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Pathogens: Nature and Transmission

Gary Ketner, PhD
Johns Hopkins University

Module 2 Introduction, by Dr. Vern Carruthers, PhD

- Module 2: pathogens and host immunity
  - Existing and emerging infectious diseases
  - Principles of microbial transmission and host responses
  - Led by Dr. Gary Ketner
    - Professor of Molecular Microbiology and Immunology

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Section A

Introduction

Infectious Disease

- Disease caused by replicating agents transmissible to humans from another person, an animal, or the environment

Infectious Disease Health Burden: All Nations

Leading Causes of Mortality from Infectious Diseases, 2001 (in Millions)	
Respiratory infections	3.9
AIDS	2.9
Diarrhoeal diseases	1.9
Tuberculosis	1.6
Malaria	1.1
All infectious diseases	~16.4 (32%)

Source: *Communicable Diseases* 2002, WHO 2003

Continued

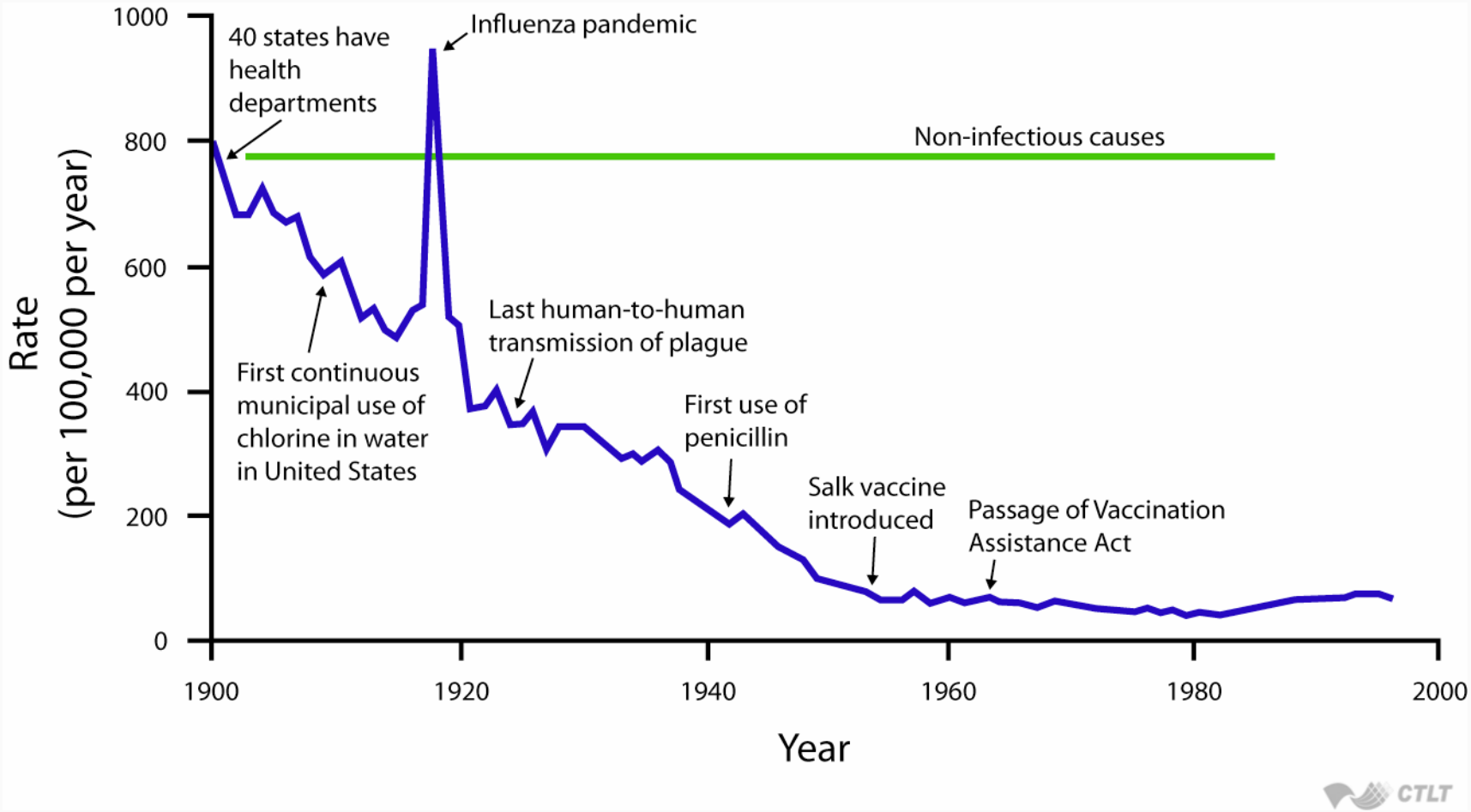
Infectious Disease Health Burden: All Nations

Leading Infectious Causes of Cancer, 2000			
Infectious agent	**Cancer site**	**Cases (number)**	**% Due to Infection**
Human papilloma virus	cervix	471,000	100%
Hepatitis B virus	liver	306,800	55%
Hepatitis C virus	liver	175,600	31%
Helicobacter pylori	stomach	442,000	50%

Source: *Communicable Diseases* 2002, WHO 2003

Encouraging Trends

- Crude death rate (infectious disease) USA 1900–1996



Source: Adapted from Armstrong et al. *JAMA.* 1999;281:6–66.

Public Health Challenges of Infectious Diseases

- We have not yet applied existing tools to many infectious diseases with effective interventions (polio, measles)
- Many of the remaining infectious disease problems are refractory to existing tools (HIV, malaria)
- Emerging diseases (HIV, SARS, new influenza strains, drug resistant pathogens) illustrate that, as a public health issue, infectious diseases will always be important

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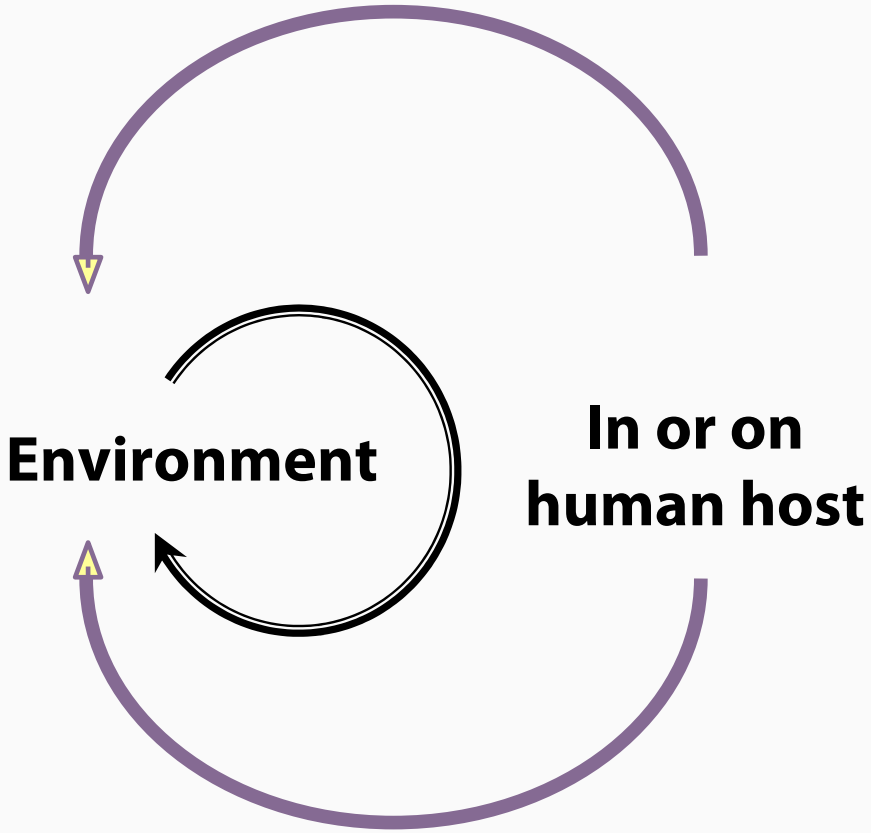
Public Health Challenges of Infectious Diseases

- Application of **existing** tools to remaining "controllable" infectious disease
- Development of **new** tools applicable controlling refractory and emerging infectious disease
  - The development of new tools will be critically dependent upon knowledge of the biology of disease and pathogen

A Generalized Infectious Cycle



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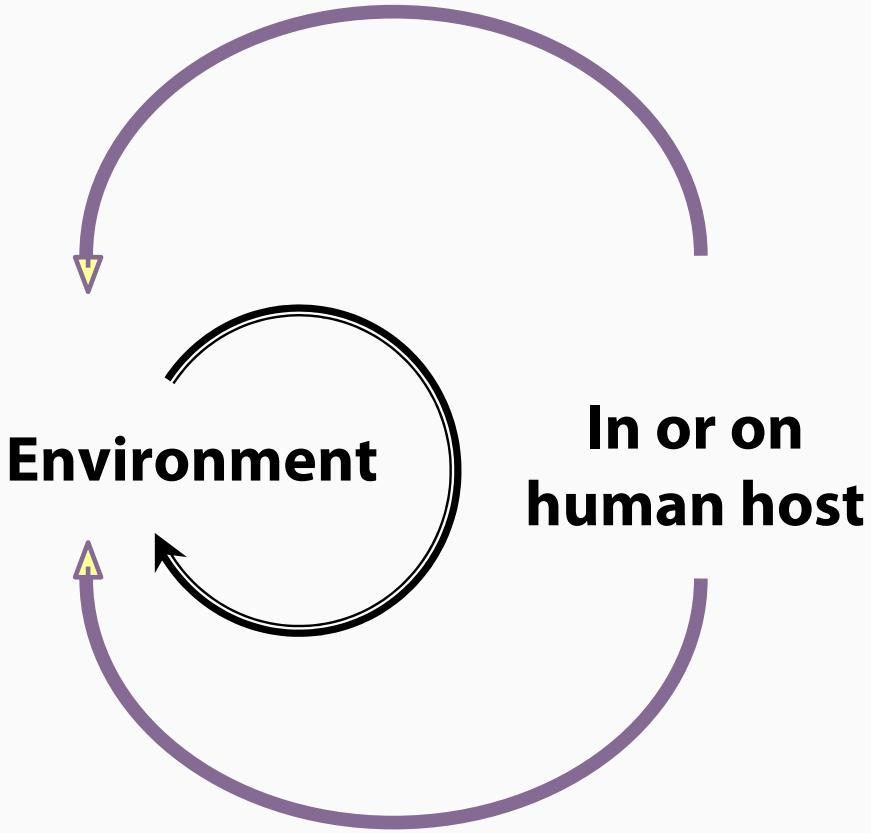
A Generalized Infectious Cycle

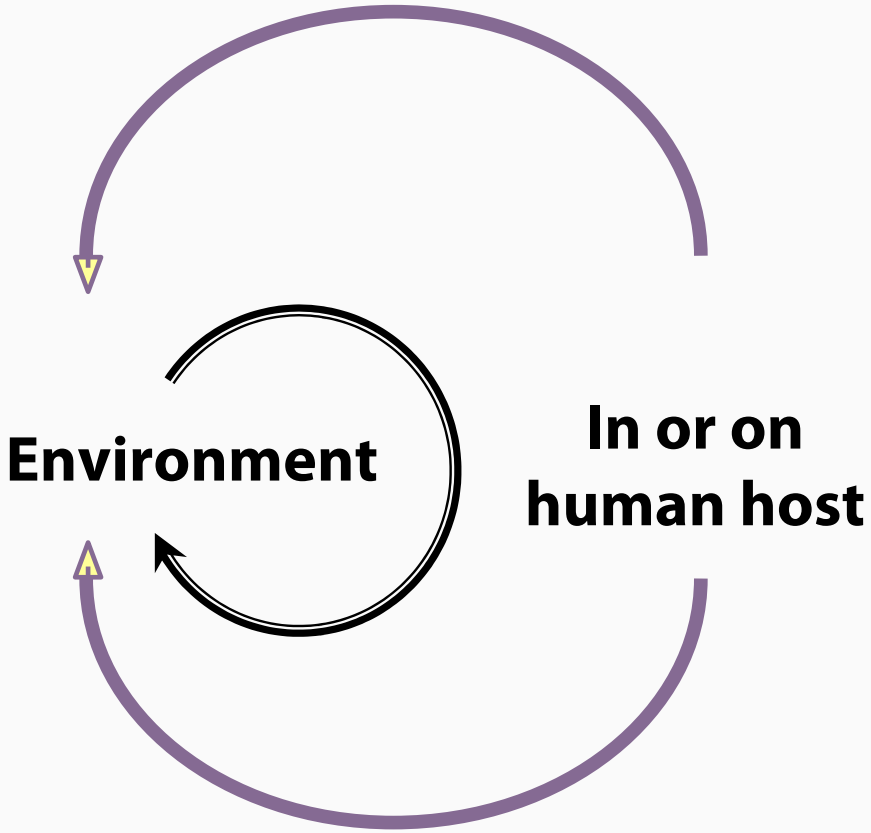


- Invasion
- Proliferation
- Evasion of defenses
- Exit
- Pathogenesis

Continued

A Generalized Infectious Cycle

- Survival
- Transit (via water, food, air, contact, vectors)
- Multiplication or development in intermediate hosts, reservoirs or environmental niches



- Invasion
- Proliferation
- Evasion of defenses
- Exit
- Pathogenesis

Infectious Agents

- Prions
- Viruses
- Bacteria (prokaryotes)
- Eukaryotes
  - Fungi
  - Protozoan parasites (single cells)
  - Metazoan parasites (multicellular)

Prions

- Creutzfeldt-Jacob disease (CJD), vCJD, Mad Cow (and early-onset Alzheimer's disease?)
- Single protein molecules (or small aggregates) (PrP)

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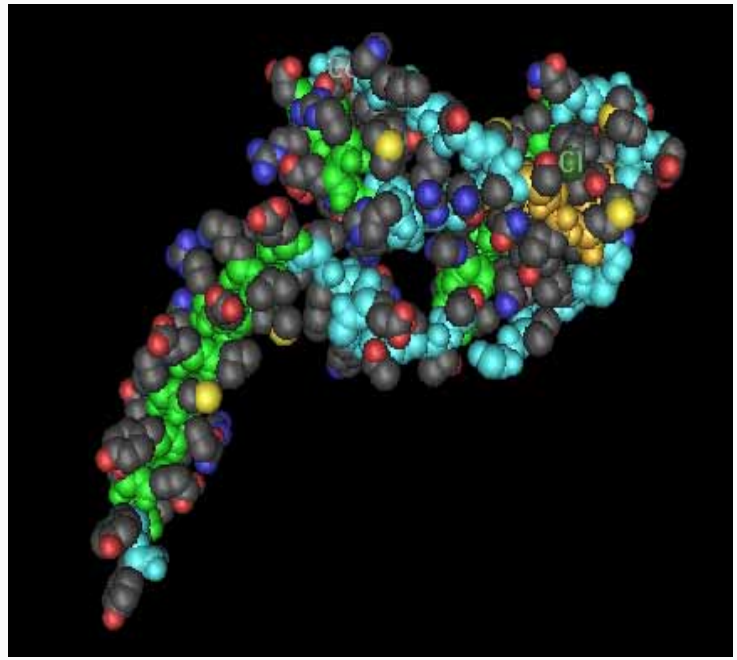
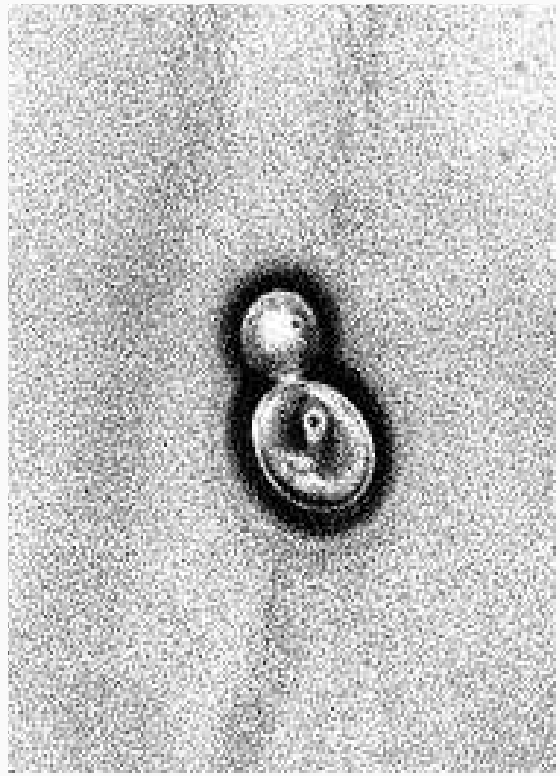
Prions

- No nucleic acid
  - Prion protein encoded by cellular DNA
- Aberrant folding propagates



Fungi

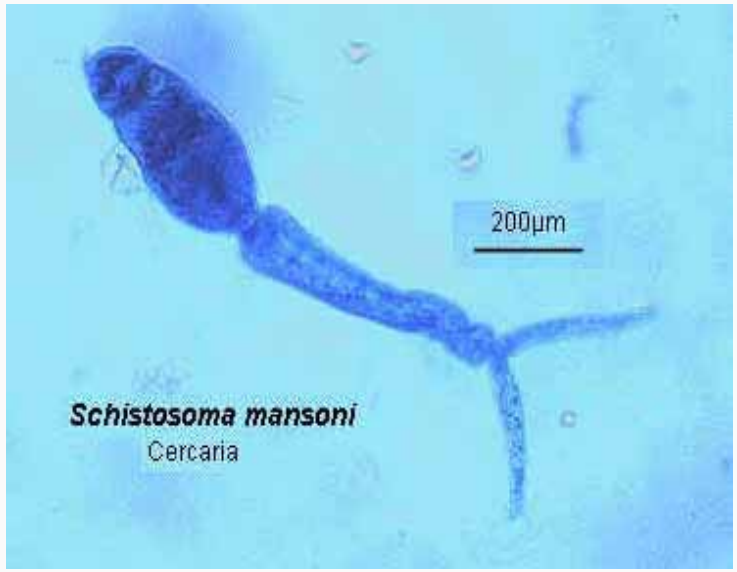
- Eukarotes (contain defined nuclei)
- Fairly closely related (biochemically) to humans
- Fungal disease comparatively rare
  - Seen mostly in the immunocompromised
- Intractable: drugs poor
- No vaccines



Candida albicans

Metazoan Parasites

- Multicellular eukaryotes
- Agents of many important diseases (mostly tropical)
  - Schistosomiasis
  - Filariasis
- Drug treatment available for a several diseases
- No vaccines
- Transmission control central



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Section B

Viral Pathogens

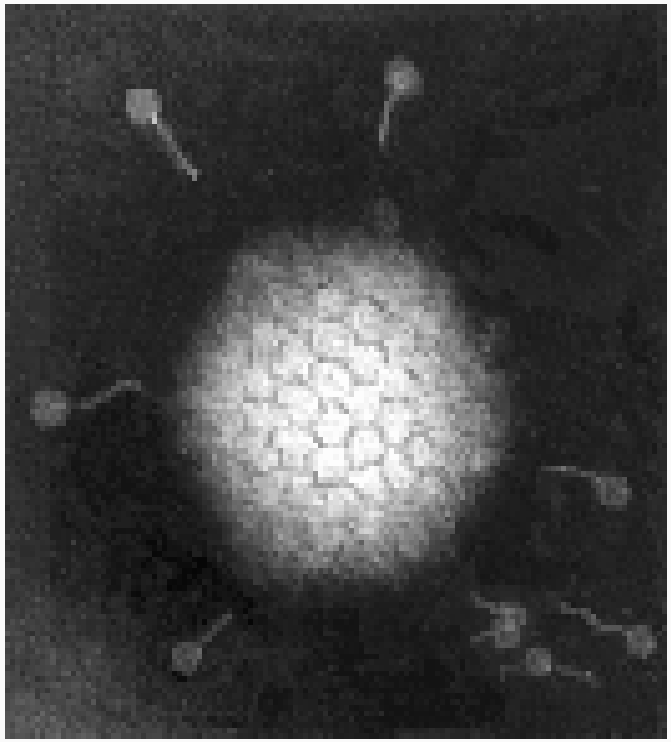
Viruses

- Obligate intracellular parasites
  - Perform replicative functions only in living cells
    - Dependent upon host cells for:
      - Energy
      - Biochemical precursors (amino acids, nucleosides)
      - Protein synthesis
      - Nucleic acid synthesis (to varying extents)
  - Transmitted as metabolically inert particles (virions)

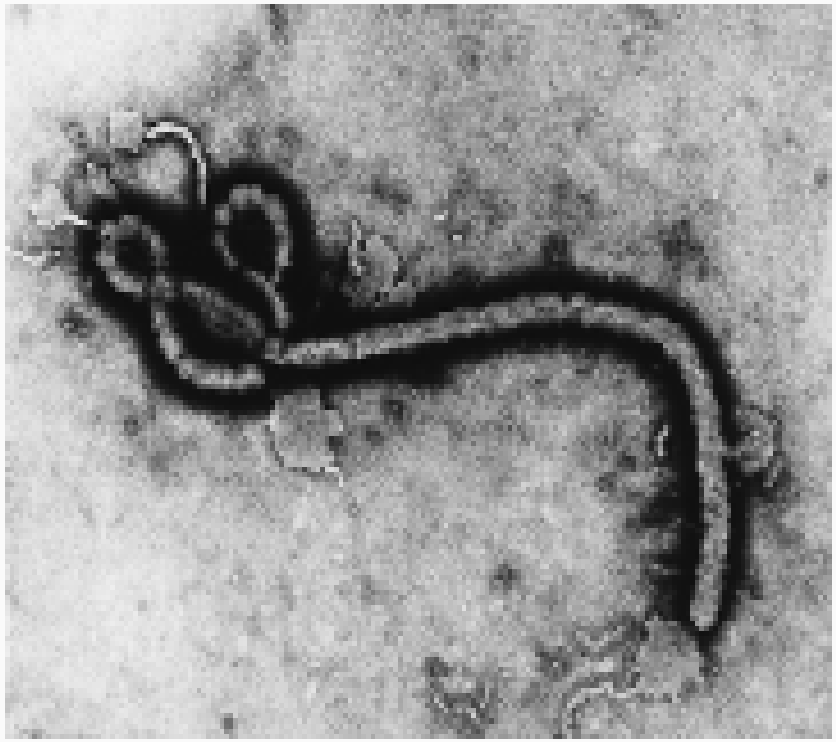
Viruses

- Virions all have:
  - Nucleic acid (DNA or RNA)
    - 5,000 to 250,000 bases (human: 3 x 10^9)
    - 3 to 100+/- genes (human: 50,000)
  - Protective protein coat
    - 1 to 50 different types, a few to 1000s of copies of each
  - Mechanism for specific attachment to host cells
    - (Commonly targets for immunity)
- And may also have:
  - Membranes derived from the host cell
  - Enzymes (HIV reverse transcriptase, for example)
  - Specialized attachment structures

Virion Morphology



Human adenovirus
(colds, DNA)



Ebola virus Zaire
(hemorrhagic fever, RNA)

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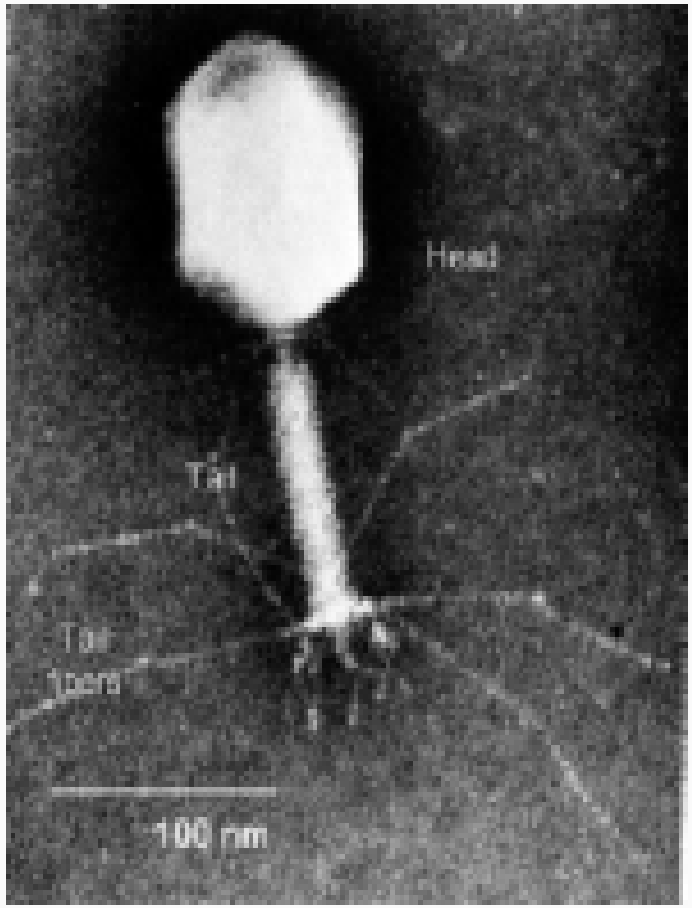
Virion Morphology



T4 virus of *E. coli,*
(DNA)

Poliovirus

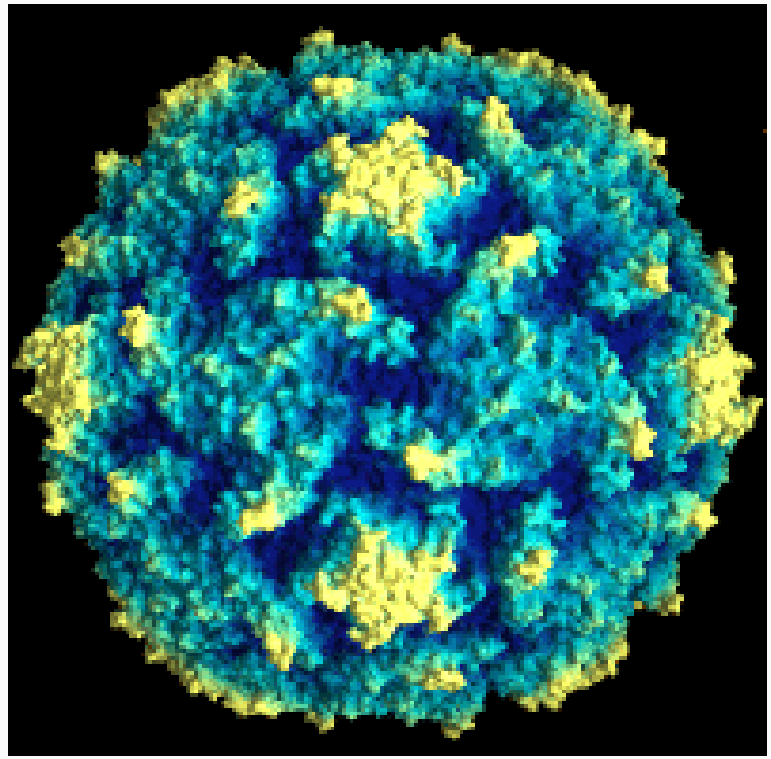
- Etiologic agent of paralytic poliomyelitis
- Wild polio eradicated in the West
  - 1,919 cases worldwide in 2002 (down from ~350,000 in 1988)
- Decrease due to effective vaccines and successful immunization campaigns

Poliovirus: Infectious Cycle

- Transmission by fecal-oral route via contaminated water
- Primary replication/multiplication is in lymphoid cells (specialized cells of the immune system) especially in the gut
- Virus shed primarily into the gut, excreted in the feces
- Some virus also enters the blood and reaches other susceptible cells
- These include anterior horn cells (motor neurons), which innervate muscle
- Destruction of these cells can result in paralysis
  - Disease is not a consequence of an essential step in the virus's life cycle

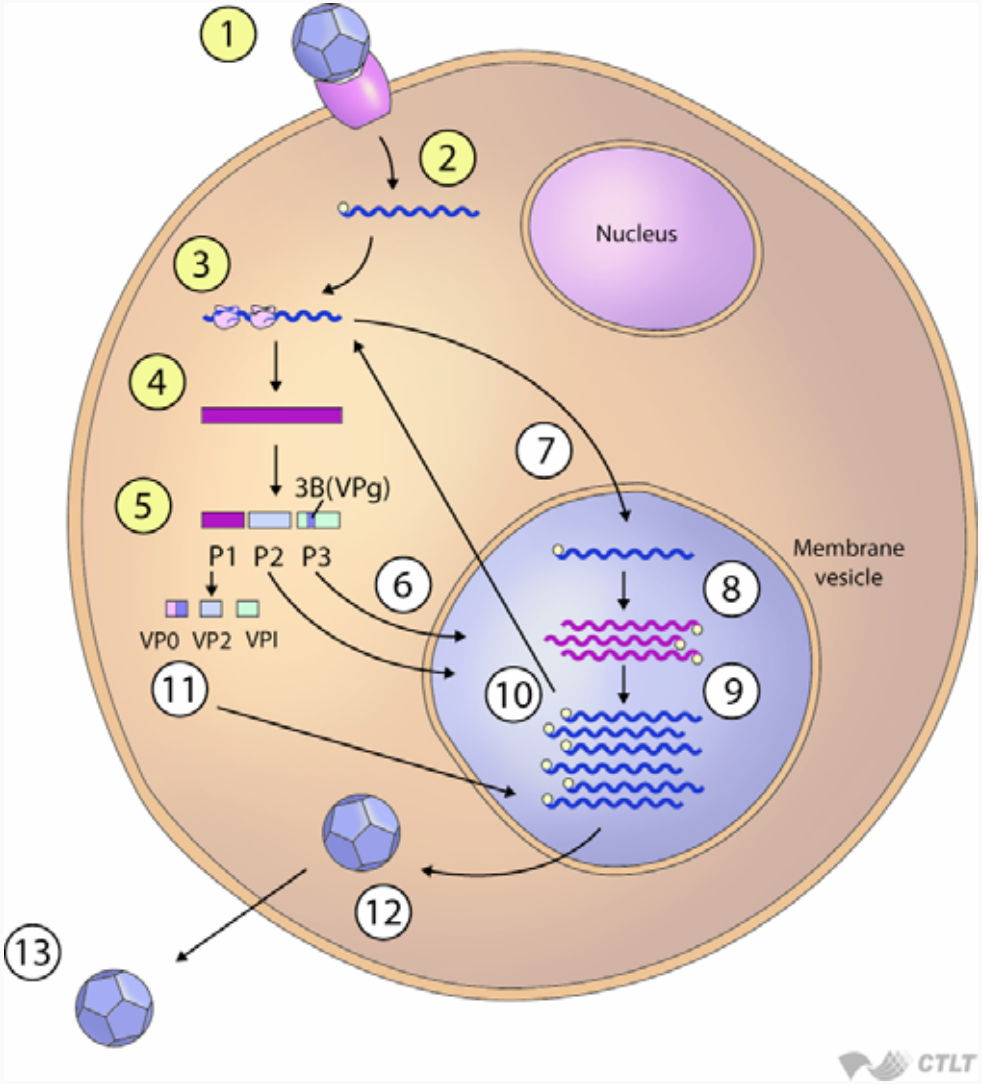
Poliovirus Virion

- 30 nM diameter virion contains 60 copies each of four proteins (encoded in the viral RNA)
- Viral RNA is a single strand with mRNA (+) polarity, is about 8000 bases long, and encodes 11 proteins
- Virion is non-enveloped and contains no enzymes



Poliovirus: Intracellular Replication

1. Attachment to cell via specific receptor (Vpr) on cell membrane
2. Virus entry (endocytosis); extrusion of RNA into cytoplasm
3–5: Translation of viral RNA; processing of polyprotein; formation of RNA replicase protein



Source: Adapted from Flint et al., *Principles of Virology,* ASM Press, 2000

Continued

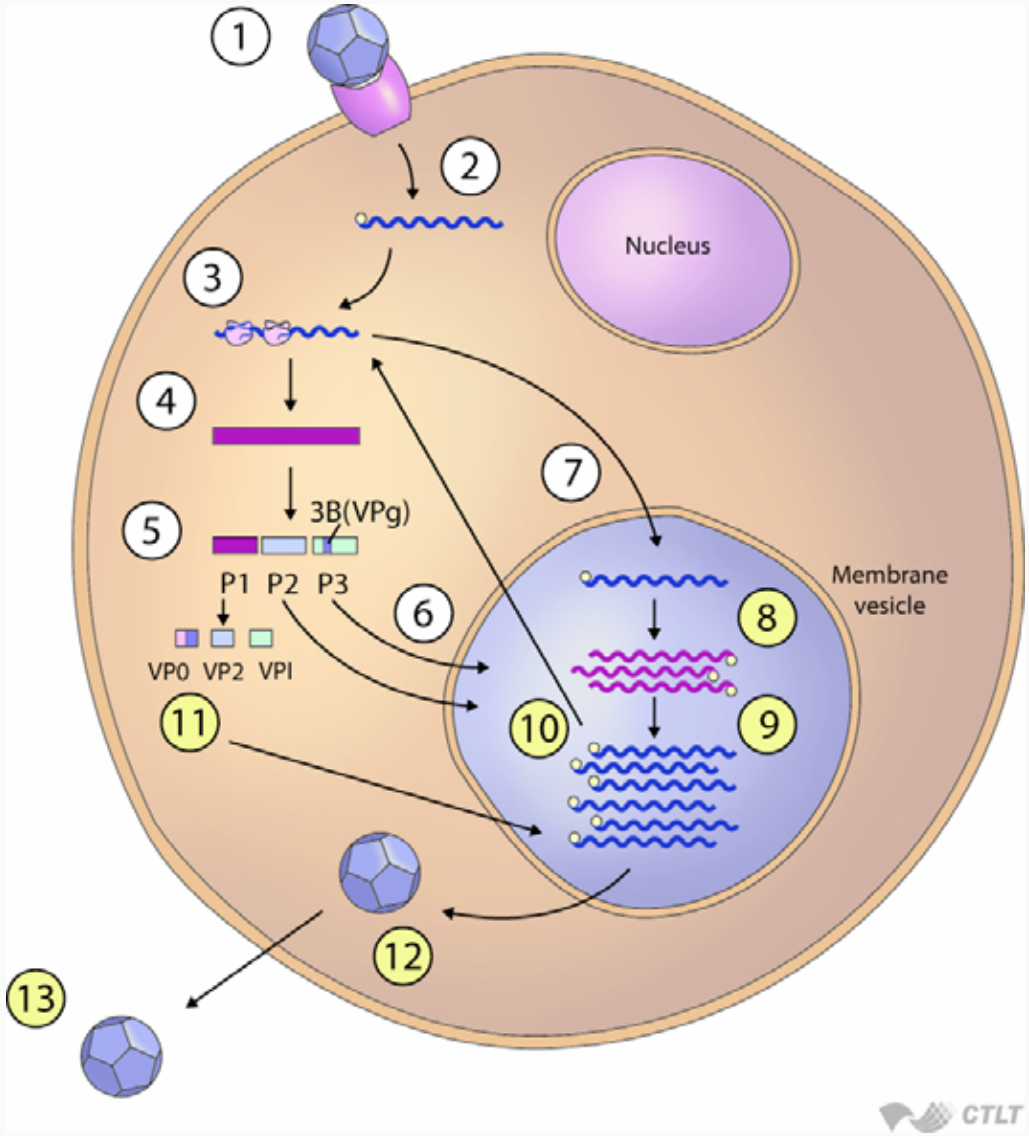
Poliovirus: Intracellular Replication

8-10. Replication of viral RNA

11. Continued translation and processing; formation of virion proteins

12. Assembly of (+) RNA and virion proteins into new virions

13. Virion release into the gut



Source: Adapted from Flint et al., *Principles of Virology,* ASM Press, 2000

Potential Targets: Viruses

- Entry
  - Immunization
- Uncoating
  - Drugs (amantadine)
- Nucleic acid synthesis
  - Drugs (nucleoside analogs—e.g., AZT)
- Translation
  - Interferons
- Protein processing
  - Drugs (protease inhibitors)

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Section C

Bacterial Pathogens

Bacteria Are Living Cells

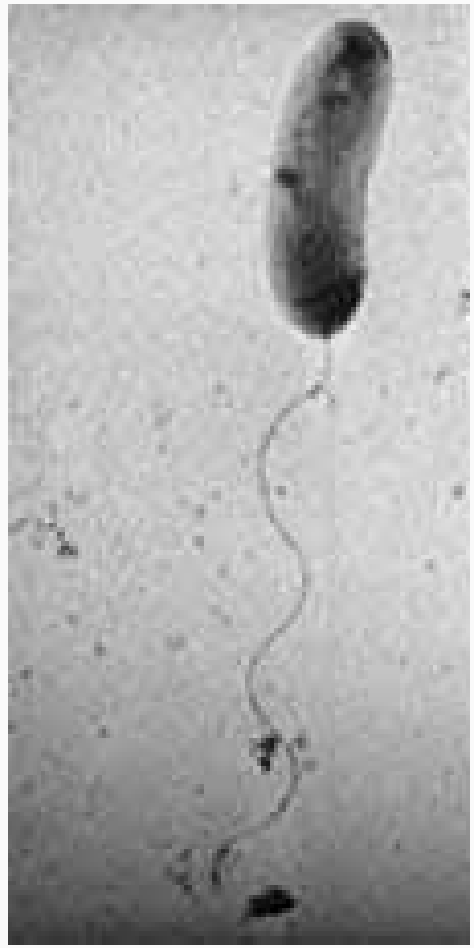
- Living cells
  - Membrane bound; with or without cell walls
  - Genetic material is DNA
  - Produce energy
  - Produce biochemical precursors
  - Produce and translate RNA to form proteins
  - Replicate DNA
  - Reproduce by cell division
  - Respond to environmental signals

Bacteria

- Bacteria are prokaryotes ("before nuclei")
  - Biochemically, they are distantly related to their eukaryotic hosts (such as humans)
    - This underlies successful antibacterial drug treatments
- Bacteria are transmitted either as metabolically active cells or dormant forms called spores
- Bacteria can be professional pathogens (e.g., TB), but many are opportunistic or facultative pathogens

Vibrio Cholerae

- Etiologic agent of cholera, a severe epidemic diarrheal disease
- There are both pathogenic and non-pathogenic *V. Cholerae* strains
  - Close relatives are normal estuarine organisms
- Transmission to humans happens occasionally from this source
  - During epidemics, transmission is fecal-oral via water
- Infection control is by cleanliness of water and food
  - A good vaccine is not available



V. Cholerae: *Infectious Cycle*

- Bacteria are ingested in contaminated water or food
- Bacteria pass through the stomach to the gut, where they colonize the surface of the small intestine
  - Ability to colonize the gut is the key determinant of ability to cause disease
  - Colonization is dependent on a number of identified and unidentified genetic "virulence factors"
  - *V. cholerae* is non-invasive and does not cause much tissue damage (differing from some other bacterial and viral pathogens)

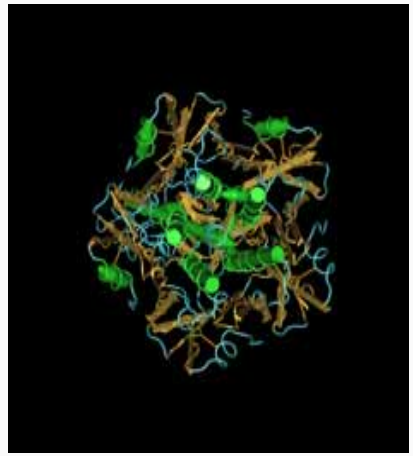
Continued

V. Cholerae: *Infectious Cycle*

- Multiplication occurs on the surface of the gut.
- Cholera toxin induces diarrhea, washing organisms out into the environment
  - Pathogenesis greatly increases efficiency of transmission
- Death is from hypotensive shock and circulatory collapse

Cholera Toxin

- Cholera toxin (CT) induces diarrhea by stimulating secretion in the intestine
  - CT secreted by *V. cholerae* in response to colonization
  - CT binds to intestinal cells (R subunit)
  - CT A subunit enters cell
  - A1 indirectly activates a cellular pump that causes Cl- and HCO_3^- secretions across epithelium; water , K+, and Na+ are secreted passively



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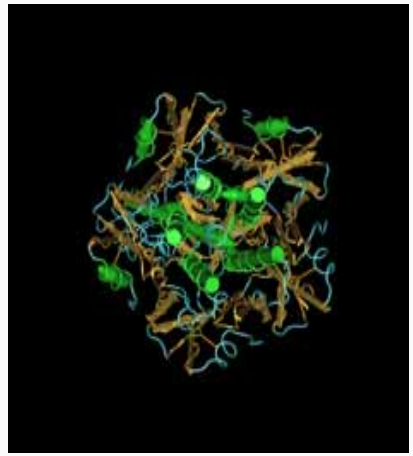
Cholera Toxin

- Effects of the toxin are independent of the continued presence of the bacteria (for a few days)
  - Antibiotics are not immediately effective in treating disease



Cholera Treatment

- Treatment is by fluid/salt replacement
- Salt solutions are not effective due to poor uptake
- IV infusions of salt solutions are effective
  - Expensive
  - Require trained personnel
  - Involve risk of other infection
- Discovery in the 1960s of glucose-dependent co-transport of Na^+ and water in the intestinal epithelium
- Oral rehydration solution now standard (glucose or starch plus appropriate salts)
  - Inexpensive
  - Skilled personnel not required
  - Immediately available (no hospitalization required)

Virulence

- Virulence is the ability of an organism to cause disease
- Most pathogens exist in forms with varying virulence
  - Not all *V. cholerae* are virulent, nor are the large number of closely related Vibrios
- A variety of genetically-determined properties of *V. cholerae* underlie virulence
  - Ability to move through mucus
  - Ability to attach to intestinal epithelium
  - Ability to produce CT
  - Ability to evade pre-existing immunity
    - For example, when El Tor *V. cholerae* was replaced with O139

Virulence Determinants

- Mechanisms/proteins/genes are of interest
  - Identification of virulent strains in the environment
  - Targets for intervention (chemotherapeutic or immune)
  - Targets for attenuation (vaccine design)
    - CVD 111 *cholerae* O1 El Tor Ogawa Δ (ctx, zot, cep, ace), ins (ctxB, mer^r)
  - Prediction of emergence of virulent strains
- Identification of virulence genes
  - Genomics: comparison of DNA sequence of known virulent and non-virulent strains
  - Construction and characterization of mutant strains in model systems

Virulence Genes

Relevant Genes Associated with Virulence and Environmental Properties in *V. Cholerae*			
Gene name	*Function*	*Location*	*Virulence (v)/(e)*
aphAB	Regulatory proteins	chromosome	v
chi genes	Chitinase homologues	chromosome	e
£rC	Flagellar transcriptional regulator	chromosome	v/e
irgA	Iron-regulated outer membrane protein	chromosome	v
msh genes	Type IV pili (mannose-sensitive hemagglutinin)	chromosome	e
ompU, T	Outer membrane porins	chromosome	v/e
rfb genes	O-antigen biosynthesis	chromosome	v/e
rtxA	"Repeats in toxin" toxin, cross-links cellular actin	chromosome	?
toxRS	Transmembrane regulatory proteins	chromosome	v/e
wav genes	LPS core oligosaccharide synthesis	chromosome	v/e
vps genes	Exopolysaccharide synthesis	chromosome	e
ace	M13 gene VI homologue formerly 'accessory enterotoxin'	CTXP phage	v
cep	M13 gene VIII homologue formerly 'core-encoded pili'	CTXP phage	v
ctxAB	CT subunits A, B	CTXP phage	v
orfU	M13 gene III homologue	CTXP phage	v
rst genes	regulation, integration, replication	CTXP phage	v
zot	M13 gene I homologue formerly 'zonula occludens toxin'	CTXP phage	v
acfABCD	Accessory colonization factors, function unknown	VPI	v
aldA	Aldehyde dehydrogenase ToxT-activated	VPI	?
tagA	ToxT-activated gene	VPI	?
tcpA	Toxin co-regulated pili major subunit (type IV pili)	VPI	v
tcpPH	Transmembrane regulatory proteins	VPI	v
toxT	Virulence transcriptional activator	VPI	v

Virulence and Mobile Genetic Elements

- Half of *V. cholerae* virulence-associated genes lie on mobile genetic elements
  - DNA sequences that can be transmitted from one strain to another (or across species barriers)
    - Vibrio Pathogenicity Island (Toxin co-regulated Pili)
    - CTX phage (CT)
- Populations that interact to produce cholera epidemics include humans, Vibrio, viruses, and other mobile genetic elements
  - Transfer of mobile virulence determinants may underlie emergence of virulent strains

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Section D

Eukaryotic Pathogens/Malaria

Eukaryotic Pathogens

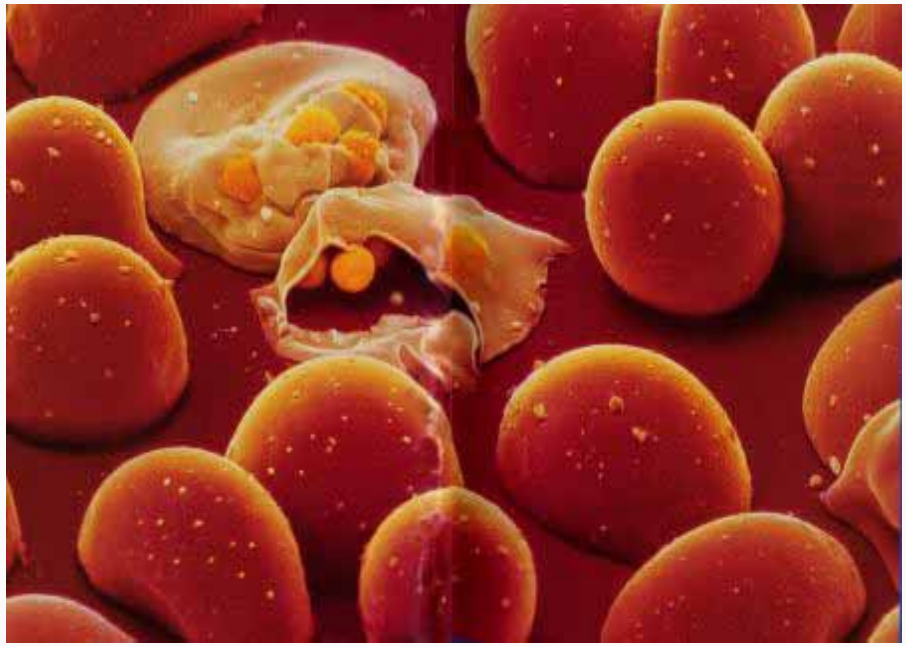
- Living cells
  - Membrane bound; with or without cell walls
  - Genetic material is DNA
  - Produce energy
  - Produce biochemical precursors
  - Transcribe and translate RNA
  - Replicate DNA
  - Reproduce by cell division
  - Respond to environmental signals
- Unlike bacteria, eukaryotic pathogens are (biochemically) rather closely related to humans
  - Complicates chemotherapy

Malaria: The Disease

- Responsible for about 300 million acute cases/year and about 1 million deaths (mostly children)
- Acute malaria includes periodic fever, diarrhea, aches
- Most deaths result from severe malaria (severe anemia, hypoglycemia, circulatory collapse) or cerebral malaria
  - The unifying pathogenic mechanism is reduced tissue oxygenation (anemia, sequestration)

Malaria: Pathology

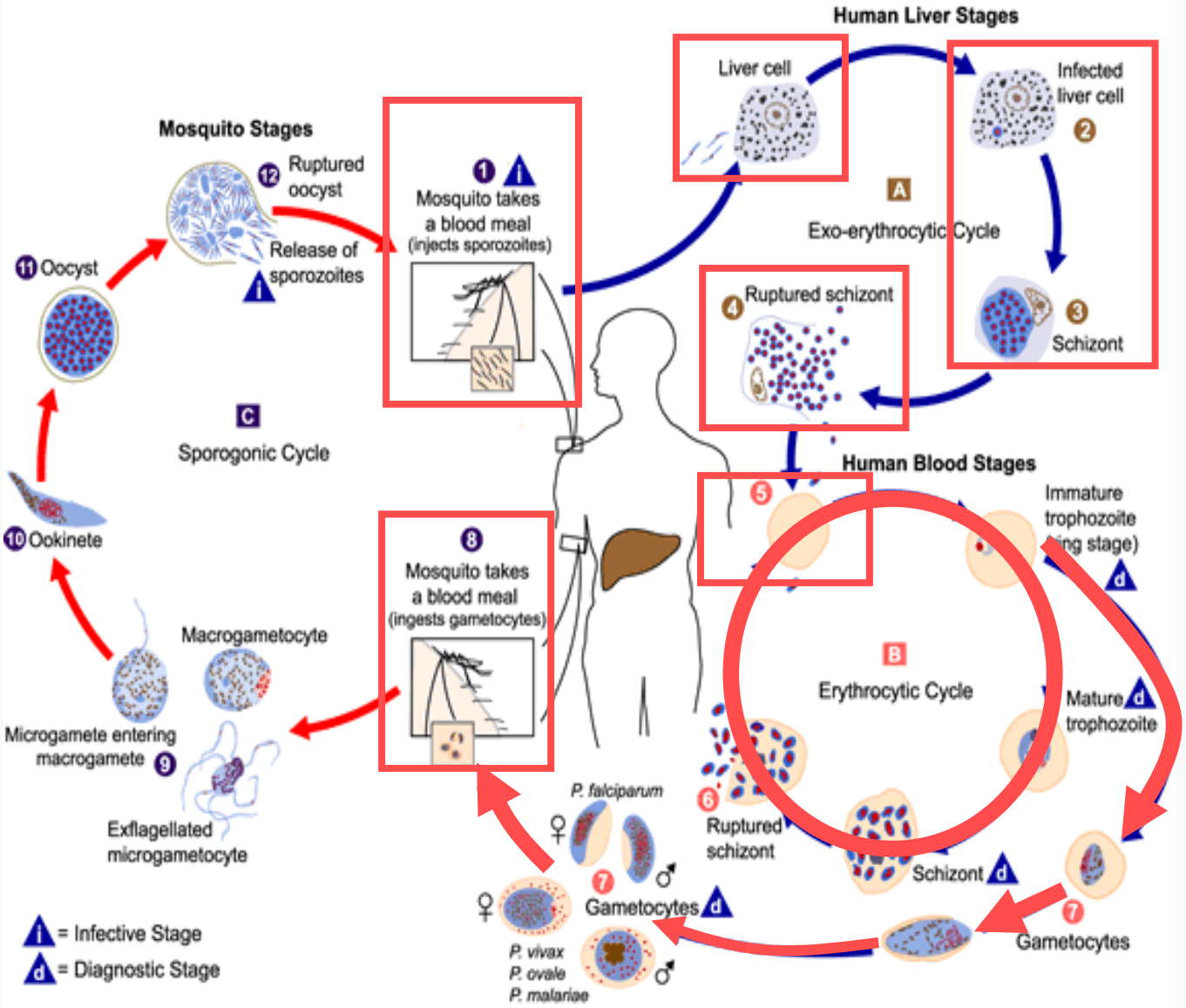
- Etiologic agent is a single-cell eukaryotic parasite of the genus *Plasmodium*
  - *Falciparum* (severe malaria)
  - *Vivax*
  - *Ovale*
  - *Malariae*



- Vector-borne
  - Transmitted by female mosquitoes of the genus *Anopheles*
- Complex life cycle

Photo source: (www.dpd.cdc.gov/dpdx/images/ParasiteImages/M-R/Malaria/malaria_LifeCycle.gif)

Malaria Life Cycle



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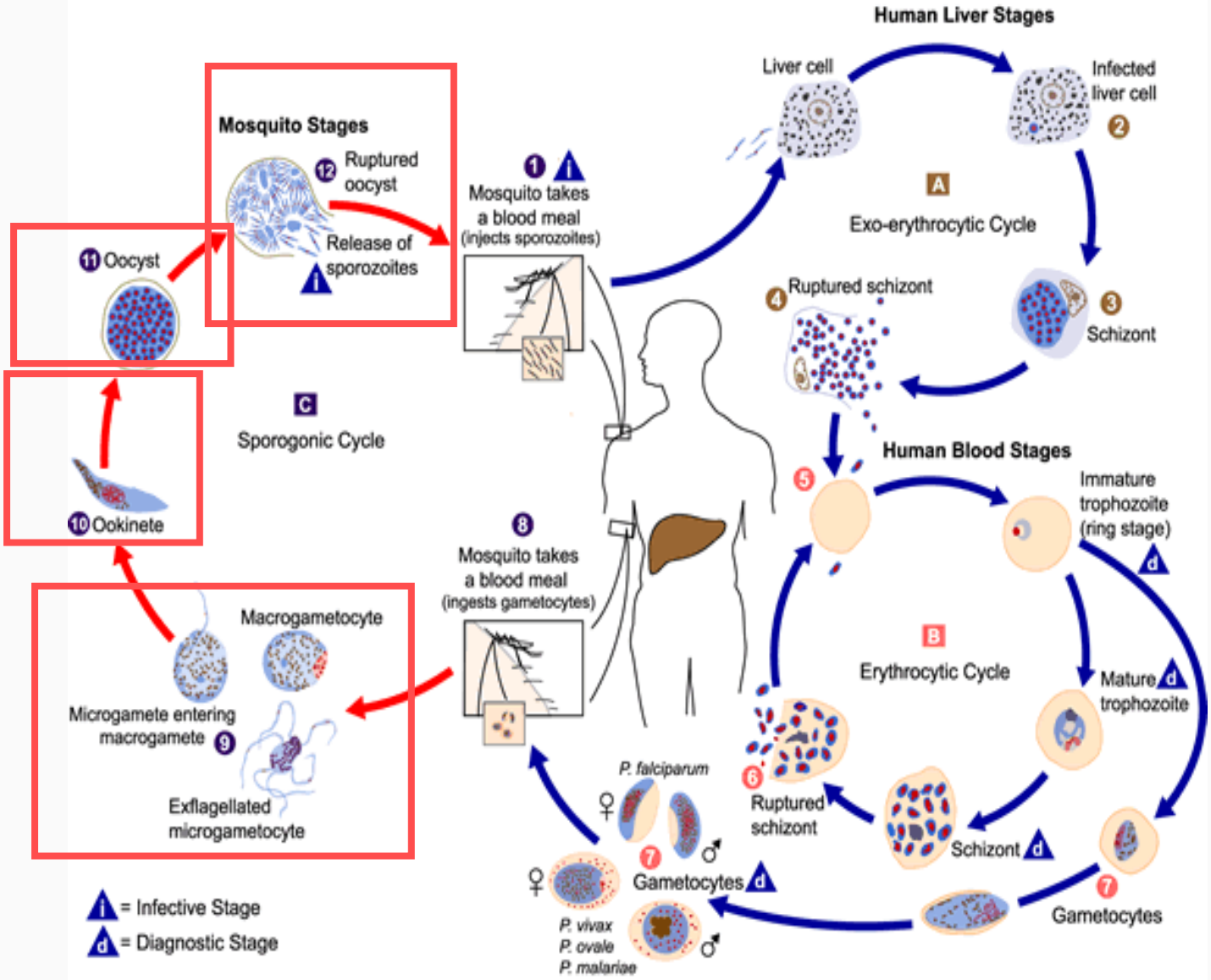
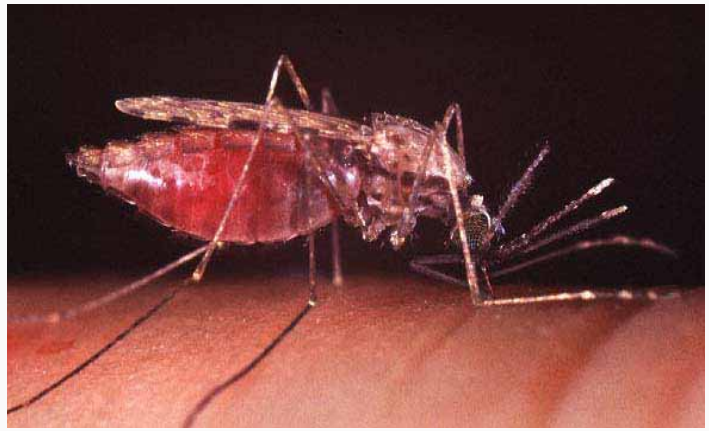
Malaria Life Cycle



Malaria Control: The Vector

- Vector essential for transmission (Ronald Ross)
  - Control of breeding sites (water)
  - Insecticide use (residual sprays in/around houses)
  - Reduction in exposure (bed nets, screens, repellants)



An. gambiae

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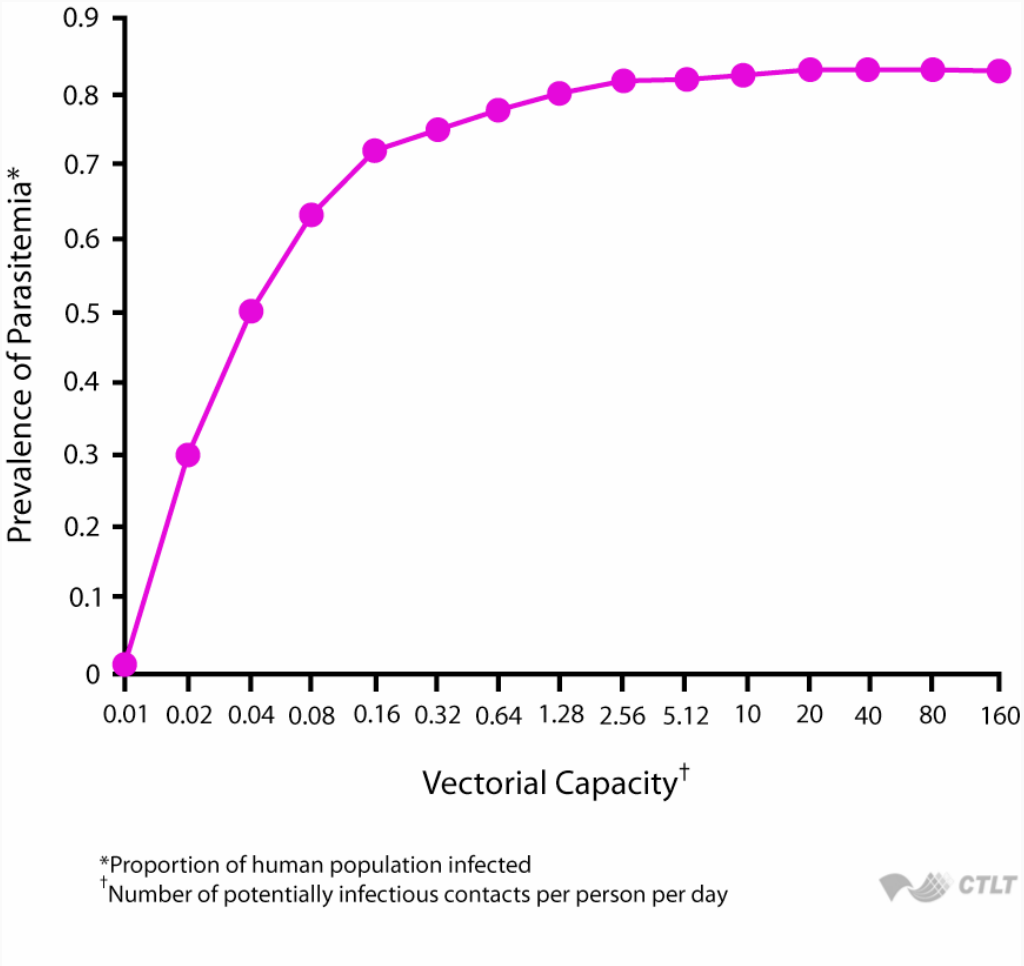
Malaria Control: The Vector

- Difficulties of target control
  - Target species must be determined and programs must be designed
    - Not all mosquitoes (nor all anopheles) transmit malaria
    - Species differ in breeding, feeding, resting behaviors
  - Costs of vector control
    - Programs are hard to sustain when the problem appears to be solved
  - Vectors develop insecticide resistance (with inappropriate use)
  - Political opposition to insecticide use/ environmental modification

Malaria Control: Vector Capacity

- Complications of vector control: in some areas, huge reductions in vector populations would be needed to affect transmission (1000x)



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Malaria Control: Drugs

- Chloroquine
  - Discovered prior to WW II
  - Cheap and effective
  - Non-toxic to humans
  - Acts against the blood stage (merozoites) by interfering with heme detoxification
  - Resistance to chloroquine developed in the 1960s, and some degree of resistance is now seen in all endemic regions in Africa
- Mefloquine
- Primaquine
- Pyrimethamine
- Artemesinin (Quinhaosu)

Malaria Control: Vaccination

- No effective malaria vaccines exist
- Major efforts to produce vaccines against multiple life stages are underway
  - Sporozoites (infection-blocking)
  - Hepatic stages (early post-infection)
  - Blood stages (reduce symptoms)
  - Gametes/gametocytes (transmission-blocking)
- Technologies include vaccination with recombinant protein, DNA, and vectored vaccines

Malaria and Infectious Disease: Summary

- Using these three strategies in combination, it should be possible to substantially reduce malaria:
  - Vaccination
  - Development of new drugs
  - Mosquito control
- The biology of infectious organisms underlies prevention and treatment strategies
- Knowledge of the ecology of infectious organisms provides insight into population-based control strategies