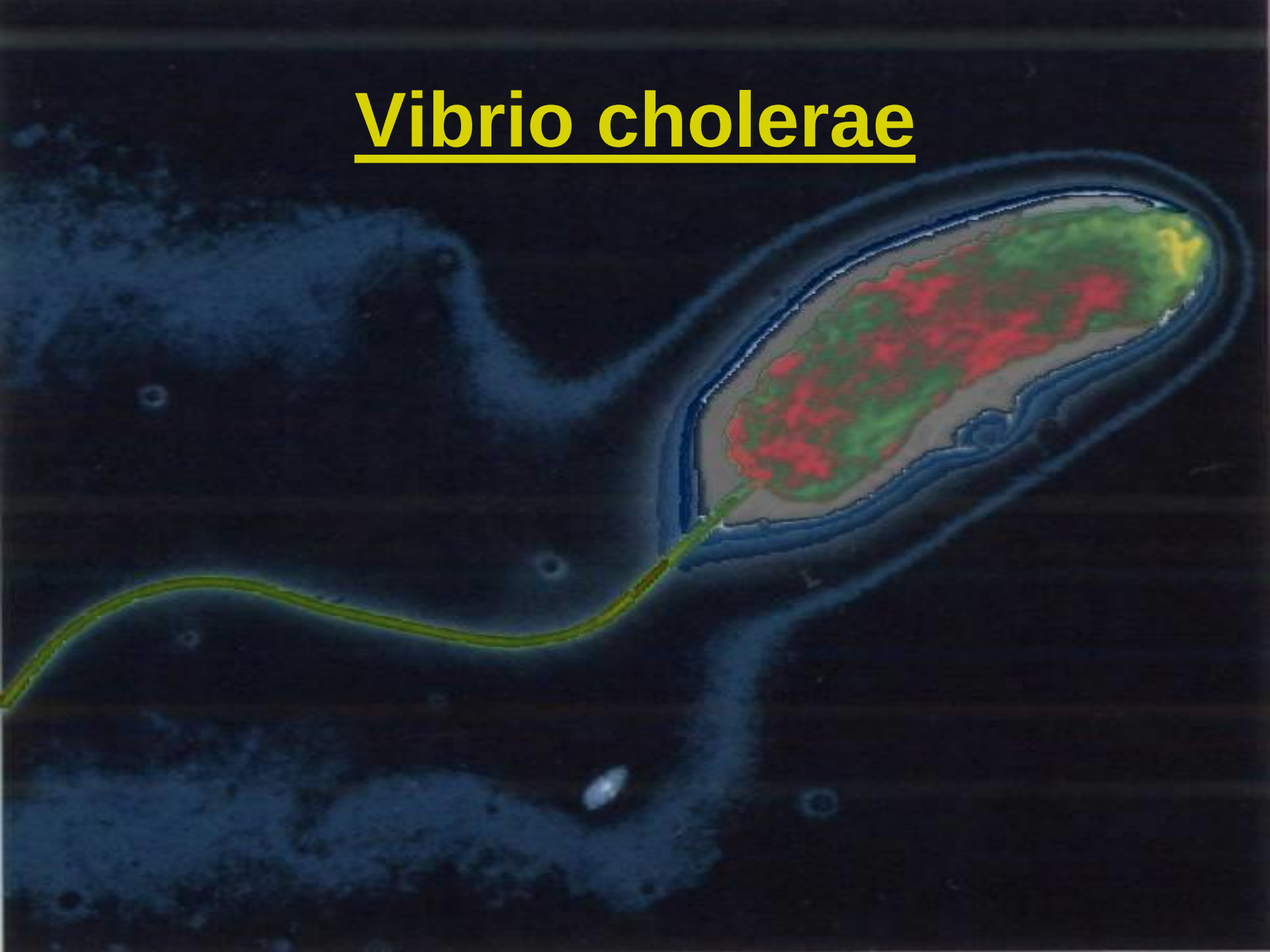
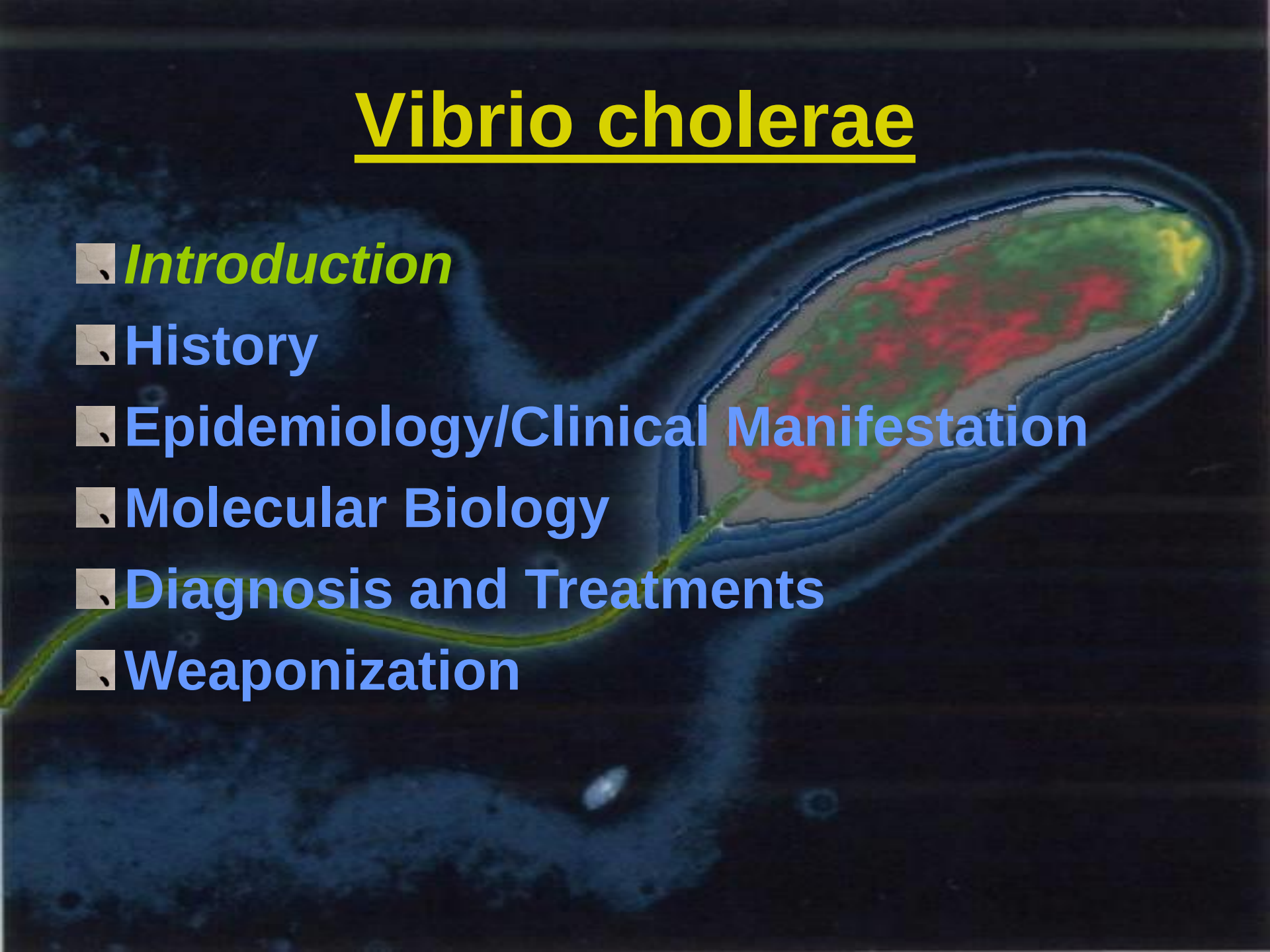
Vibrio cholerae

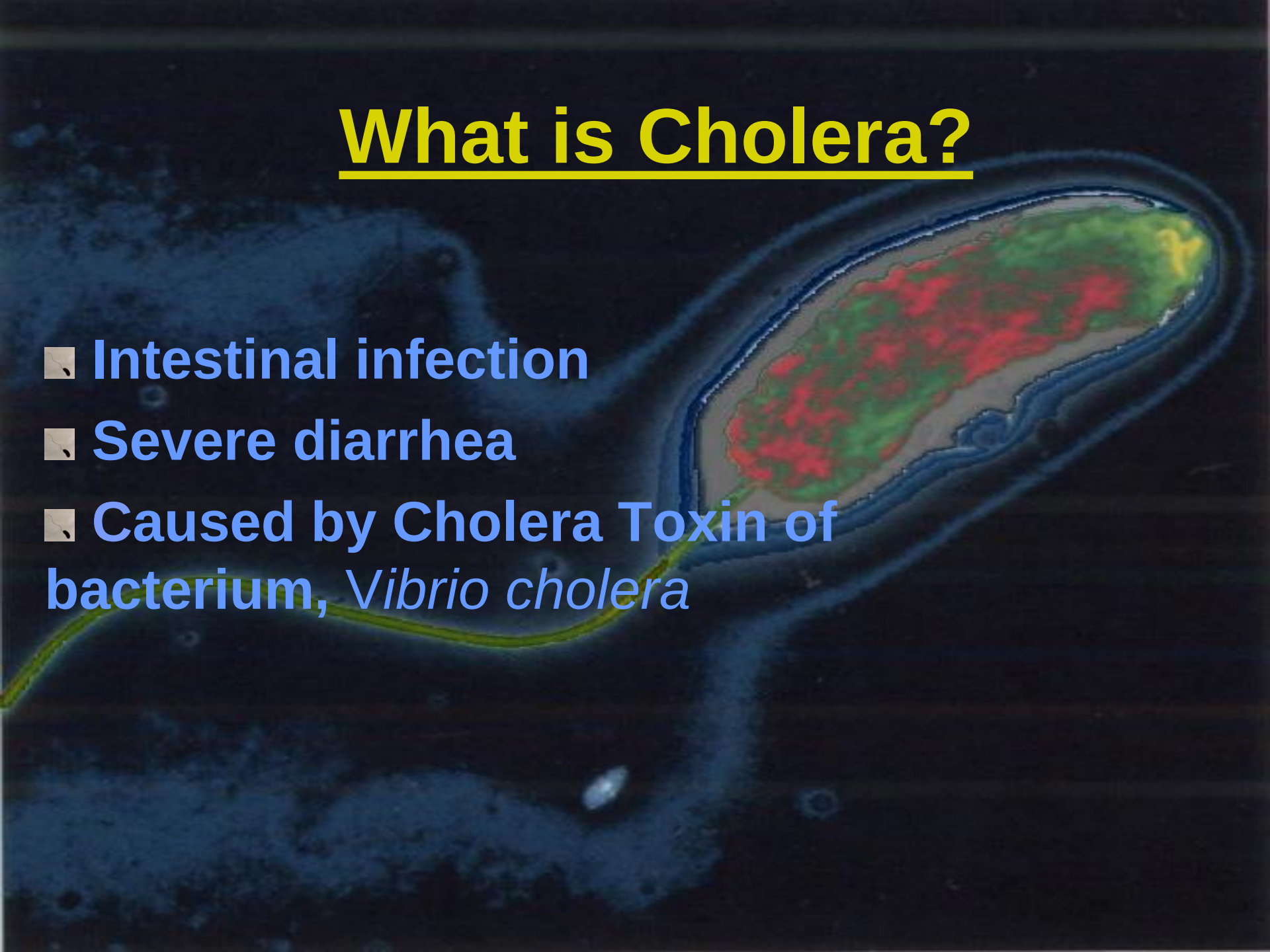


Vibrio cholerae



- *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

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

What's In a Name?

"The appelation 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*

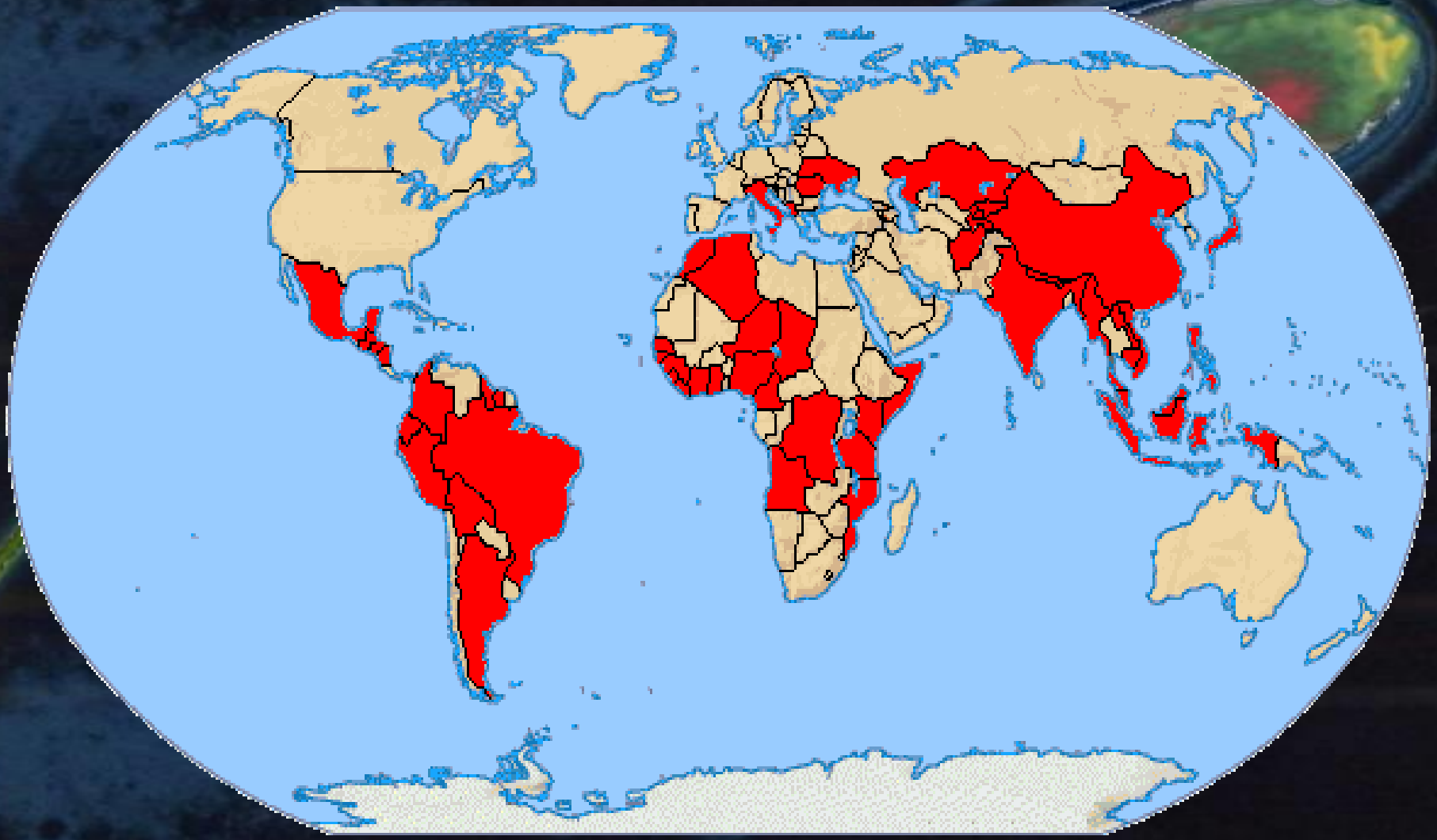
Continent	Total Cases	Total Deaths
Africa	118,932	4,610
America(s)	3,101	40
Asia**	11,246	232
Europe	35	0
Oceania	3,757	26
Total	**137,071**	**4,908**

*Data published in August, 2001

**Does not include Bangladesh, Pakistan and other cou

Source: World Health Organ

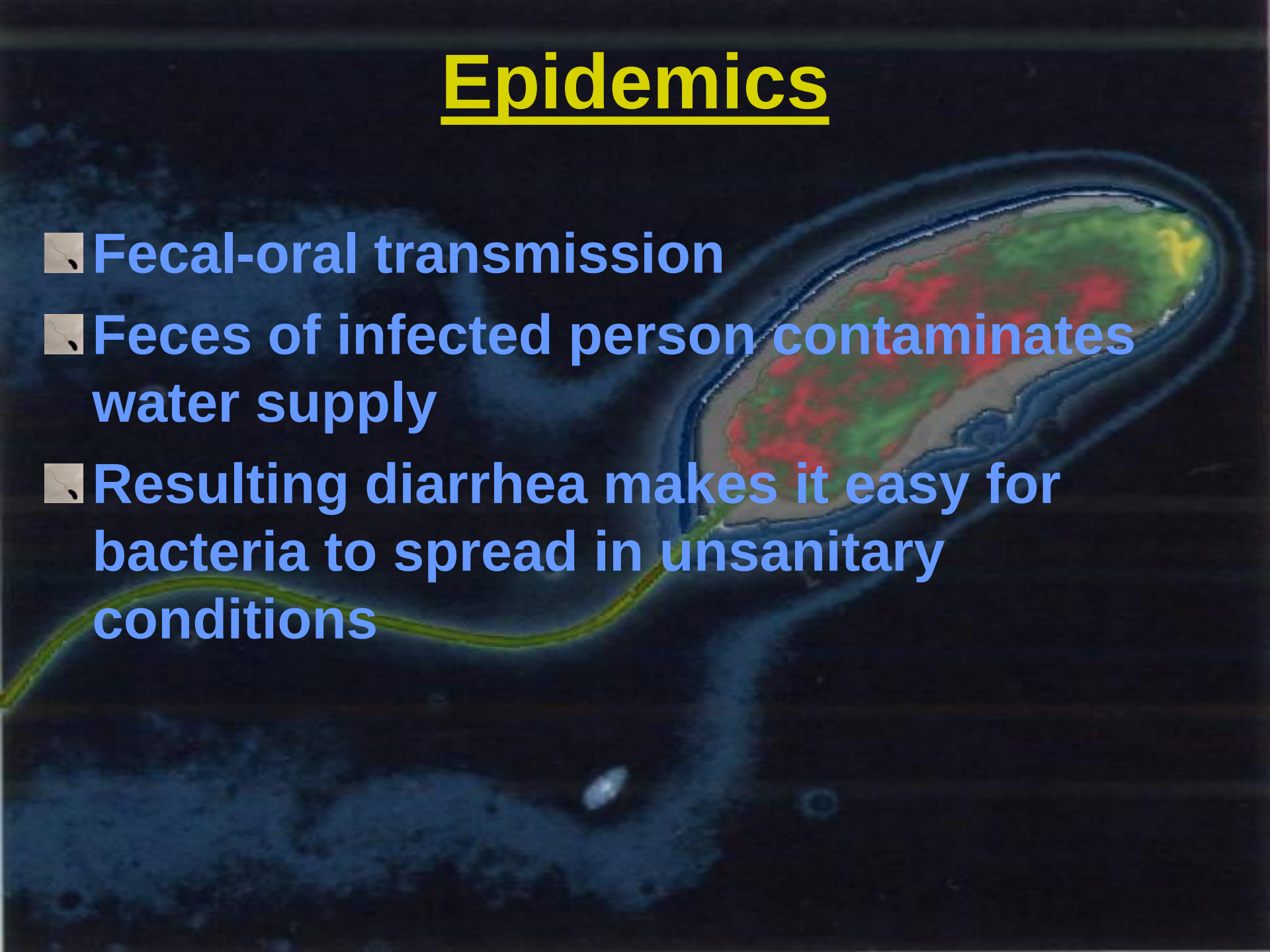
V. Cholerae Afflicted Areas (2000)



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

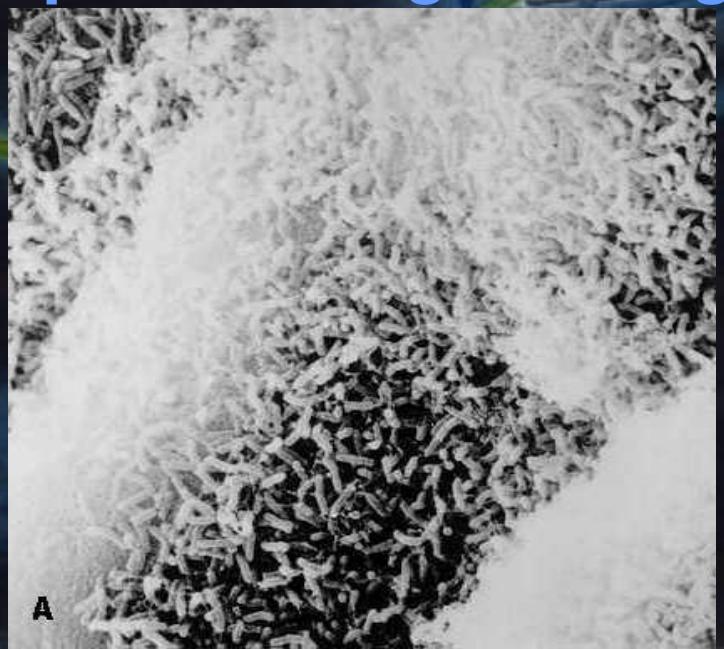
- People with low gastric acid levels
  - Children: 10x more susceptible than adults
  - Elderly
- Blood types
  - O>> B > A > AB

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?

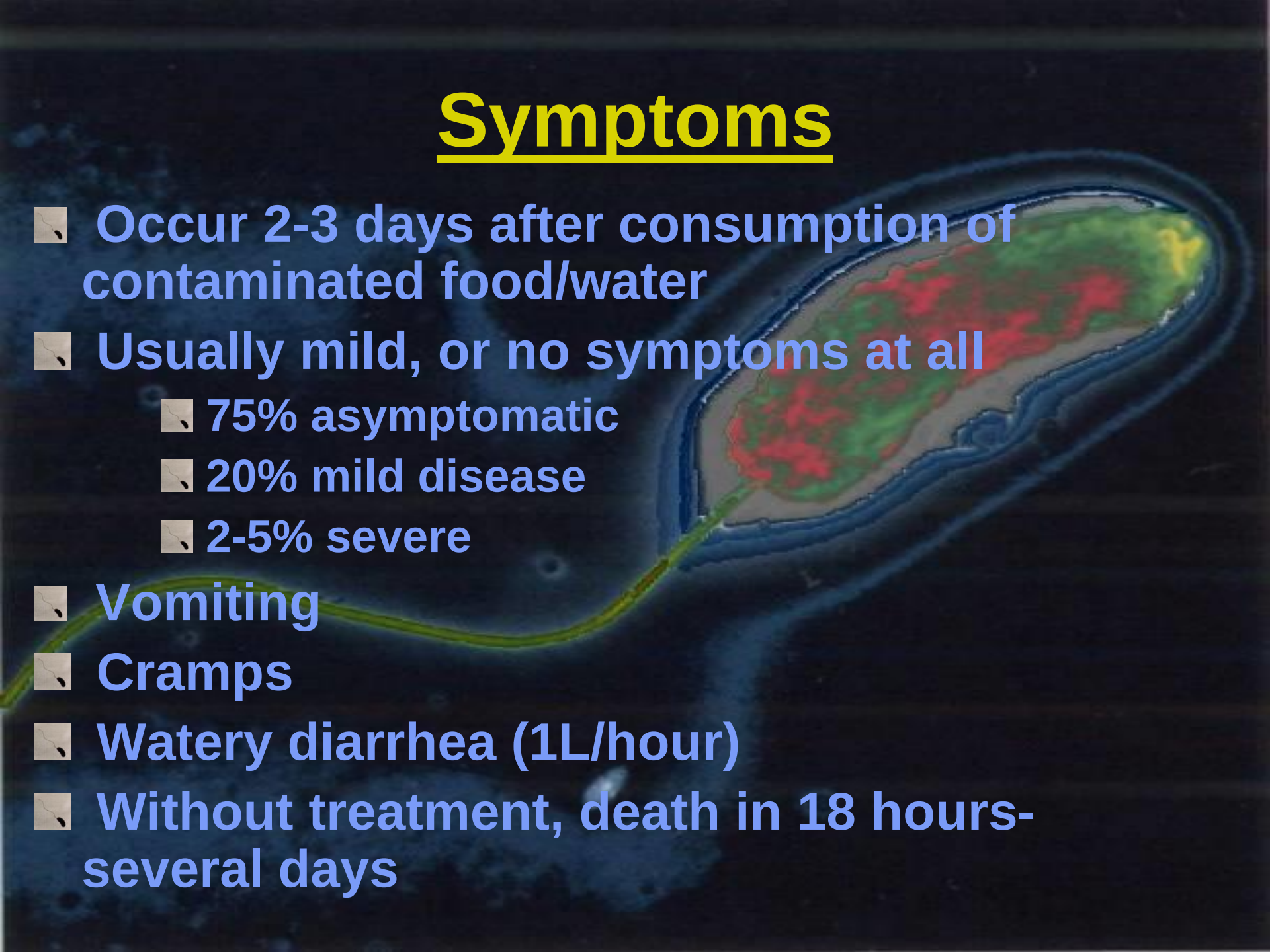
- Inactivates GTPase function of G-protein coupled receptors in intestinal cells
- G proteins stuck in “On” position
- 100 fold increase in cAMP
- Activation of ion channels
- Ions flow out and water follows
- animation

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

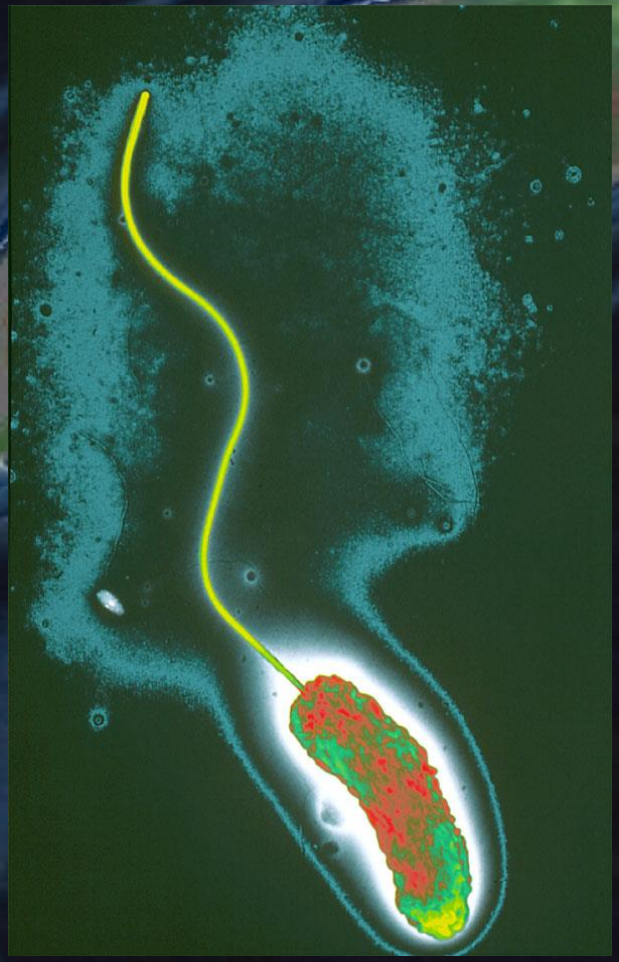
- Intravascular volume depletion
- Severe metabolic acidosis
- Hypokalemia
- Cardiac and renal failure
- Sunken eyes, decreased skin turgor
- Almost no urine production

Mortality Rate

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

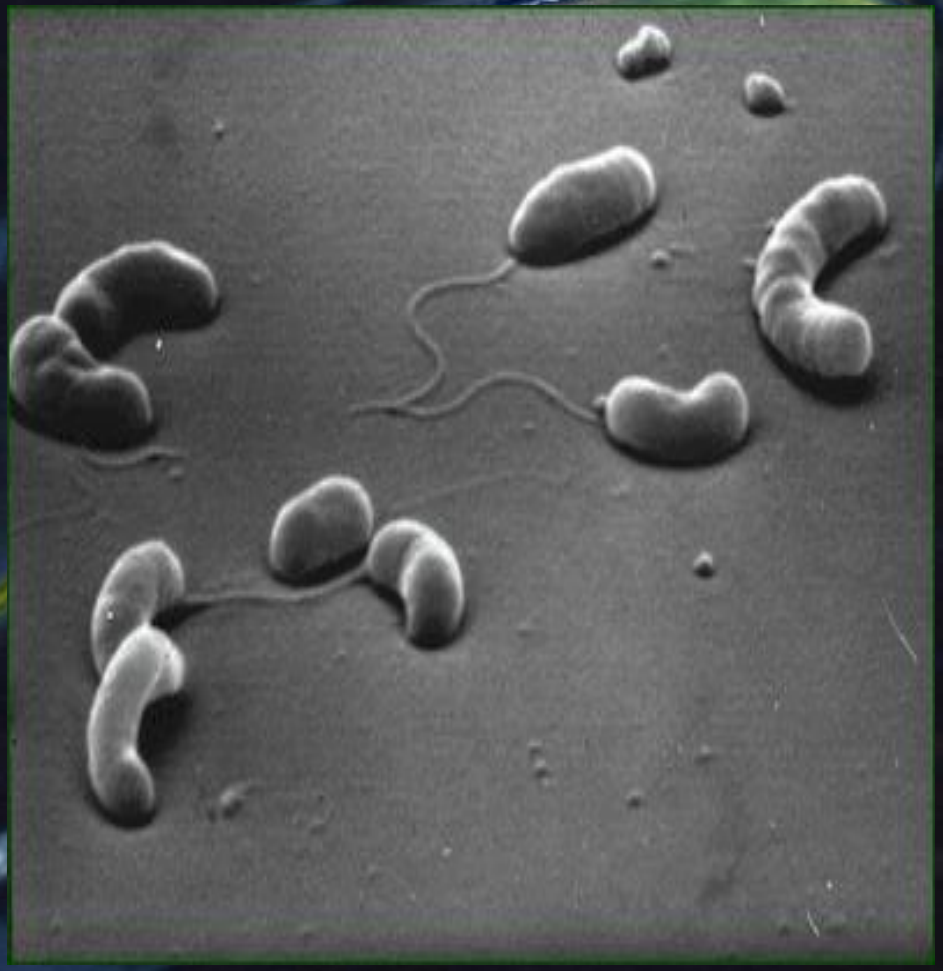
Molecular Biology of Vibrio cholerae

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

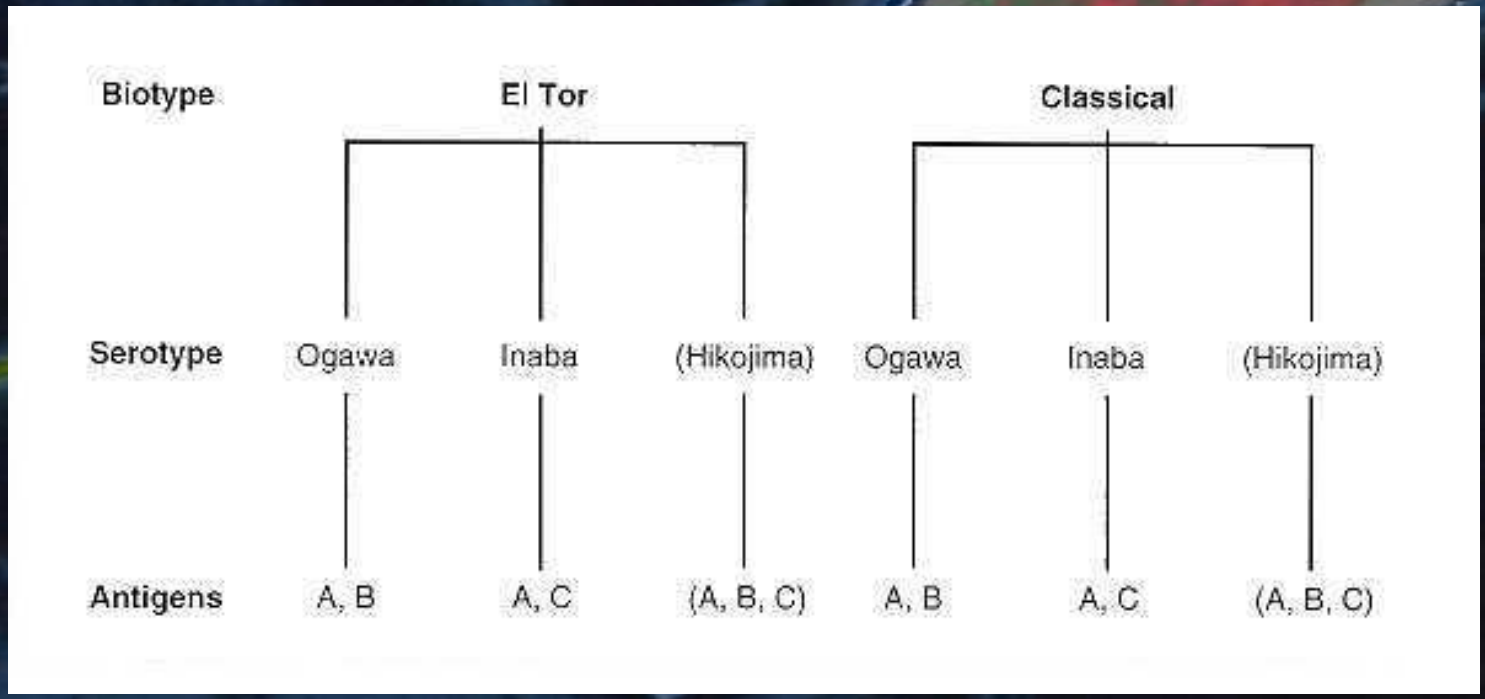


Identification

- 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.



Classification: O1 Antigen

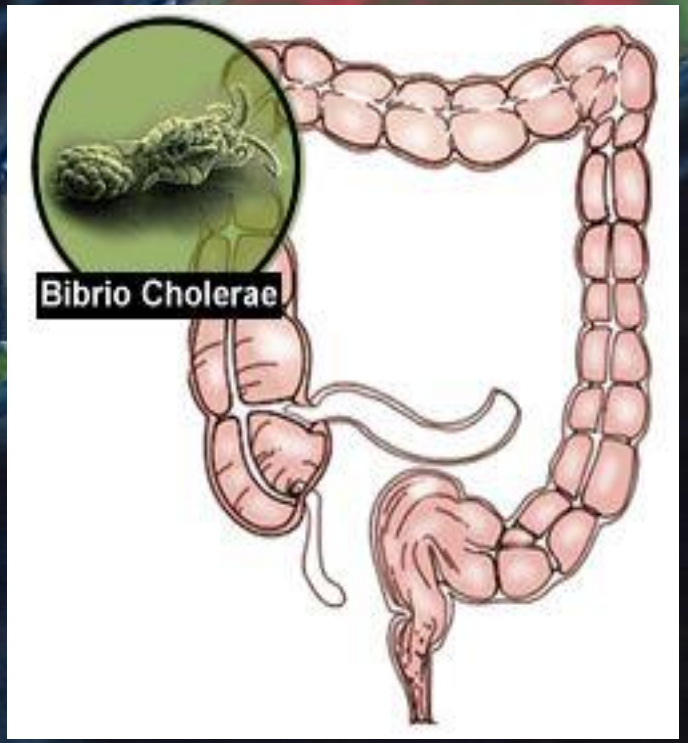


Classification: Other antigens

- 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.

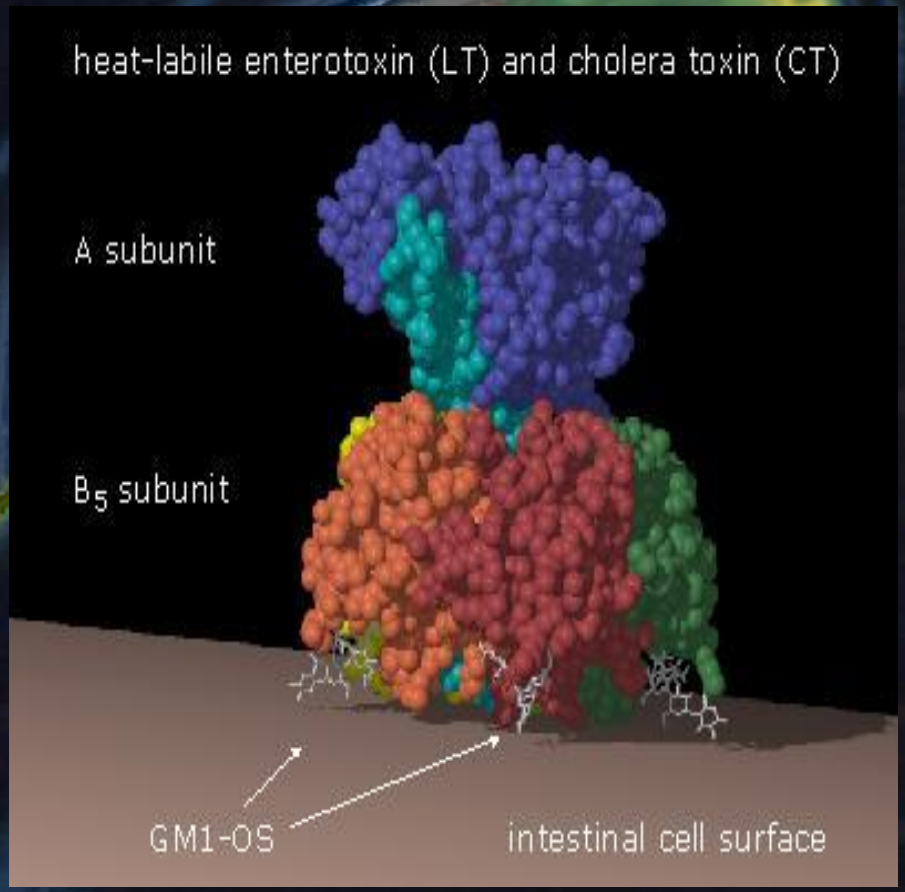
Pathogenesis: Overview

- 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: 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.

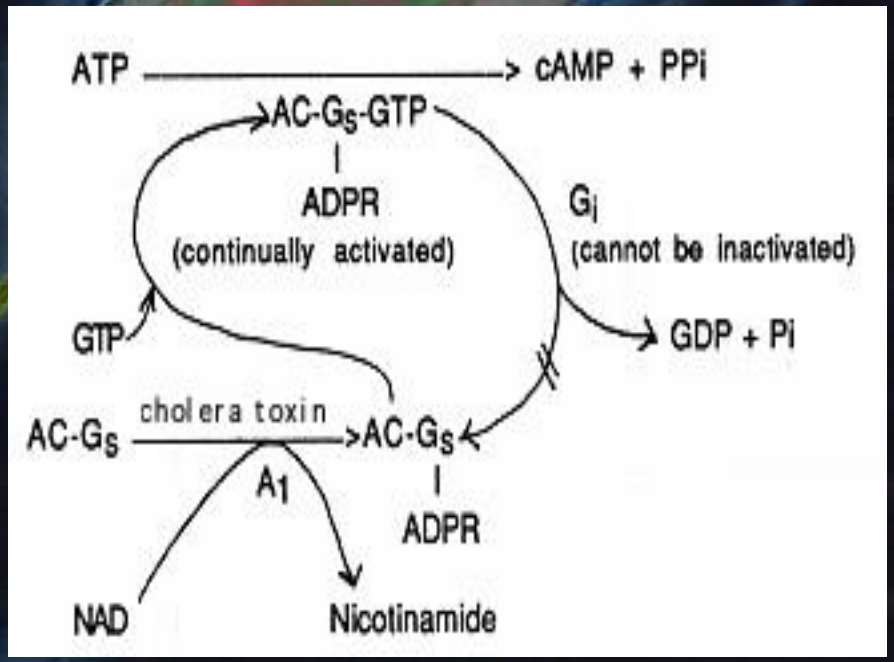


B subunits bind to GM1 Receptor

Pathogenesis: Mechanism of Action cont.

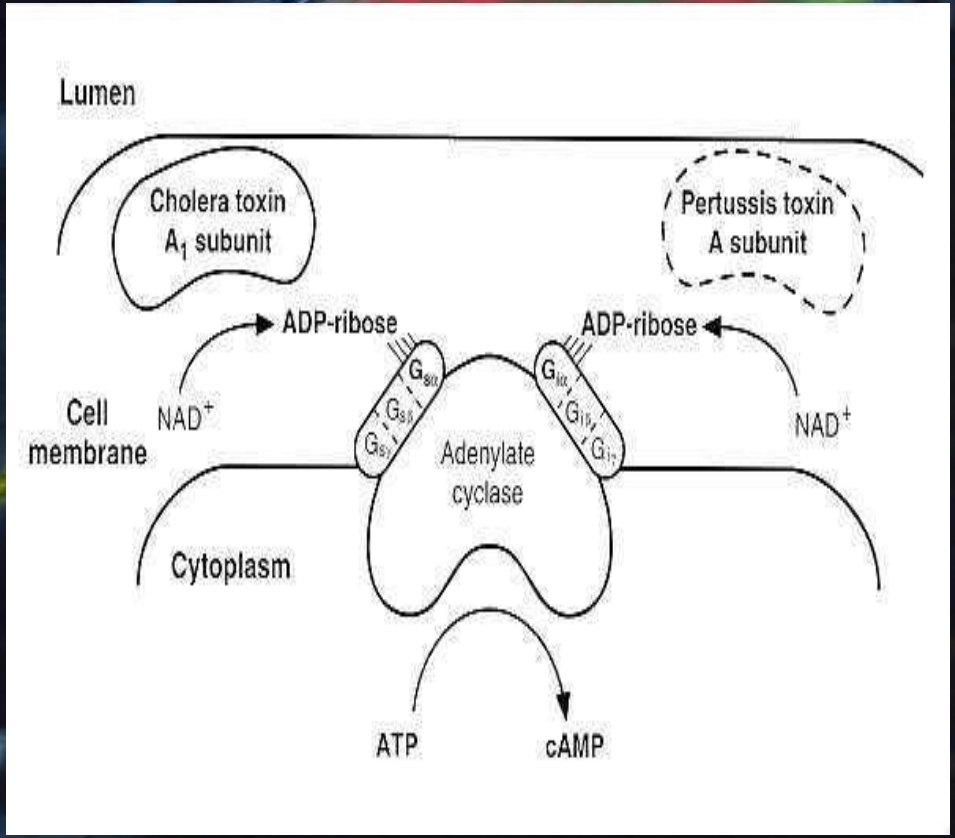
- Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system.
  - The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed.
- Since GTP hydrolysis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

CHOLERA



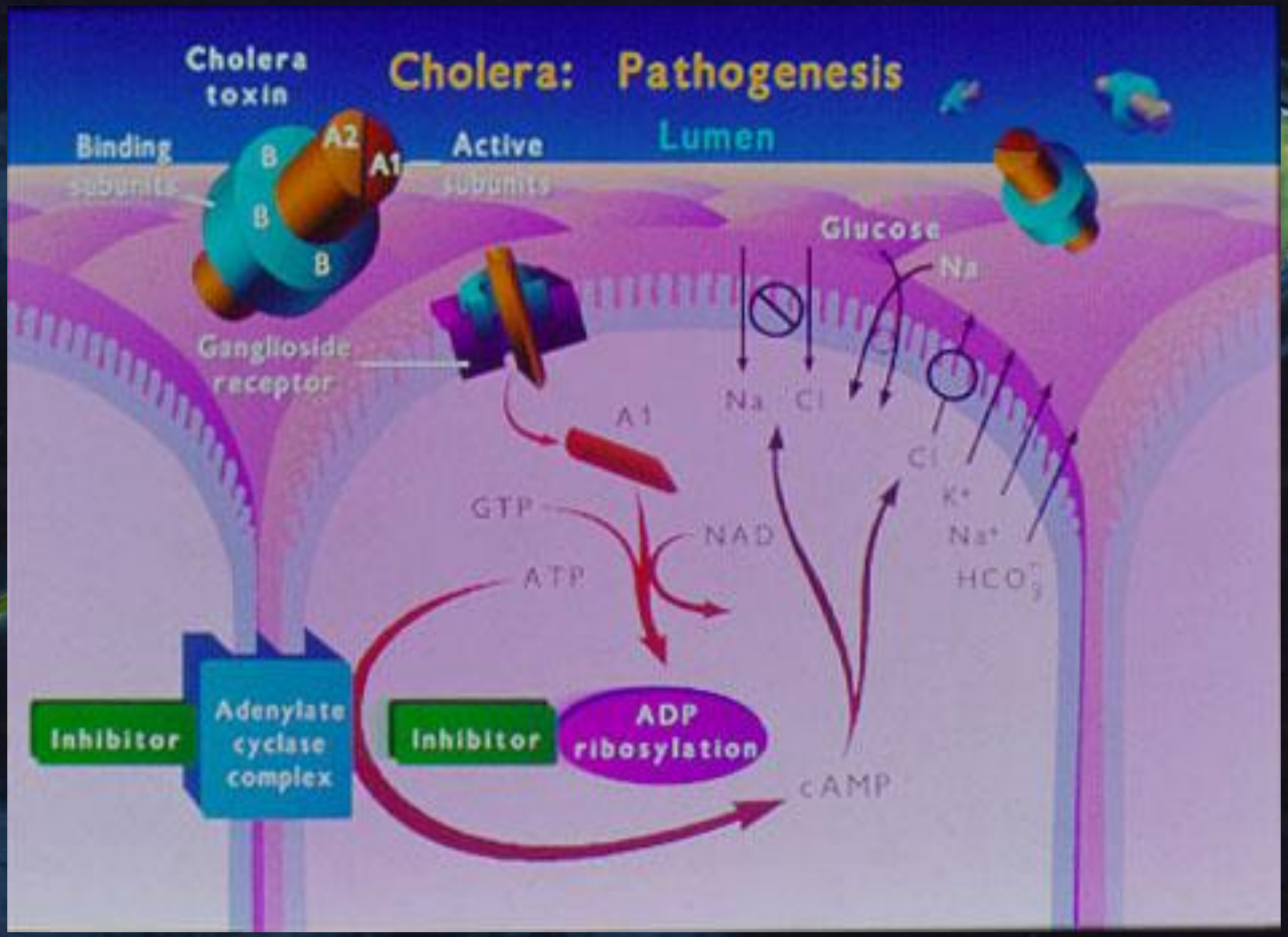
Pathogenesis: Mechanism of Action cont.

- 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.


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

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

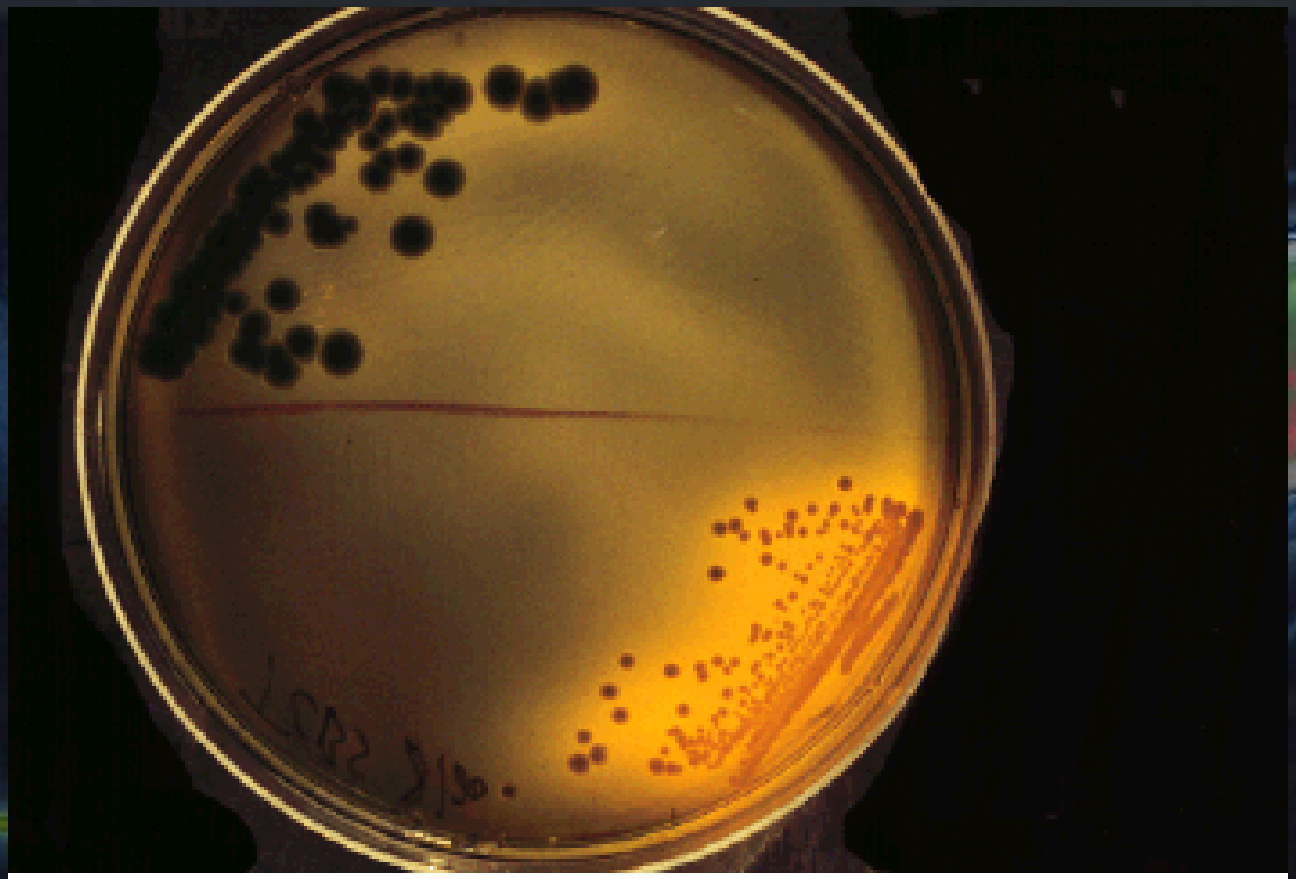
- No clinical manifestations help distinguish cholera from other causes of severe diarrhea:
  - Enterotoxigenic e. coli
  - Viral gastroenteritis
  - Bacterial food poisoning

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 Thiosulphate bile salt 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: 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

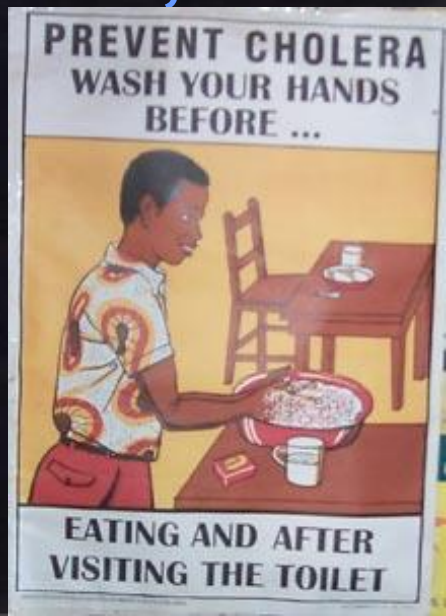
- Ringer's Lactate
  - Commercial product
  - Has necessary concentrations of electrolytes
- Alternative options
  - Saline
  - Sugar and water
  - Do not replace potassium, sodium, bicarbonate

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

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

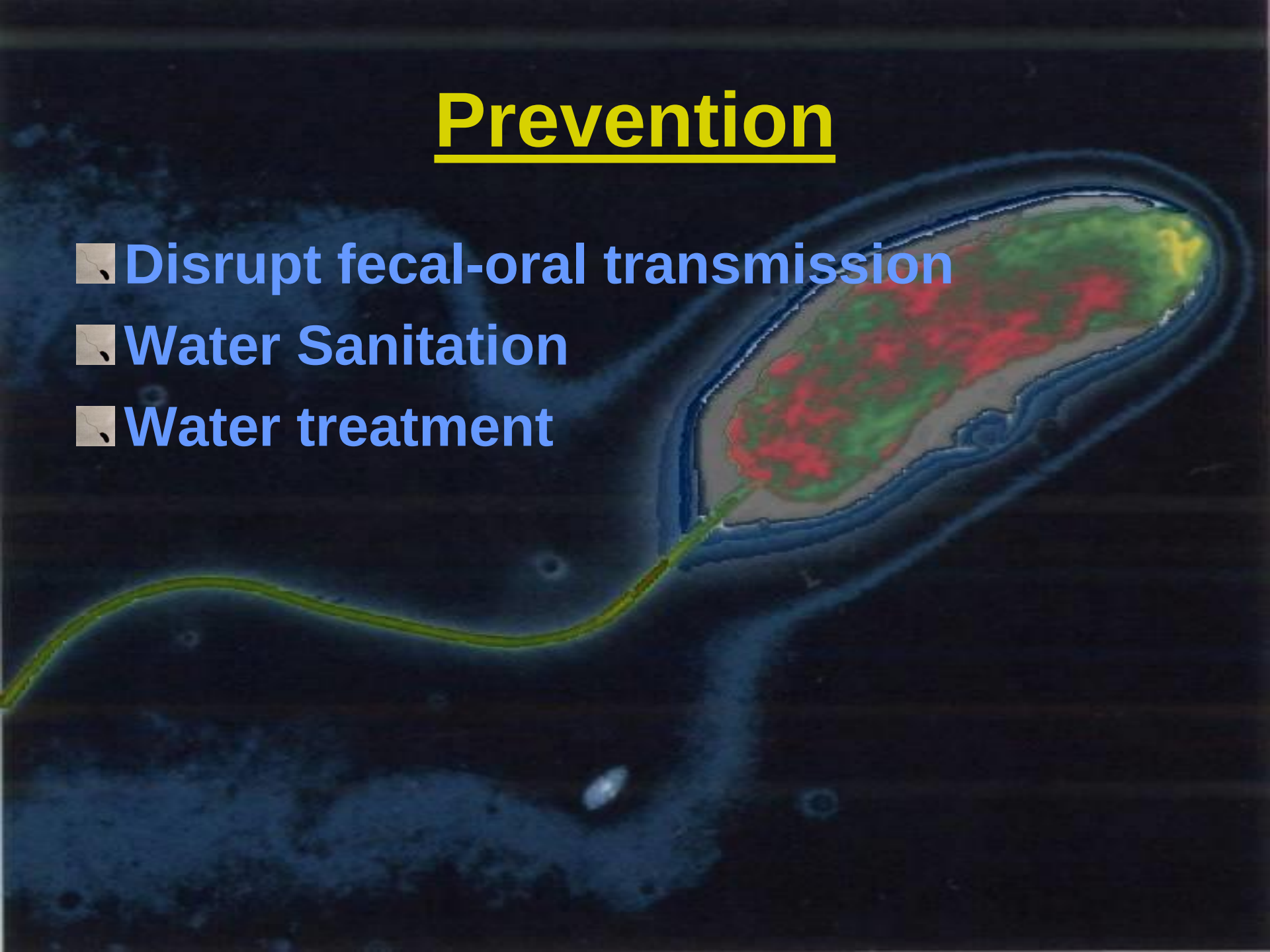
Killed Whole-cell Vaccines: Disadvantages

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

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

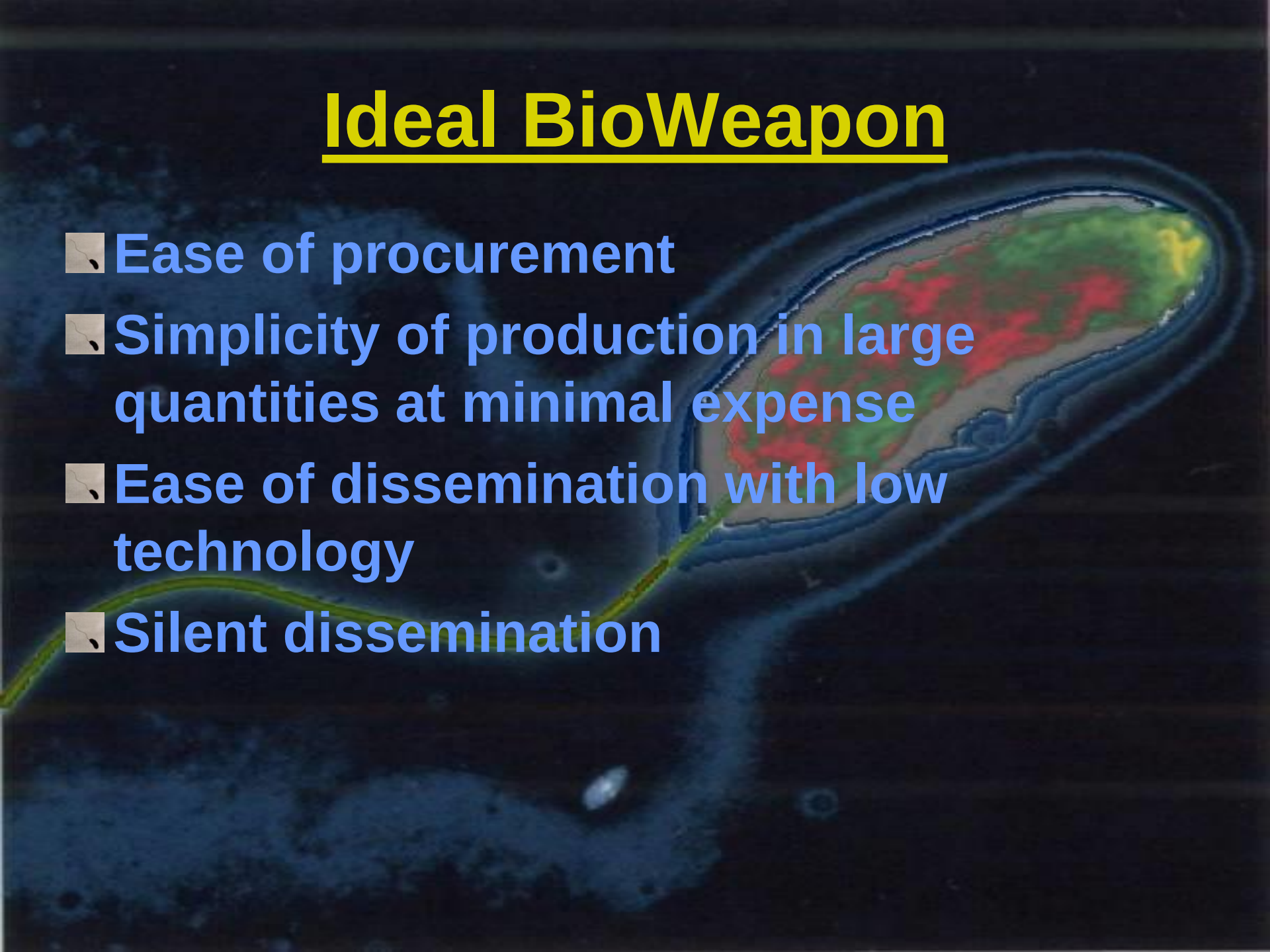
Prevention



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

Ideal BioWeapon

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



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

- 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