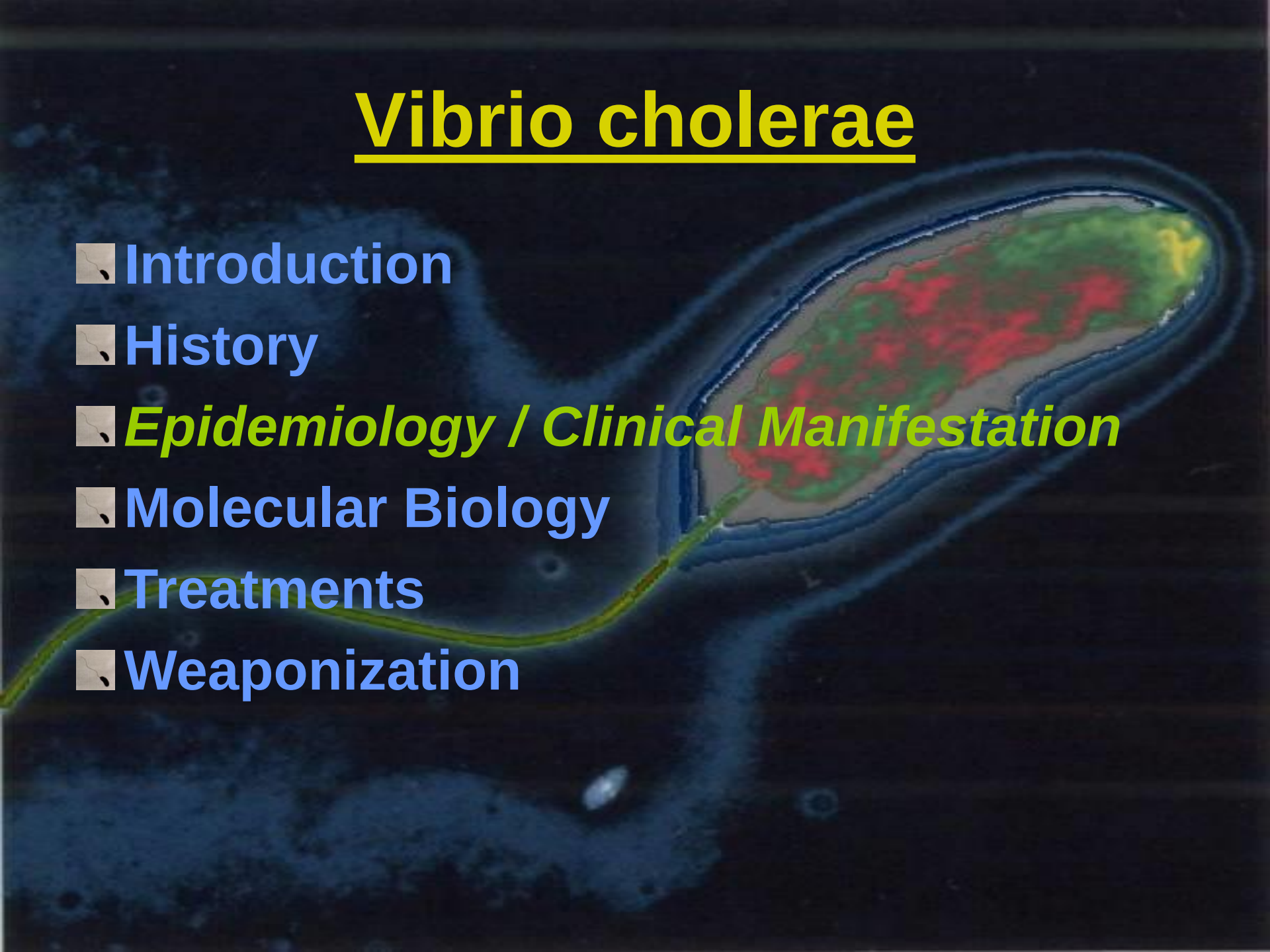
7th Pandemic: 1961-present

- Caused by El Tor strain
- From Pacific Islands to Asia, Bangladesh, India, USSR, Iran, Iraq
- 1970: reemerged in Africa after 100 years
- 1991: Latin America (4,000 dead of 400,000 cases)
- 1993: O139 serogroup (“Bengal”) – may be start of 8th pandemic

Genome

- Aug 2000: published complete DNA sequence of V. cholerae, El Tor strain
- Unusual - 2 distinct chromosomes
- Hope that genome will be useful in creating an effective vaccine

Vibrio cholerae



- Introduction
- History
- *Epidemiology / Clinical Manifestation*
- Molecular Biology
- Treatments
- Weaponization

What's In a Name?

"The appellation cholera probably derives from the Greek word for the gutter of a roof, comparing the deluge of water following a rainstorm to that from the anus of an infected person."

- Dr. Jean-Pierre Raufman

American Journal of Medicine

Profile of *vibrio cholerae*

- Gram-negative
- Highly motile; polar flagellum
- Brackish rivers, coastal waters
  - Associate with plankton and algae
- Proliferate in summers
- Cholera toxin
- Pathogenic and nonpathogenic strains
  - 206 serogroups

Strains Causing Epidemics

- 2 main serogroups carry set of virulence genes necessary for pathogenesis
- O1
  - Classical: 1 case per 30-100 infections
  - El Tor: 1 case per 2-4 infections
- O139
  - Contained in India, Bangladesh

Epidemiology

- Responsible for seven global pandemics over the past two centuries
- Common in India, Sub-Saharan Africa, Southern Asia
- Very rare in industrialized countries

Cholera Statistics, 2000*

Continent	Total Cases	Total Deaths
Africa	118,932	4,610
America(s)	3,101	40
Asia**	11,246	232
Europe	35	0
Oceania	3,757	26
Total	**137,071**	**4,908**

*Data published in August, 2001

**Does not include Bangladesh, Pakistan and other cou

Source: World Health Organ

Cholera, 2010

Since 2000, the incidence of cholera has increased steadily, culminating in 317 534 reported cases worldwide, including 7543 deaths, in 2010, with a case-fatality rate (CFR) of 2.38%

Overall, in 2010 the cumulative number of cases represented an increase of 43% compared to the number in 2009, and an increase of 130% compared to that in 2000

This increase is the result of a large outbreak that started in Haiti in October 2010.

For the first time since 1995, the worldwide proportion of cases reported to WHO from the African continent shifted from >90% to <50% in a given year

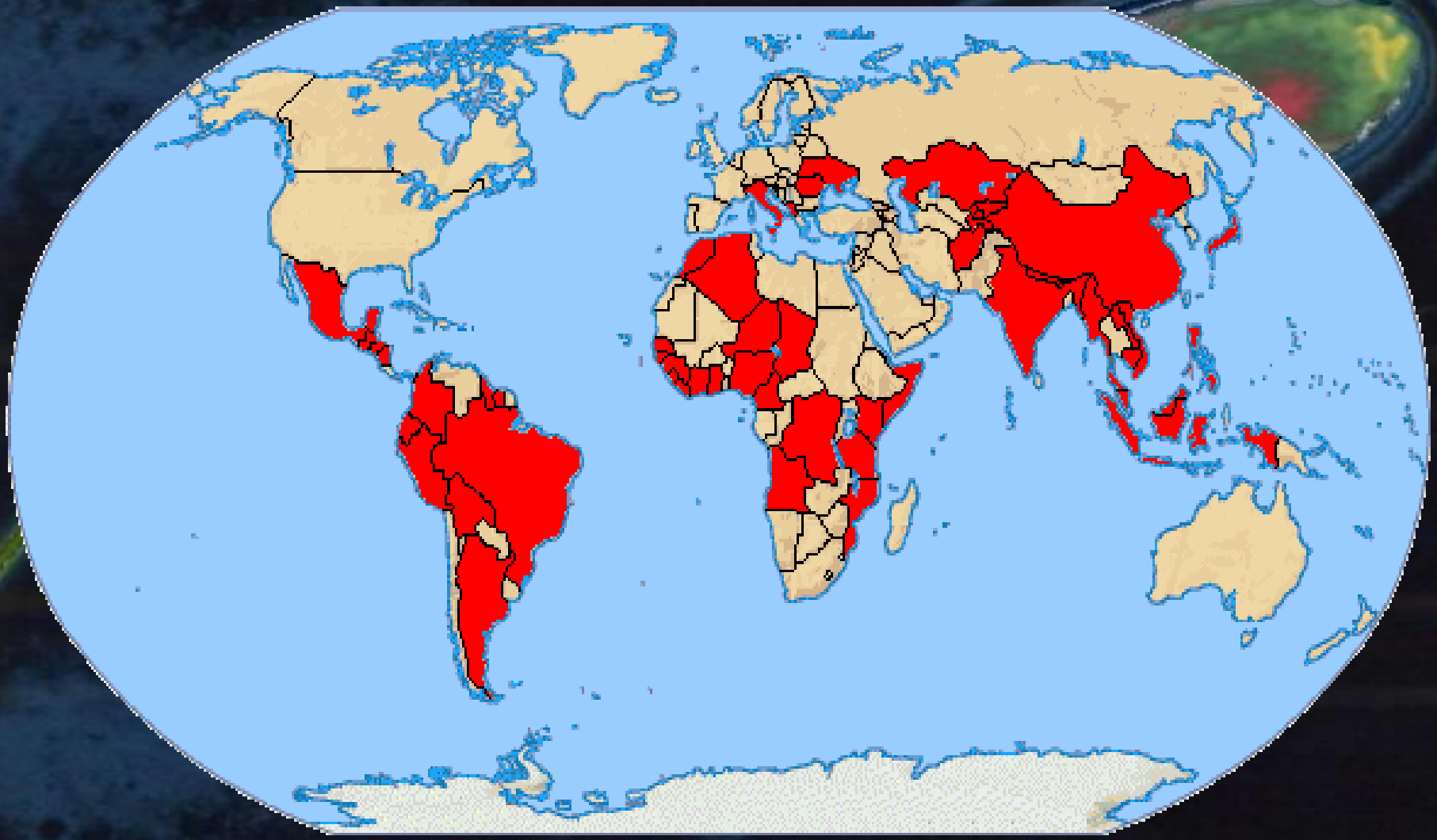
In May 2011, the World Health Assembly recognized the re-emergence of cholera as a significant global public health problem and adopted resolution WHA 64.15, calling for implementation of an integrated and comprehensive global approach to cholera control.

Globally, the number of deaths from cholera rose from 4948 in 2009 to 7543 in 2010, an increase of 52% with an overall CFR of 2.38%.

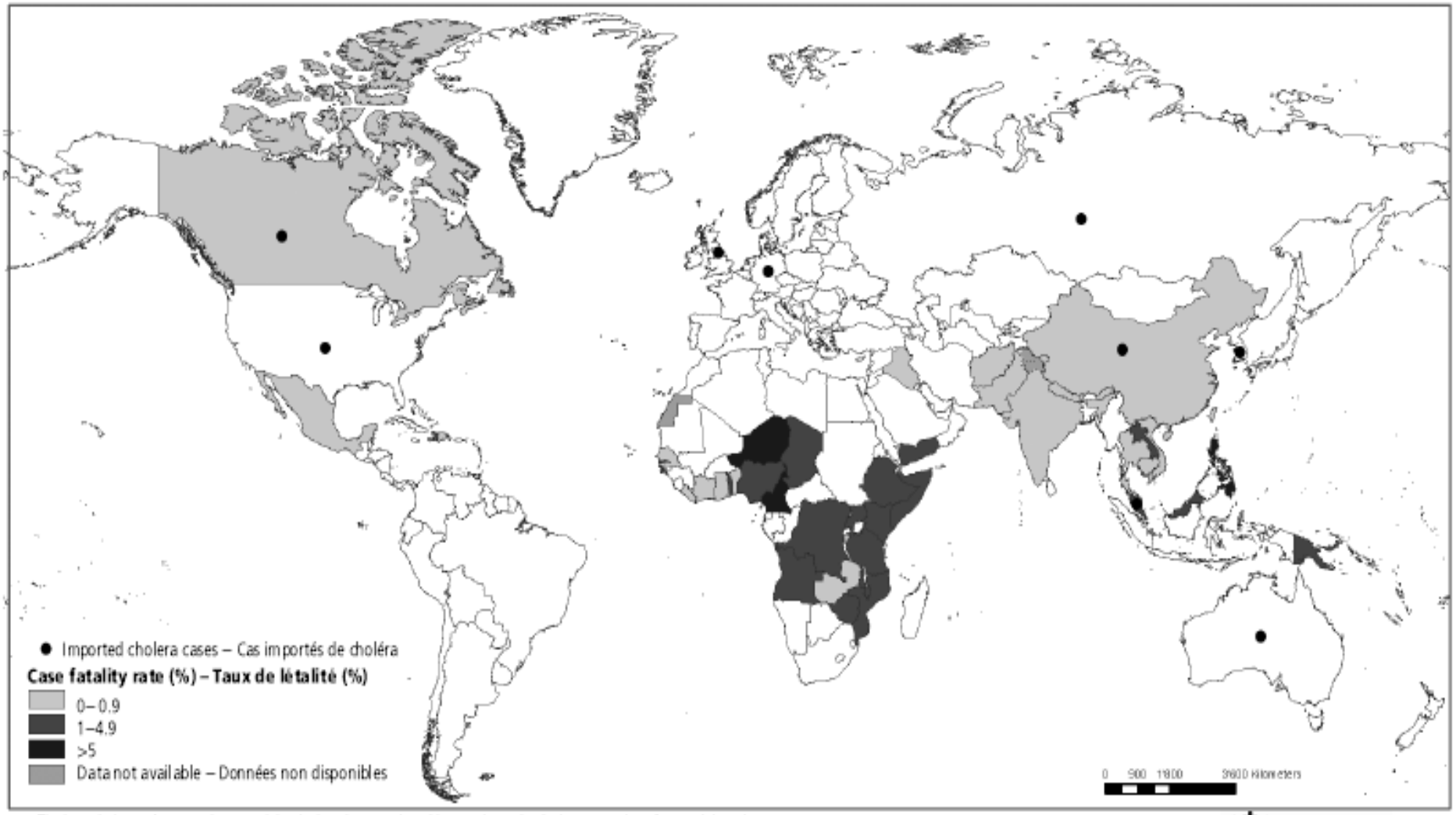
Of the 32 countries that reported deaths from cholera, 20 were on the African continent: these countries accounted for 3397 deaths and 45% of the global total.

In the Americas, Haiti reported 3990 deaths, accounting for 53% of the global total; these deaths occurred over a period of 70 days

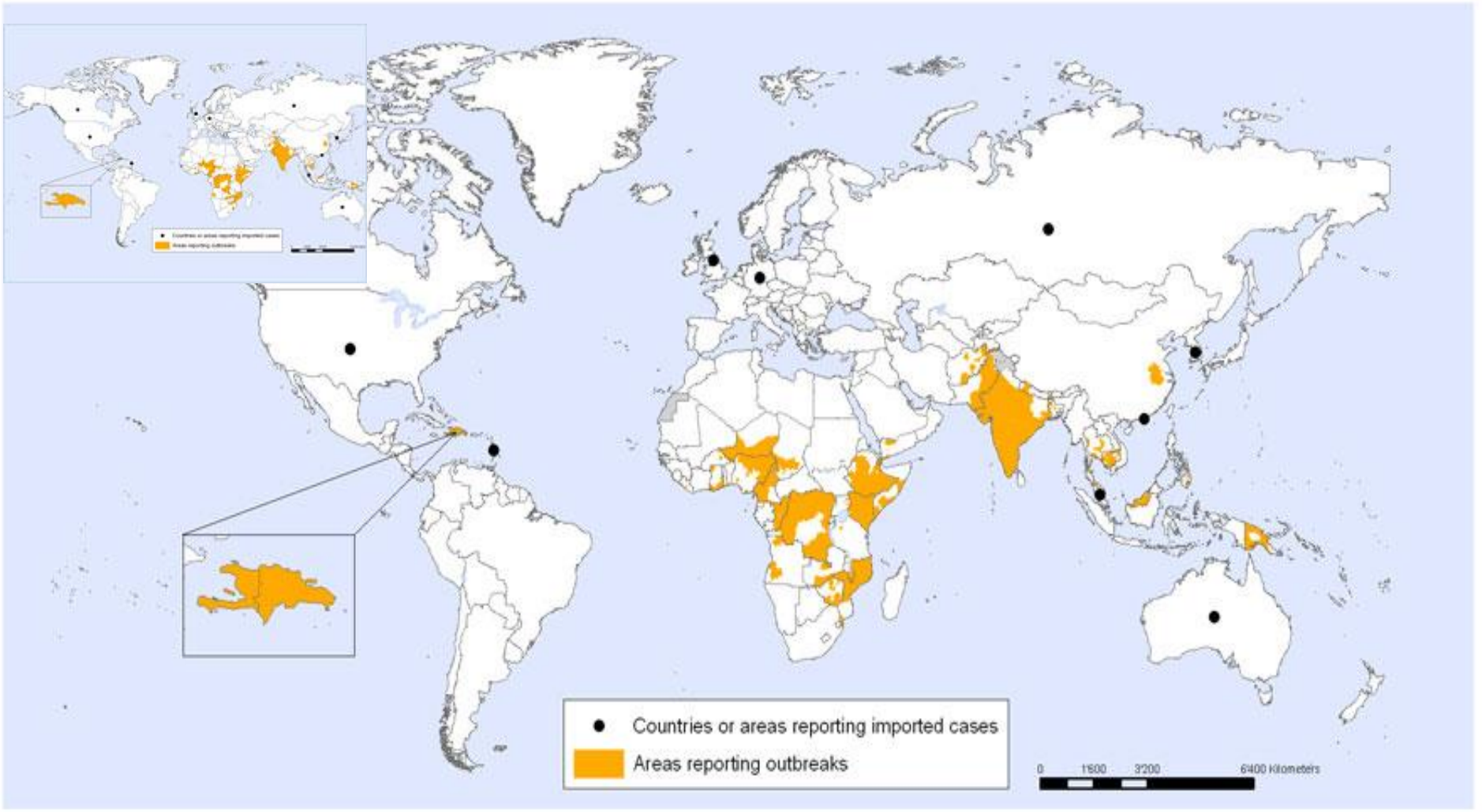
V. Cholerae Afflicted Areas (2000)



Map 1 **Countries reporting cholera in 2010**
Carte 1 **Pays ayant déclaré des cas de choléra en 2010**



Cholera, areas reporting outbreaks, 2010–2011



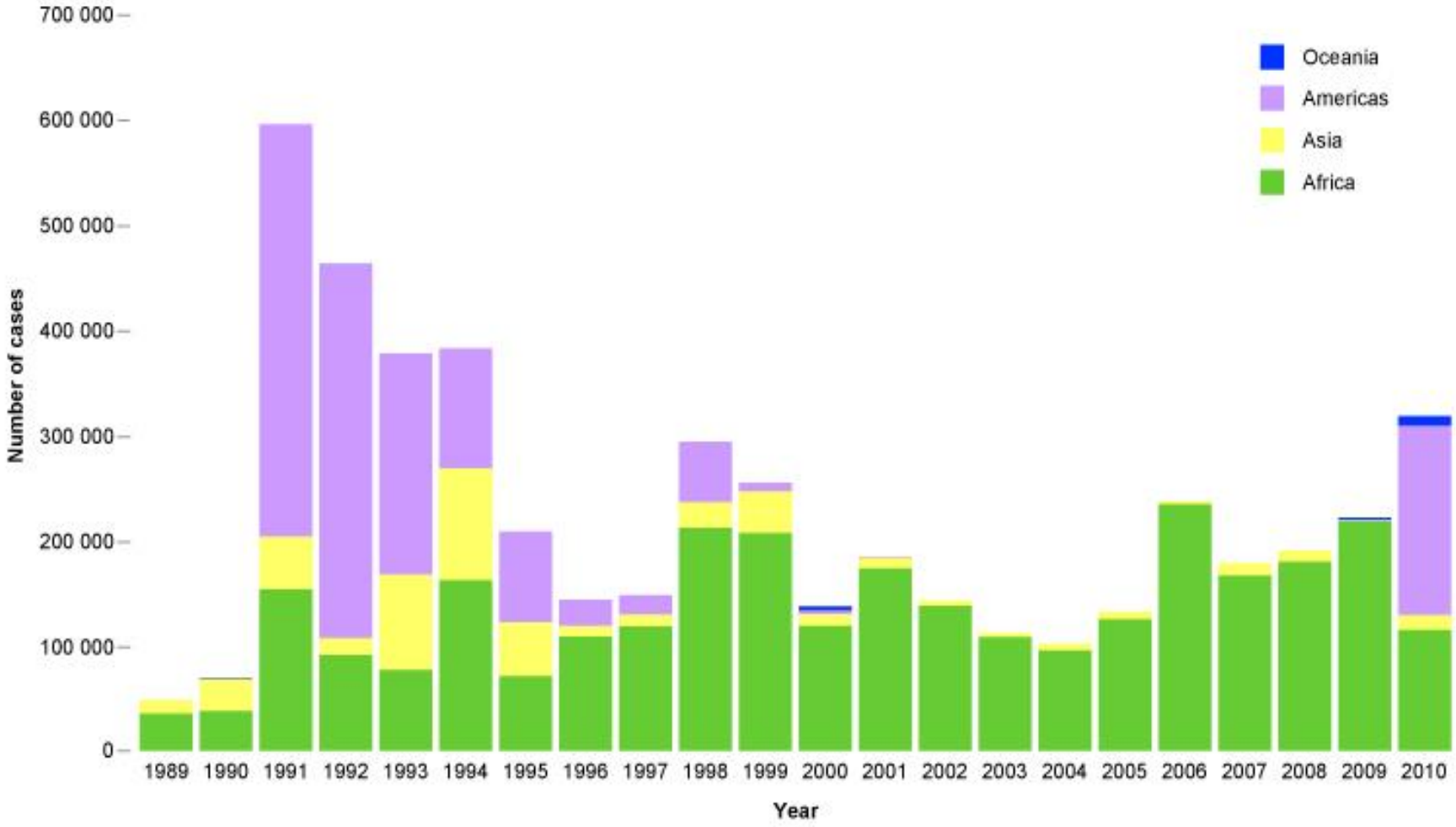
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

World Health Organization

Cholera cases reported to WHO by year and by continent 1989–2010



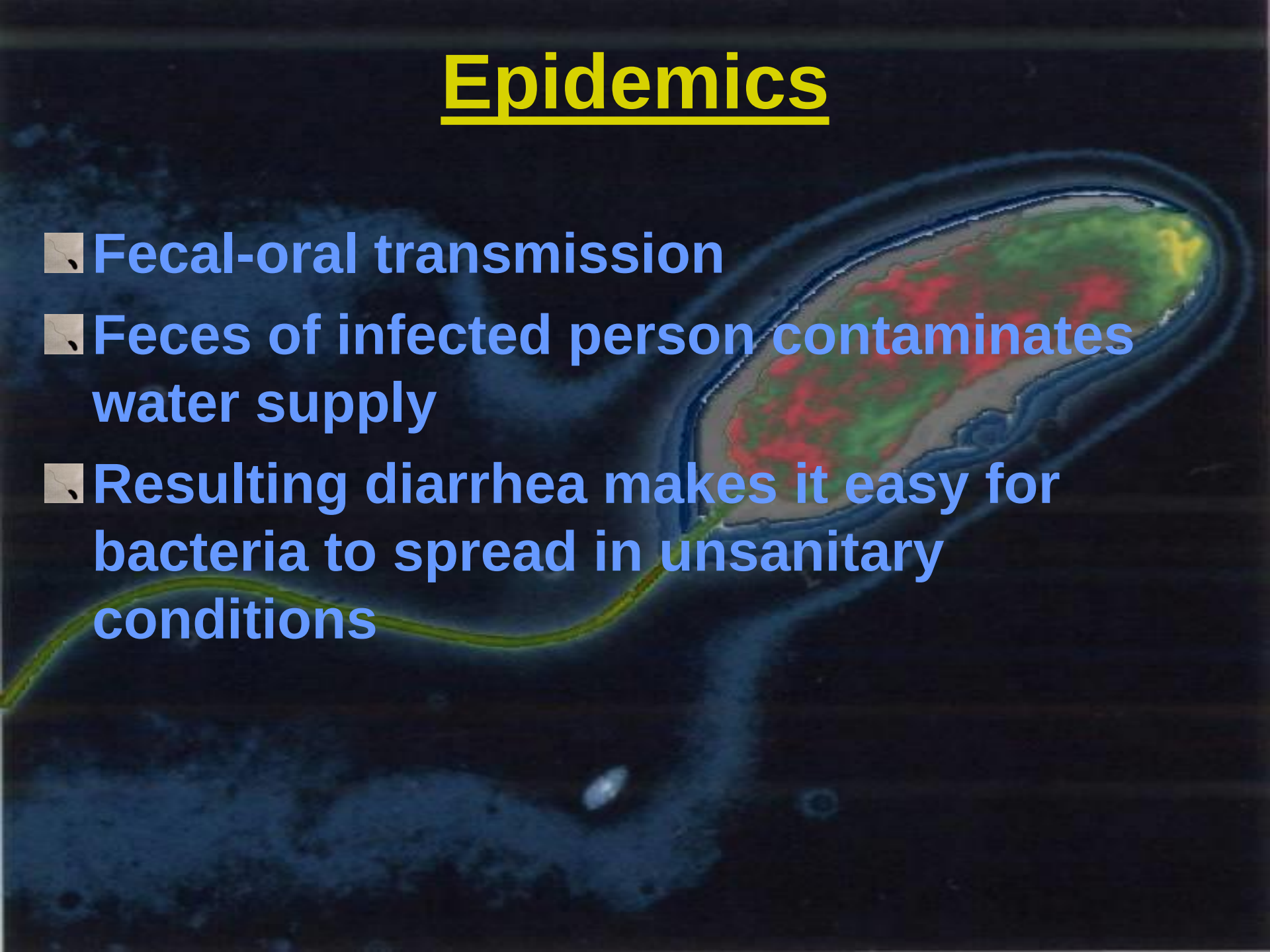
Source: WHO Weekly Epidemiological Record no. 31, 2011, 86, 325–340

Transmission

- Contaminated food or water
  - Inadequate sewage treatment
  - Lack of water treatment
  - Improperly cooked shellfish
- Transmission by casual contact unlikely

Epidemics

- Fecal-oral transmission
- Feces of infected person contaminates water supply
- Resulting diarrhea makes it easy for bacteria to spread in unsanitary conditions



- Hanging latrine on Meghna River, Nepal

People Most at Risk

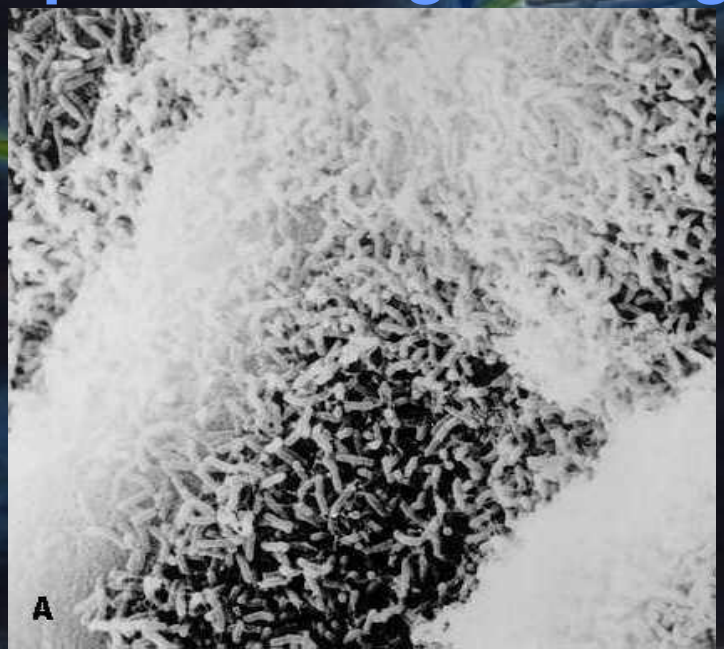
- People with low gastric acid levels
  - Children: 10x more susceptible than adults
  - Elderly
- Blood types
  - O>> B > A > AB

Period of Communicability

- During acute stage
- A few days after recovery
- By end of week, 70% of patients non-infectious
- By end of third week, 98% non-infectious

Incubation

- Ranges from a few hours to 5 days
- Average is 1-3 days
- Shorter incubation period:
  - High gastric pH (from use of antacids)
  - Consumption of high dosage of cholera



How Does Cholera Toxin Work?

- Inactivates GTPase function of G-protein coupled receptors in intestinal cells
- G proteins stuck in “On” position
- 100 fold increase in cAMP
- Activation of ion channels
- Ions flow out and water follows

Infectious Dose

- 10^6-10^{11} colony-forming units
- Why such a high dosage?
  - Series of changes as moves from aquatic environment to intestine
    - Temperature, acidity
  - Acidic environment of stomach
  - Intestinal environment
    - Bile salts, organic acids, complement inhibit bacteria growth
    - Must penetrate mucous lining of intestinal epithelial cells

Symptoms

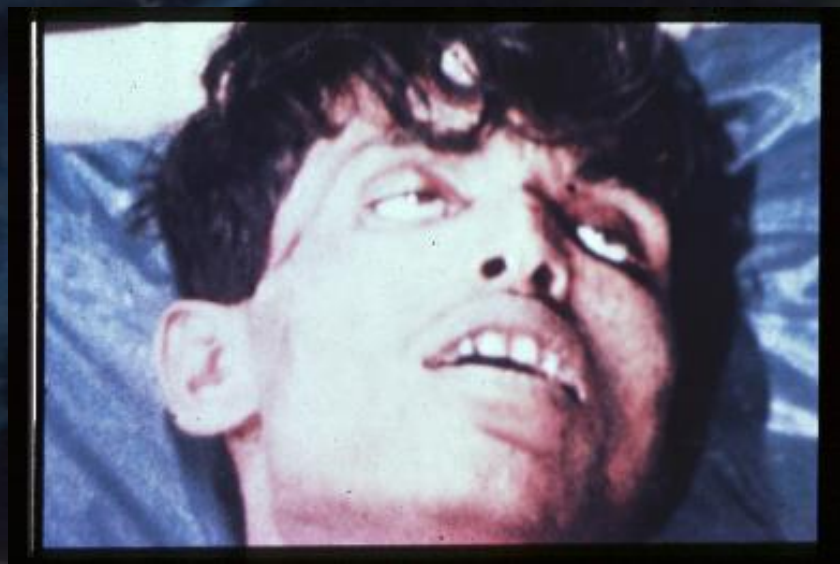
- Occur 2-3 days after consumption of contaminated food/water
- Usually mild, or no symptoms at all
  - 75% asymptomatic
  - 20% mild disease
  - 2-5% severe
- Vomiting
- Cramps
- Watery diarrhea (1L/hour)
- Without treatment, death in 18 hours-several days

Cholera Gravis

- More severe symptoms
- Rapid loss of body fluids
  - 6 liters/hour
  - 10^7 vibrios/mL
- Rapidly lose more than 10% of bodyweight
- Dehydration and shock
- Death within 12 hours or less
- Death can occur within 2-3 hours

Consequences of Severe Dehydration

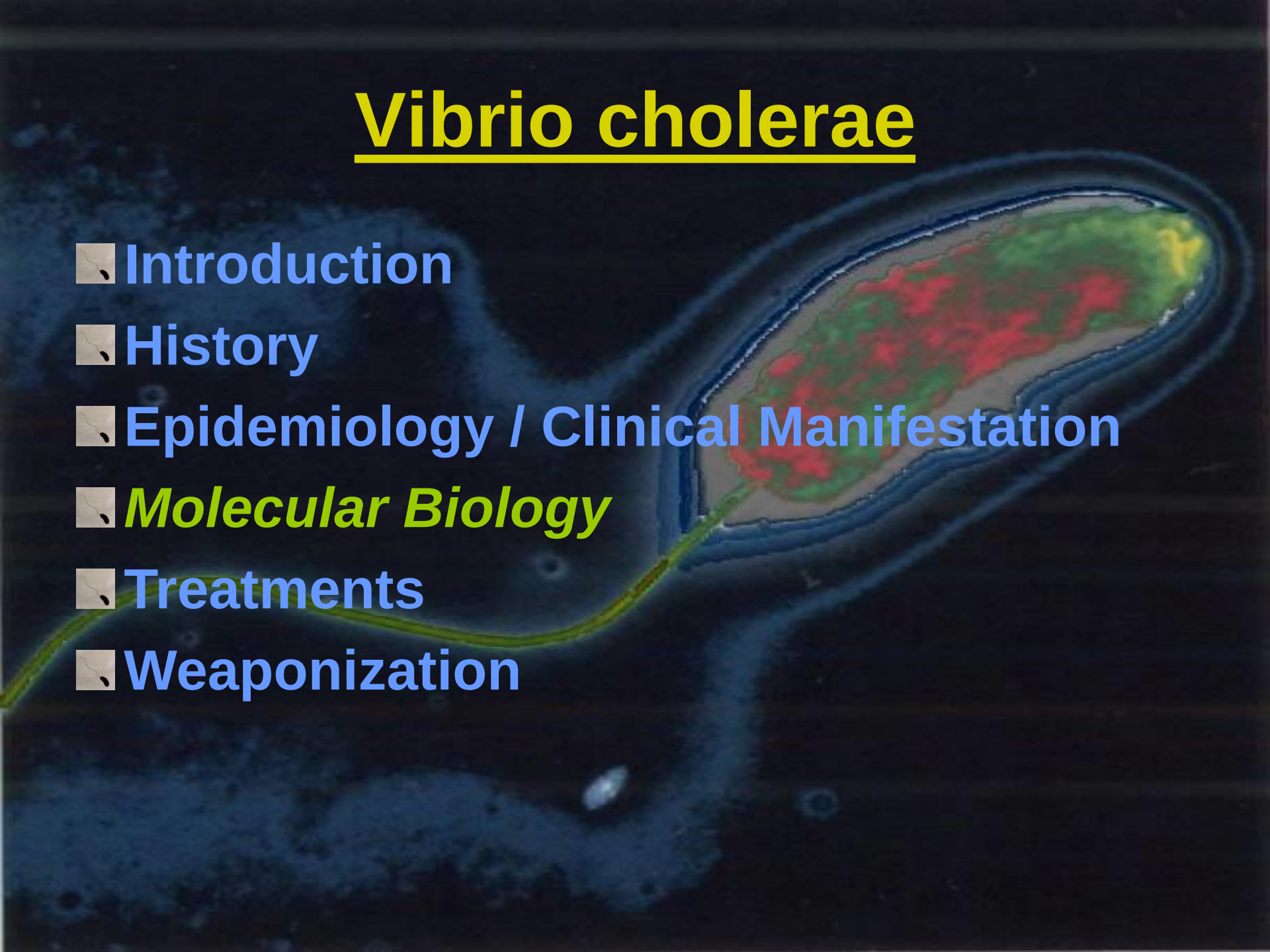
- Intravascular volume depletion
- Severe metabolic acidosis
- Hypokalemia
- Cardiac and renal failure
- Sunken eyes, decreased skin turgor
- Almost no urine production



Mortality Rate

- Causes 120,000 deaths/year worldwide
- With prompt rehydration: <1%
- Without treatment: 50%-60%

Vibrio cholerae



- Introduction
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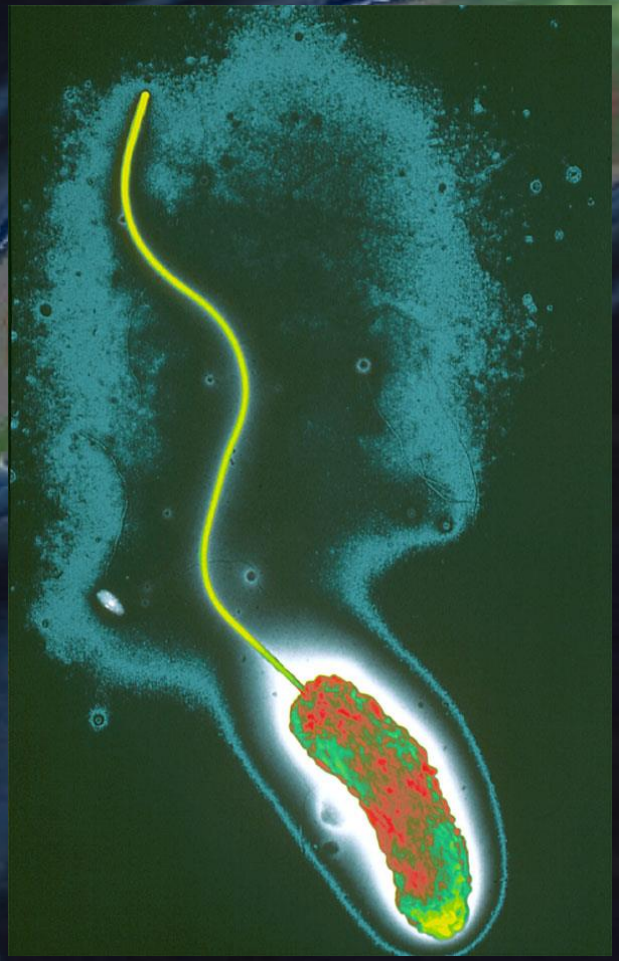
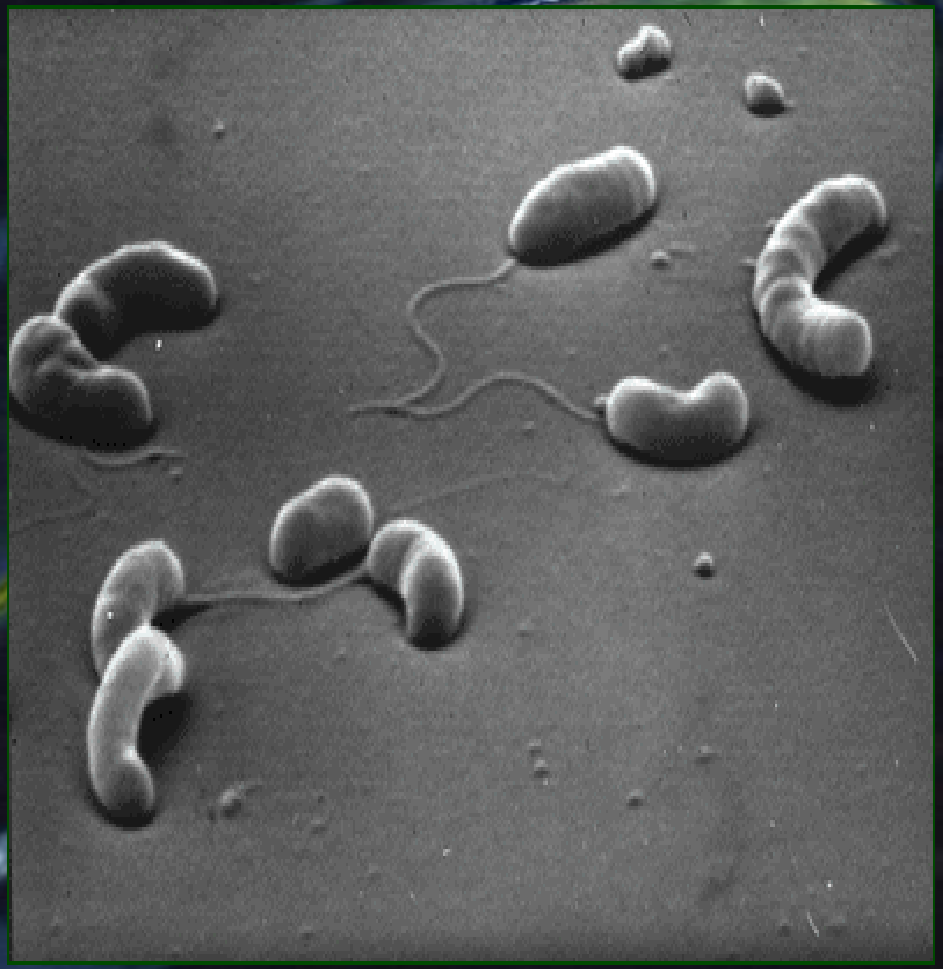
Molecular Biology of Vibrio cholerae

- **Identification & Classification (serogroups)**
- Genomic Structure
- Pathogenesis (mechanism of action)



Identification

- Vibrios are highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum, whose natural habitat is usually salt or fresh water.

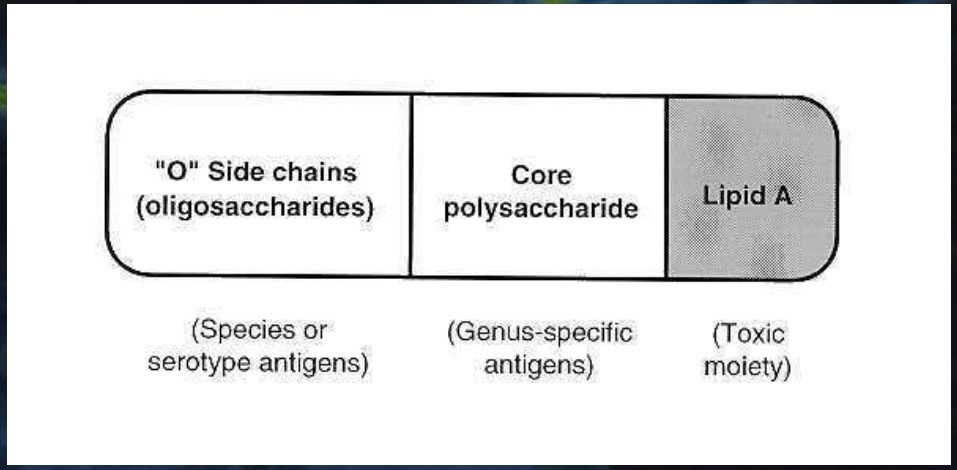


Identification

- Fresh isolates are prototrophic (i.e., they grow in media containing an inorganic nitrogen source, a utilizable carbohydrate, and appropriate minerals).
- In adequate media, they grow rapidly with a generation time of less than 30 minutes.
- Although they reach higher population densities when grown with vigorous aeration, they can also grow anaerobically.
- Vibrios are sensitive to low pH and die rapidly in solutions below pH 6; however, they are quite tolerant of alkaline conditions.

Classification: Serogroups and Biotypes

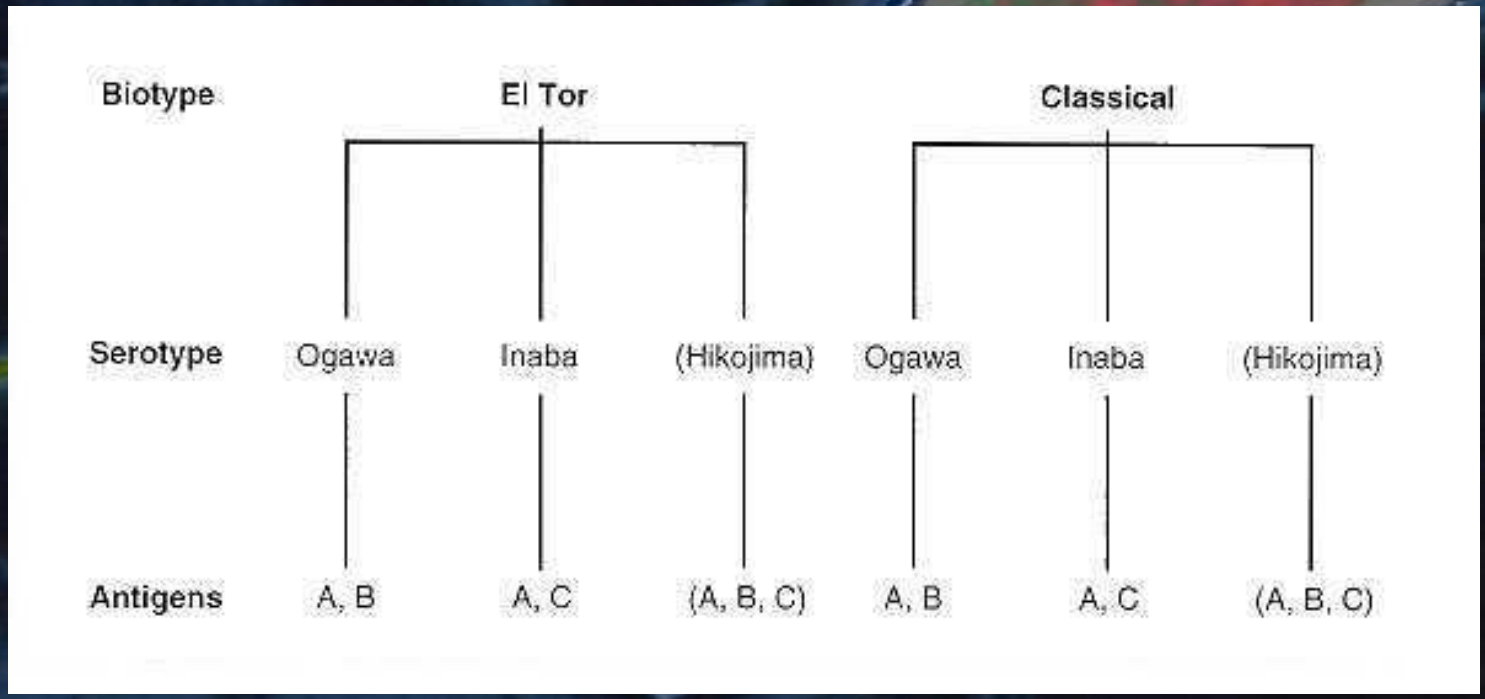
- The species *V. cholerae* can be sub-classified into 200 serogroups based on the O antigen of LPS (lipopolysaccharide).
  - Only O1 and O139 strains have been implicated in the cholera syndrome.



Classification: O1 Serogroup

- 2 Biotypes: El Tor and Classical
- V. cholerae O1 are further divided into 2 major subserotypes (Inaba and Ogawa).
- The basis for subtyping is 3 antigenic determinants of the O antigen structure of their LPS.
  - These serotypes are differentiated in agglutination and vibriocidal antibody tests on the basis of their dominant heat-stable lipopolysaccharide somatic antigens.
- The serotypes share one determinant known as the A antigen.
- In addition, Inaba strains express the C antigen whereas Ogawa strains express the B antigen .

Classification: O1 Antigen



Classification: O1 Serogroup

- O1 cholera almost always fall into the Heiberg I fermentation pattern; that is, they ferment sucrose and mannose but not arabinose, and they produce acid but not gas.
- Vibrio cholera also possesses lysine and ornithine decarboxylase, but not arginine dihydrolase.
- Freshly isolated agar-grown vibrios of the ***El Tor*** biotype, in contrast to ***classical*** V. cholerae, produce a cell-associated mannose-sensitive hemagglutinin which is found active in chicken erythrocytes.
- Strains of the ***El Tor*** biotype, however, produce less cholera toxin, but appear to colonize intestinal epithelium better than vibrios of the ***classical*** variety.
- Also, they seem some what more resistant to environmental factors. Thus, ***El Tor*** strains have a higher tendency to become endemic and exhibit a higher infection-to-case ratio than the ***classical*** biotype.

Classification: Other antigens

- O139 Serogroup
  - In 1993, the emergence of an entirely new serogroup (O139) was the cause an epidemic in Bangladesh.
  - O139 organisms produce a polysaccharide capsule but do not produce O1 LPS or O1 antigen.
  - Toxigenic O139 cholera arose through the acquisition of a large block of genes encoding the O139 antigen by O1 El Tor.
- Non-O1, Non-O139 Serogroup
  - Most are CT (cholera toxin) negative and are not associated with epidemic disease.

Viability of Cholera Vibrio outside the body

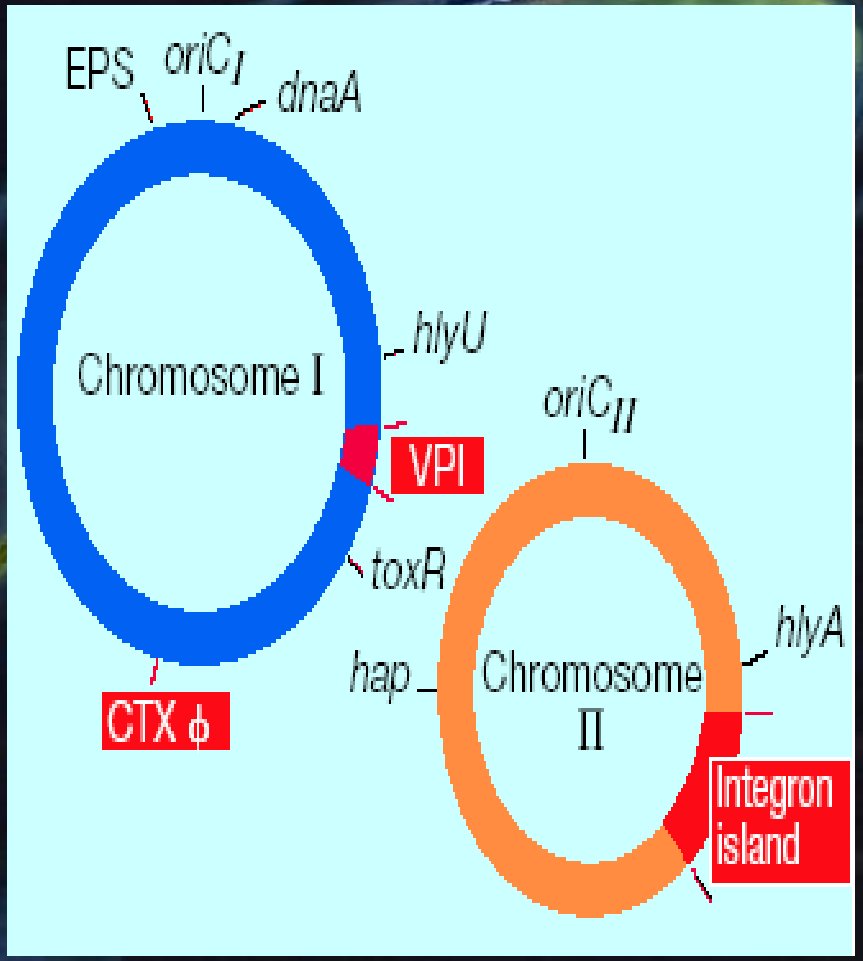
- In tap water (contam. with faeces)= 5 days
- In stool: (in summer) = 2 days
- In stool: (in winter) = 8 days
- In corpses = 4 wks
- In clothing = 2-6 days
- In dates (peelings) = 3 days
- In fish = 2-10 days
- In milk (raw) = 3 days
- In milk (boiled) = 10 days

Molecular Biology of Vibrio cholerae

- Identification & Classification (serogroups)
- **Genomic Structure**
- Pathogenesis (mechanism of action)

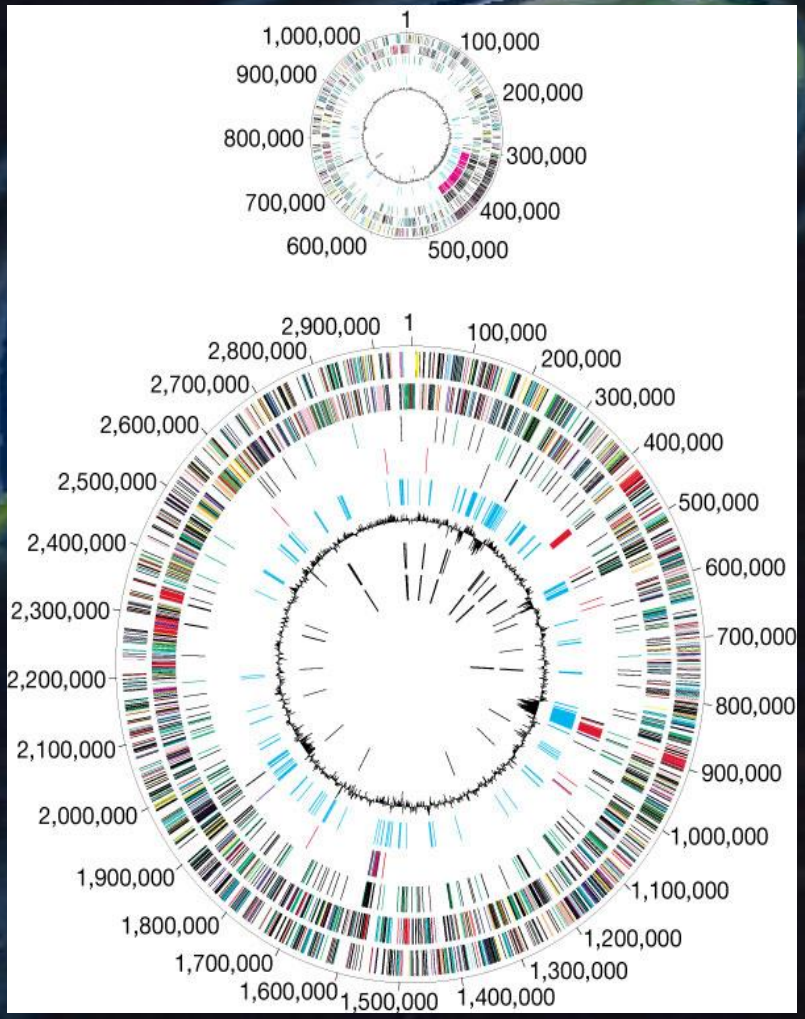
Genomic Structure

- The cholera genome contains 2 circular chromosomes.
  - The genome is approximately 4.0Mb, in which the *classical strain* is divided between a 2.4Mb large chromosome and a 1.6 Mb small chromosome.
  - In the *El Tor strain*, the large chromosome contains 2.96Mb and the small chromosome contains 1.07Mb



Genomic Structure: Circular representation of the *V. cholerae* genome

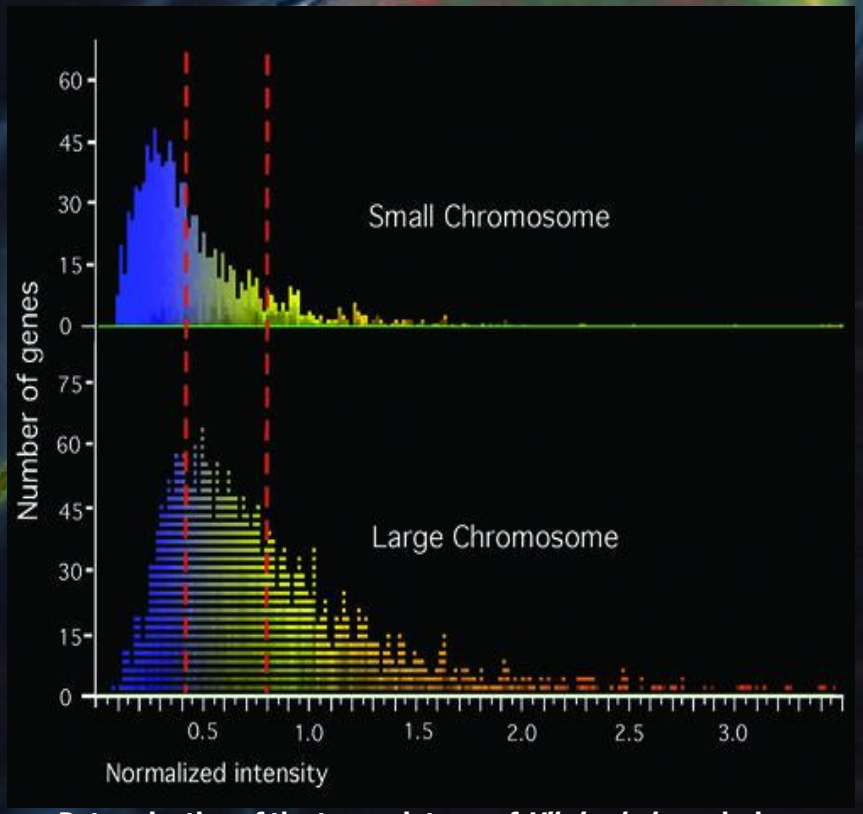
- From the outside inward: the first and second circles show predicted protein-coding regions on the plus and minus strand (unknown and hypothetical proteins are in black).
- The third circle shows recently duplicated genes on the same chromosome (black) and on different chromosomes (green).
- The fourth circle shows transposon-related (black), phage-related (blue), VCRs (pink) and pathogenesis genes (red).
- The fifth circle shows regions with significant X^2 values for trinucleotide composition in a 2,000-bp window.
- The sixth circle shows percentage G+C in relation to mean G+C for the chromosome.
- The seventh and eighth circles are tRNAs and rRNAs, respectively.



DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*
John F. Heidelberg et. al

Genomic Structure

- Graphical representation of *V. cholerae* gene expression in LB.
- All 3890 genes were analyzed by using GENESPRING, and the expression levels of these genes are represented by normalized intensities.
- The *V. cholerae* genome contains 3,890 genes distributed between a large and a small chromosome. Although the large chromosome encodes the majority of recognizable gene products and virulence determinants, the small chromosome carries a disproportionate number of hypothetical genes.
- 285 of the 300 most highly expressed genes resided on the large chromosome.



Determination of the transcriptome of *Vibrio cholerae* during intraintestinal growth and midexponential phase *in vitro*
Mekalonos et. al

Genomic Structure: Mobile Elements (PLASMIDS)

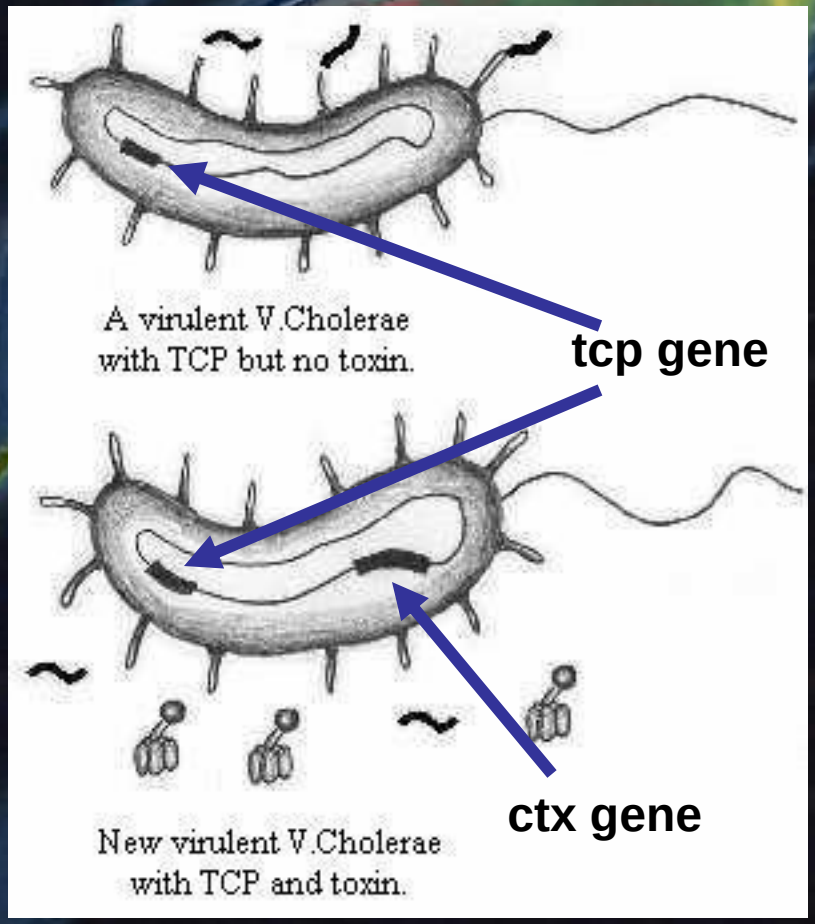
- Although several plasmids have been isolated, none appear to be involved in pathogenesis.
- A 4.7Kb cryptic plasmid is present in all ctx-positive strains.
- A 6.8Kb plasmid (P factor) is capable of mobilizing chromosomal genes but less efficiently than the F factor in E. Coli.

Genomic Structure: Bacteriophage

- In 1996 Matthew K. Waldor and John J. Mekalanos reported a stunning discovery about the toxin.
- The toxin was for the first time shown to be not a part of the bacterium but actually that of a virus that got integrated into the V. cholerae genome.
- Normally this virus remains silent within V. cholerae but during infection it gets activated.
- The major virulence factor of cholera, CT (cholera toxin) is encoded on a filamentous phage (ctxΦ) that is capable of transducing the ctx gene into other cholera strains.
- The released phages specifically attach to the bacterium and enter it. Vigorous viral multiplication results in the production of large amounts of toxin causing severe diarrhea.

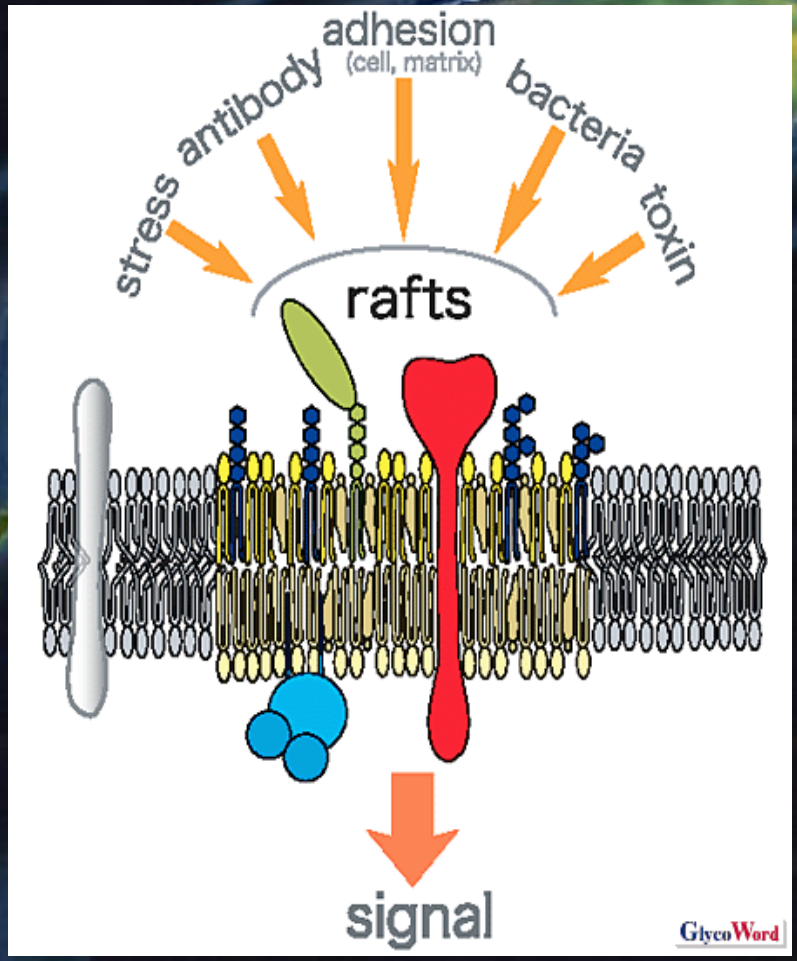
Genomic Structure: Pathogenicity Islands (PAI)

- Upon transduction, the bacteriophage (ctxΦ) brings the toxin and a specific pilus called toxin-co-regulated pilus (TCP).
- The important genes involved in intestinal colonization (tcp) and virulence gene regulation (toxT) are encoded in a 40Kb pathogenicity island.
  - This PAI is present in pathogenic cholera strains.



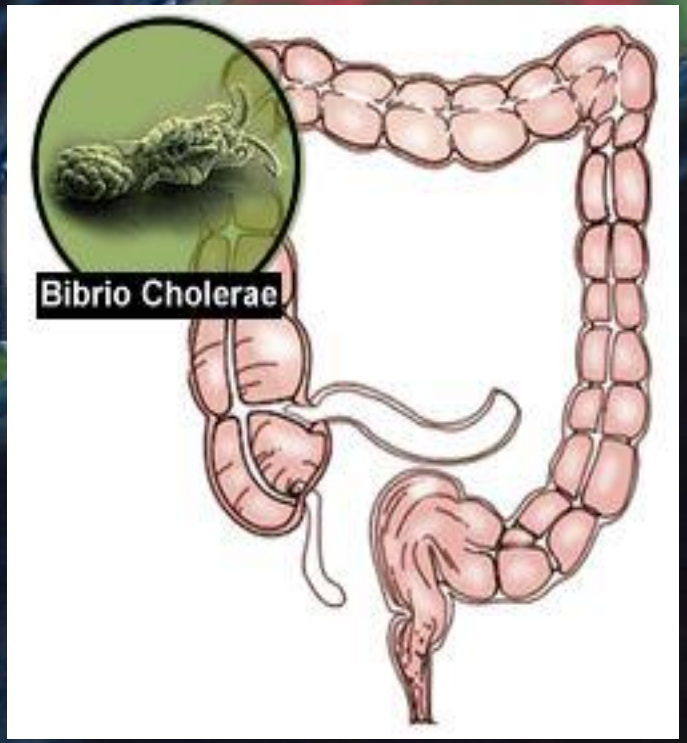
Molecular Biology of Vibrio cholerae

- Identification & Classification (serogroups)
- Genomic Structure
- **Pathogenesis (mechanism of action)**



Pathogenesis: Overview

- To establish disease, V. cholerae must be ingested in contaminated food or water and survive passage through the gastric barrier of the stomach.
- On reaching the lumen of the small intestine, the bacteria must overcome the clearing mechanism of the intestine (peristalsis), penetrate the mucous layer and establish contact with the epithelial cell layer.



Pathogenesis: Overview cont.

- Colonization of the intestinal microvilli and the subsequent production and release of cholera toxin, lead to the purging diarrhea.

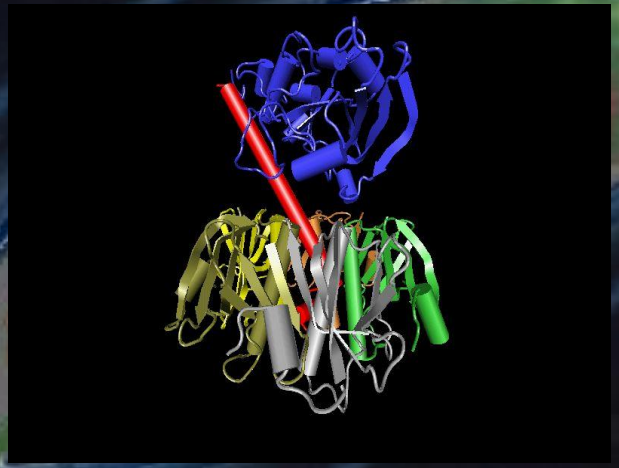
- This complex progression of events appears to involve tightly regulated differential gene expression by the bacteria.
  - This is because expression of intestinal colonization factors is unlikely to be of advantage to the bacterium in its salt/fresh water environment niche.

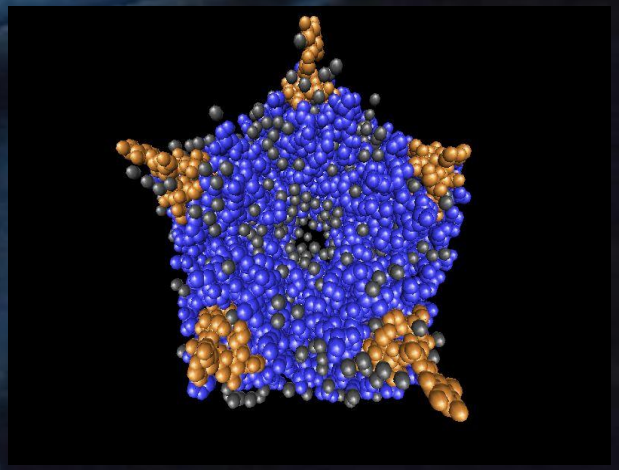
Pathogenesis: Cholera Toxin (CT)

- In 1983, by administering purified CT to volunteers, Levin et al. were able to conclusively demonstrate that the toxin is the major mediator of the cholera syndrome.
  - Ingestion of only 5µg of purified toxin resulted in production of 1-6L of diarrheal stool.
- CT elicits vigorous mucosal immune responses in the absence of a conventional adjuvant.
- Direct immunomodulatory effects of CT on leukocytes include induction of CD25 and class II MHC on B cells, apoptosis of CD8+ T cells, and activation of macrophages with release of IL-10.

Pathogenesis: Cholera Toxin (CT) Structure

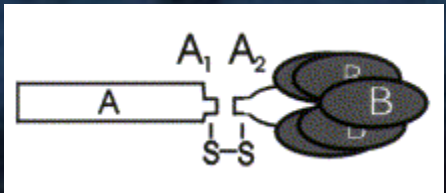
- CT is a prototype A/B subunit toxin, consisting of one A subunit and 5 B subunits.
- The B subunit weighs 11.6kDa each and multimerize to form a pentameric ring, which binds the holotoxin to a eukaryotic cell surface receptor.





Pathogenesis: Cholera Toxin (CT) Structure cont.

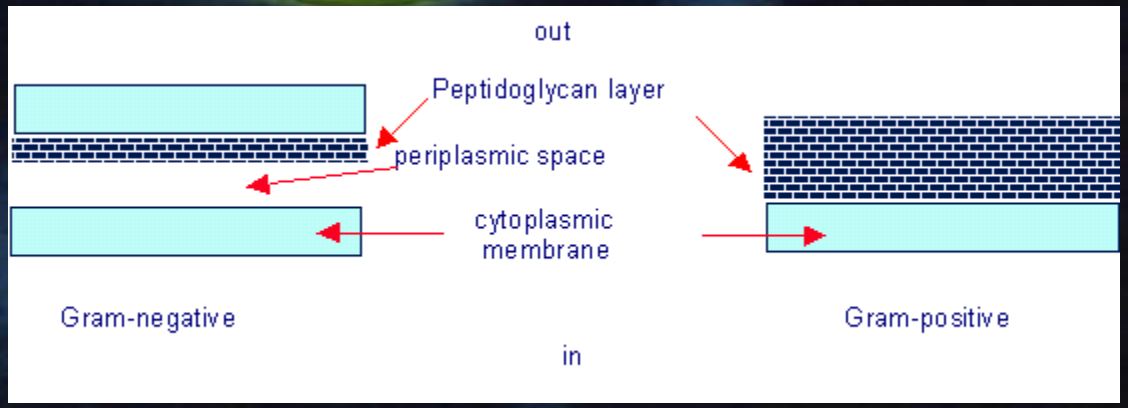
- The A subunit contains an intracellular ADP-ribosyltransferase activity.
- The mature A subunit is proteolytically cleaved to produce a 21.8kDa A1 polypeptide, which contains the intracellular enzymatic activity, and a 5.4kDa A2 polypeptide



- After cleavage, the A1 and A2 polypeptides remain linked by a disulphide bond.
- The crystal structure of CT revealed that the A and B subunits are connected through the C-terminus of the A2 subunit, which is inserted through the central pore of the B pentamer.

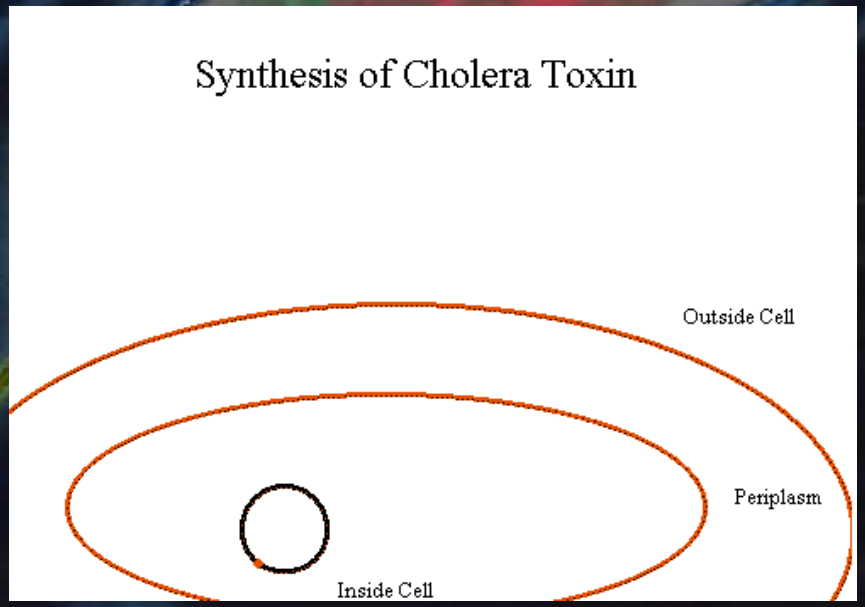
Pathogenesis: Cholera Toxin (CT) Structure cont.

- CT must be assembled for activity, as neither the A nor B subunit individually can cause secretory diarrhea.
- CT holotoxin is assembled in the periplasmic space.
- The subunits are exported individually into the periplasm through the cytoplasmic membrane via the general secretion pathway; both the A and B protein subunits contain normal sequences at their N-terminus.



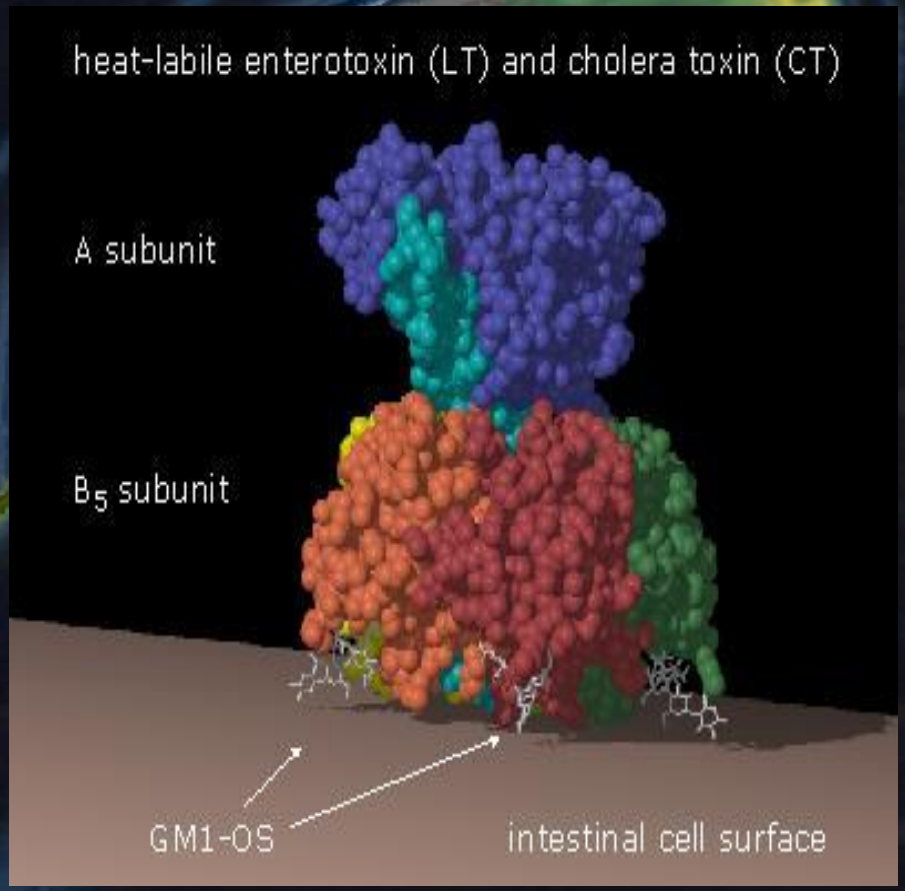
Pathogenesis: Cholera Toxin (CT) Structure cont.

- Once in the periplasm, both subunits must undergo modification by the periplasmic enzyme DsbA, which is responsible for disulphide bond formation.
- Again, once the holotoxin is secreted from the bacterium, the A subunit must be cleaved to generate separate A1 and A2 peptides for maximum toxin activity.



Pathogenesis: Mechanism of Action cont.

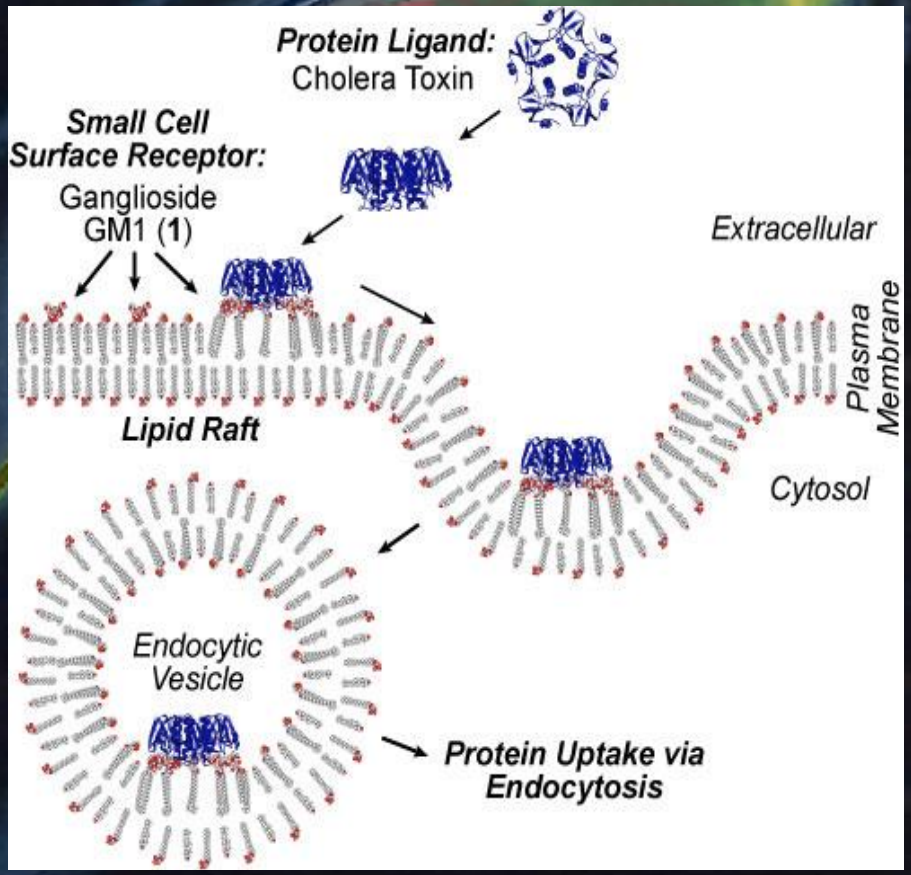
- The biological activity of CT is dependent on binding of the holotoxin B pentamer to specific receptors on the eukaryotic cell.
- The B oligomer binds with high affinity exclusively to GM1 ganglioside.



B subunits bind to GM1 Receptor

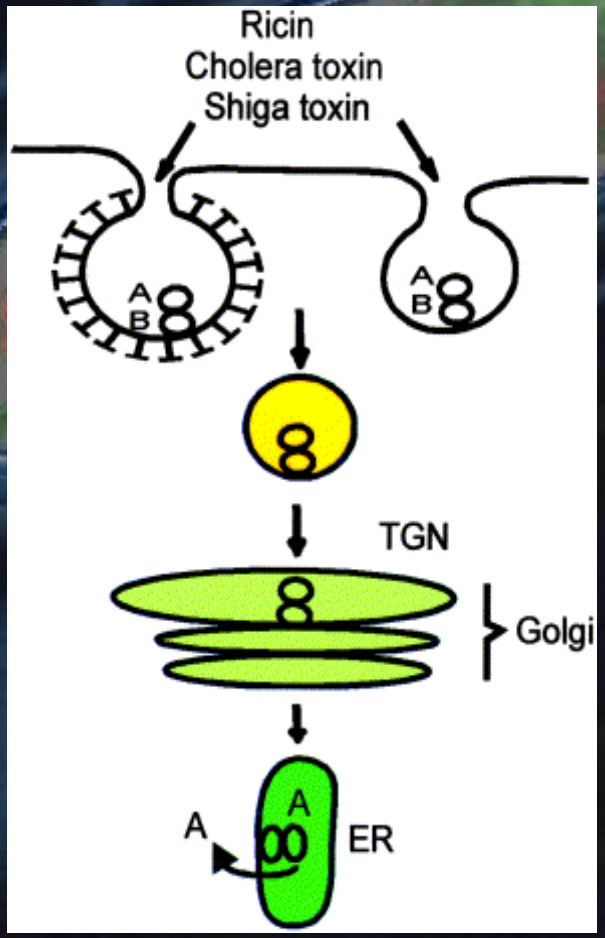
Pathogenesis: Mechanism of Action cont.

- Internalization is initiated once CT-GM1 complexes cluster which then invaginate to form apical endocytic vesicles.



Pathogenesis: Mechanism of Action cont.

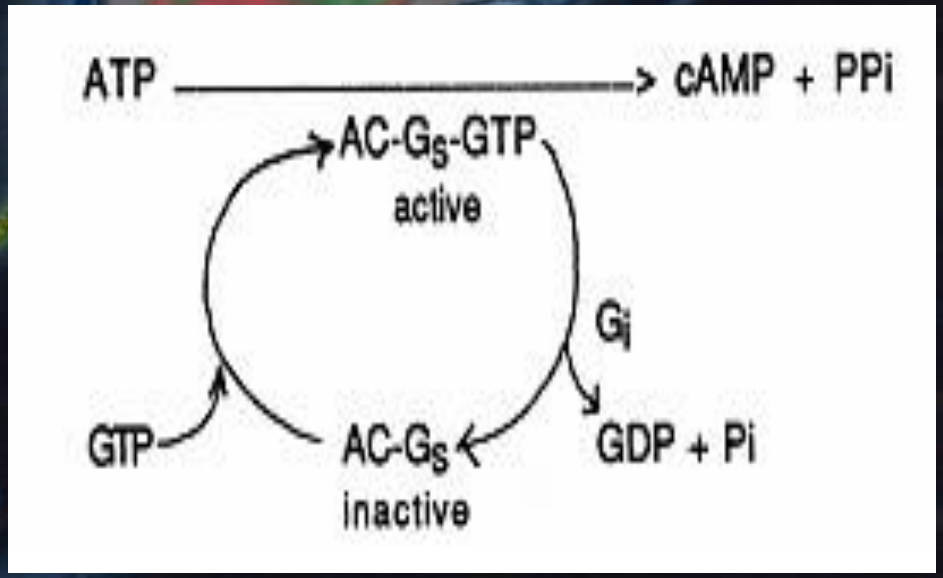
- These vesicles enter cellular trafficking pathways leading to the trans-Golgi network (TGN).
- The toxin then moves retrograde via the Golgi cistern to the ER.
- Once in the ER, CT is processed to activate the A1 peptide, which then targets the basolateral membrane (heterotrimeric GTPase and adenylate cyclase (AC)).



Pathogenesis: Mechanism of Action cont.

- Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP.

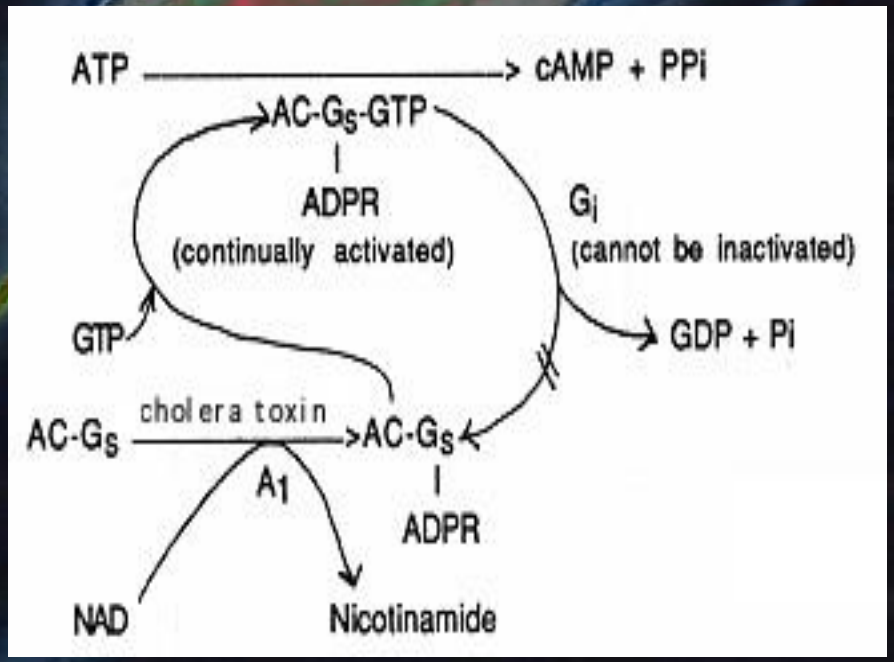
NORMAL CONDITION



Pathogenesis: Mechanism of Action cont.

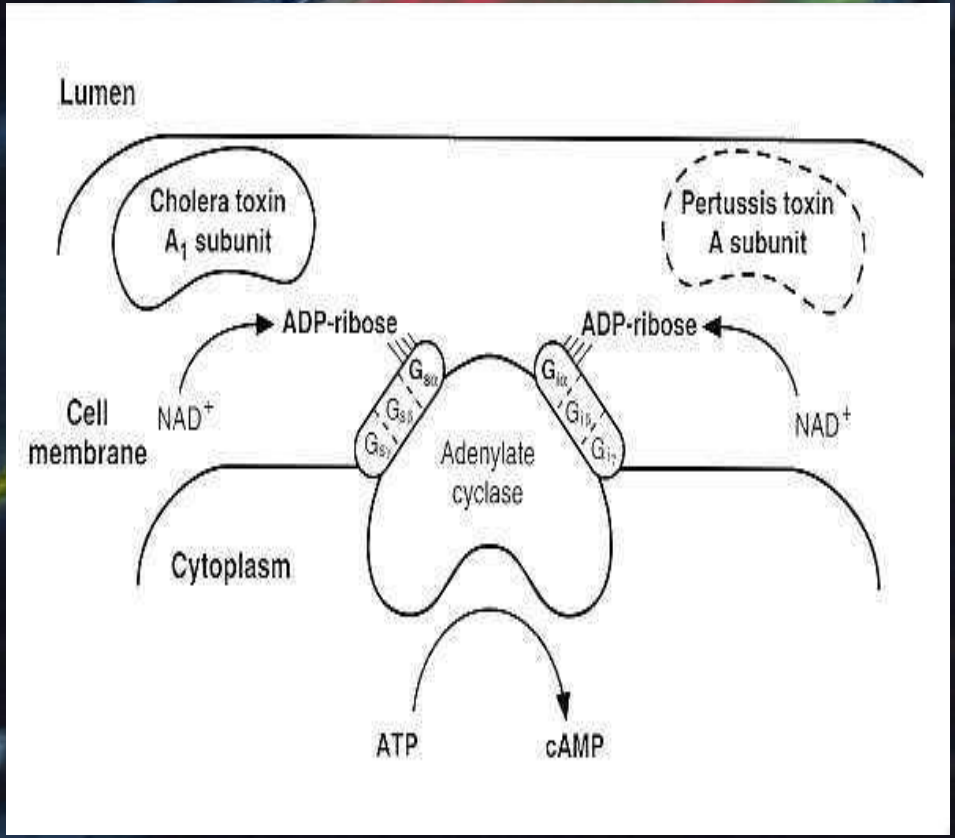
- Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system.
  - The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed.
- Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated.

CHOLERA



Pathogenesis: Mechanism of Action cont.

- Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl- into the intestinal contents.

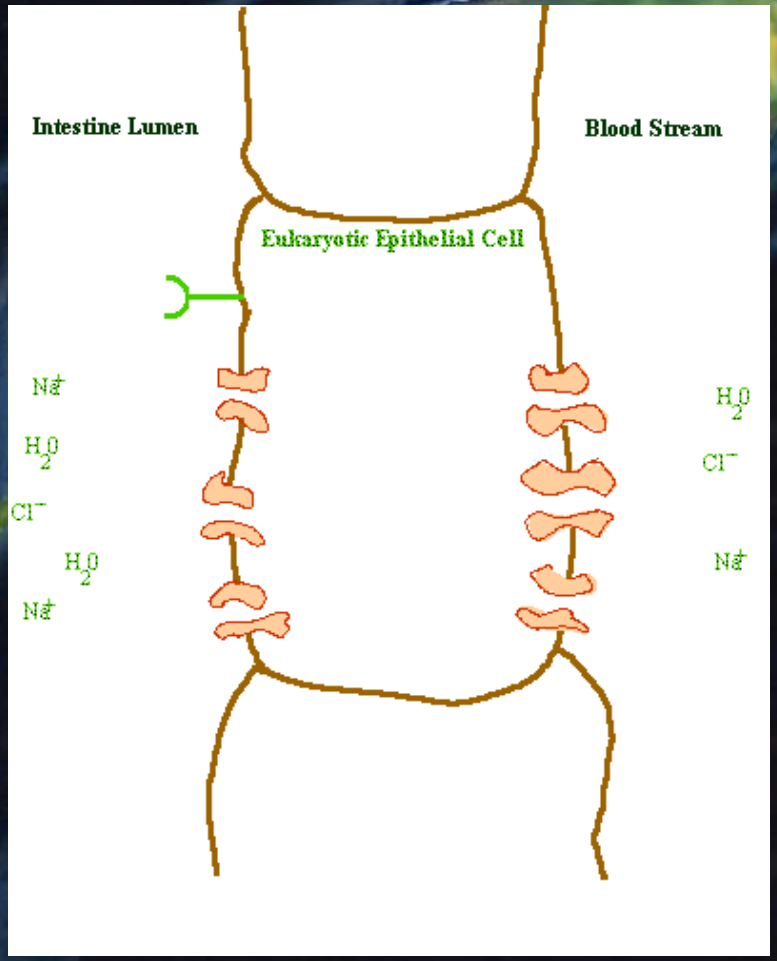


Pathogenesis: Mechanism of Action cont.

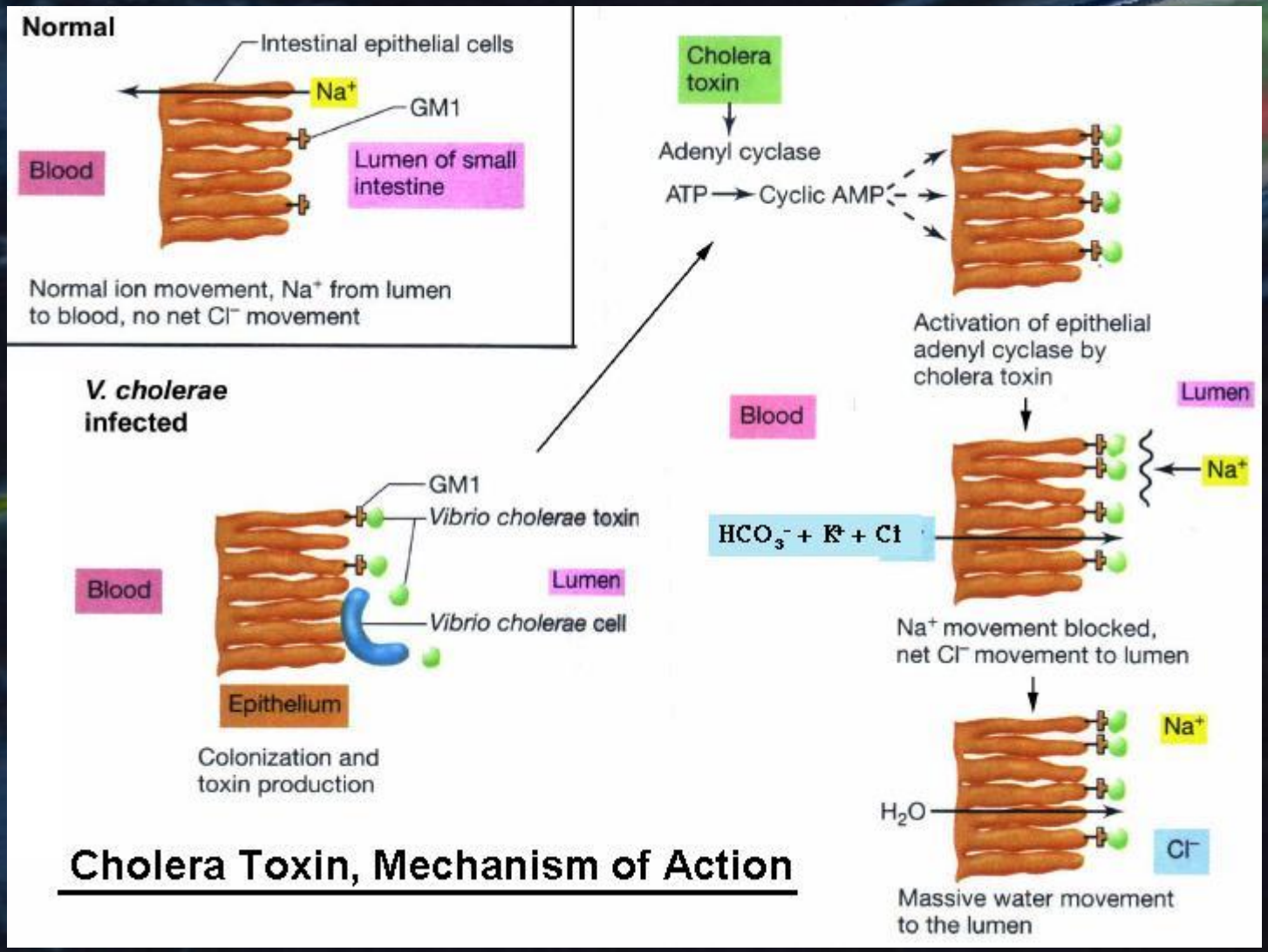
- H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-.
- The lost H_2O and electrolytes in mucosal cells are replaced from the blood.
- Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.

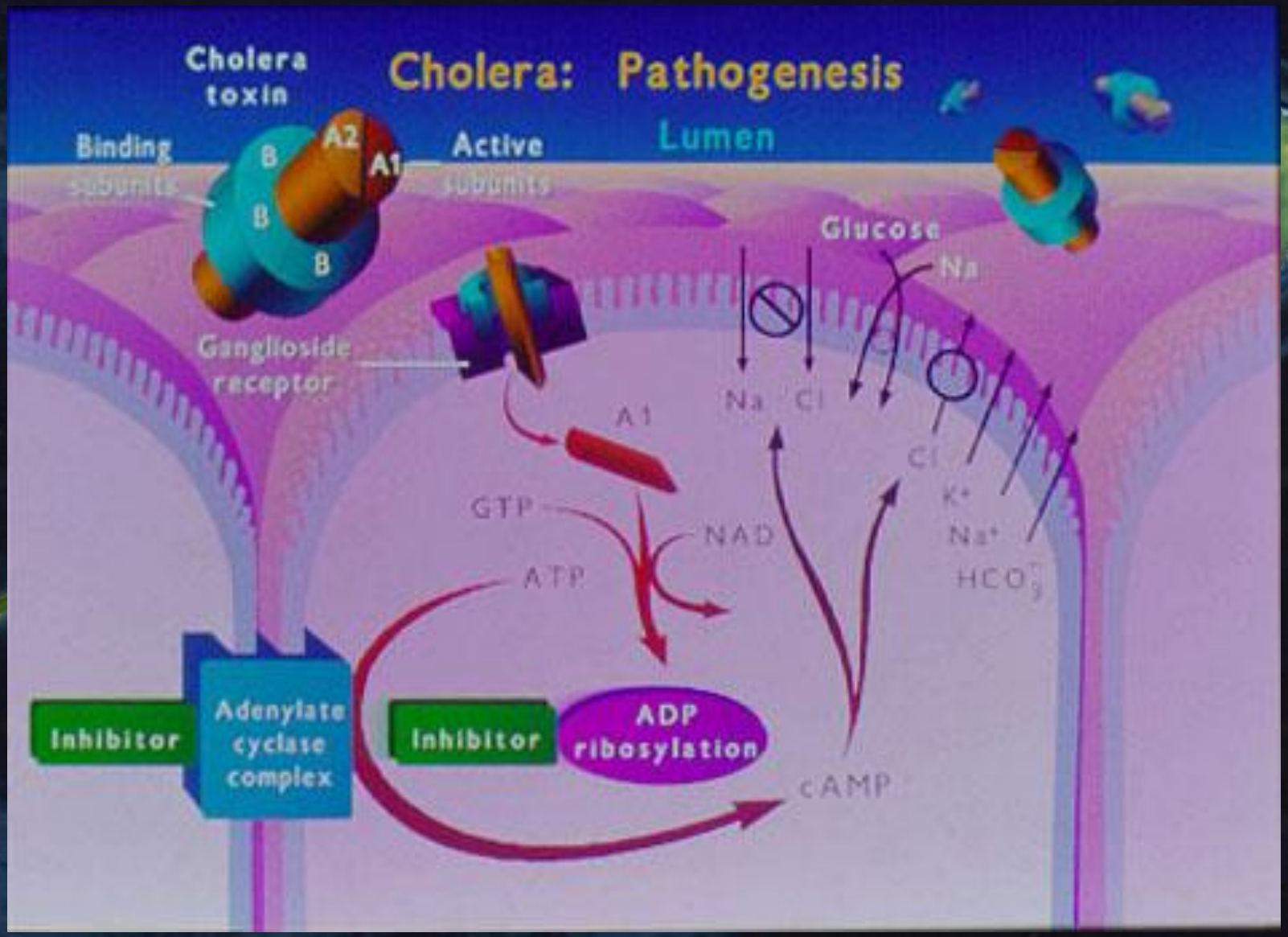
Pathogenesis: Mechanism of Action cont.

- Normally, the epithelial cells of the inner lining of the intestines (lumen) transfer sodium and chloride ions from the inside of the intestines to the blood stream.
- The "B" subunit of cholera toxin is bound by a host receptor (like a specific "landing pad") allowing the "A" subunit to enter the cell.
- Once inside the cell the "A" subunit causes a change in the regulation of the cells genes and as a result, the flow of ions and water is reversed.

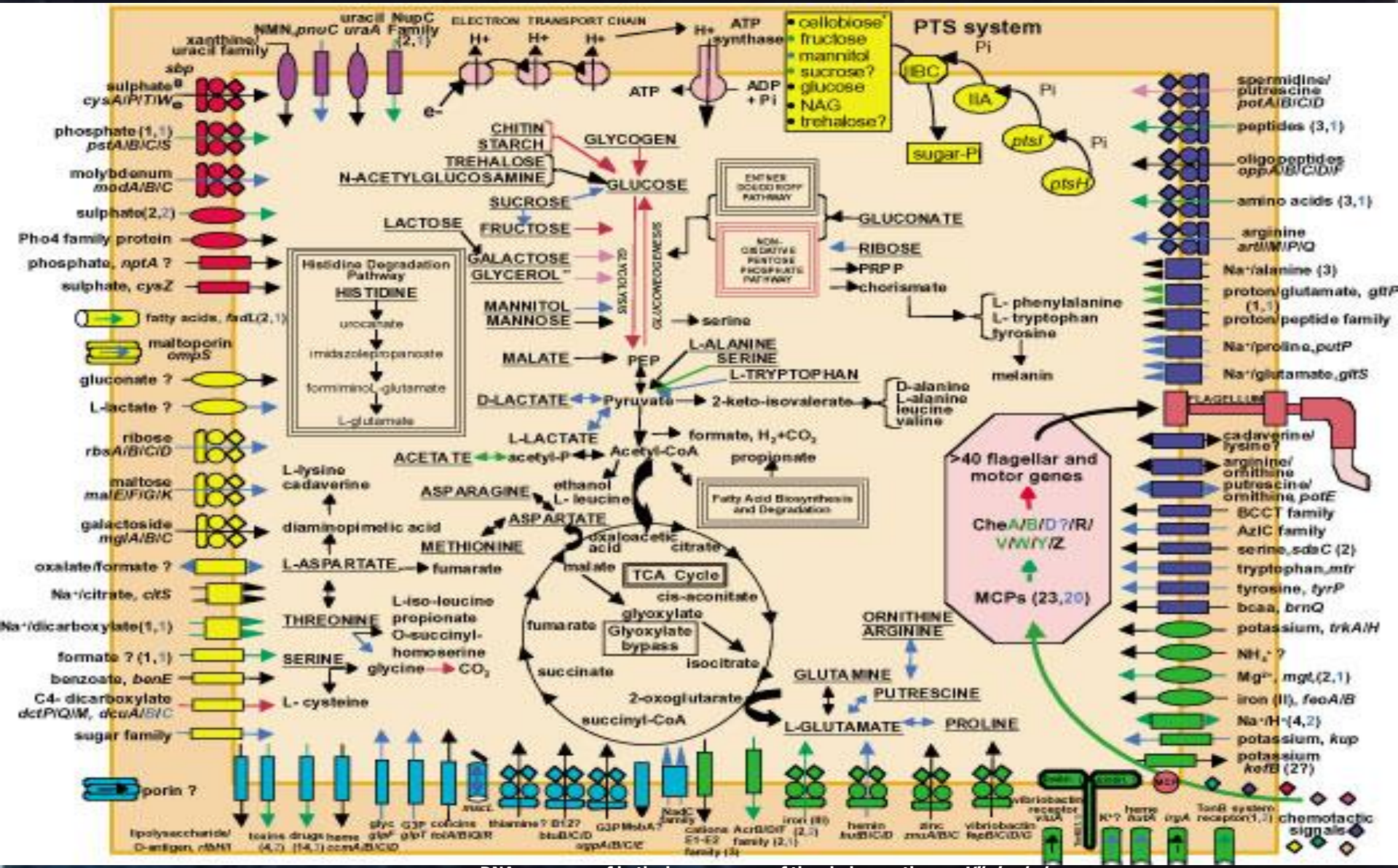


Pathogenesis: Mechanism of Action: Overview




Cholera toxin
Cholera: Pathogenesis
Lumen
Binding subunits
B
B
B
A2
A1
Active subunits
Glucose
Na
Ganglioside receptor
A1
Na Cl
Cl
K+
Na+
HCO3-
GTP
NAD
ATP
Inhibitor
Adenylate cyclase complex
Inhibitor
ADP ribosylation
cAMP

Overview of metabolism and transport in *V. cholerae*



DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*
John F. Heidelberg et. al

Vibrio cholerae

- Introduction
- History
- Epidemiology / Clinical Manifestation
- Molecular Biology
- *Diagnosis/Treatments/Prevention*
- Weaponization

Diagnosis

- Cholera should be suspected when patients present with watery diarrhea, severe dehydration
- Based on clinical presentation and confirmed by isolation of *vibrio cholera* from stool

Diagnosis

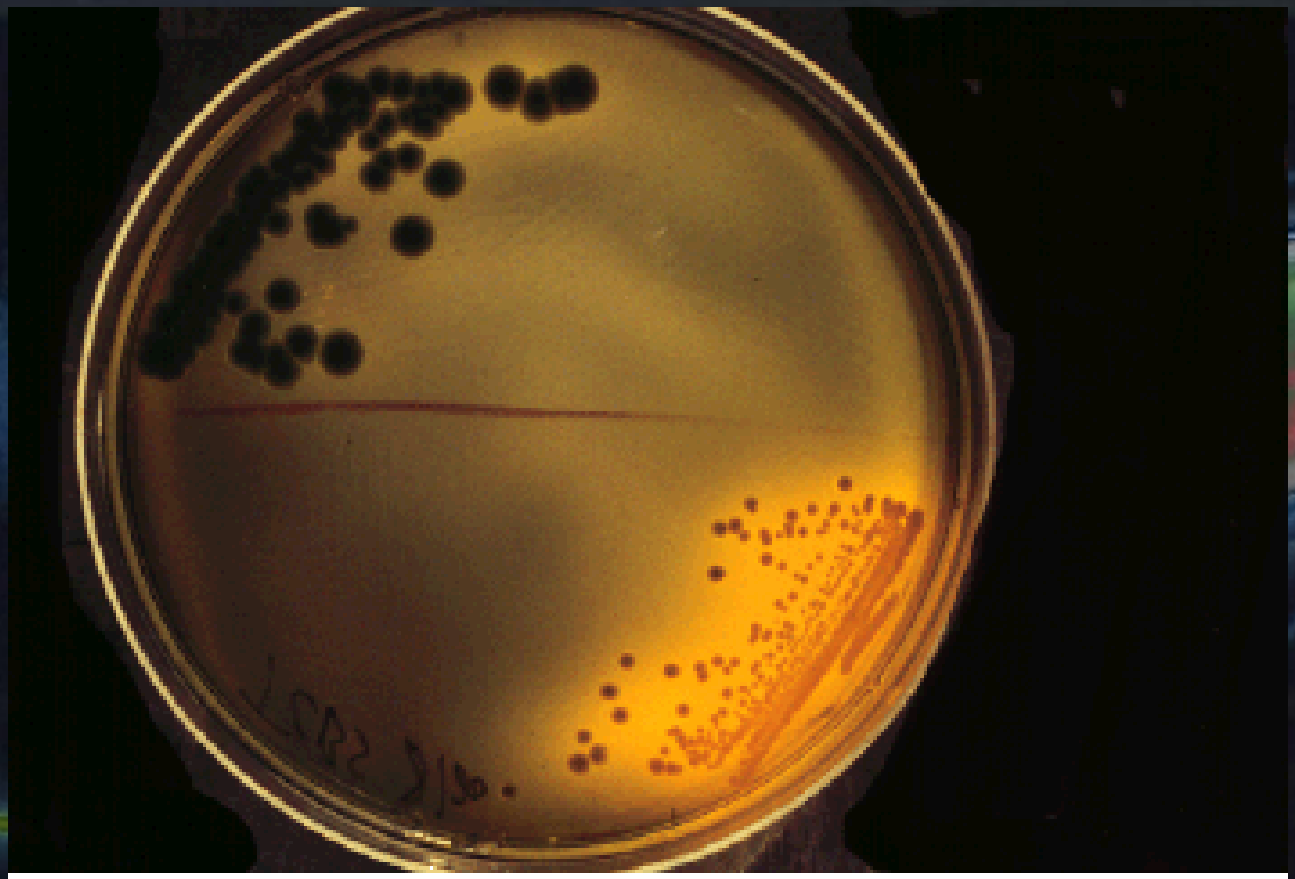
- No clinical manifestations help distinguish cholera from other causes of severe diarrhea:
  - Enterotoxigenic e. coli
  - Viral gastroenteritis
  - Bacterial food poisoning

Diagnosis: Visible Symptoms

- Decreased skin turgor
- Sunken eyes, cheeks
- Almost no urine production
- Dry mucous membranes
- Watery diarrhea consists of:
  - fluid *without* RBC, proteins
  - electrolytes
  - enormous numbers of vibrio cholera (10^7 vibrios/mL)

Laboratory Diagnosis

- Visualization by dark field or phase microscopy
  - Look like “shooting stars”
- Gram Stain
  - Red, curved rods of bacteria
- Isolate V. cholerae from patient’s stool
  - Plate on sucrose agar
  - Yellow colonies form



Vibrio species on TCBS agar Vibrio species can be selectively recovered from stool by culture on thiosulfate-citrate-bile salts-sucrose (TCBS) agar. On this medium, V. parahaemolyticus usually produces a green colony and V. cholerae a yellow colony (indicative of the fermentation of sucrose). Courtesy of Harriet Provine.

Treatment

Even before identifying cause of disease, rehydration therapy must begin Immediately because death can occur within hours

- Oral rehydration
- Intravenous rehydration
- Antimicrobial therapy

Treatment: Oral Rehydration

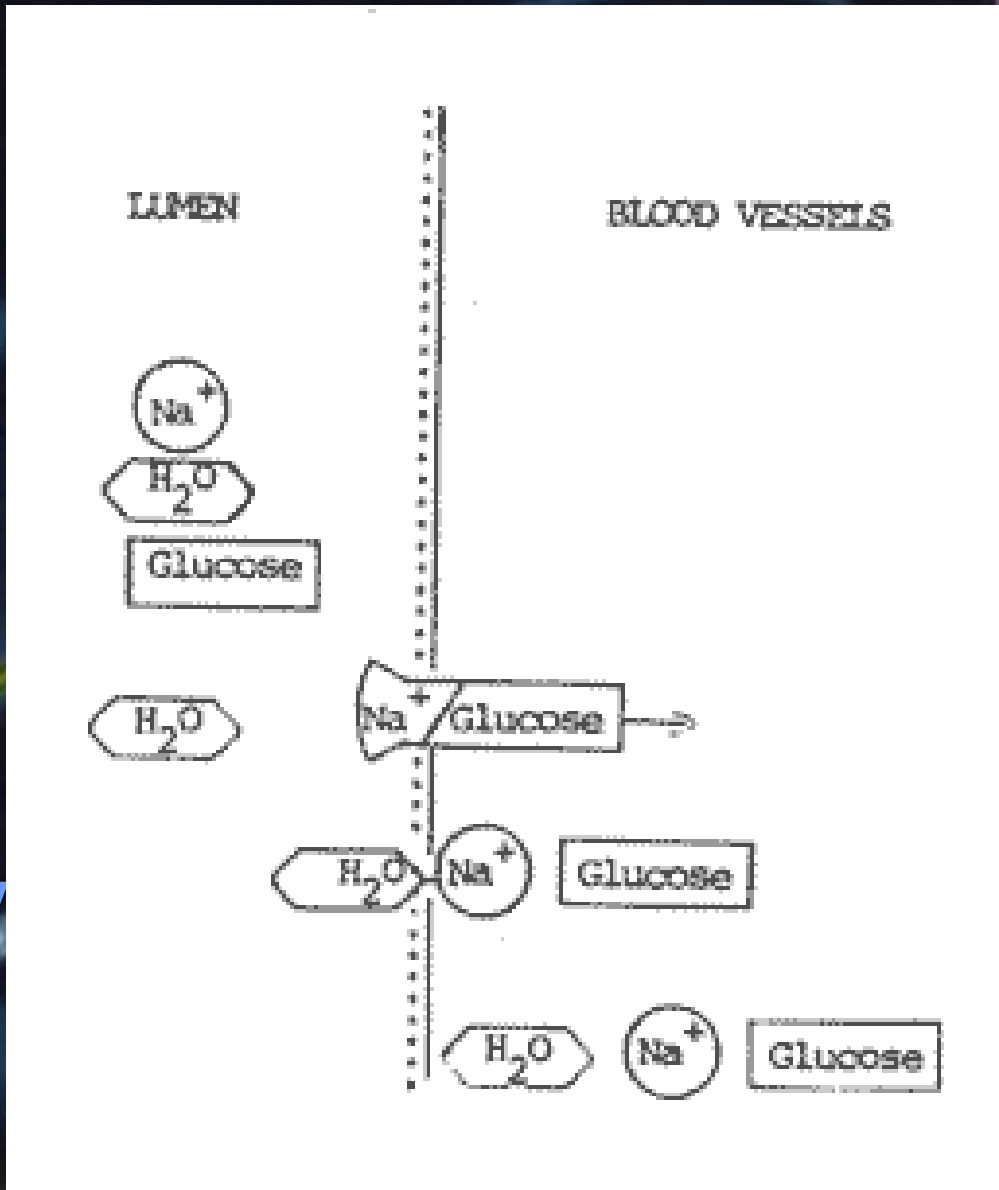
- Reduces mortality rate from over 50% to less than 1%
- Recover within 3-6 days
- Should administer at least 1.5x amount of liquid lost in stools
- Use when less than 10% of bodyweight lost in dehydration

Treatment: Oral Rehydration Salts (ORS)

- Reduces mortality from over 50% to less than 1%
- Packets of Oral Rehydration Salts
  - Distributed by WHO, UNICEF
  - Dissolve in 1 L water
  - NaCl, KCl, $NaHCO_3$, glucose

Treatment: How ORS Works

- Na^+ transport coupled to glucose transport in small intestine
- Glucose enables more efficient absorption of fluids and salts
- Potassium passively absorbed



Treatment: ORS in United States?

- American doctors skeptical of such simple, inexpensive treatment
- Cost
  - ORS: $270/infant
  - IV: $2,300/infant
  - $1 billion/year for IV treatment for rehydrating children
- Insurance companies do not reimburse for ORS
- 600 American children die unnecessarily from dehydration each year
- Hospitals consider IV more time efficient
  - Less personal attention required

Treatment: Intravenous Rehydration

- Used when patients have lost more than 10% bodyweight from dehydration
- Unable to drink due to vomiting
- Only treatment for severe dehydration

Treatment: Intravenous Rehydration

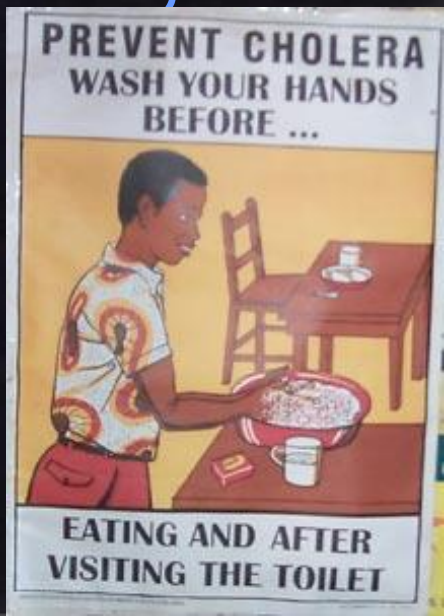
- Ringer's Lactate
  - Commercial product
  - Has necessary concentrations of electrolytes
- Alternative options
  - Saline
  - Sugar and water
  - Do not replace potassium, sodium, bicarbonate

Treatment: Antibiotics

- Adjunct to oral rehydration
- Reduce fluid loss by half
- Reduce recovery time by half
  - 2-3 days instead of 4-6
- Tetracycline, Doxycycline
- Not recommended
  - Short duration of illness
  - Antibiotic resistance
  - Limited gain from usage

Traveling Precautions

- Boil or treat water with chlorine or iodine
- No ice
- Cook everything
- Rule of thumb: “Boil it, cook it, peel it, or forget it.”
- Wash hands frequently



Vaccines

- Need localized mucosal immune response
  - Oral Vaccine
- Not recommended
  - Travelers have very low risk of contracting disease: 1-2 cases per million international trips
  - Not cost-effective to administer vaccines in endemic regions
    - Brief and incomplete immunity
- Two types approved for humans:
  - Killed whole-cell
  - Live-attenuated

Vaccines: Killed Whole-cell Vaccines

- Provides antigens to evoke protective antitoxic and antibacterial immunity
- Contains:
  - 1 x 10^{11} heat inactivated bacteria
  - Mixture of *V. cholerae* O1 El Tor and classical strains
  - 1 mg of B subunit of cholera toxin

Killed Whole-cell Vaccines: Disadvantages

- 50% protection for 6 months to adults
- Gives less than 25% protection to children aged 2-5
- Need for multiple doses of nonliving antigens

Vaccines: Live-Attenuated

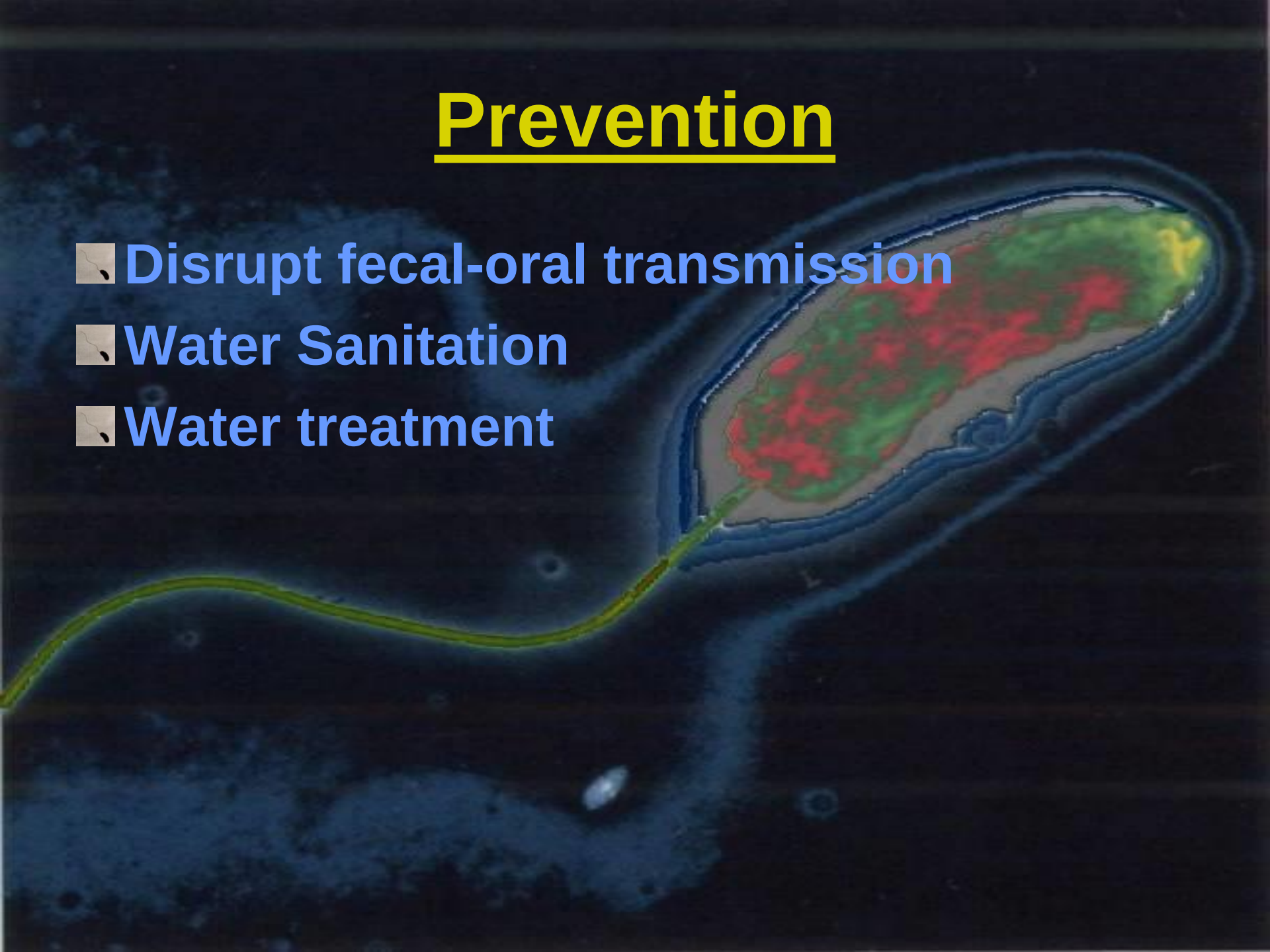
- Eliminates need for multiple doses of non-living antigens
- Ensures that crucial antigens potentially altered during killing process would be retained
  - Expected to mimic broad immunity conferred by natural infection
- 85-90% protection against classical biovar
- 65-80% protection against El Tor biovar

Live Attenuated Vaccines: Disadvantages

- In children, protection rapidly declines after 6 months
- In adults, only receive 60% protection for 2 years
- Live vaccine induces mild cholera symptoms
  - Mild diarrhea, abdominal cramping

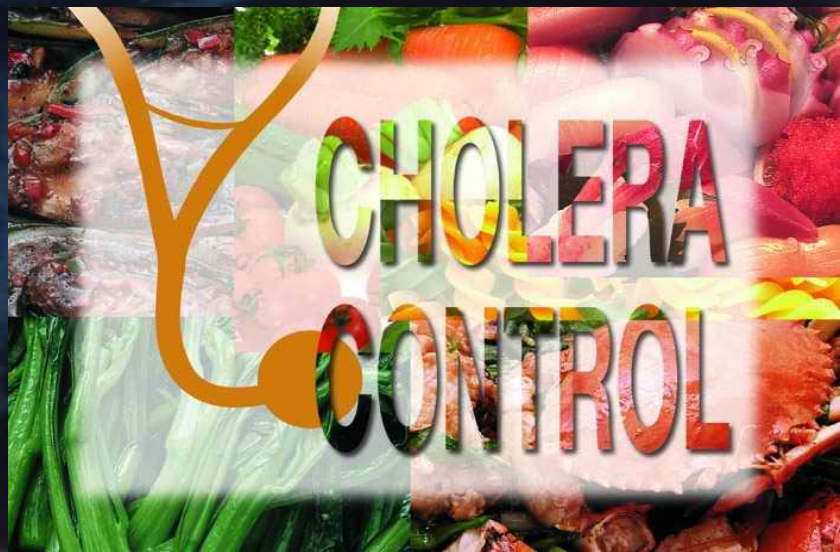
Prevention

- Disrupt fecal-oral transmission
- Water Sanitation
- Water treatment

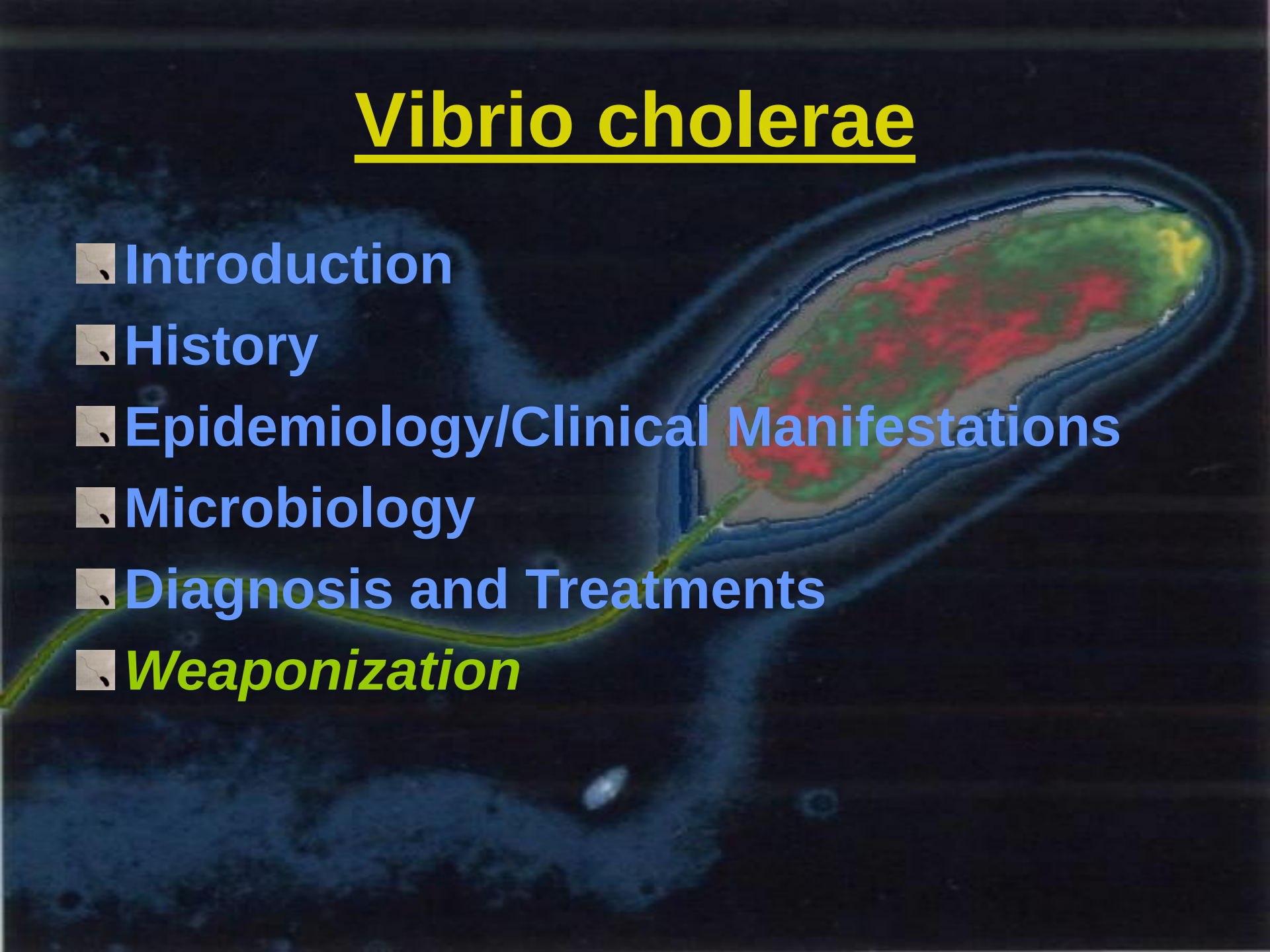


Precautions Taken in US

- EPA works closely with water and sewage treatment operators
- FDA
  - Tests imported shellfish
  - Controls US shellfish sanitation program



Vibrio cholerae



- Introduction
- History
- Epidemiology/Clinical Manifestations
- Microbiology
- Diagnosis and Treatments
- *Weaponization*

Ideal BioWeapon

- Ease of procurement
- Simplicity of production in large quantities at minimal expense
- Ease of dissemination with low technology
- Silent dissemination

Ideal BioWeapon

- Potential to overwhelm medical system with large number of casualties
- Incubation period allows terrorists to escape, but short enough to kill before medical treatment can help
- Causes widespread panic
- Causes economic difficulties (high costs of treatment and preventions overwhelm available finances)

V. cholerae as a BioWeapon

- Easy to obtain samples for growth from environment, easy to grow in lab
- Inexpensive to procure and produce
- Presence of O139 means that other infectious serogroups may appear in future
- Can be used to contaminate food/water directly or be aerosolized and sprayed to contaminated large water sources

V. cholerae as a BioWeapon

- Short incubation period (avg. 1-3 days) and can be shortened with higher dosage of bacteria or higher gastric pH
- ORS not used because not covered by insurance - cause deaths in US
  - 600 kids die/year with ORS instead of IV
- Would need large system of intravenous rehydration for those unable to drink water – would overwhelm hospital resources and staff

V. cholerae as a BioWeapon

- Need enough antibiotics
- Effective vaccine does not exist
- Severely debilitates victims quickly
- Would cause widespread panic and raid on clean water resources
- Severe economic losses
  - 1991: Peru lost $770 million in tourism and trade
  - 1994: India lost $2 billion

V. cholerae as a BioWeapon

- Threat to world leaders because they are older and more susceptible
- Can be genetically modified to produce toxin with harsher effects
- Can be used in conjunction with another BioWeapon (i.e. anthrax, etc.) to debilitate before other disease shows symptoms
  - Show choleric symptoms 2-3 days after ingestion of V. cholerae, symptoms of anthrax occur within 7 days

Means to Increase Virulence

- Amplify and insert toxin producing portion of genome into a more infectious agent – try to make Cholera contagious
  - Spread of new agent that could infect people without need for ingestion of contaminated food/water

Ineffective BioWeapon

- 1% mortality rate with treatment
- Treatment is simple and inexpensive - rehydration
- Many groups present that combat water-borne diseases: CDC, FDA, EPA, WHO
  - Difficult to adequately infect water supply and food due to various protective measures (food recall, water treatment)

Ineffective BioWeapon

- Infectious dose is large: 10^6-10^{11} colony-forming units
  - Difficult to ingest that amount because of extensive water treatment and services to prevent water-borne diseases
- Unlikely that terrorists have expertise to conduct research or the resources to increase virulence of V. cholerae
- Unlikely they have the money or means to bypass water treatment measures that protect populace

Current Weaponization Efforts

- Countries that have done research on Cholera as a BioWeapon: France, Iraq, Japan, Germany, N. Korea, S. Africa
- Japan: 1930s – Infamous Unit 731 under Dr. Shiro Iishi
  - Experimented on prisoners
  - Practiced contaminating food, water, and aerosolizing/spraying over crops and water
  - 1941 – used in China, but ended up killing thousands of Japanese soldiers as well

Current Weaponization Efforts

- Germany: WWII
  - German Offensive biological weapons program
  - Studied natural history of disease and vaccine development in experimentally infected prisoners in Nazi concentration camps
- S. Africa: 1980-1993
  - military allegedly used V. cholerae to contaminate water supplies
- Iraq – Cholera studied at the Al Hazen Institute
  - Little known about production or weaponization

Threat to New York City

- Reservoir/aqueduct system serves 1.3 billion gallons of water daily to 9 million people
- Not a large threat
  - Extensive water treatment facilities

Water Treatment Process

- Intake: water from source into plant
  - Plants, logs, fish screened out at intake or by soil (for groundwater)
- Water sampled and tested throughout plant to check if processes are working
- Chemical addition: aluminum sulfate, polymers, and/or chlorine added
  - Kill pathogens, improve taste and odor, help settle solids still in water

Water Treatment Process

- Coagulation and Flocculation: added chemical stick to particles already in water (coagulation) and form larger particles called floc (flocculation)
- Sedimentation Basin: floc settles to the bottom and is removed
- Filtration: remaining particles removed as water passes through layers of sand and gravel

Water Treatment Process

- Disinfection: chlorine added to kill remaining pathogens (only treatment given to water systems with groundwater sources)
- Storage: put in closed tank or reservoir (clear well)
  - Allows chlorine to mix and disinfect all water
- Distribution

Prevention Efforts

- US Agency for International Development: provides medical supplies to affected countries
- EPA: prevents contamination of water with sewage and water treatment facilities
- FDA: Shellfish sanitation program
  - Tests imported and domestic shellfish
  - Monitors health of US shellfish beds
- Aid to countries with Cholera lowers risk of Cholera in US

Prevention Efforts

- WHO: Global Task Force on Cholera Control
  - Reduce mortality and morbidity
  - Provide aid for social and economic consequences of Cholera
- CDC
- U.N.: GEMS/Water
  - Global Water Quality Monitoring Project
  - Addresses global issues of water quality with monitoring stations on all continents

Industrialized vs. Third World

- Attack with only V. cholerae more likely to severely affect Third world nations where Cholera is already endemic
  - Industrialized nations have treatment facilities that prevent V. cholerae from water sources from ever reaching people
  - Nations where Cholera is endemic lack water treatment systems and the ability to treat current patients (do not have resources to treat bioterrorism attack as well)