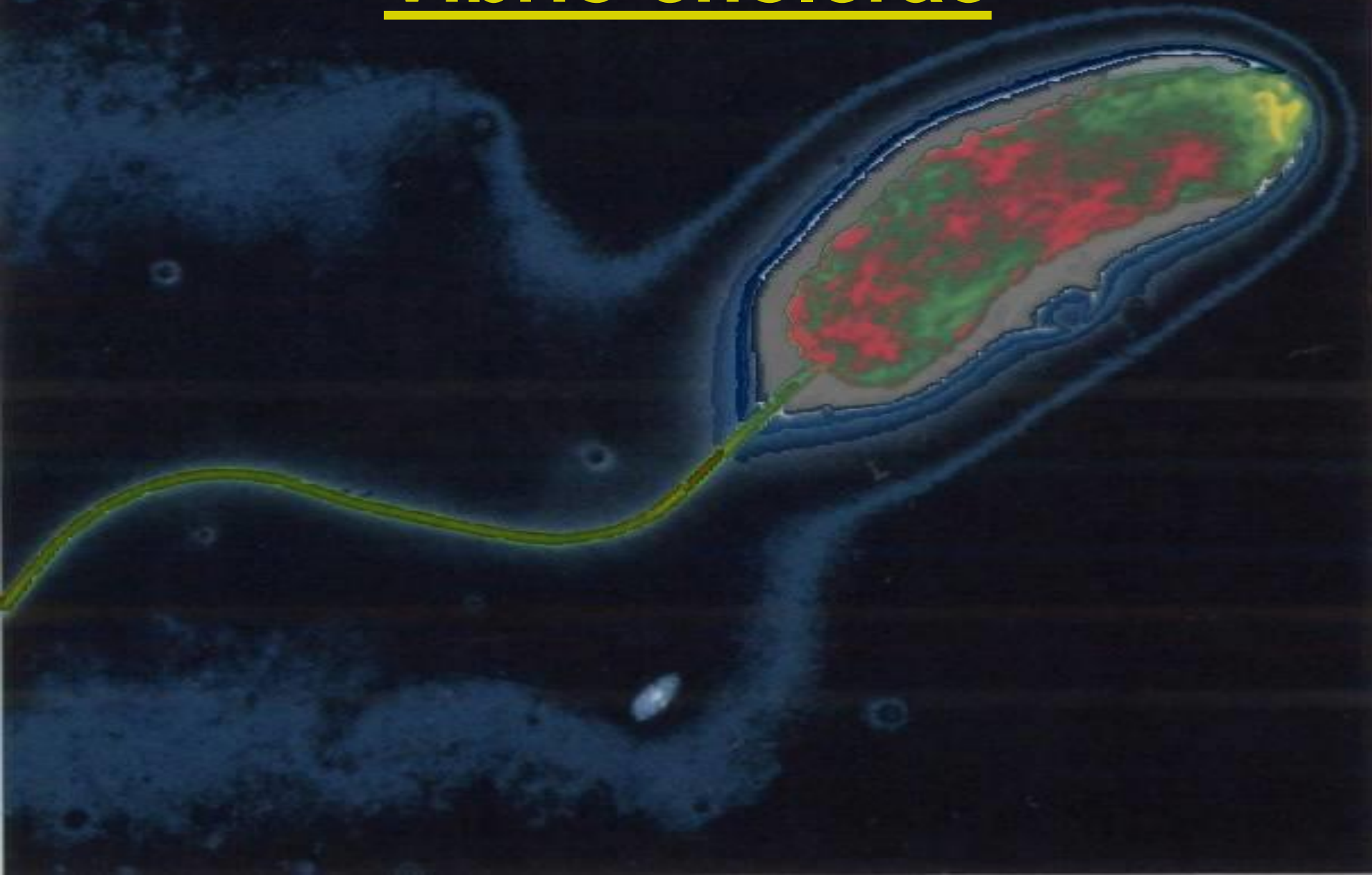


Vibrio cholerae



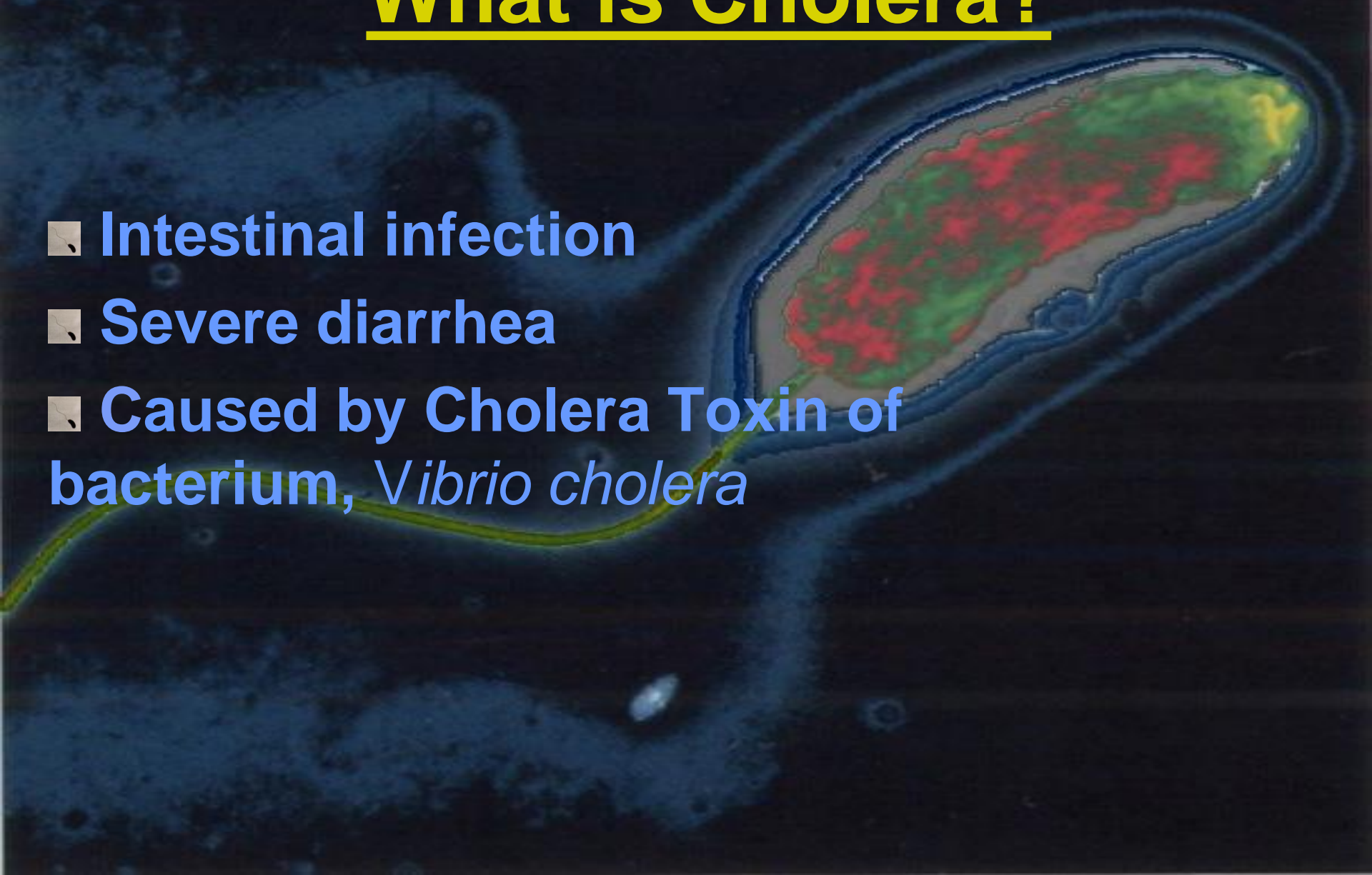
Vibrio cholerae

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

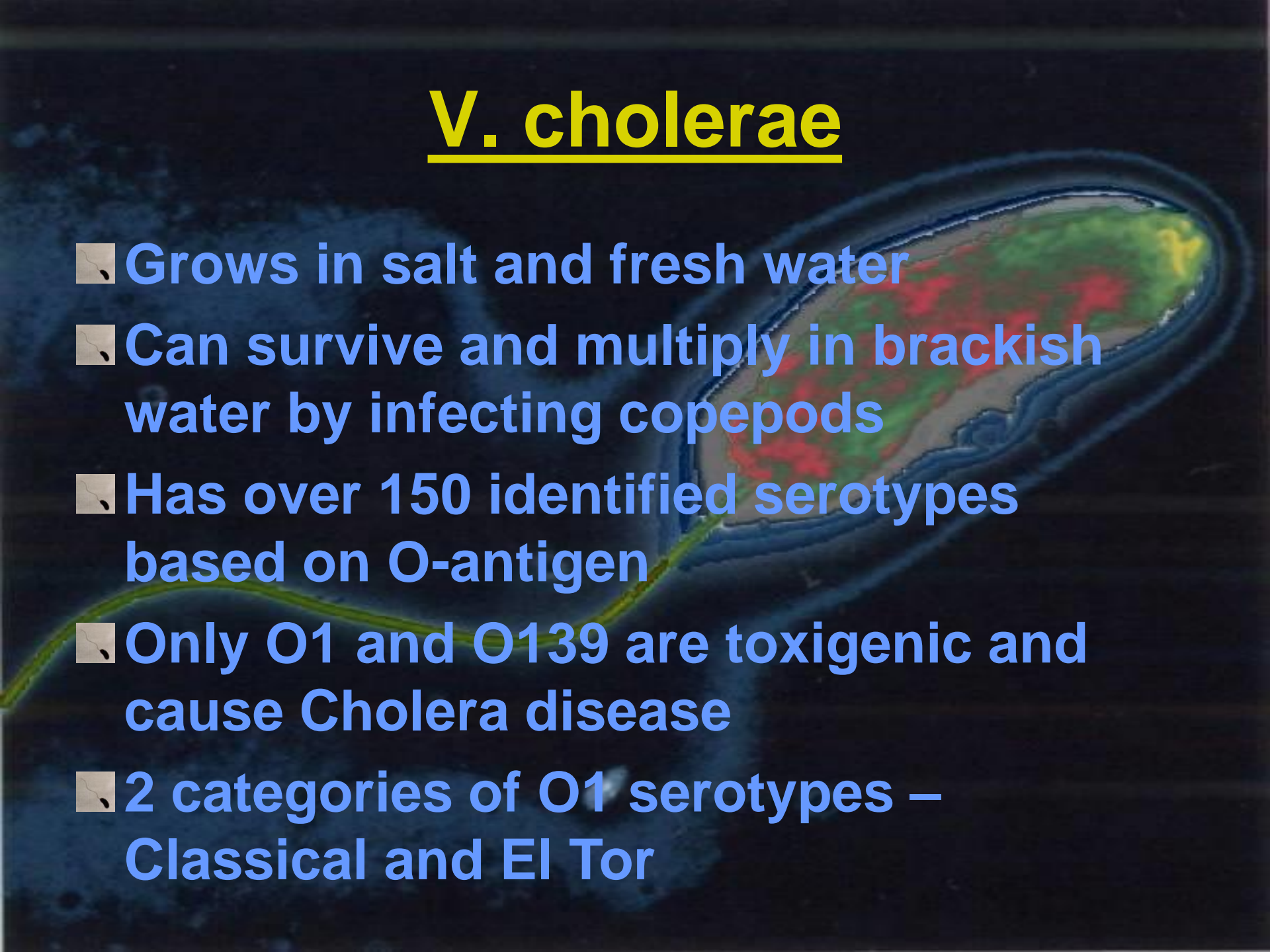


What is Cholera?

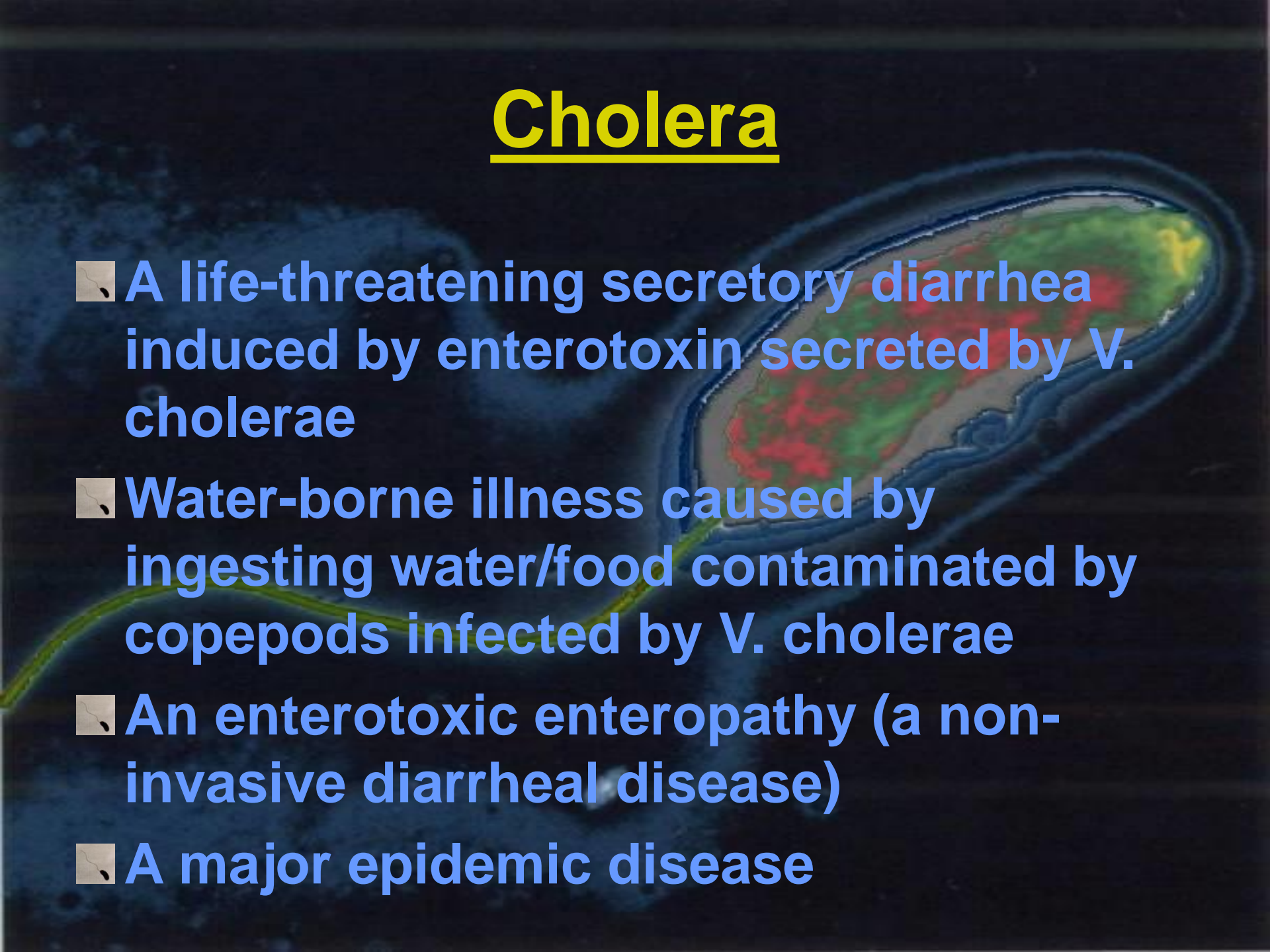
- Intestinal infection
- Severe diarrhea
- Caused by Cholera Toxin of bacterium, *Vibrio cholera*



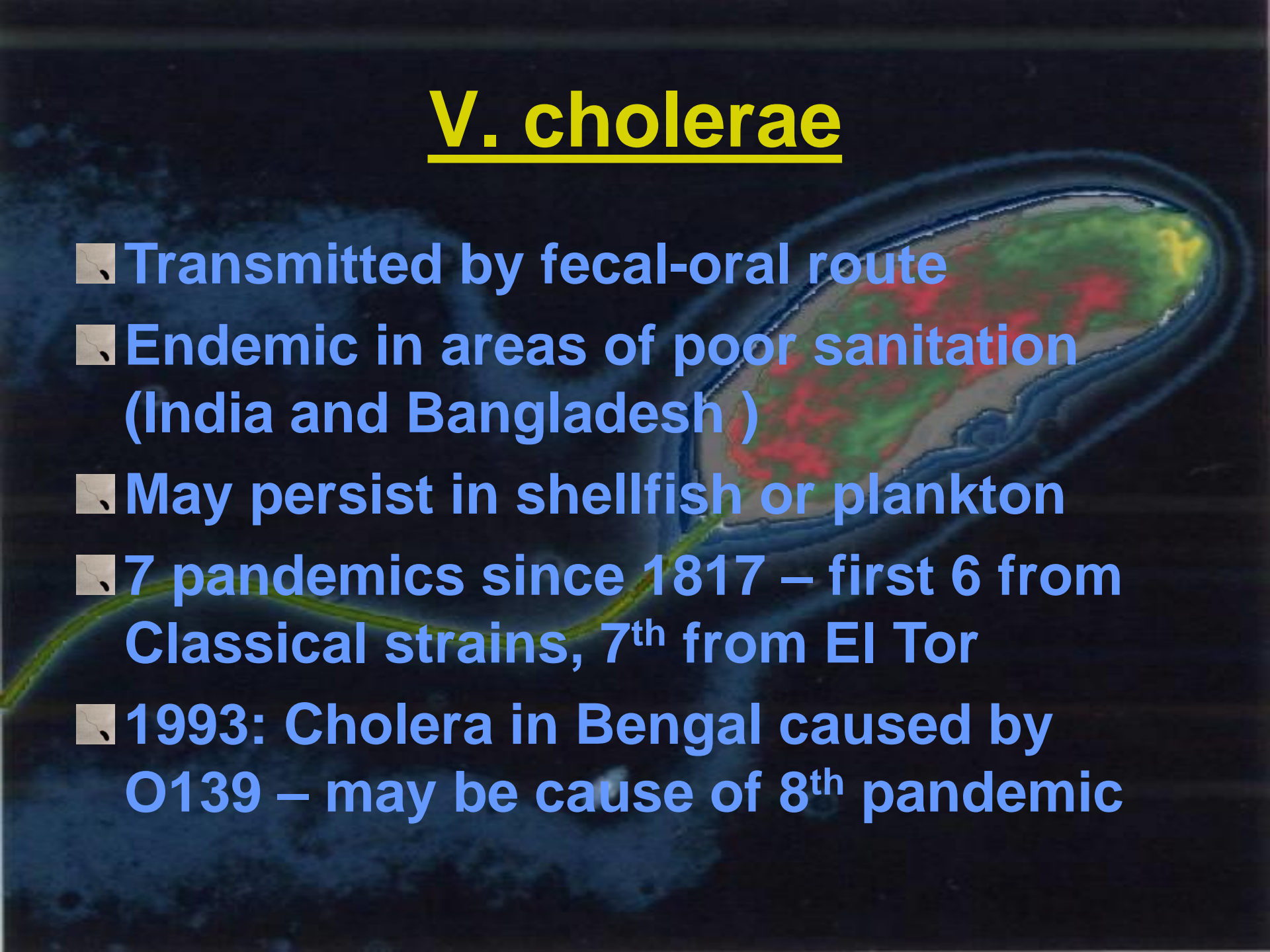
V. cholerae

- 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
- 
- A large, detailed microscopic image of a Vibrio cholerae bacterium is positioned on the right side of the slide. The bacterium is comma-shaped with a long, thin flagellum extending from one end. The cell body is filled with internal structures, and the flagellum is shown in a curved path across the lower left portion of the slide.

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
- 

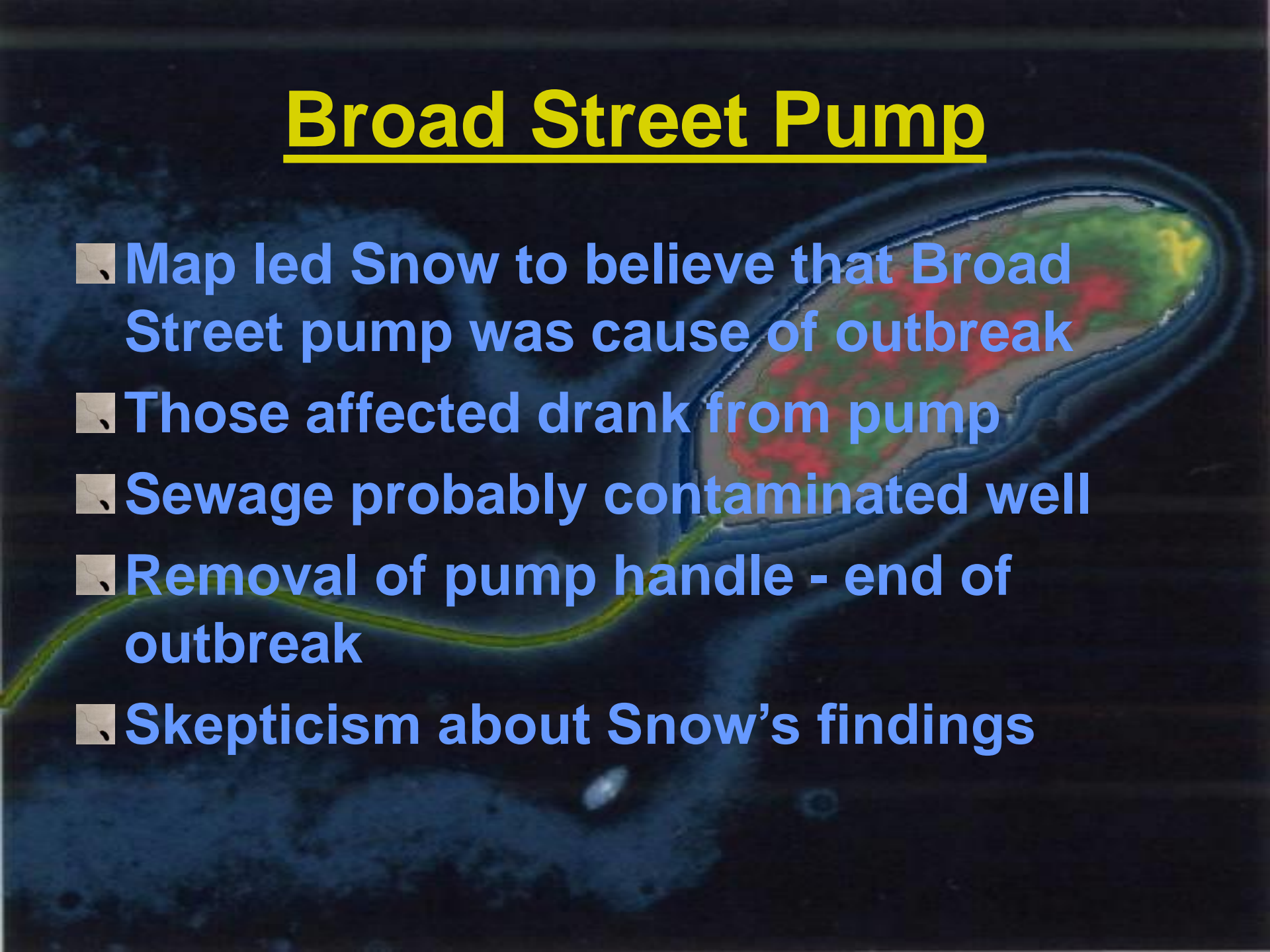
John Snow – Record of Locations of Cholera Cases in London, 1854



X 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 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*

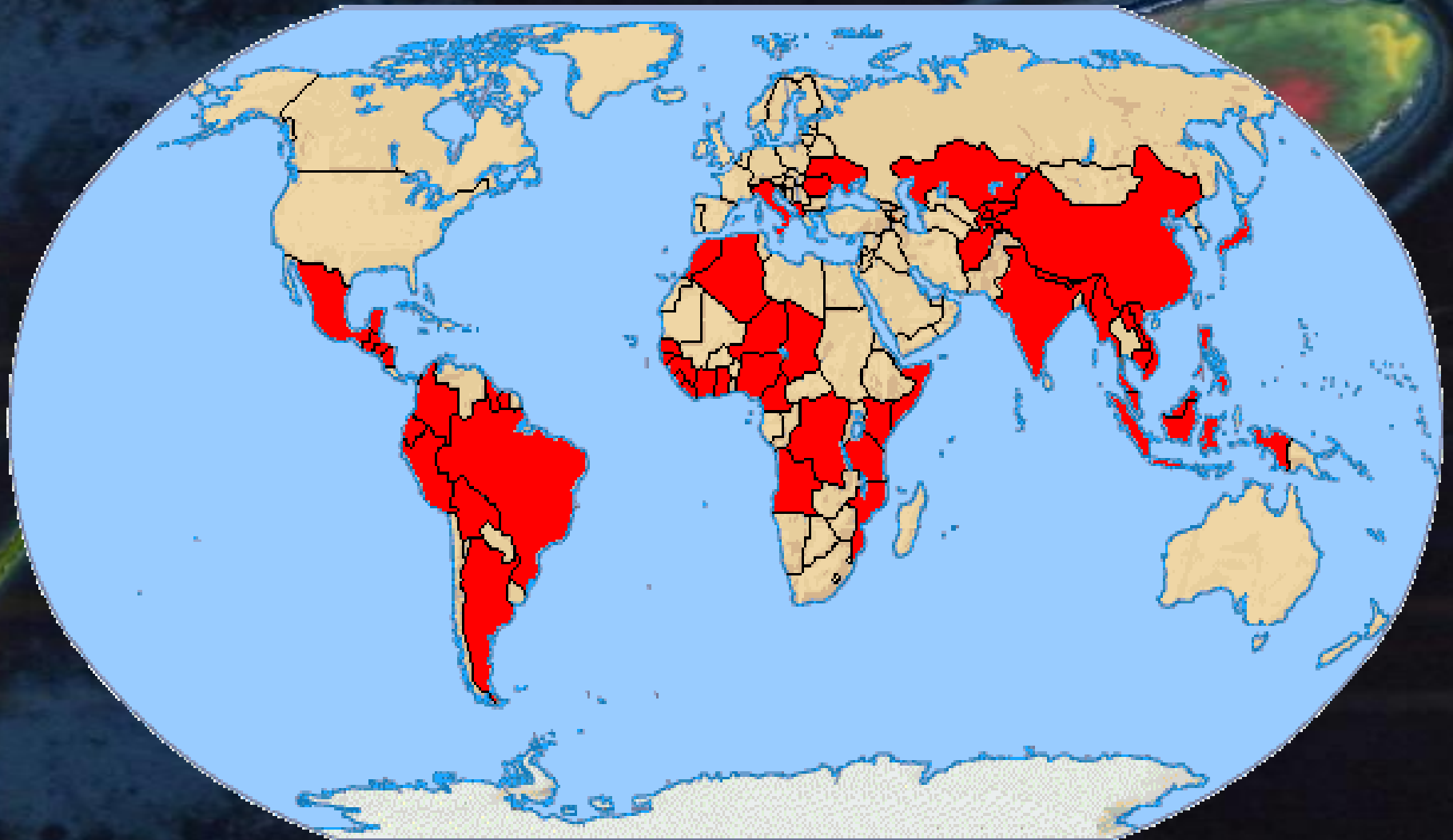
| <u>Continent</u> | <u>Total Cases</u> | <u>Total Deaths</u> |
|------------------|--------------------|---------------------|
| Africa | 118,932 | 4,610 |
| America(s) | 3,101 | 40 |
| Asia** | 11,246 | 232 |
| Europe | 35 | 0 |
| Oceania | 3,757 | 26 |
| Total | 137,071 | 4,908 |

*Data published in August, 2001

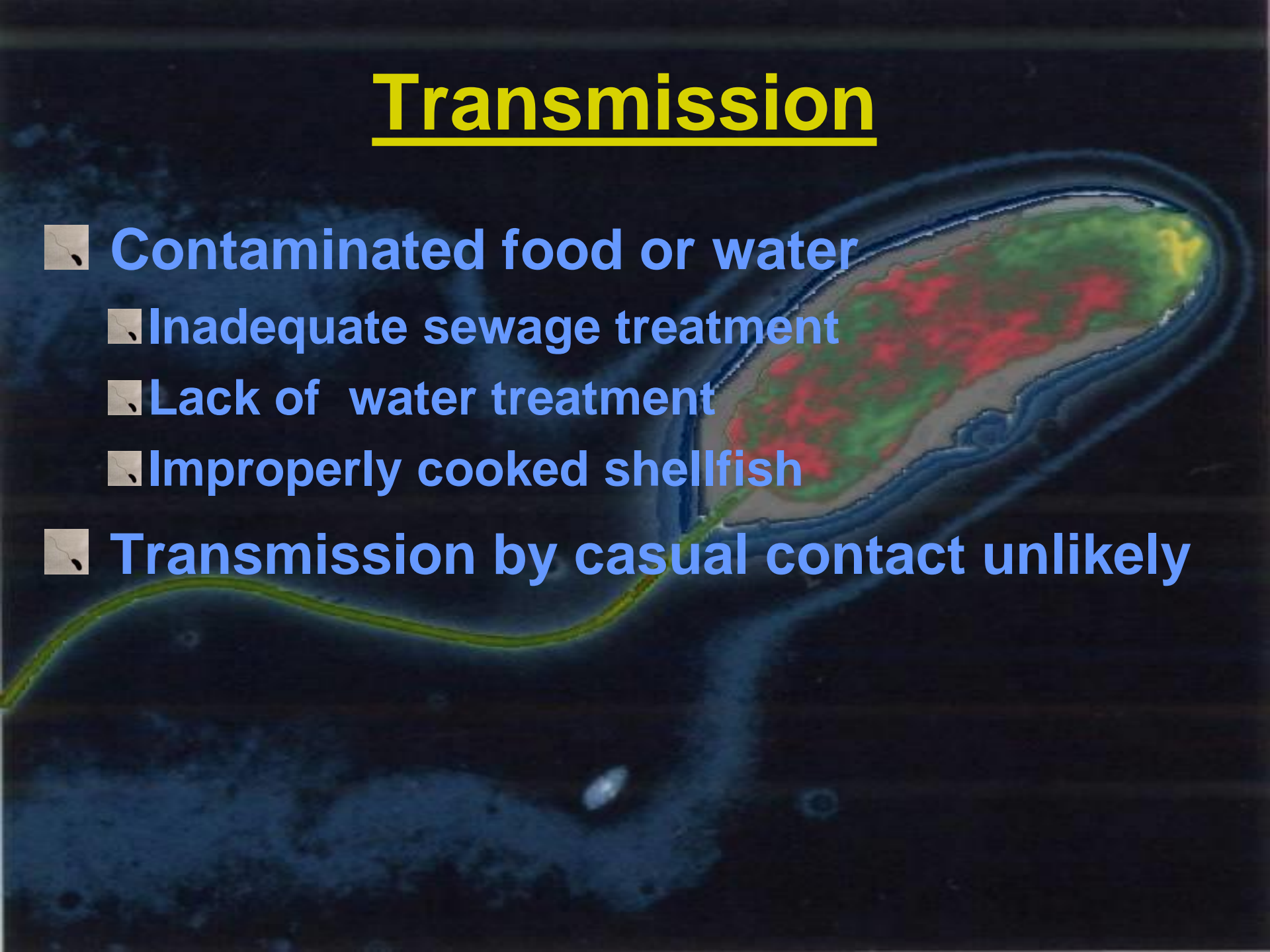
**Does not include Bangladesh, Pakistan and other countries

Source: World Health Organization


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
- 
- An aerial photograph of a coastal region. A large, irregularly shaped landmass is visible, colored in shades of green and red, suggesting different vegetation or land use. The landmass is surrounded by a dark blue body of water. A thin, winding road or path is visible on the landmass, extending from the bottom left towards the center. The overall scene is captured from a high angle, providing a clear view of the coastline and surrounding waters.

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

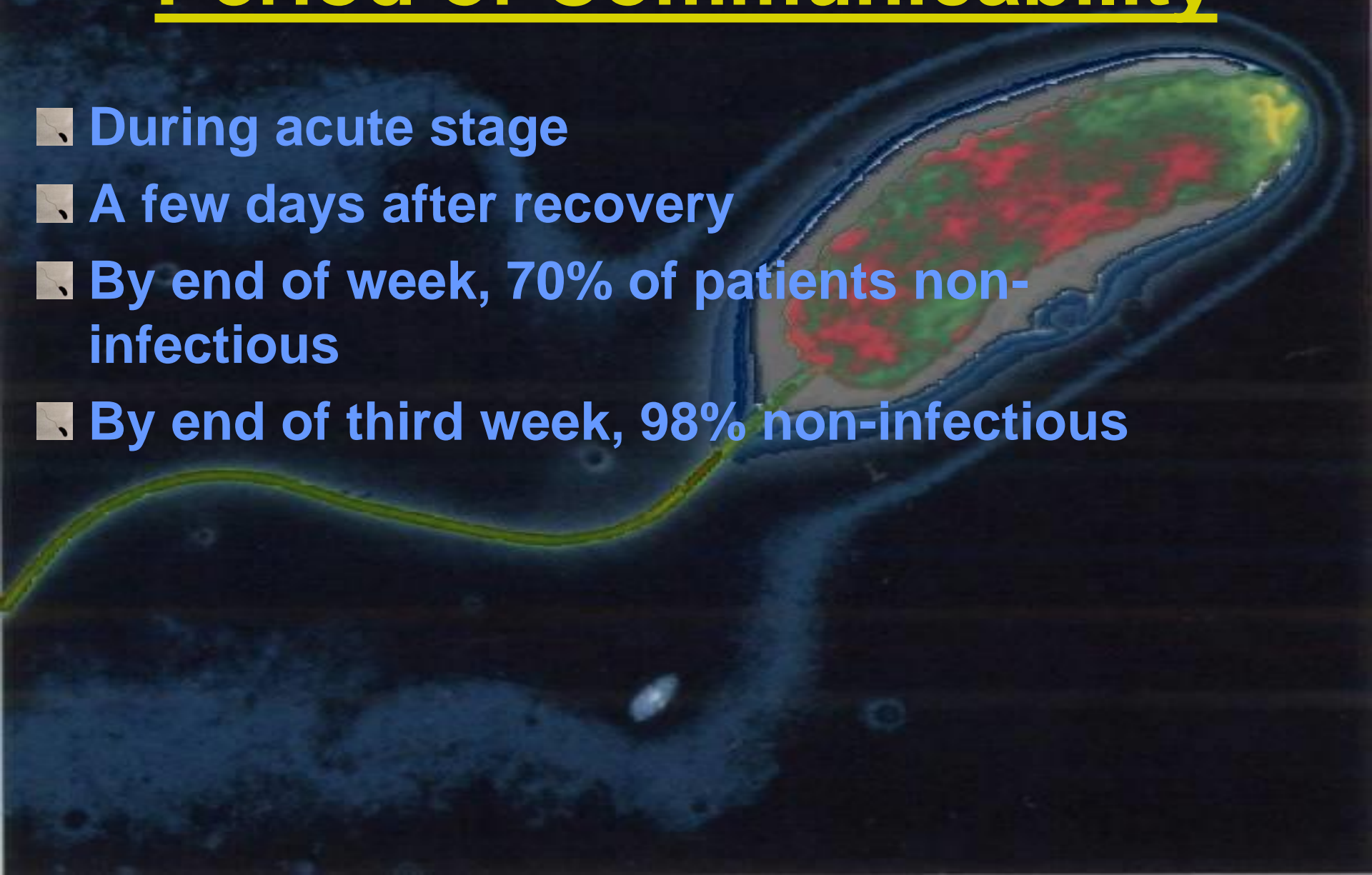
People Most at Risk

- 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

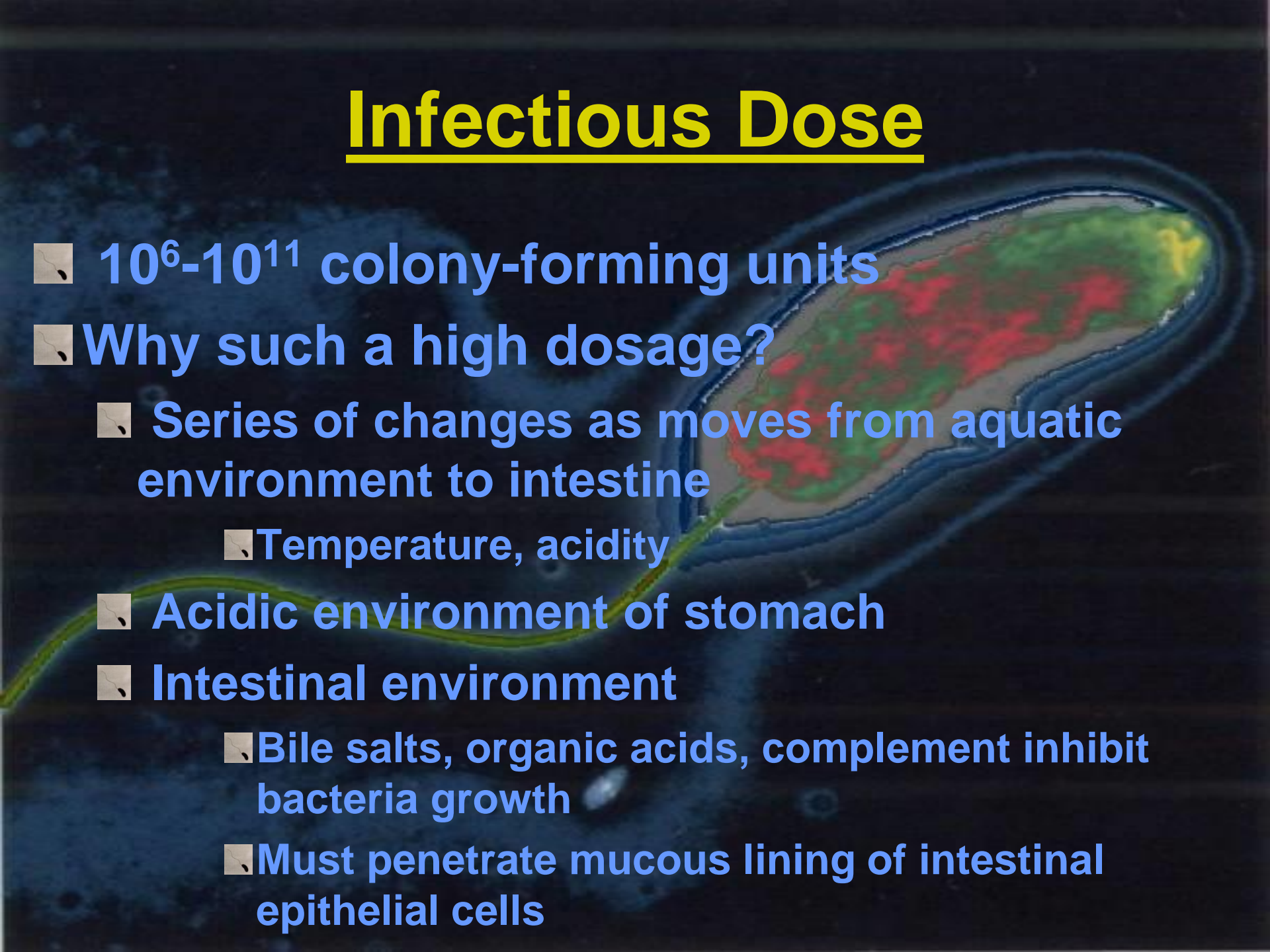
- Ranges from a few hours to 5 days
- Average is 1-3 days
- Shorter incubation period:
 - High gastric pH (from use of antacids)
 - Consumption of high dosage of cholera



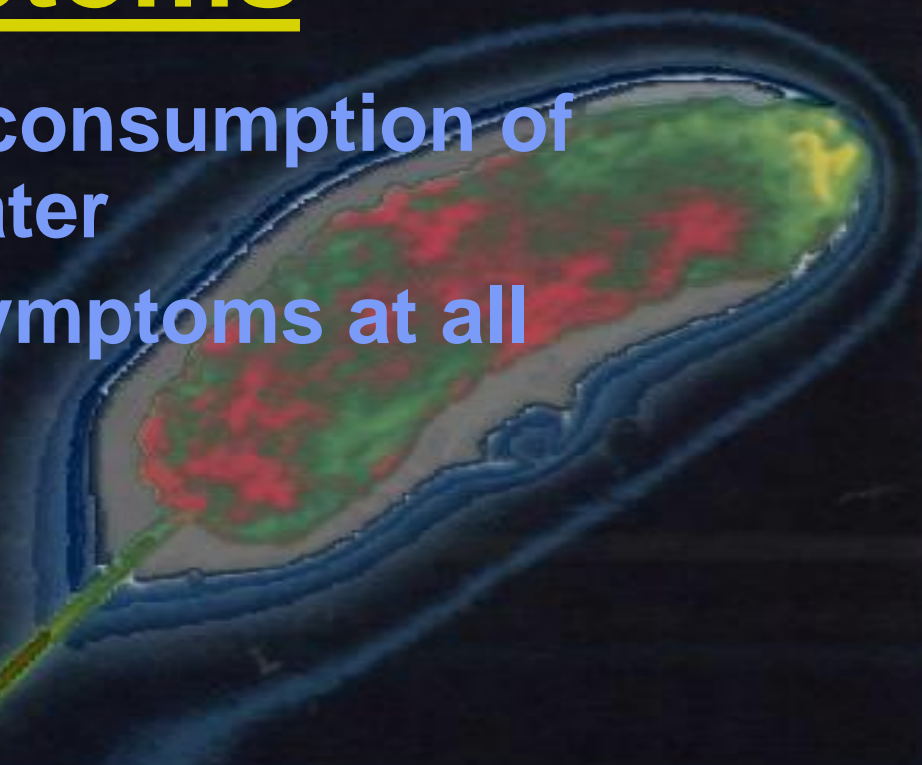
How Does Cholera Toxin Work?

- 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
- 
- A microscopic image of a bacterium, likely a rod-shaped organism, with a prominent green filament extending from one end. The bacterium has a multi-layered structure, possibly a cell wall or capsule, and internal structures are visible in shades of red and green. The background is dark and grainy, typical of a micrograph.

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
- 

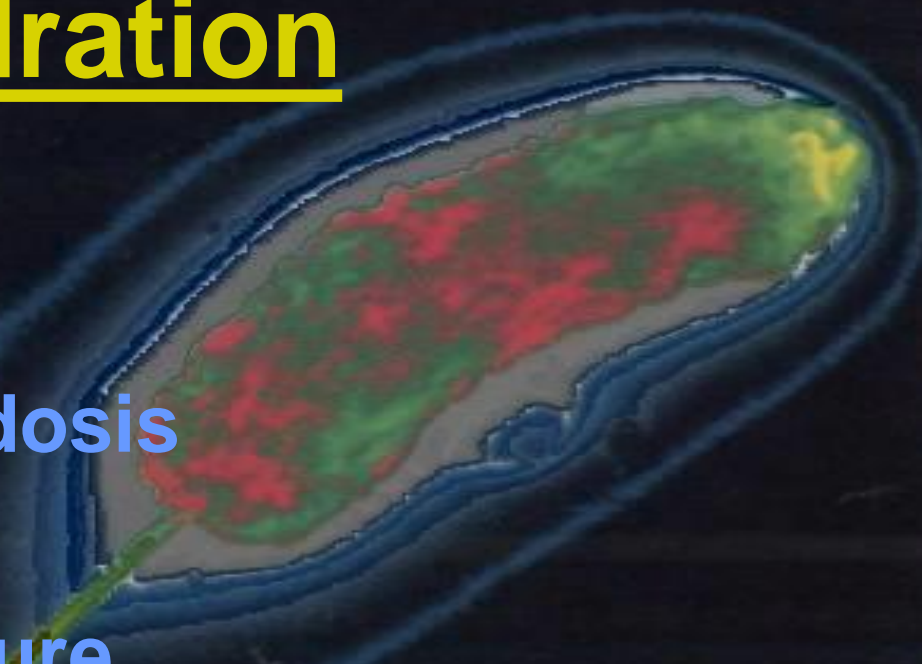
Cholera Gravis

- 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



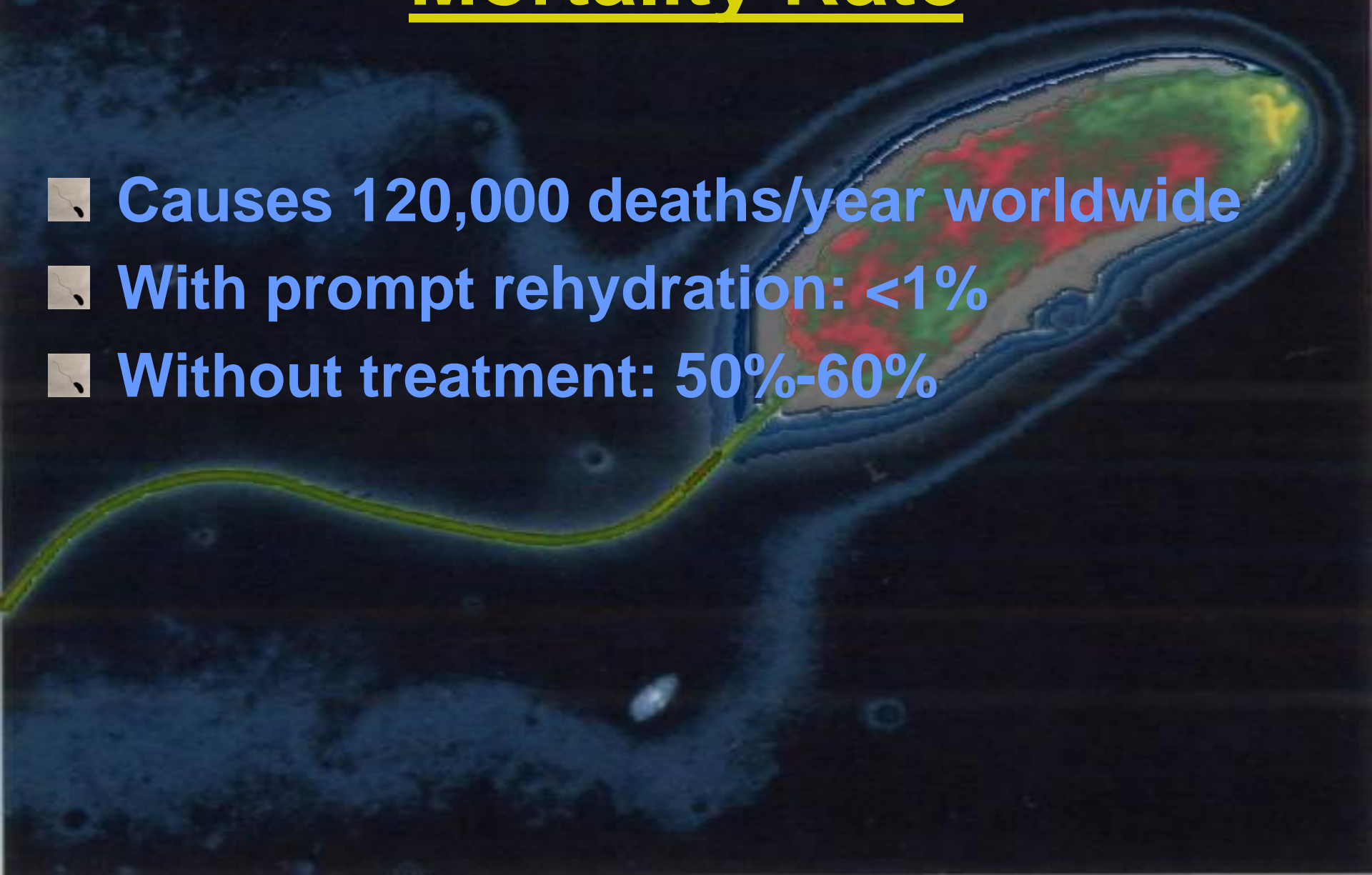
Consequences of Severe Dehydration

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



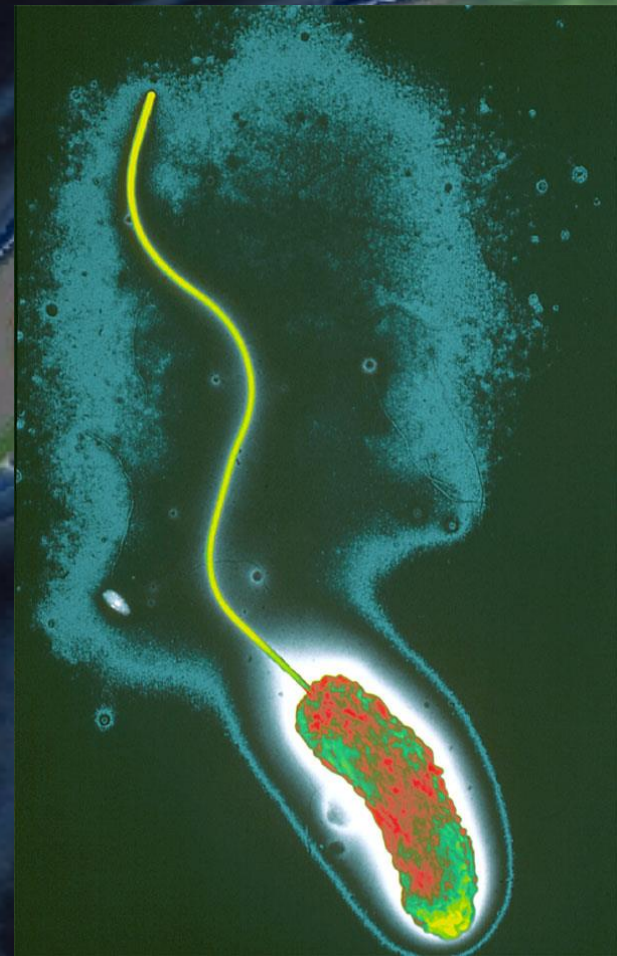
Mortality Rate

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



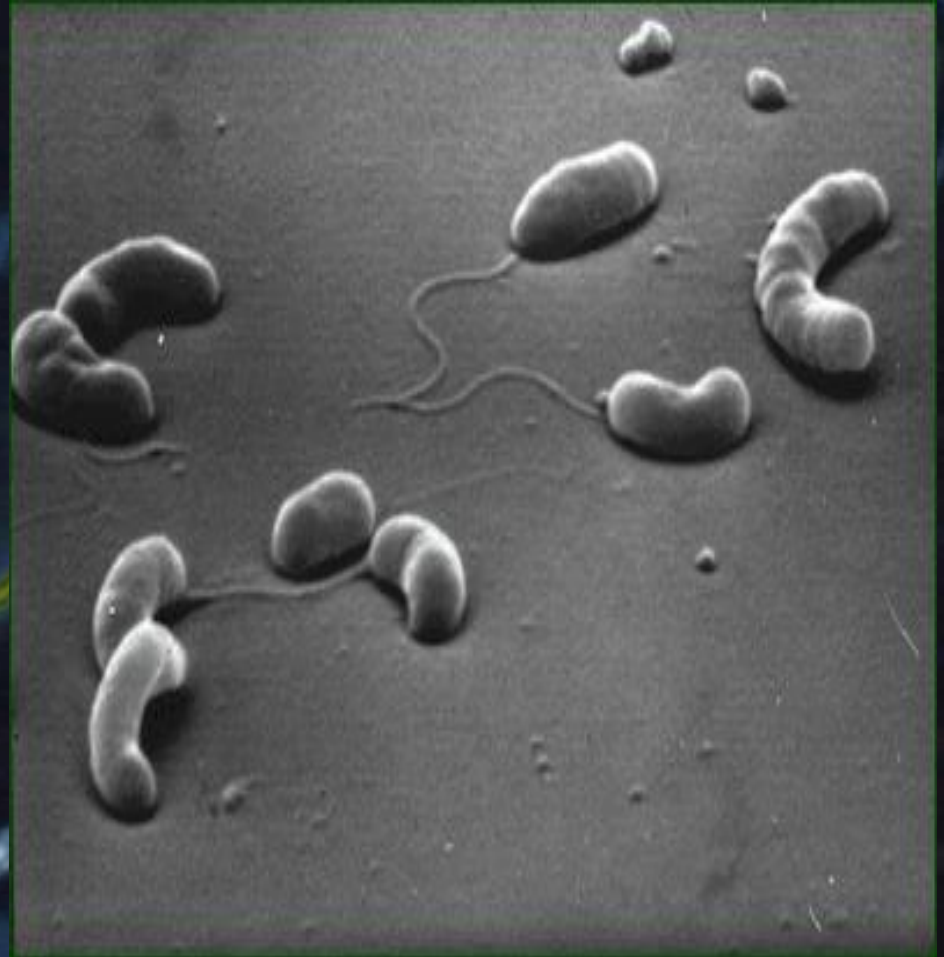
Molecular Biology of Vibrio cholerae

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

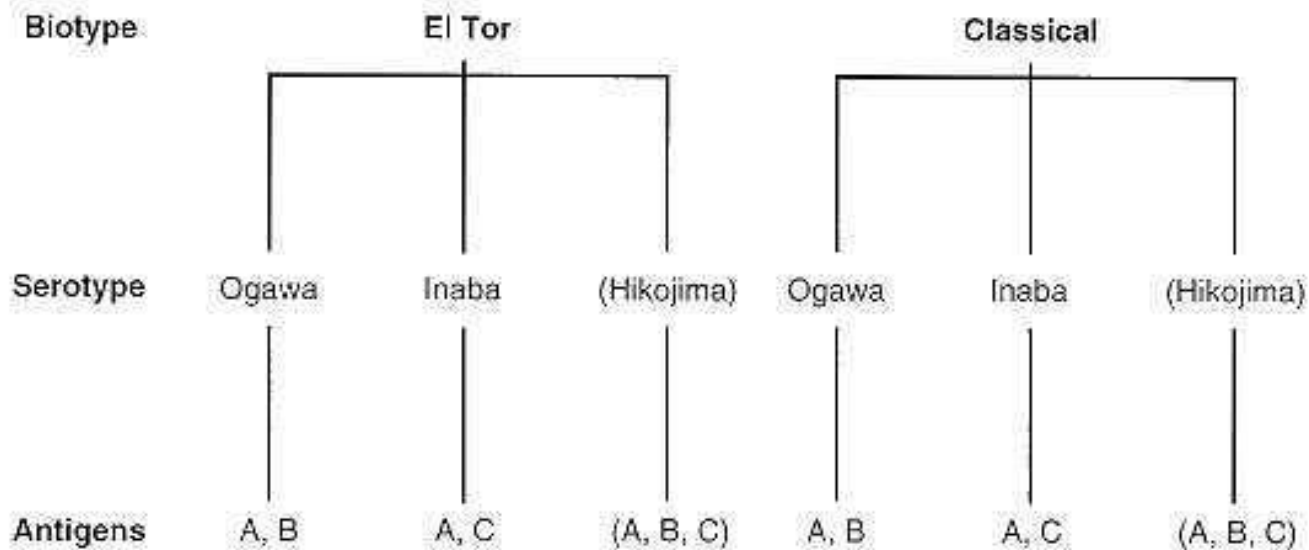


Identification

- 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



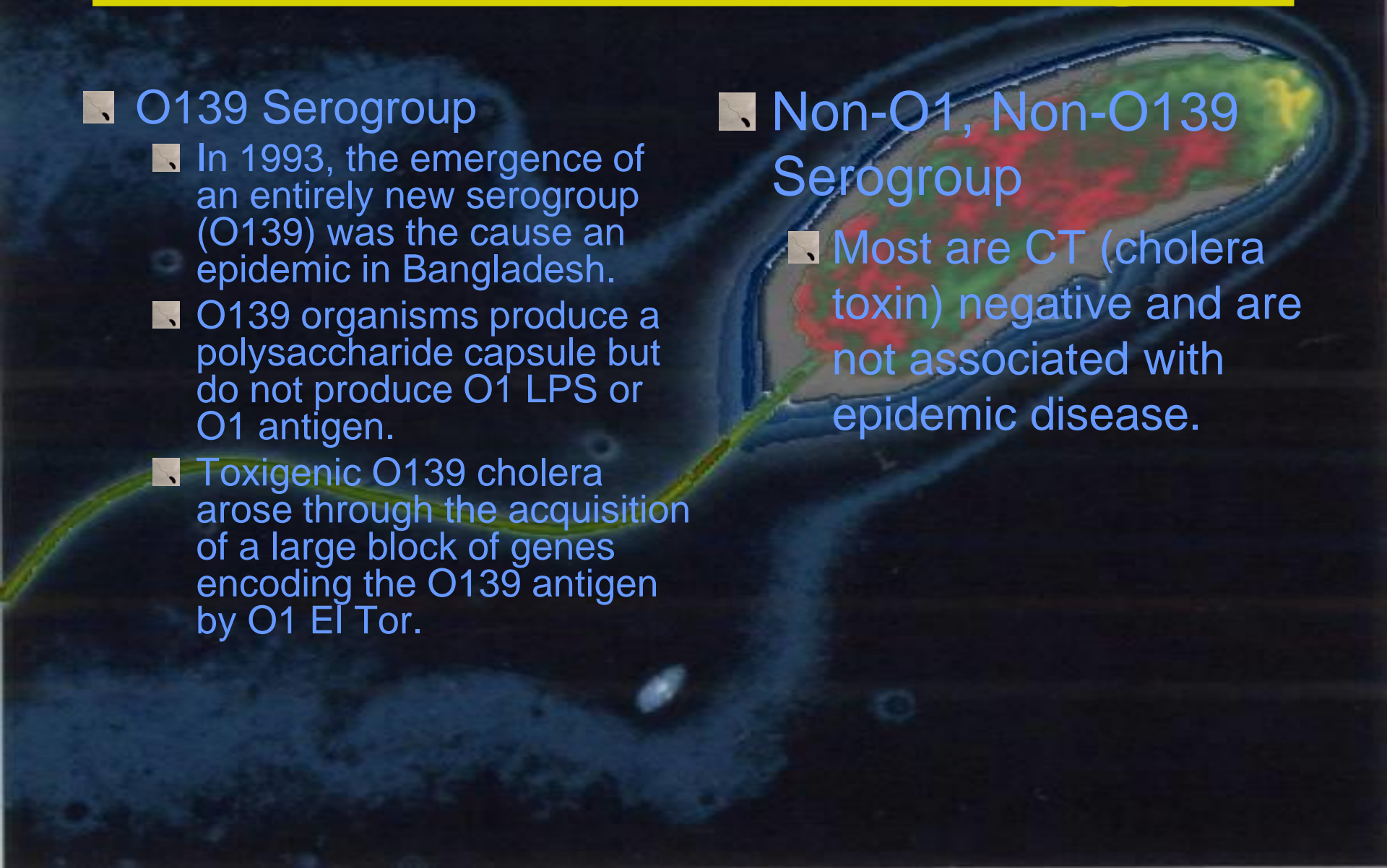
Classification: Other antigens

■ O139 Serogroup

- In 1993, the emergence of an entirely new serogroup (O139) was the cause of 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.



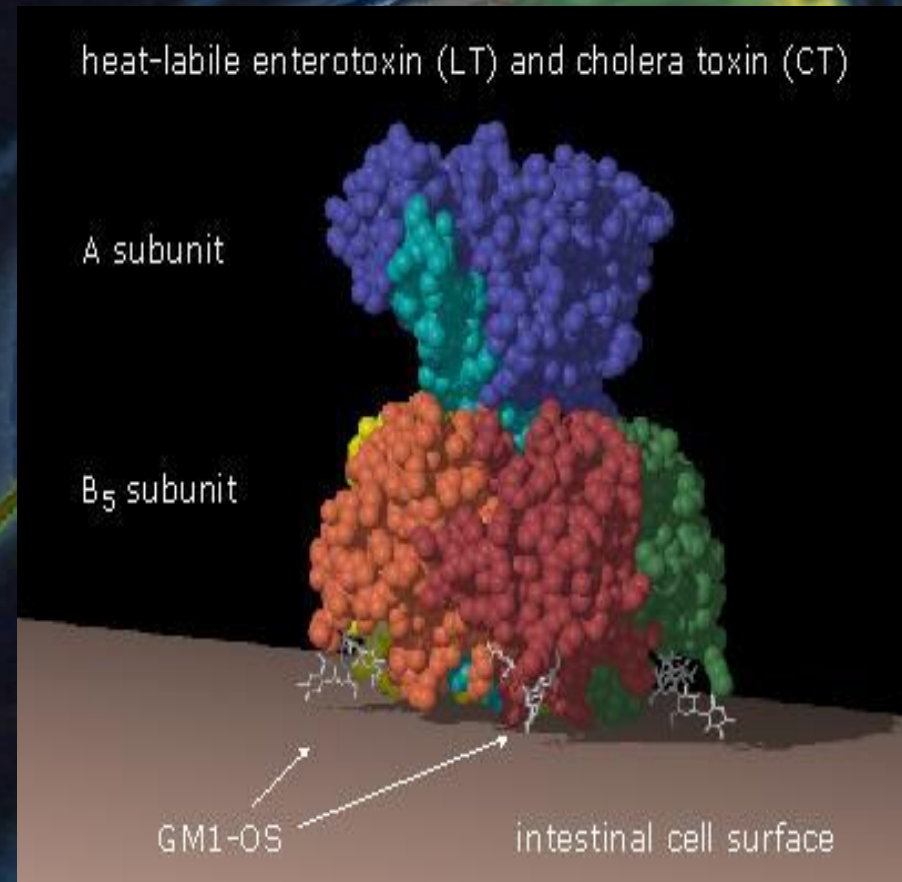
Pathogenesis: Overview

- 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.

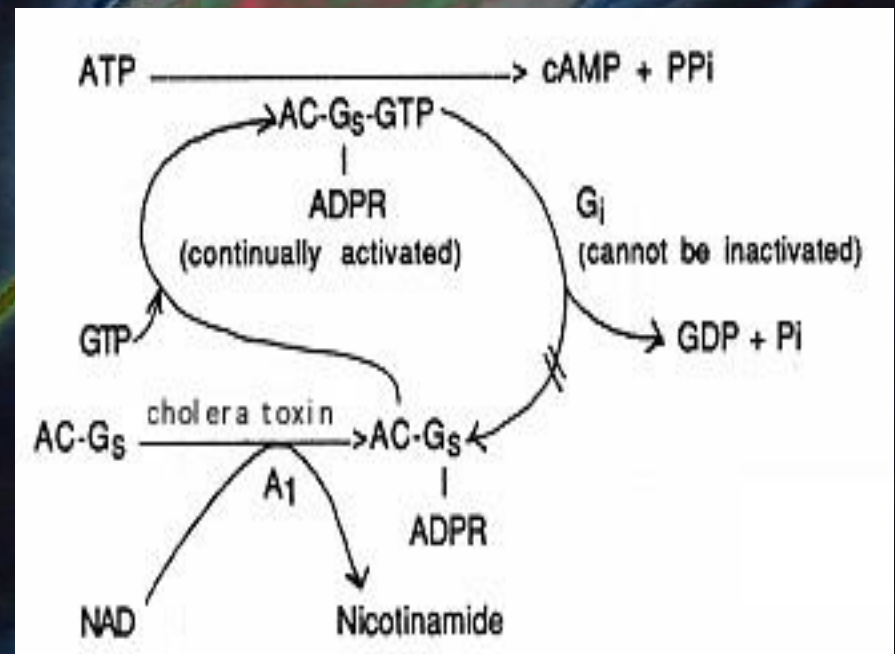


B subunits bind to GM1 Receptor

Pathogenesis: Mechanism of Action cont.

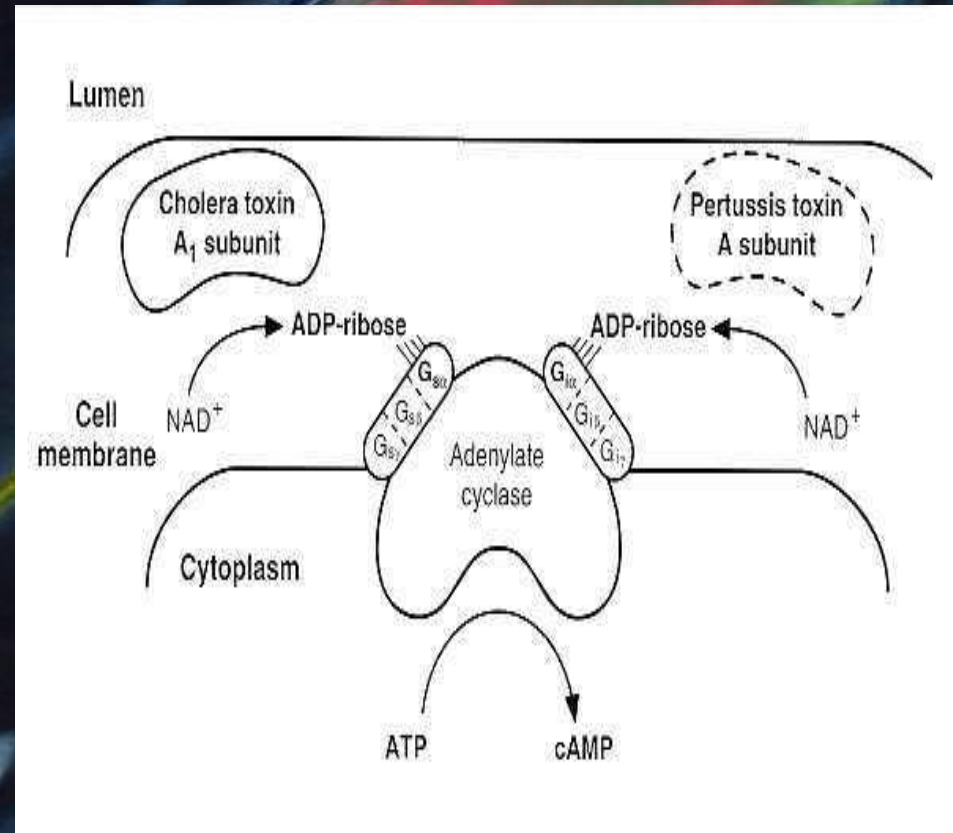
- Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system.
 - The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed.
- Since GTP hydrolysis is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

CHOLERA

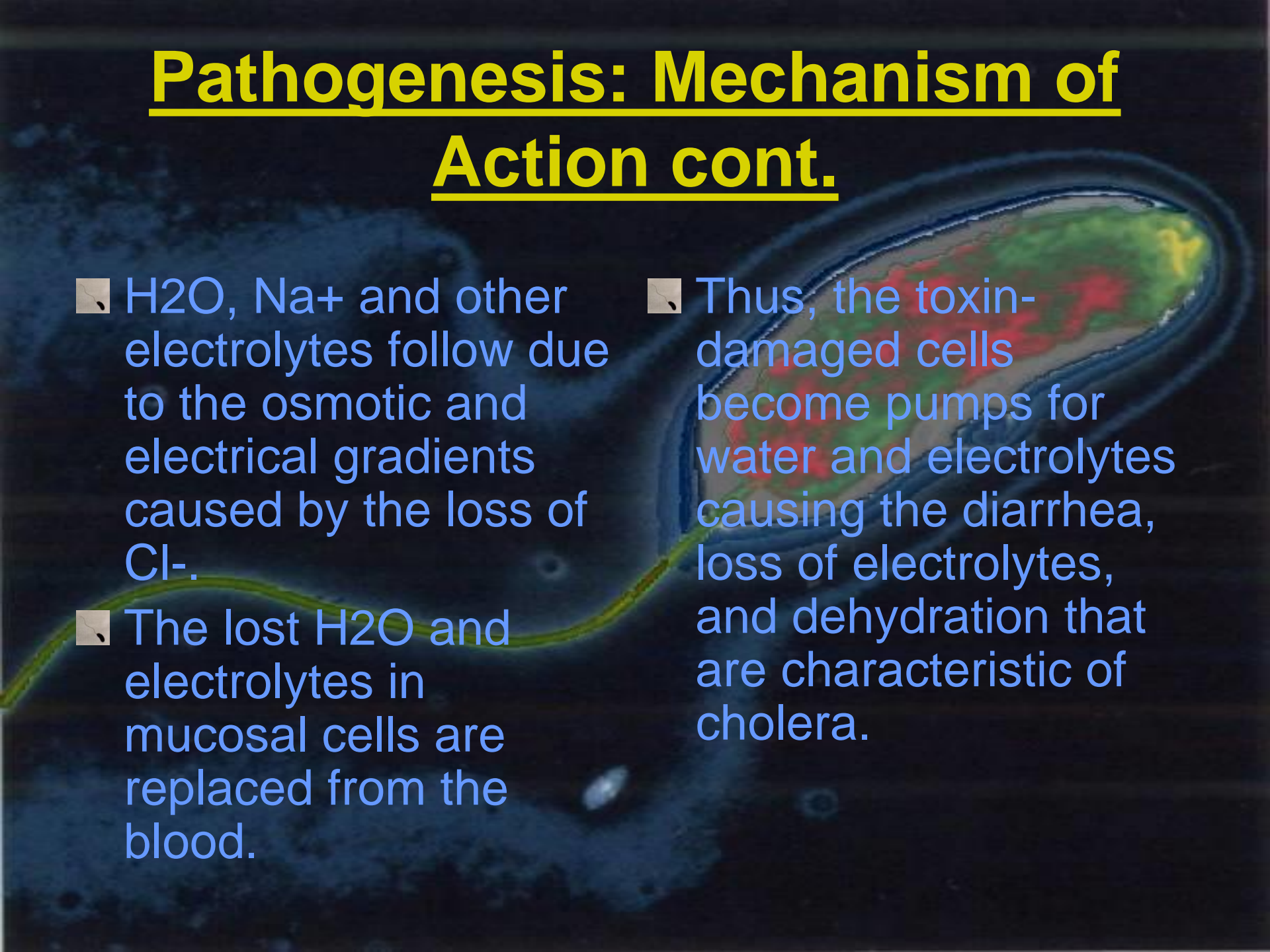


Pathogenesis: Mechanism of Action cont.

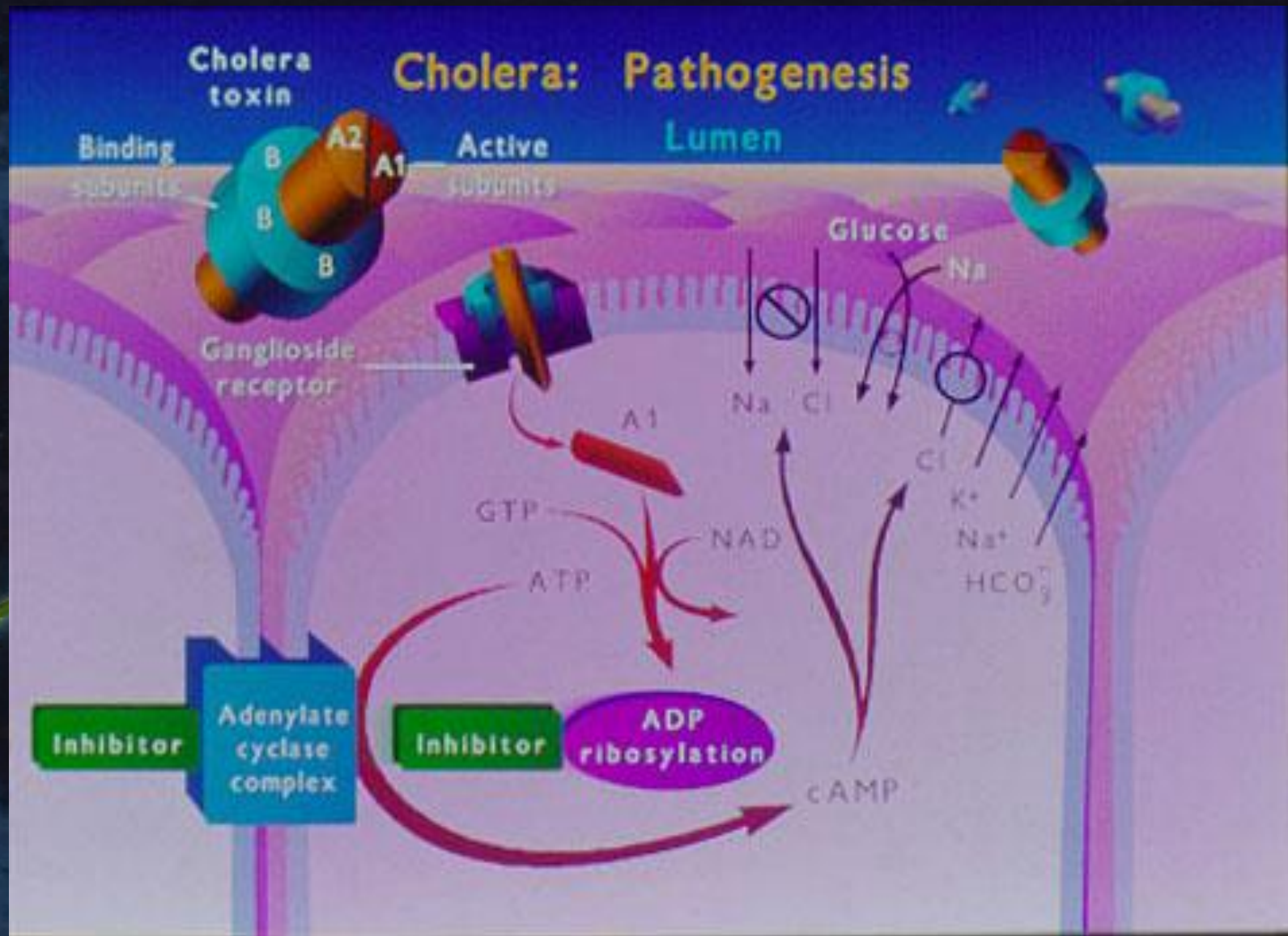
- Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents.



Pathogenesis: Mechanism of Action cont.

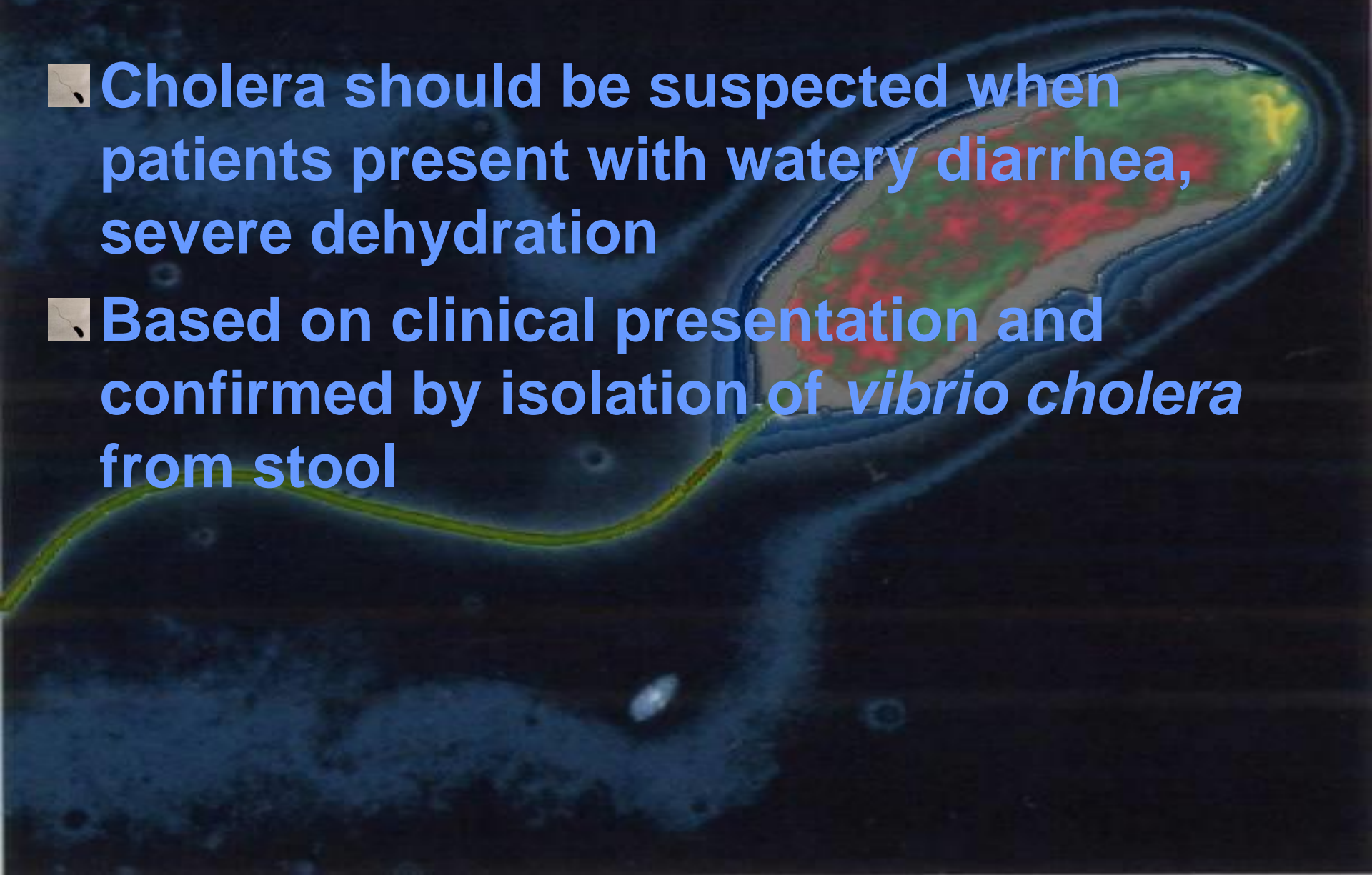
- H₂O, Na⁺ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl⁻.
 - The lost H₂O and electrolytes in mucosal cells are replaced from the blood.
 - Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.
- 
- A microscopic image of a cell, likely a mucosal cell, showing a green filamentous structure extending from the cell. The cell is stained with various colors, including blue, green, and red, highlighting different internal components. The background is dark, making the cell and its structures stand out.

Cholera: Pathogenesis

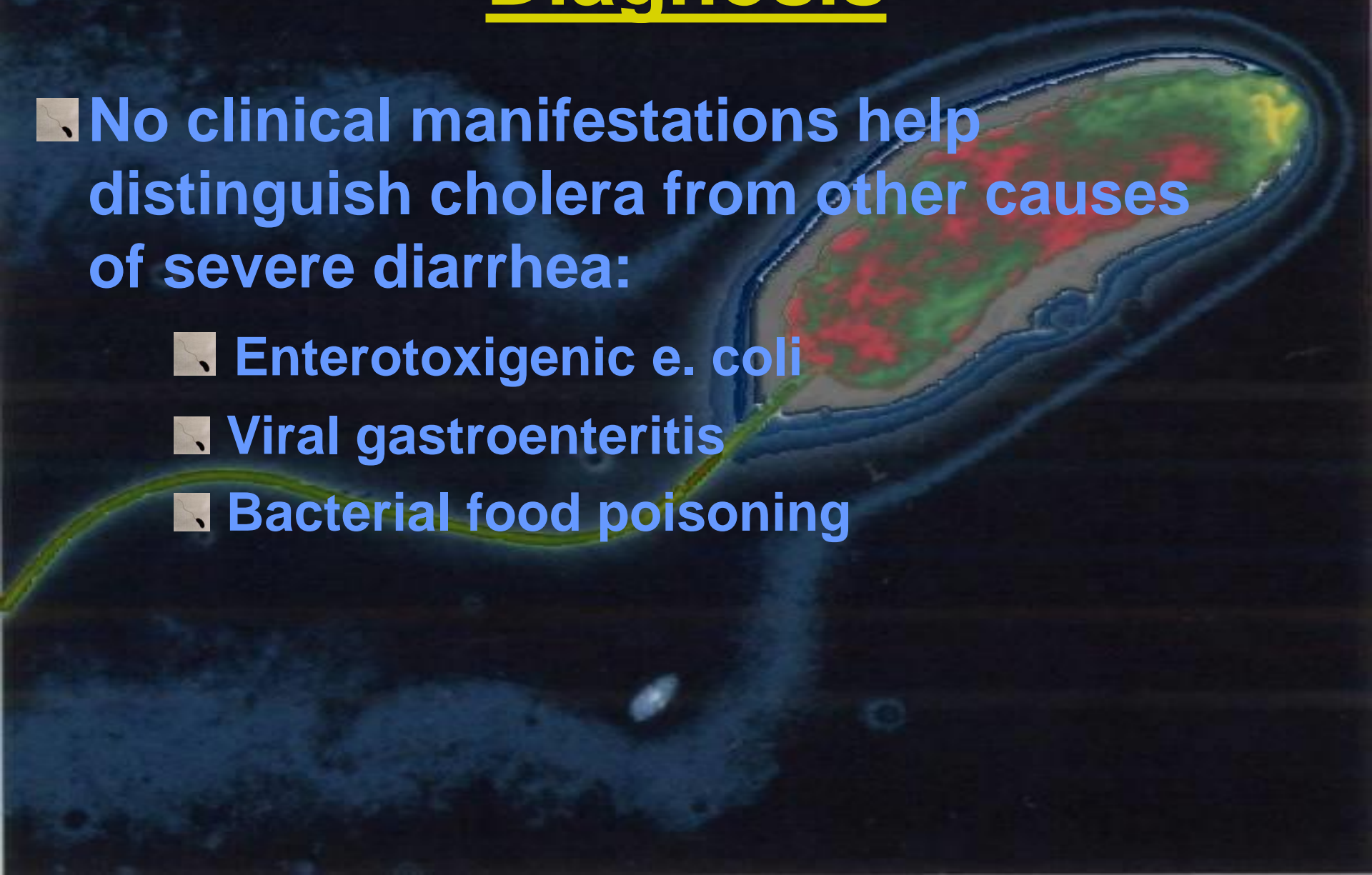


Diagnosis

- 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

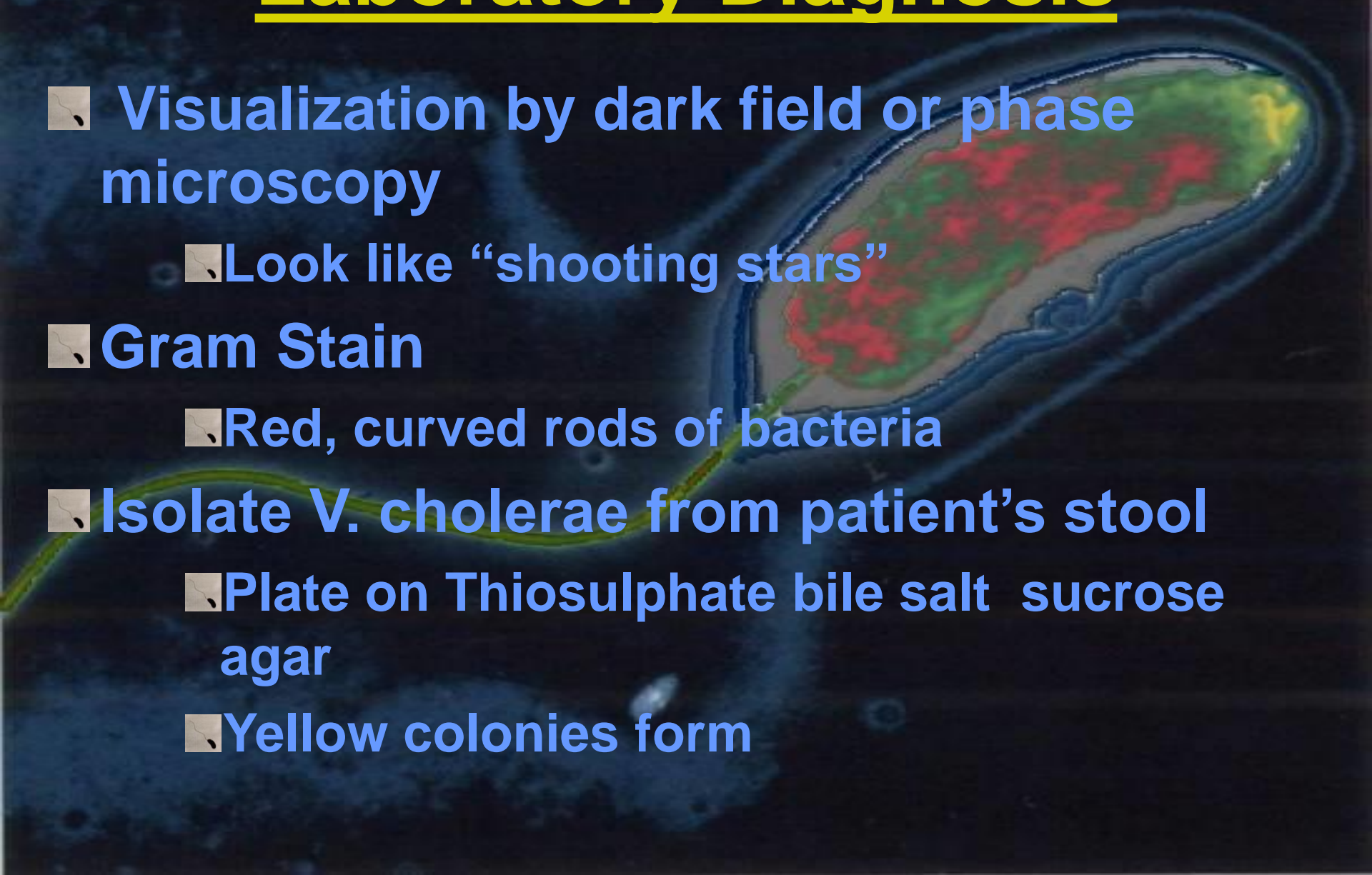
- No clinical manifestations help distinguish cholera from other causes of severe diarrhea:
 - Enterotoxigenic e. coli
 - Viral gastroenteritis
 - Bacterial food poisoning
- 
- A microscopic image of a bacterium, likely a vibrio, showing a long, wavy flagellum extending from one end. The cell body is oval-shaped and contains internal structures, including a prominent red and green area. The background is dark blue.

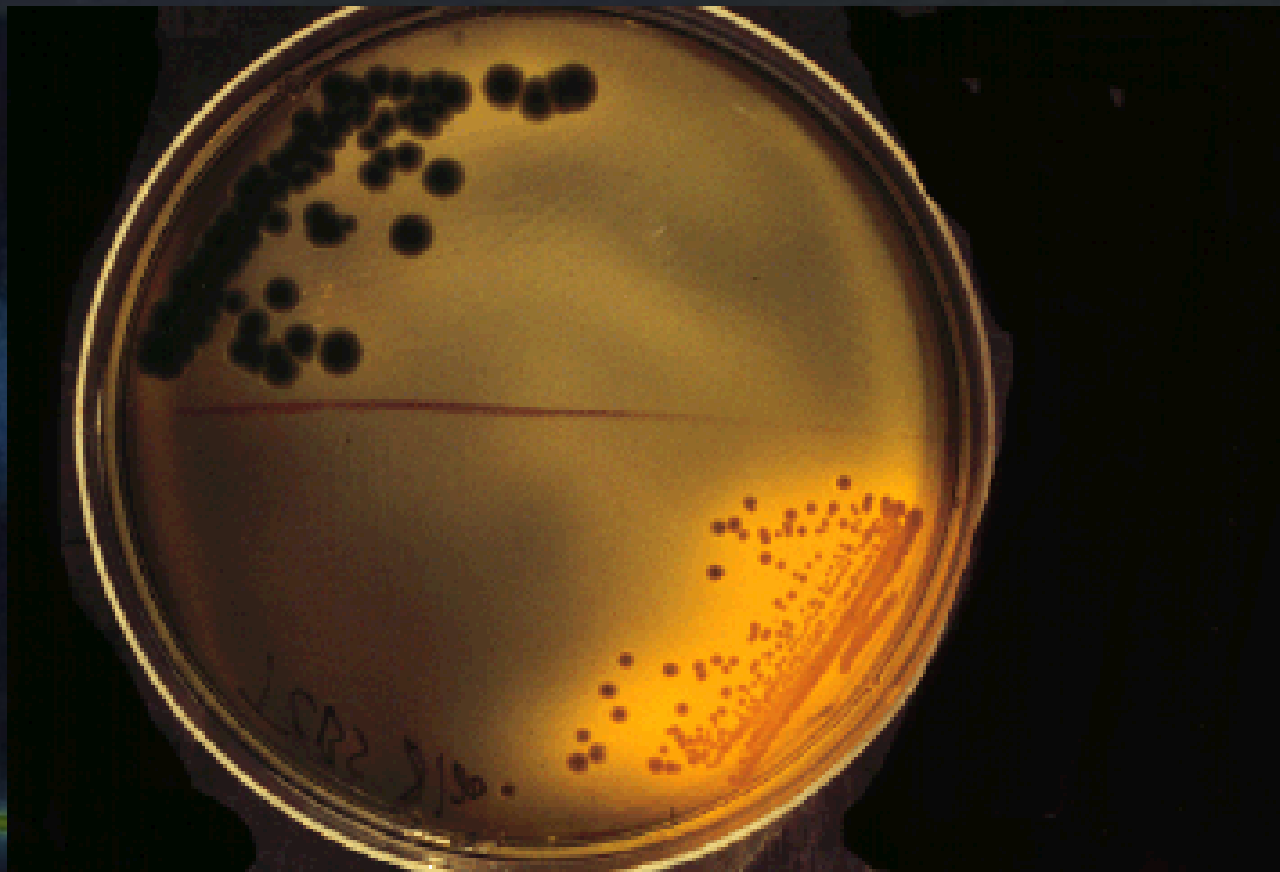
Diagnosis: Visible Symptoms

- 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
- 



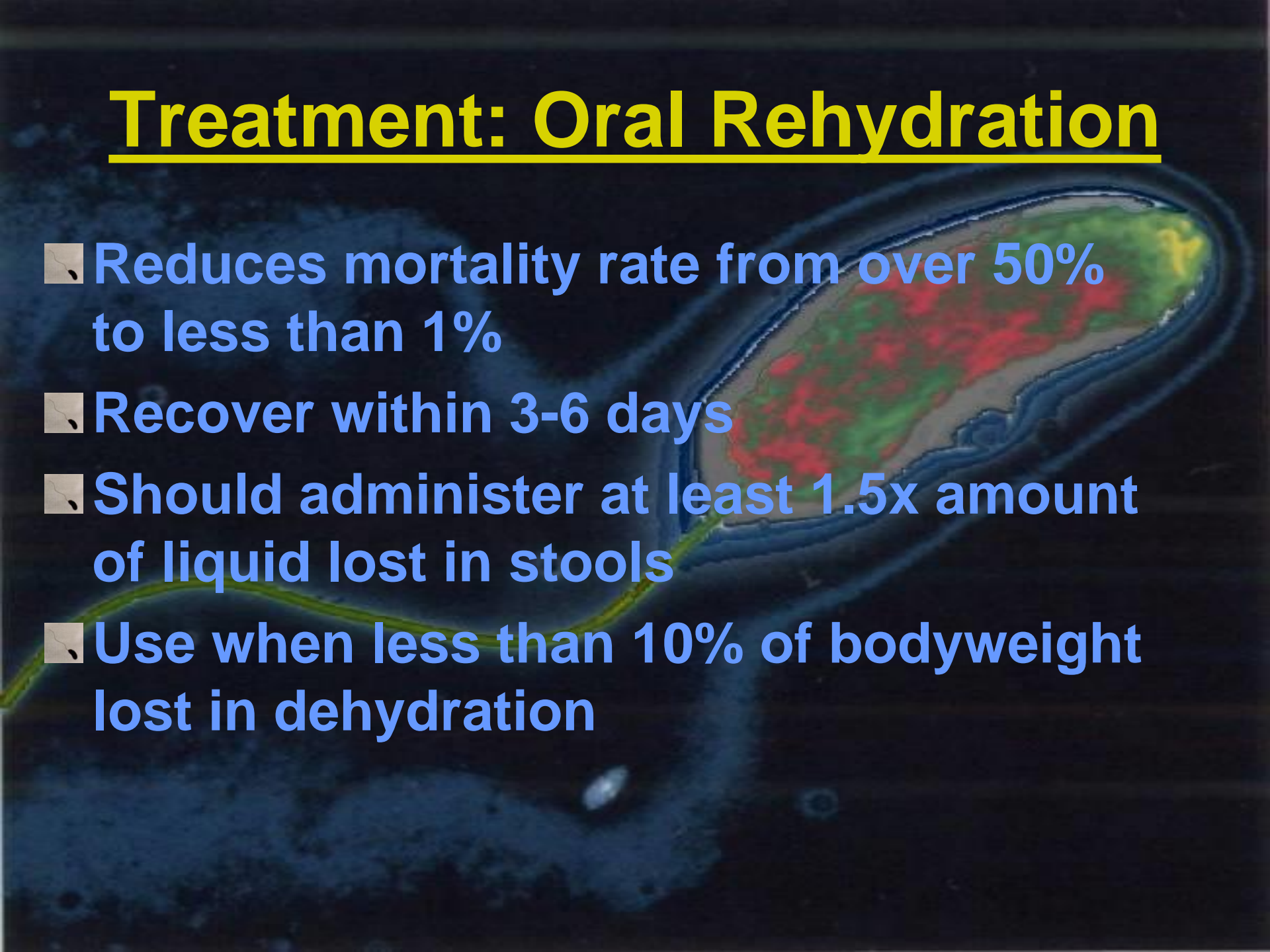
Vibrio species on TCBS agar *Vibrio* species can be selectively recovered from stool by culture on thiosulfate-citrate-bile salts-sucrose (TCBS) agar. On this medium, *V. parahaemolyticus* usually produces a green colony and *V. cholerae* a yellow colony (indicative of the fermentation of sucrose). Courtesy 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
- 
- An aerial photograph of a tropical island, likely in the South Pacific, showing a vibrant coral reef with red and green patches, a blue lagoon, and a sandy beach. The island is surrounded by deep blue ocean water. The image is used as a background for the text.

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, NaHCO₃, glucose



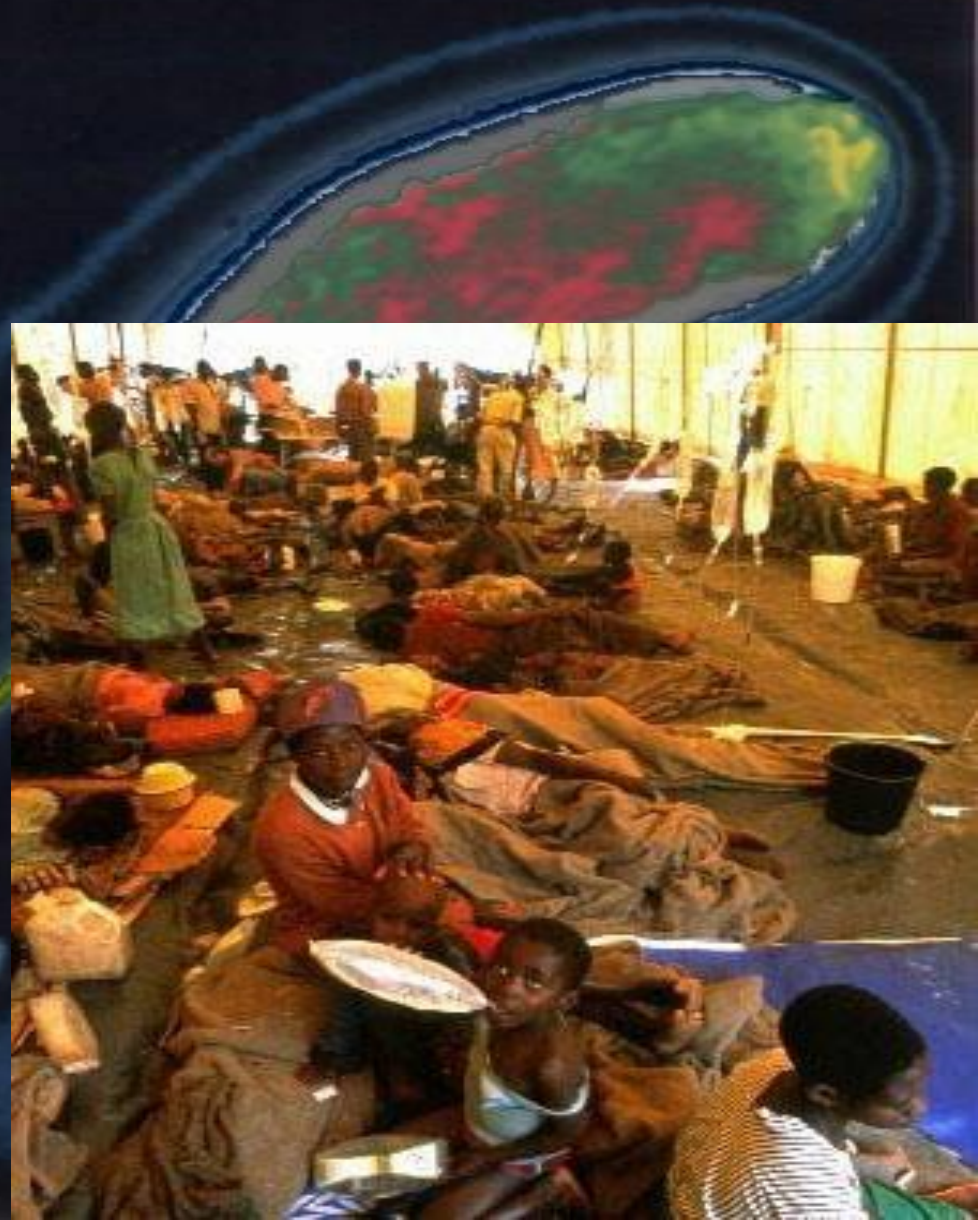
Treatment: Intravenous Rehydration

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



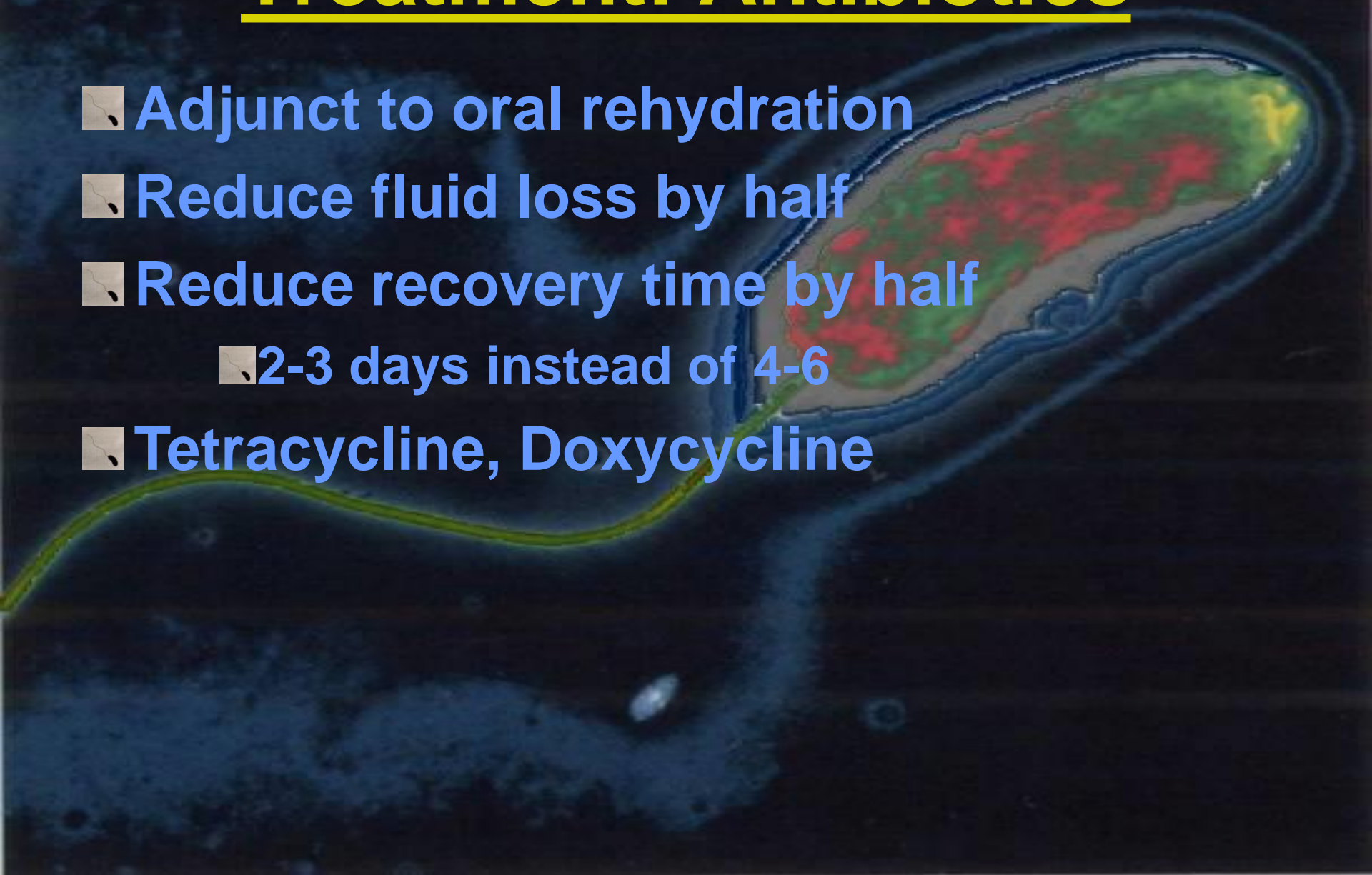
Treatment: Intravenous Rehydration

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



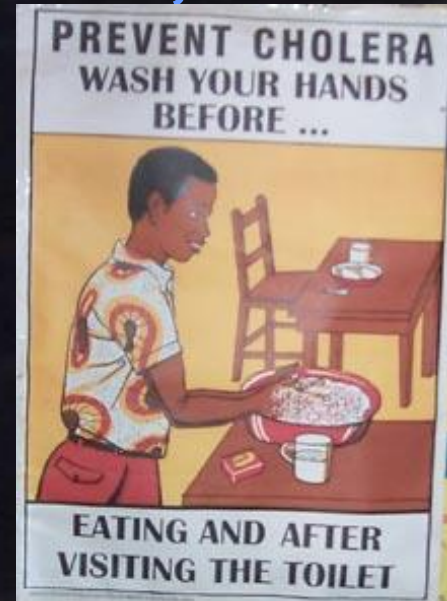
Treatment: Antibiotics

- Adjunct to oral rehydration
- Reduce fluid loss by half
- Reduce recovery time by half
 - 2-3 days instead of 4-6
- Tetracycline, Doxycycline

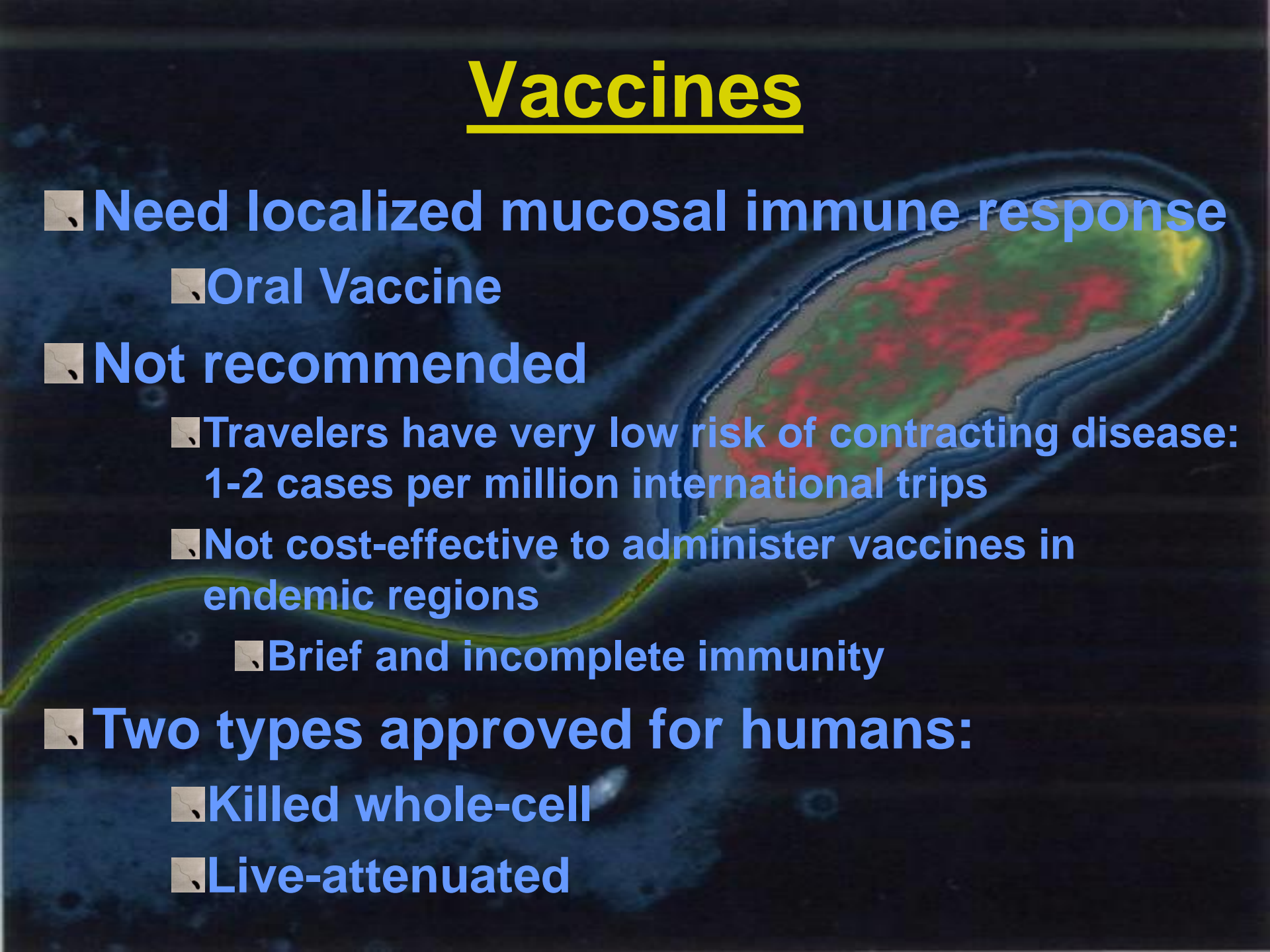


Traveling Precautions

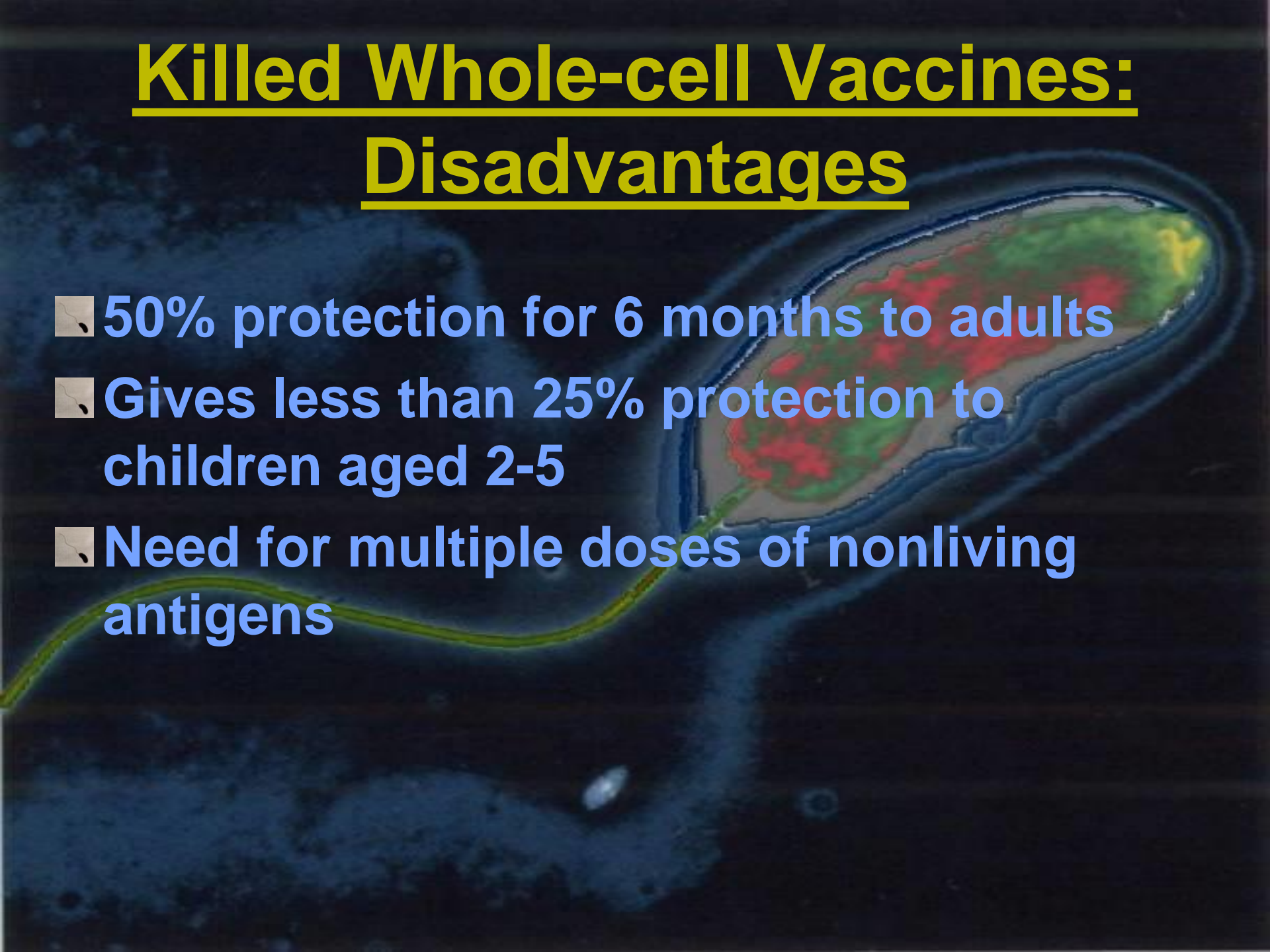
- Boil or treat water with chlorine or iodine
- No ice
- Cook everything
- Rule of thumb: “Boil it, cook it, peel it, or forget it.”
- Wash hands frequently



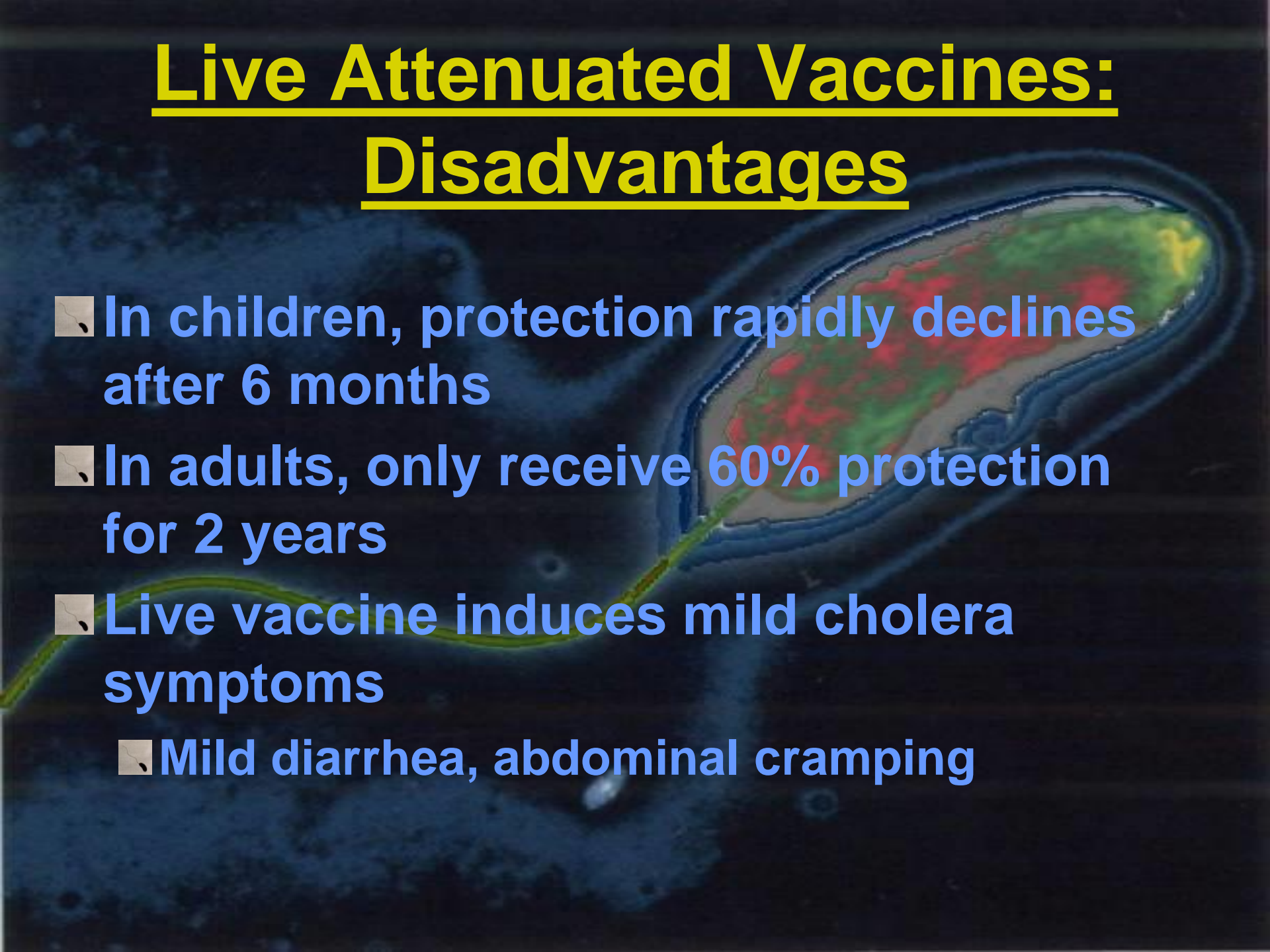
Vaccines

- 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
- 
- A microscopic image of a cell, possibly a bacterium or a large eukaryotic cell, showing a complex internal structure. The cell is roughly oval-shaped with a dark blue outer boundary. Inside, there are various colored regions: a large red area, a green area, and a yellow area. A thin, green, filamentous structure extends from the cell towards the bottom left.

Killed Whole-cell Vaccines: Disadvantages

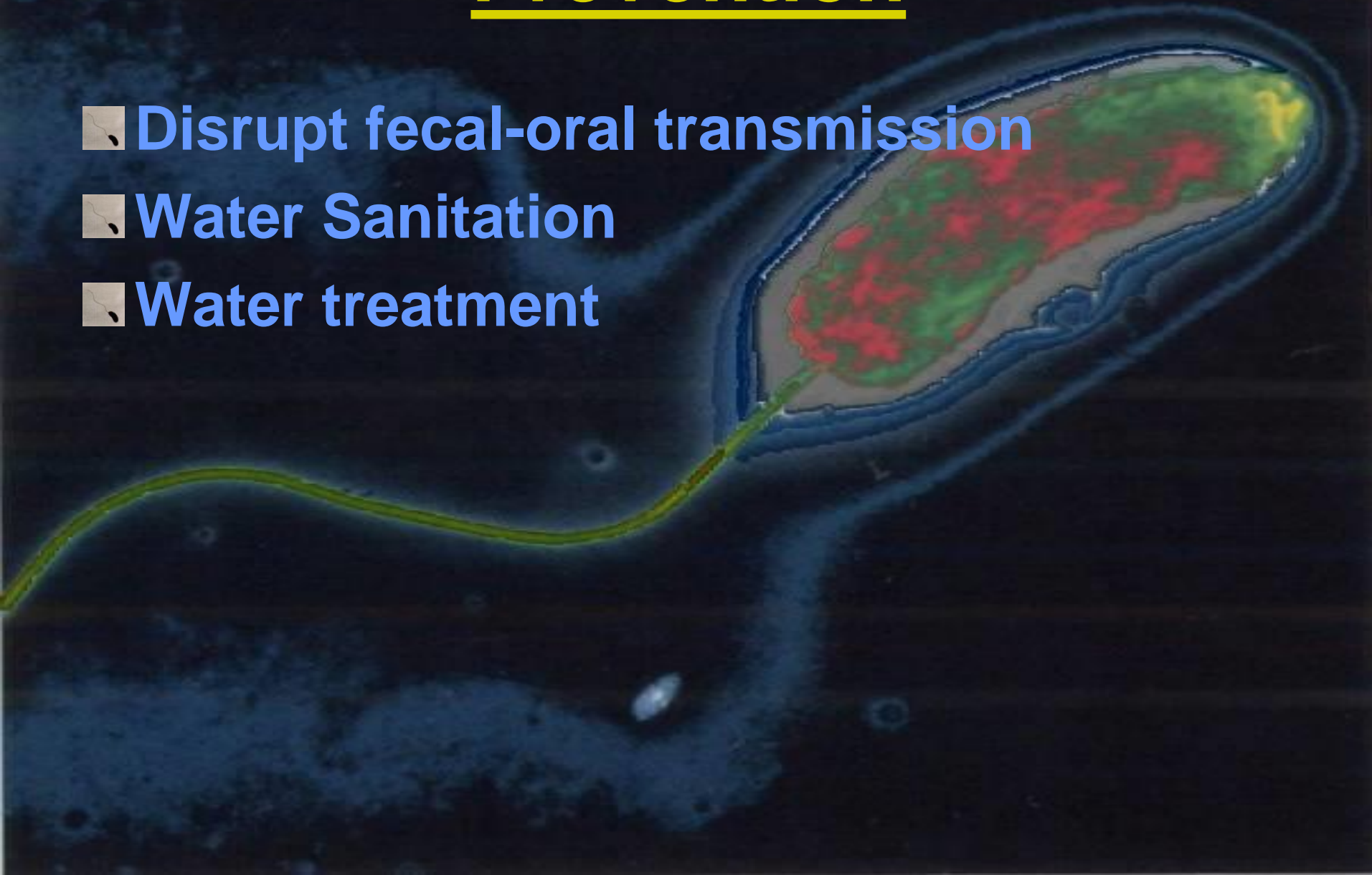
- 50% protection for 6 months to adults
 - Gives less than 25% protection to children aged 2-5
 - Need for multiple doses of nonliving antigens
- 
- A microscopic image of a bacterium, likely a Gram-negative rod, showing a complex internal structure. The cell is stained with various colors: a blue outer layer, a greenish-yellow cytoplasm, and a prominent red structure, possibly a nucleus or a specific organelle. The background is dark blue.

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
- 
- A microscopic image of a bacterium, likely Vibrio cholerae, showing a cross-section with a colorful internal structure. The bacterium is elongated and has a distinct outer membrane and a central core. The core is divided into regions of red, green, and yellow, possibly representing different cellular components or organelles. The background is dark blue with some faint, out-of-focus structures.

Prevention

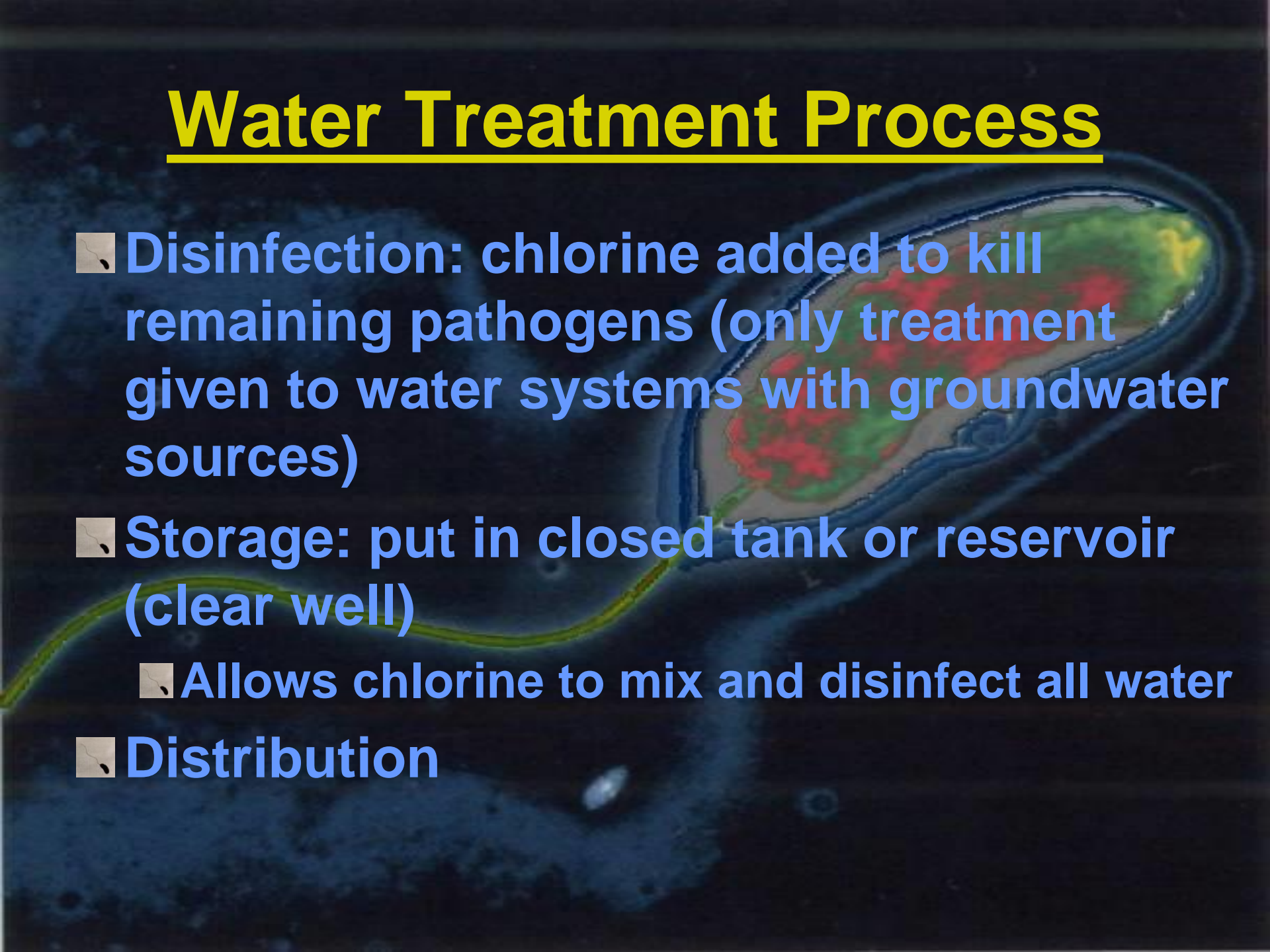
- Disrupt fecal-oral transmission
- Water Sanitation
- Water treatment



Ideal BioWeapon

- Ease of procurement
 - Simplicity of production in large quantities at minimal expense
 - Ease of dissemination with low technology
 - Silent dissemination
- 
- A satellite-style image of a tropical island, possibly a lagoon or atoll, with a green and red color overlay. The green areas represent land, and the red areas represent water or a specific region of interest. The image is set against a dark blue background, suggesting a night or low-light satellite view.

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**
- 
- An aerial photograph of a large, irregularly shaped reservoir. The water is a deep blue color. In the center of the reservoir, there is a large, elongated island with a mix of green and brown vegetation. A thin, light-colored dam or barrier runs across the middle of the reservoir, separating the island from the rest of the water body. The surrounding area is dark and appears to be a natural landscape.

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

