Environmental Change In Bangladesh

Effects On The Evolution And Epidemiology Of Cholera

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Conceptual Models Of An Infectious Disease

- **Medical Perspective**: the disease
- **Epidemiology Perspective**: reservoirs and epidemic spread
- **Ecology Perspective**: ecological factors and their effects on the evolution and epidemiology of an infectious agent.
- **Environmental Model** of Cholera in South Asia and Agro-economic Impacts
- **From Knowledge to Intervention**: Implications of the Environmental Model for Public Health
Cholera

The Disease
Fluid Depleted Cholera Patient
Prior to Death
Rice Water Stool From Cholera Patient
Rice Water Stool
Gram Stain

$10^6 - 10^8$ bacteria / ml

Vibrio cholerae
The Hanging Latrine Of Bangladesh
TRANSMISSION OF *Vibrio cholerae* IN NATURE

CHOLERA PATIENTS

CONTAMINATED WATER ← CONTAMINATED FECES → CONTAMINATED FOODS

AQUATIC ENVIRONMENT
Geography Of Endemic Cholera
In South Asia
A 100 Year Portrait
Annual Climate Cycle And Cholera Case Rates

Cholera Cases

Post-Monsoon

Region-Wide Synchronous Outbreaks

Pre-Monsoon

Cholera Cases

October - December

June - September

January - May

Monsoon

Synchronous
Monsoons and Annual Cholera Outbreaks
In Bangladesh

**Climate**

Acting on aquatic reservoirs
where *V. cholerae* resides
causes it to emerge as a pathogen
and
spread through human communities
Cholera
Ecology
Environmental and Ecological Events That Precede Or Coincide With Cholera Outbreaks In Bangladesh
Algal Blooms Precede Cholera Outbreaks In Bangladesh
Sunlight + Nitrogen Lead To Algal Blooms

Monsoon rains or irrigation create runoff that carries nitrogen-rich chemical fertilizers and human/animal wastes into Aquatic Habitats.
Vibrio cholerae Persists and Replicates For Months In the Mucilagenous Sheath of Anabaena variabilis, Subsisting Entirely on the Products of Photosynthesis
Copepod Bloom

Algal Bloom

Vibrios move from algae to copepods

Copepod Blooms Follow Algal Blooms
Copepods Belong to the Class *Crustacea*

- Large (crab, shrimp)
- And
- Small (copepodia)

*Harbor Vibrio cholerae*
Copepod—subclass of crustacean zooplankton
“The most numerous animal on earth”

Chitin-containing exoskeleton

SEM
Copepod
* Tigriopus californicus
Monterey Bay, CA
Dorsal Surface
Chitin

Polymer of β-1,4-N-acetylglucosamine

- Most abundant polymer in nature after cellulose
- About $10^{11}$ tons of chitin are produced annually in aquatic biosphere
  - $10^9$ tons from copepods alone
- This highly insoluble polymer is converted into biologically useful material by chitin degrading bacteria
- ‘Rain of chitin’ to the ocean floor would deplete the oceans for C and N if chitin was not degraded
Stage I:
Vertical association of Vibrio via one pole
Stage II:
Longitudinal association of Vibrio, sprouting of polar fibers
Stage III

Microcolonies of *Vibrio* form on the chitin surface of the copepod exoskeleton
Dungeness Crab Shell Surface
Scanning Electron Micrograph
Dungeness Crab Shell Surface
Digested By Vibrio cholerae
Chitin utilization involves three steps:

1. **Sense and move**
2. **Attach**
3. **Degrade**

**Chitin Utilization By Vibrio cholerae**
A Complex Genetic Program
>100 Genes
**V. cholerae** Growth on Chitin Films Requires Extracellular Chitinases ChiA1 and ChiA2

*V. cholerae*: GFP labeled (Fluoresces Green)

Chitin: Auto-fluoresces Red
Biofilm on a Chitin Surface
Composed of Different *V. cholerae* Genotypes

Chitin Surface

**Nutrient Function**

- Assimilation Of Chitin As a Source Of Carbon, Nitrogen, Energy
- Growth Occurs

**Genomic Function**

- Induces Competence For Natural Transformation
- New Genes Acquired Genome is Diversified
Chitin Induces Competence For Natural Transformation

Transformation: Uptake of “naked” DNA by competent bacteria

A Mechanism For Horizontal Gene Transfer

Donor

DNA

Recipient

Donor Releases DNA
Lysis or Secretion

Competent Recipient

Incoming DNA is incorporated Into the Chromosome By Homologous Recombination
How Growth and Gene Uptake Are Coordinated On Chitin Surfaces

Growth and Chitin-Dependent DNA Uptake Occur on the Same Chitin Surface But At Different Times and Under Different Conditions

DNA As A Source Of Nucleotides For Growth

DNA As A Source Of Genes For Genome Diversification
What Decides The Fate Of Released DNA?

- Lysis
- DNA Release
  - Chitin
  - Secretion
- Gene Acquisition
  - Natural Competence
  - Gene Acquisition
- DNA Degradation
  - Nucleotide Repletion
Early Events:
Few Cells
Nuclease Secreted
DNA Degraded
Nucleotides Assimilated
Rapid Growth Occurs

Late Events:
Many Cells
Nuclease Repressed
DNA Intact
Genes Acquired
Transformants Escape From Surface

Chitin Surface
Cell Density Dependent Switch

Early Late
Population Density Dependent Dispersal Of *Vibrio cholerae* From A Chitin Surface

Events between the 18th and 48th hour of growth on a chitin surface
Time Lapse Recording For 30 hours
Disruption of the Quorum Sensing System Prevents Bacterial Dispersal

Events between the 18th and 48th hour of growth on a chitin surface
Time Lapse Recording For 30 hours
Chitin-dependent Competence Occurs In A Niche and Seasonally-Specific Manner And Is Controlled By Nutrient Availability and Population Density

Genetically-diverse *V. cholerae* From different niches swim toward and adhere to a new chitin surface in the environment. This is most likely a copepod molt → Rapid replication ensues → At a critical population density gene exchange occurs → A genetically diversified ensemble disperses for new niches
Biofilm on a Chitin Surface
Composed of Different *V. cholerae* Genotypes
What kinds of genes are acquired?

- New Metabolic Pathway
- Environmental Niche
- New Pathogenic Trait
- Host Niche
Spread Of *Vibrio cholerae* O139 Bengal Across Asia

Acquisition of a New Gene Cluster

Encoding a New Serogroup Antigen and Capsule

Spread of *Vibrio cholerae* O139 (1992 to 2005)

*Vibrio cholerae* O139 Bengal-10 years on.

Reviews in Medical Microbiology. 16(4):135-143, October 2005.

Albert M. John and Nair G. Balakrish
When Did It Happen? An Environmental Explanation

Massive Cyclone Strikes Bangladesh Spring 1992
>100,000 Deaths Due To Costal Flooding

Environmental Perturbations Correlated With Emergence Of Genetic Variants
How Did It Happen?
A Genetic Explanation

O1 Serogroup Cluster Replaced By O139 Cluster

V. cholerae O1
El Tor

V. cholerae O139
Bengal

Chitin Surface

O1 Serogroup Cluster

O139 Serogroup Cluster
Where Did It Happen?

Most likely site: on the surface of a copepod molts in an estuary of the Ganges River delta. The surface of the copepod likely harbored a mixed population of *V. cholerae* of different serogroups, including pathogenic O1 *Vibrio cholerae* and non-O1 environmental *Vibrio cholerae* strains.
Why did the O139 Bengal Strain Emerge and Spread In Asia?

- **Advantages in the Environment:** O139 Bengal is resistant to predation by phage in the environment, especially those lytic for the ancestral O1 El Tor biotype.

- **Advantages in the Host:** O139 is not neutralized by pre-existing antibodies to the 7th pandemic O1 El Tor biotype; antibodies of this kind are commonly found in persons living in south Asia owing to repeated exposures to the O1 serogroup.
Cholera

The Environmental Model
The Natural History Of *Vibrio cholerae*
Evolutionary Perspective: **Stages & Tasks**

1. **Algal Association** → \(V_1\)
2. **Copepod Association** → \(V_1\) → \(V_2\) → \(V_3\)
3. **Transmission To Humans**

- **Biomass Amplification**
- **Creation Of Genetic Diversity**
- **Natural Selection**
- **Biomass Amplification**

The Controlling Variable
A Model Of How The Green Revolution Drives the Epidemiology and Evolution of Cholera

Deforestation → Human, Animal Waste → Runoff

Irrigation → Chemical Fertilizers

Monsoon Rains

Regional And Global Climate Change

Less Knowledge Gradient More

Runoff → Algal Bloom

Copepod Bloom → Transmission To Human Host → Infection And Disease

And Disease
The Green Revolution In South Asia

- 1960’s Transformation of Agriculture
  - High Yield Hybrid Rice and Wheat
  - Irrigation
  - Chemical Fertilizer Use
    (now exceeds totals in Western Europe)

- Food Independence Banishes Famines

- Unintended Consequences: Adverse Ecological and Public Health Consequences
Can an Economic Decision by a Bangladeshi Rice Farmer To Purchase Fertilizer Spawn an Epidemic?
Bangladesh Rice Farming
Intensive Fertilizer Use and Irrigation
Gives Rise To Algal Blooms and May Drive
The Evolution and Epidemiology of Cholera
Crossing Scales

Regional and Global to Molecular and Genetic

South Asian Agricultural Practices

V. cholerae genome
Crossing Disciplines

Together they can understand complex phenomena and devise interventions

- Economics
- Ecology
- Epidemiology
- Agronomy
- Environmental Sciences
- Molecular Genetics
- Public Health
The Environmental Model of Cholera Leads To The Identification Of Potential Interventions

Knowledge → Public Health Policy → Intervention → Disease Control