Legionella, *Bordetella* and *Haemophilus*

Gram Negative Rods of the Respiratory Tract

LEGIONELLA

LEGIONELLAE
Overview

- **Facultative intracellular pathogen**
- **Gram negative rod**
- **Requires specialized media to grow**
- **Stains poorly with gram stain**
- **Transmitted via contaminated aerosols**
- **No person to person transmission**

2 Species of Clinical Importance

- ***Legionella***
  - One genus
  - 50 species
  - ½ of species implicated in human disease
- ***Legionella pneumophila***
  - Causes ~ 90% of all cases of legionellosis
  - Majority of all confirmed cases are caused by serogroups 1-6
- ***Legionella micdade***
  - Most common after *L. pneumophila*

Legionella micdadei

- **Caution:**
  - This strain can stain weakly acid fast on primary isolation, but loses this property when grown in vitro.
  - **NO** RELATIONSHIP TO MYCOBACTERIA

Microbiology

Will not grow on standard Sheep Blood Agar

Buffered Charcoal Yeast Extract Agar (BCYE)

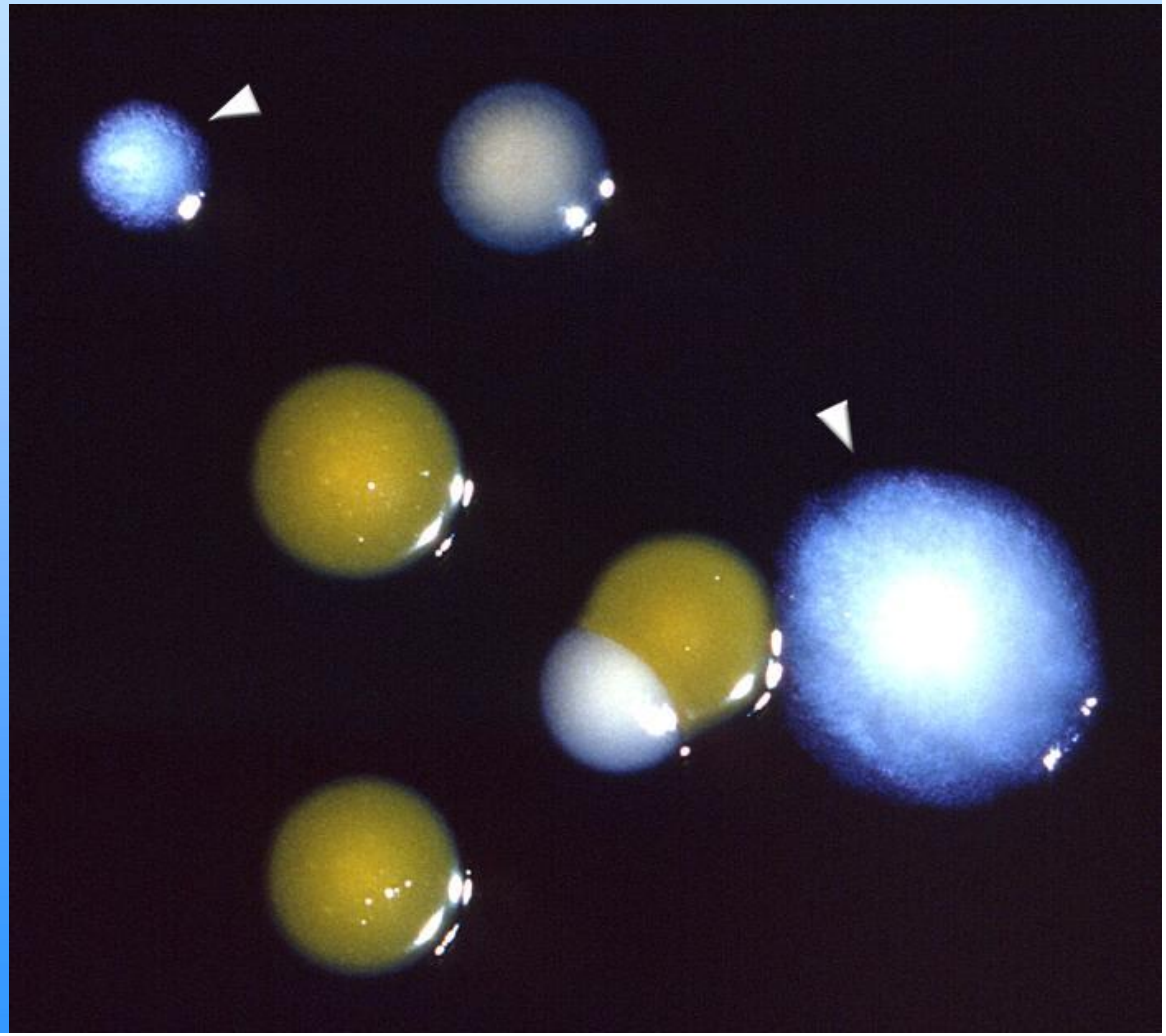
1. Cysteine is essential for growth
2. Iron is essential for growth

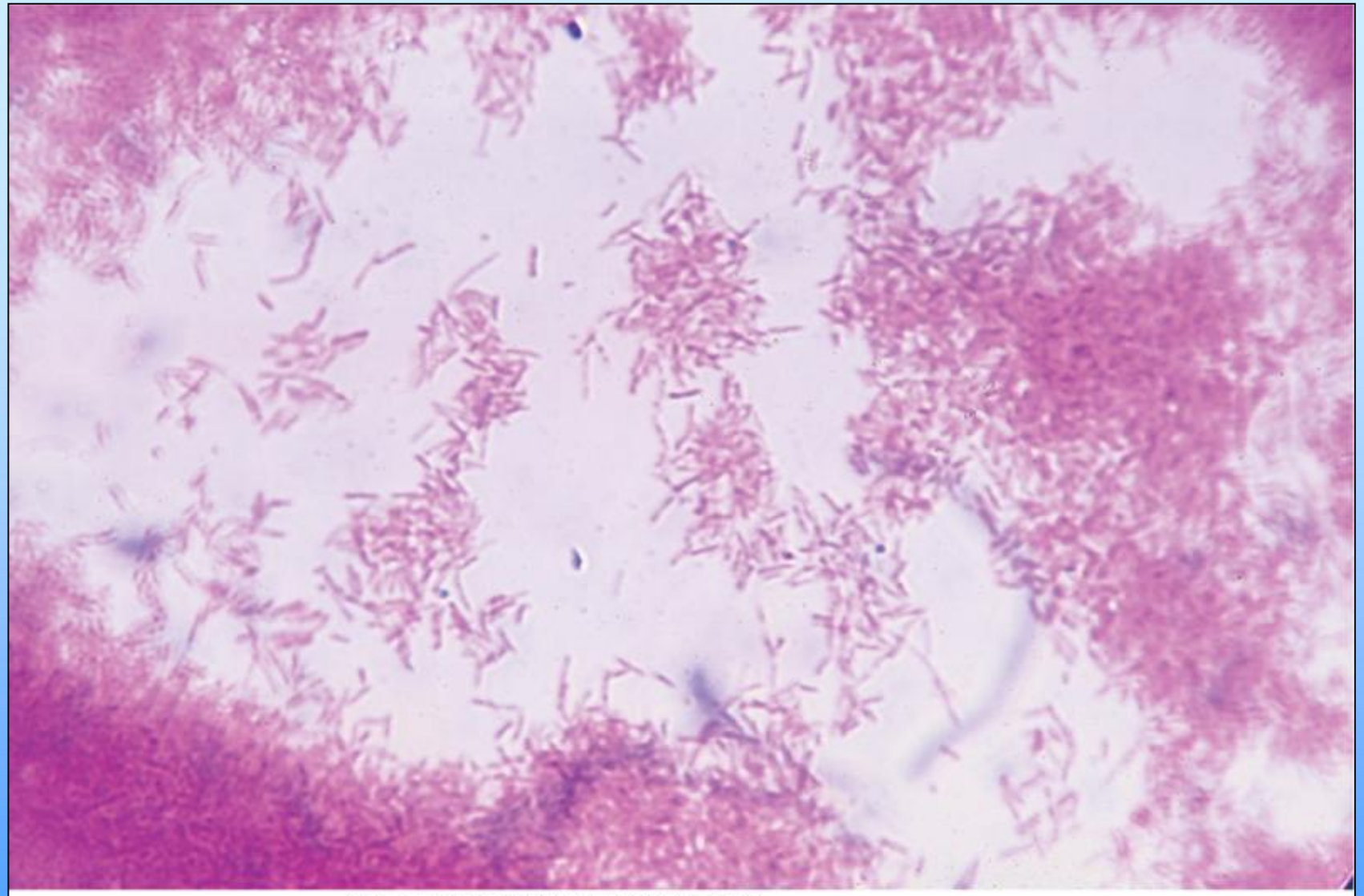
Growth conditions:

1. 35^0 C
2. 3-7 days

Colony Appearance:

- **Ground glass**
- **Small 1-3 mm**





Murray et al: Medical Microbiology, 6th Edition.

Gram staining show *Legionella* are poorly staining, slender, rods

Laboratory Diagnosis of *Legionella*

- **Culture of *Legionella* organism from normally sterile tissue**
- **Detection of *L. pneumophila* antigen in urine**
- **Seroconversion: 4 fold or greater rise in specific serum antibody titer *L. pneumophila***
- **Direct fluorescent antibody (DFA) staining**

Legionnaires disease- Public Health

- Disease - Worldwide
  - Sporadic
  - Epidemic community-acquired pneumonia
  - Nosocomial infections
- Exposure - Water-based aerosols
  - Air conditioning cooling towers
  - Whirlpool spas
  - sauna or mister
- Survival – Environment
  - Amoebae
  - biofilms

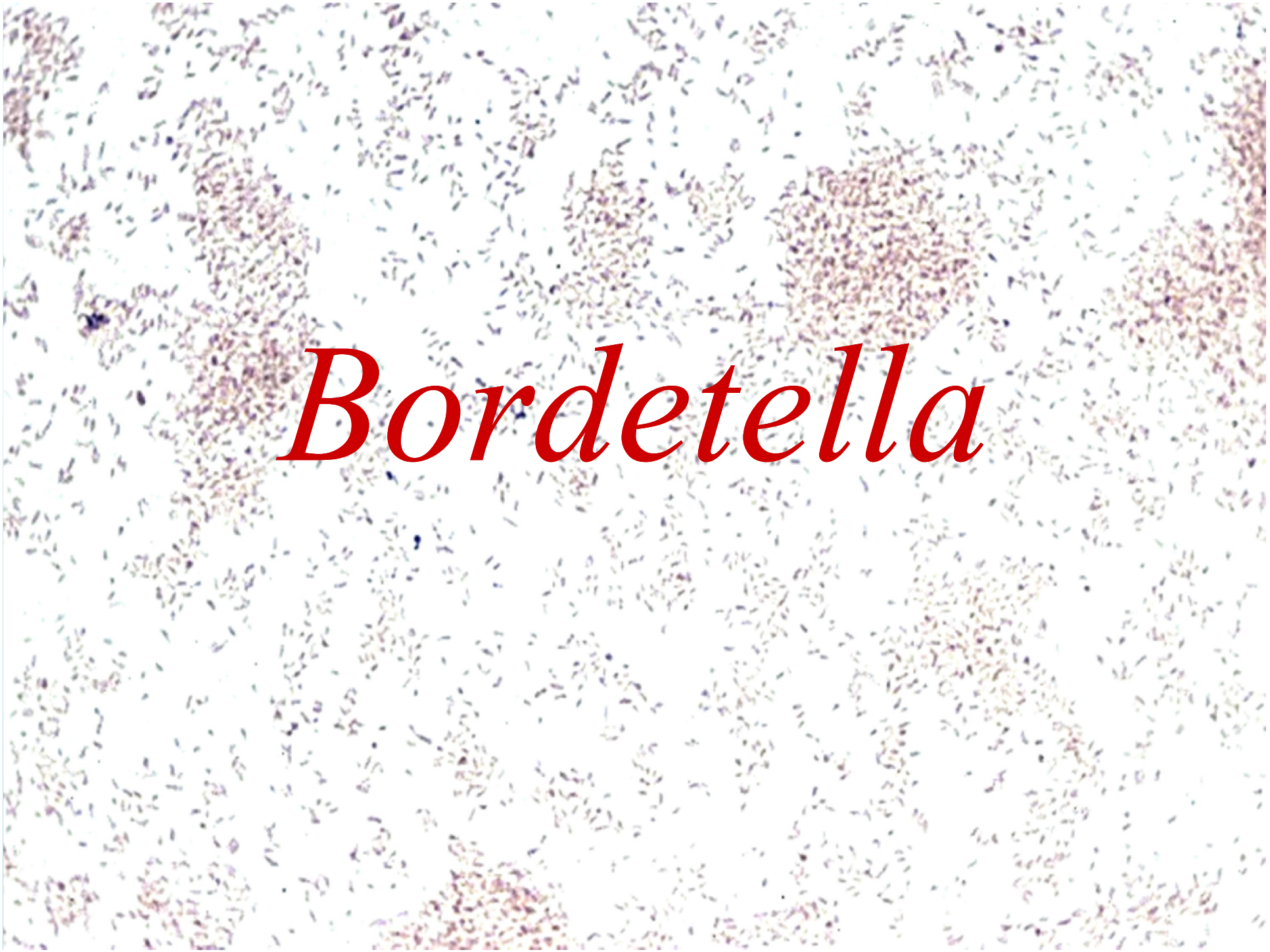
2 Clinical Presentations

- **Legionnaire's disease**
  - **Incubation period 2-10 days**
  - **pneumonia**
  - **15-75% mortality**
  - **erythromycin**

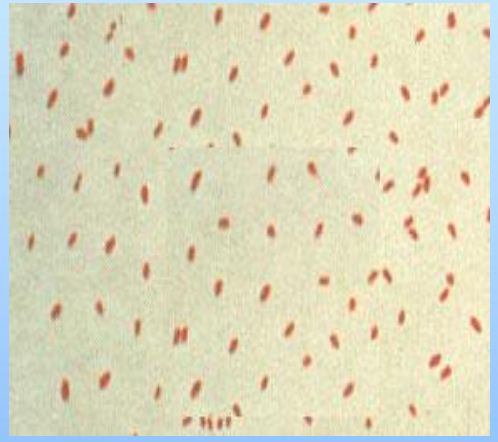
- **Pontiac fever**
  - **Incubation period 1-2 days**
  - **flu-like**
  - **milder (no mortality)**
  - **self-limiting**

PATHOGENESIS OF *LEGIONELLA*

- **Phagocytosis into the monocytes**
  - **–binding to complement receptors**
- **Inhibition of phagolysosome fusion**
- **Replication within the phagosome**
- **Lysis of the phagosome leads to apoptosis and release of the organism**
- **TH1 cells and IFN-γ**


Bordetella

Bordetella pertussis



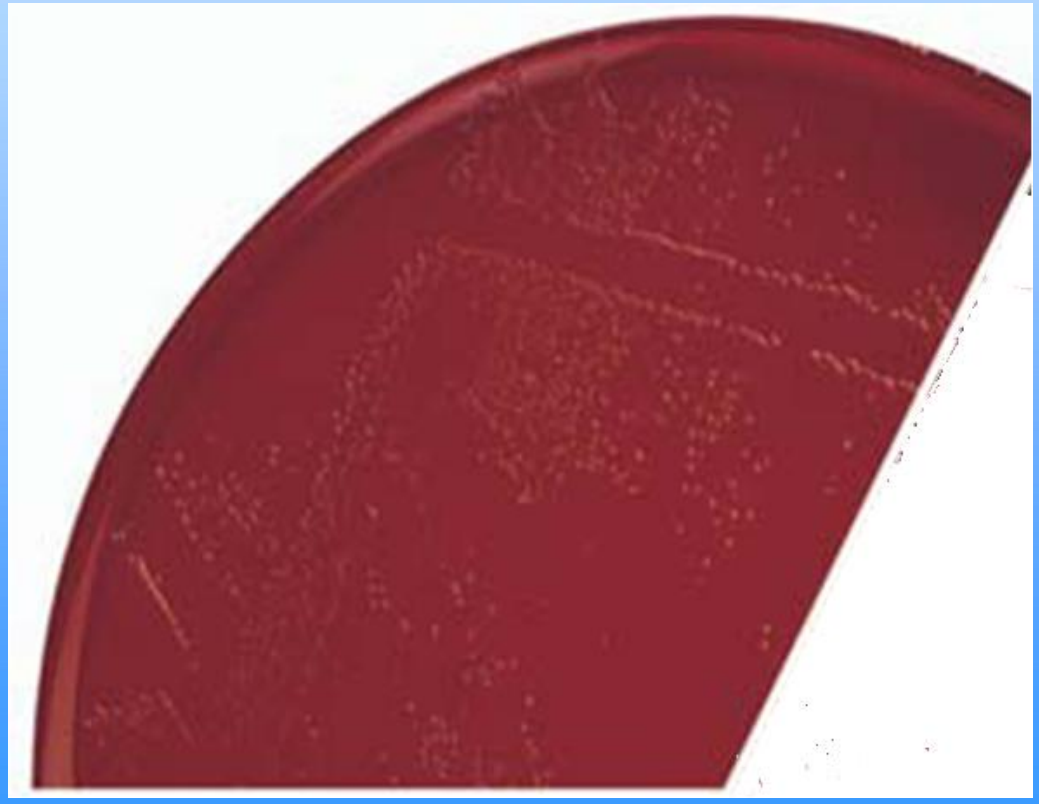
- **Strict aerobe**
- **Gram negative**
- **Small Coccobacillus -singly or in pairs**
- **Transmission by aerosolized droplets**
- **Non-invasive**
- **Strictly human pathogen**

DIFFERENTIATION OF *BORDETELLA* SPECIES

	Growth on common lab media (SBA, MacConkey)	Growth on Bordet-Gengou agar	Urease	Oxidase	Motility
B. pertussis	-	+	-	+	-
B. parapertussis	+		+	-	-
B. bronchiseptica	+		+	+	+

B. pertussis

Small, transparent hemolytic colonies on Bordet-Gengou medium

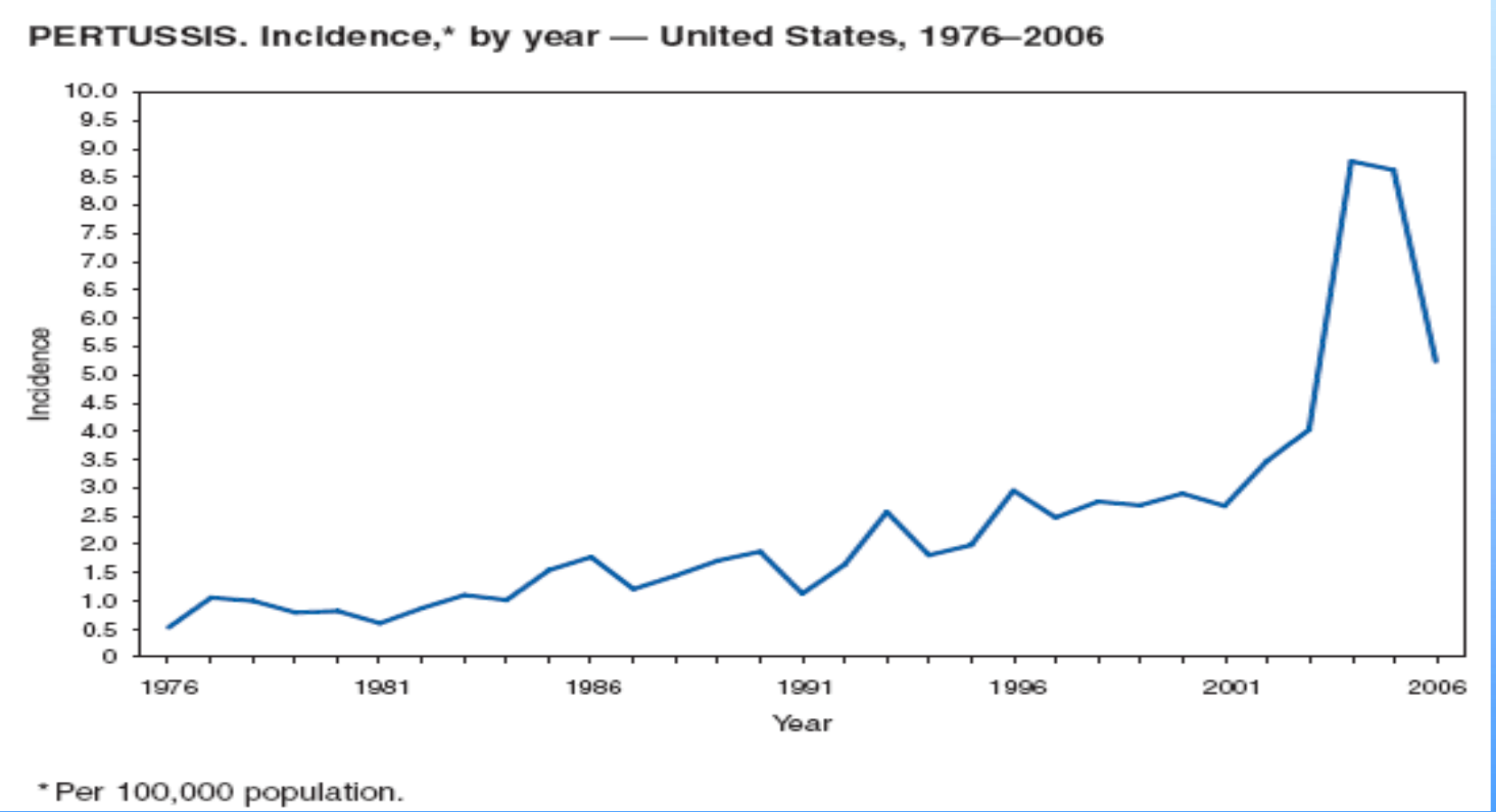


Diagnosis

- **Based on symptoms**
- **Culture of respiratory secretions on Bordet-Gengou medium**
- **Direct fluorescent antibody testing**
- **PCR**
- **Slide agglutination**

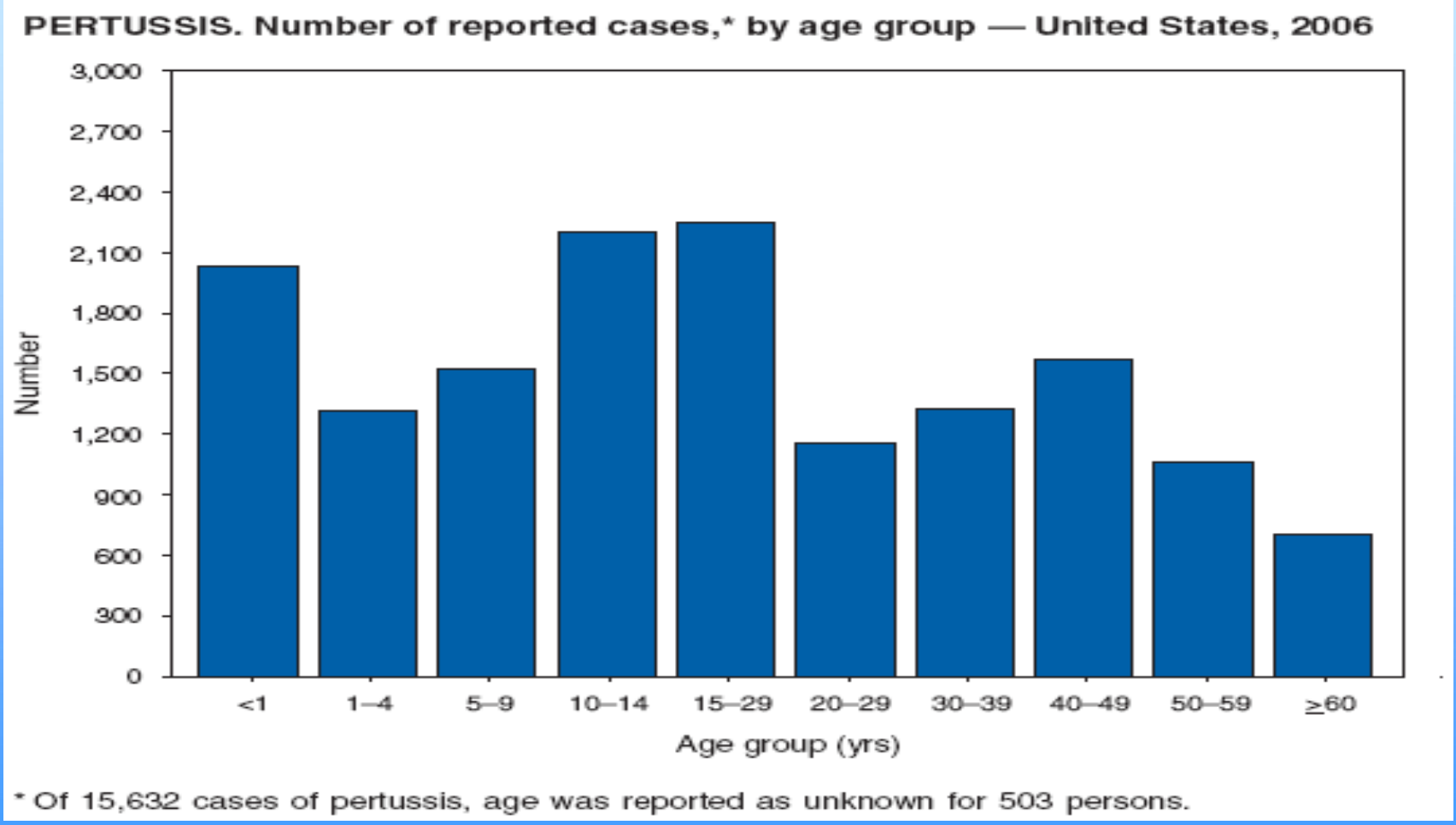
Public Health Aspects of *B. pertussis* (*Whooping Cough*)

Reported Pertussis, 1976-2006



The decrease in reported pertussis incidence in 2006 is unlikely to be related to use of Tdap and is more likely related to the cyclical nature of disease.

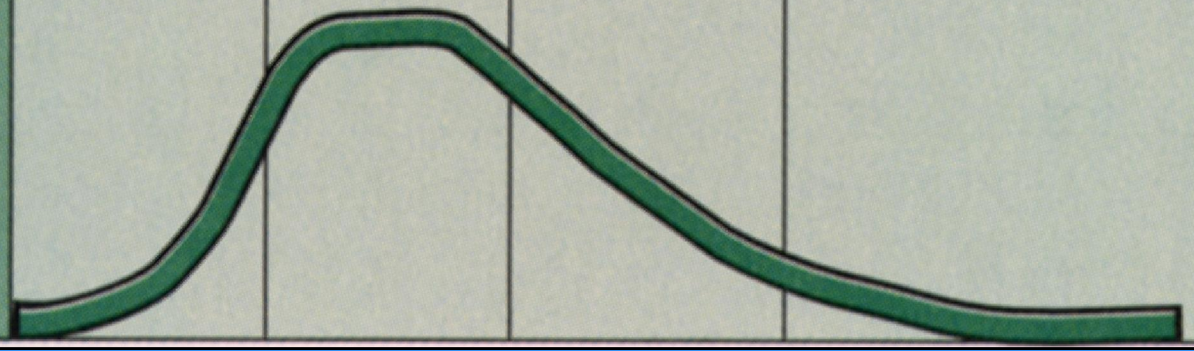
Reported Pertussis by Age Group, 2006



Infants aged <6 months (too young to be fully vaccinated), had the highest reported rate of pertussis.

Adolescents aged 10–19 years and adults aged >20 years contributed the greatest number of reported cases.

Pertussis Clinical Progression

	Incubation	Catarrhal	Paroxysmal	Convalescent
Duration	7–10 days	1–2 weeks	2–4 weeks	3–4 weeks (or longer)
Symptoms	None	Rhinorrhea, malaise, fever, sneezing, anorexia	Repetitive cough with whoops, vomiting, leukocytosis	Diminished paroxysmal cough, development of secondary complications (pneumonia, seizures, encephalopathy)
Bacterial culture				

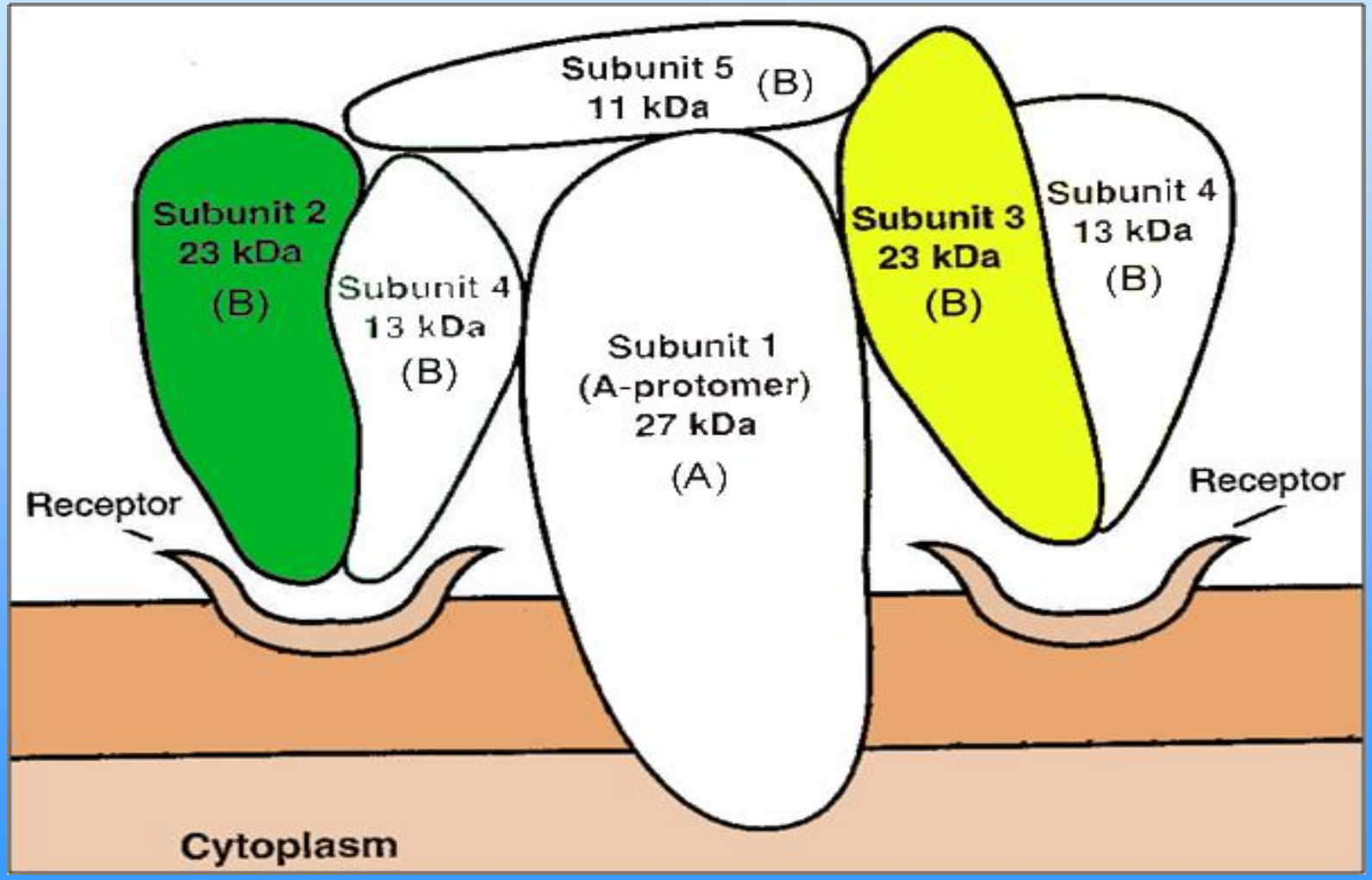
Pertussis Pathogenesis

- **Two-stage process of disease**
  - **Respiratory colonization**
    - **7-10 days**
    - **NO symptoms**
    - **Positive cultures toward the end of this stage**
  - **Toxin-mediated disease**

Colonization

- Fimbriae are NOT involved.
- Attachment requires 2 factors
  - Pertussis Toxin
  - Filamentous hemagglutinin

Pertussis Toxin
AB-toxin (6 protein subunits)



Pertussis- Disease

- **Primarily a toxin-mediated disease**
- **Exotoxins are controlled by central locus**
  - ***BvgAS* two-component signal transduction system to sense the environment and regulate gene expression**

Pertussis- Disease

- **Inflammation interferes with clearance of pulmonary secretions**
  - **Cough progresses from mild (catarrhal stage) to sever (paroxysmal stage)**
  - **Resolves slowly**
- **Evasion of host defenses**
  - **lymphocytosis**

Bordetella pertussis
Toxins

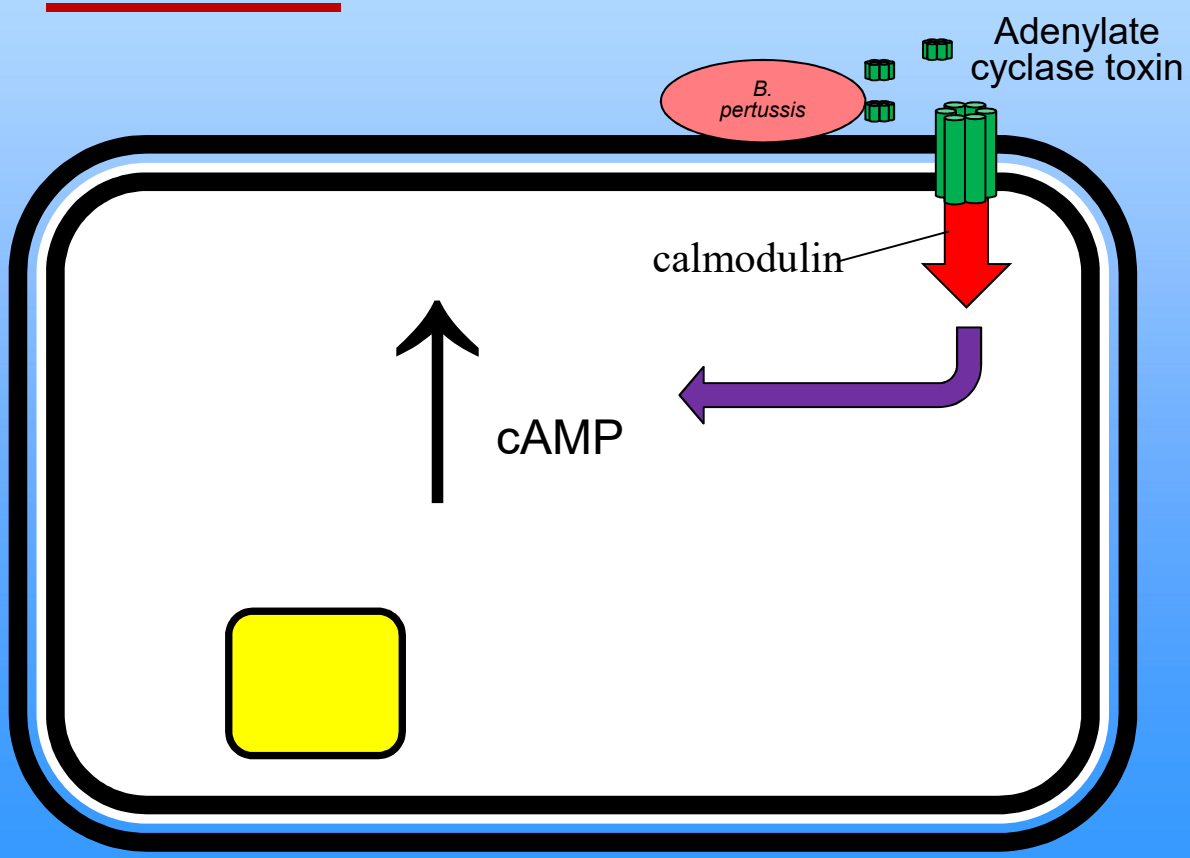
FIVE
DIFFERENT TOXINS

Systemic effects of Pertussis Toxin

- **Systemic effects**
  - **T cell Lymphocytosis with ↓ mitogenicity**
  - **↑ insulin and histamine production**
  - **↑ IgE production**
  - **Impaired phagocyte functions**

Adenylate cyclase Toxin

- **Both adenylate cyclase and hemolysin**
- **Secreted invasive toxin**



Other Toxins:

1. Dermonecrotic toxin (lethal toxin) –

Strong vasoconstrictor

2. Tracheal cytotoxin –

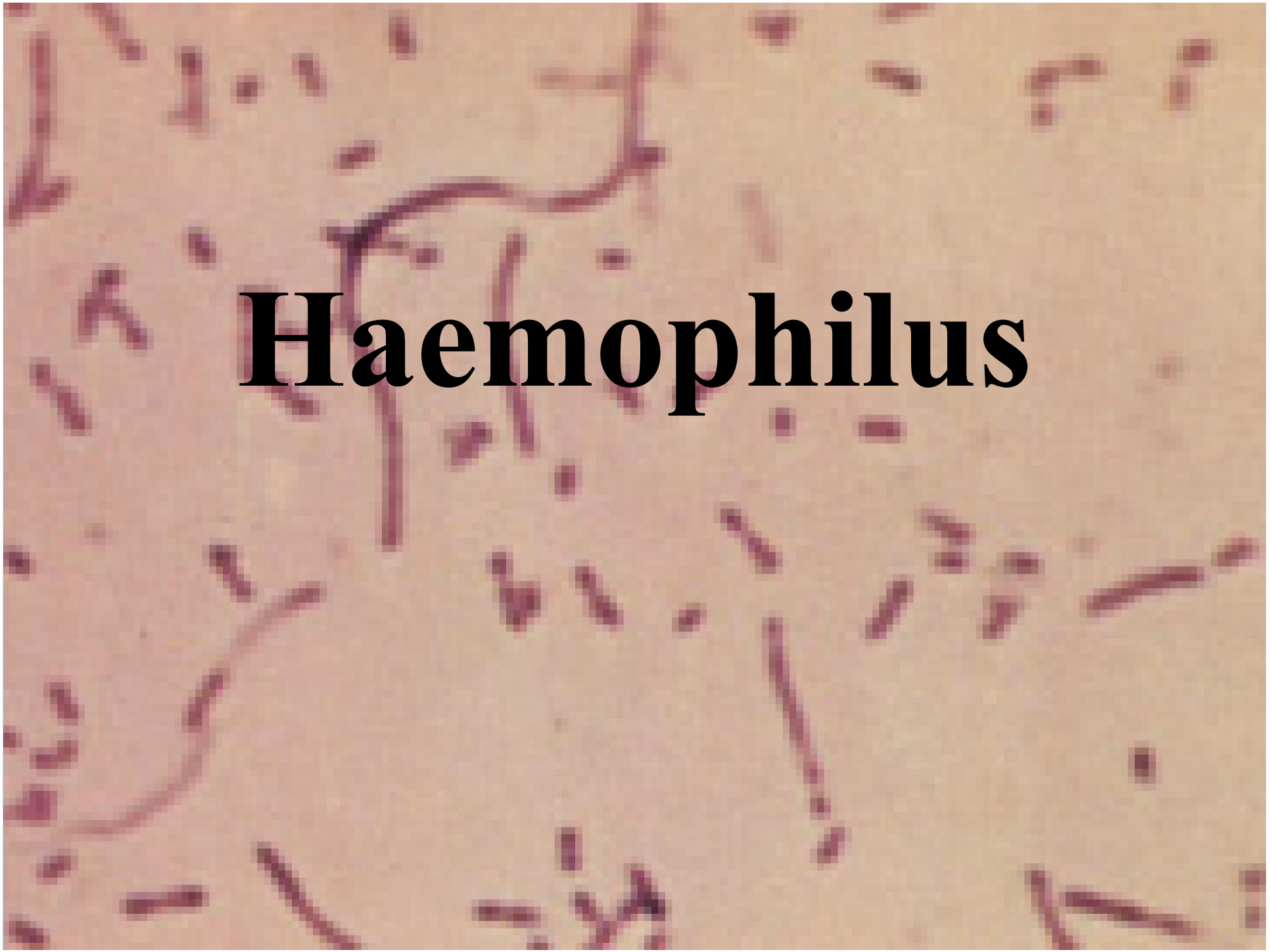
Prevents ciliated epithelial cells from beating

3. Lipopolysaccharide -

endotoxin

Treatment

- **Erythromycin**
- **Vaccine**
  - **killed bacterial cell suspension -DTP vaccine**
  - **Vaccine- induced immunity wanes after five to ten years**
  - **acellular vaccines**
    - **Multicomponent acellular vaccines**


Haemophilus

Overview- Haemophilus

- Small
- Non-motile
- Gram-negative rods
- Transmitted via respiratory droplets, or direct contact with contaminated secretions
- Normal flora of the human respiratory tract and oral cavity.

Haemophilus species of clinical importance

1. *H. influenzae*

   -type b is an important human pathogen

2. *H. ducreyi*

   -sexually transmitted pathogen (chancroid)

3. Other *Haemophilus* are normal flora

   - *H. parainfluenzae* – pneumonia & endocarditis

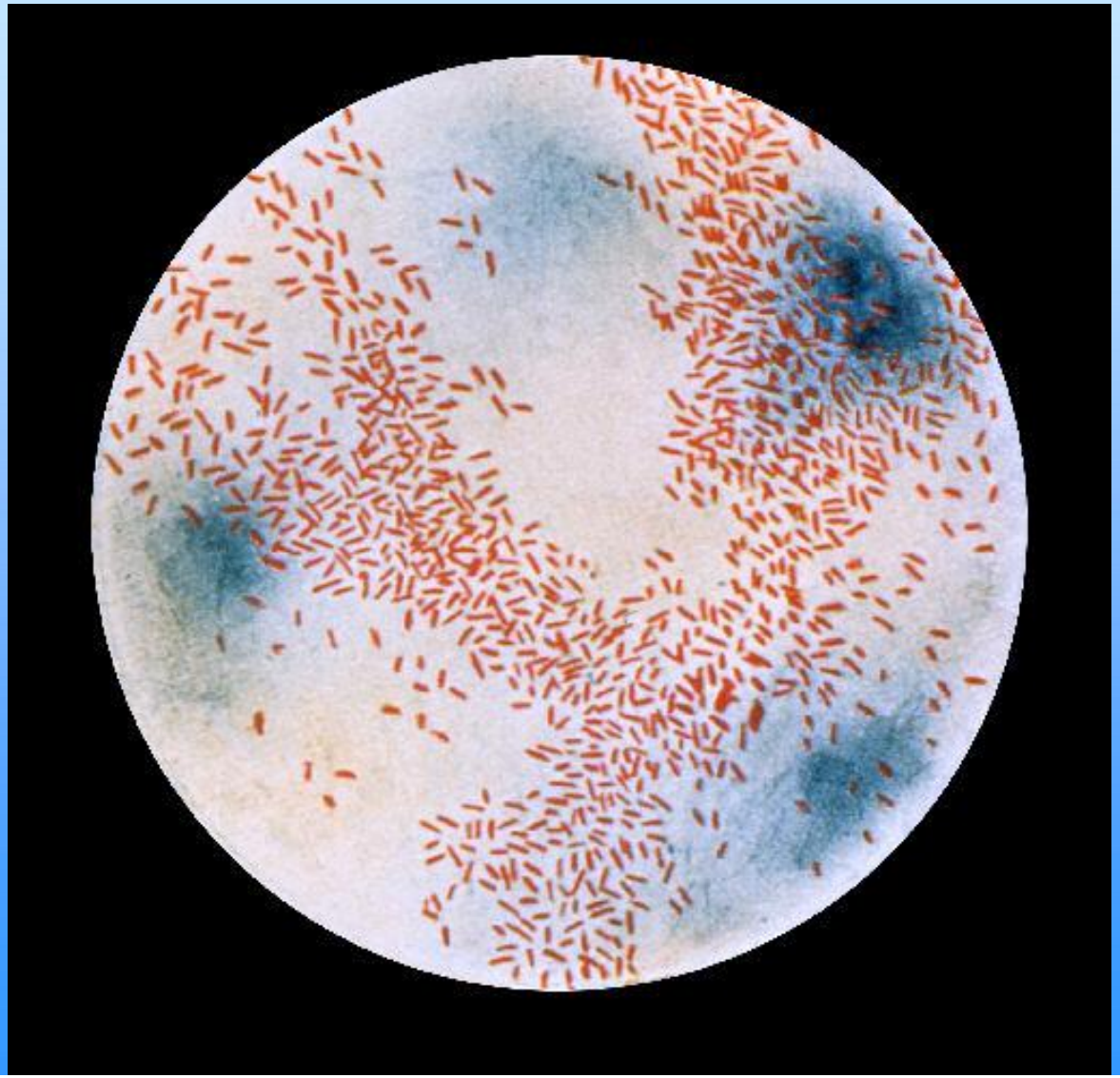
   - *H. aphrophilus* – pneumonia & endocarditis

   - *H. aegyptius* – pink eye (purulent conjunctivitis)

Differentiation of Species

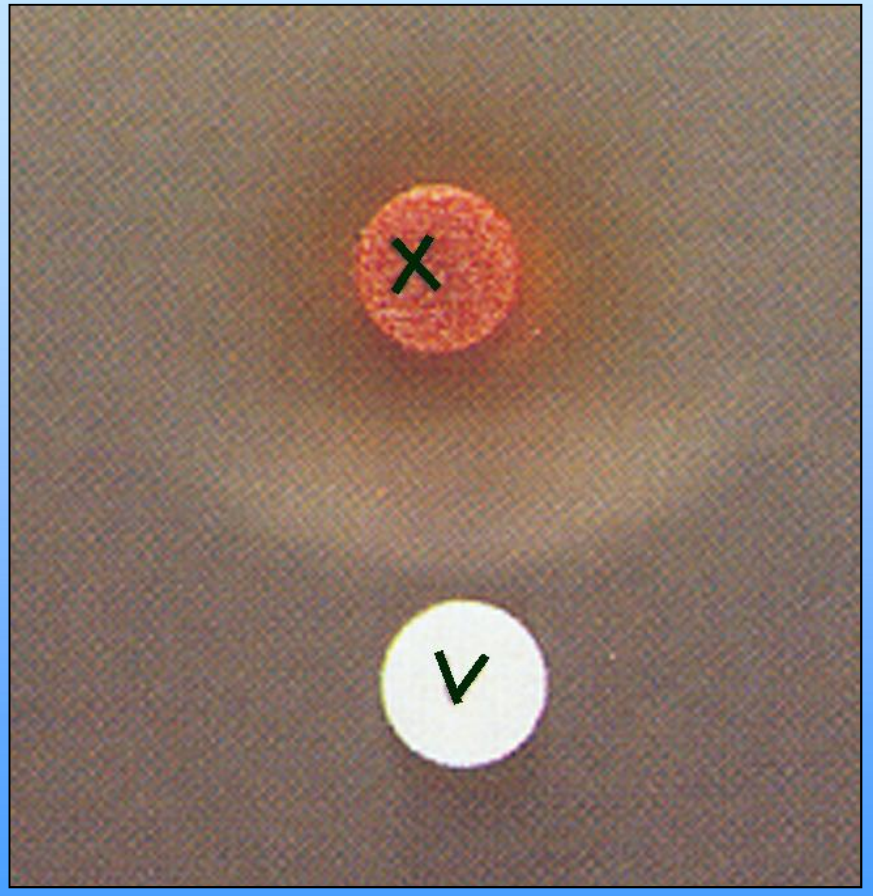
	Hemolysis	Growth Factor	
		X	Y
H. influenzae	-	+	+
H. aegyptius	-	+	+
H. ducreyi	-	+	-
H. parainfluenzae	+	-	+
H. aphrophilus	-	-	-

Haemophilus influenzae



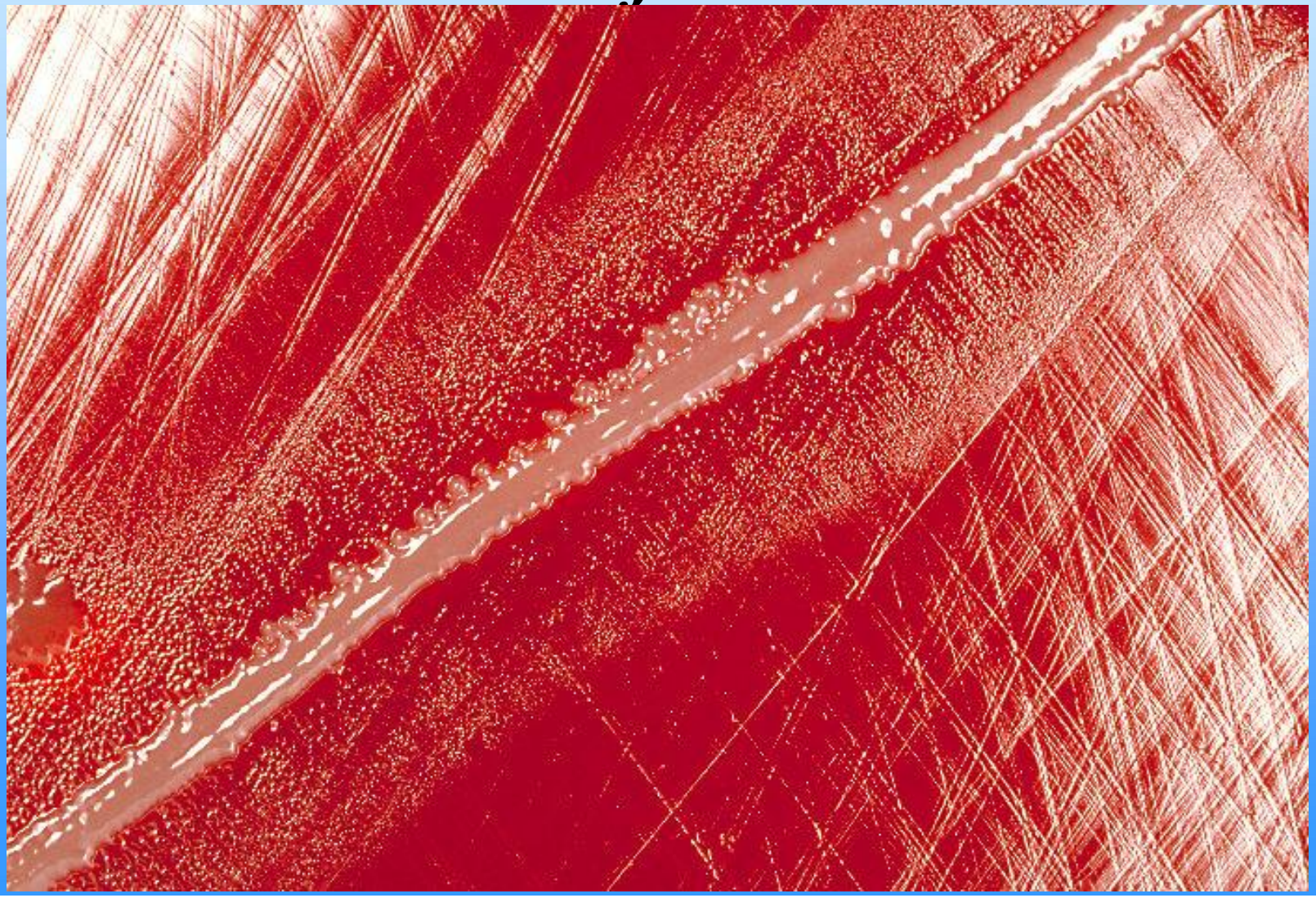
Haemophilus influenzae

- **IsoVitaleX-enriched <u>chocolate agar</u>**
- **Requires 2 erythrocyte factors for growth: X (hemin) and V (NAD).**
- **X & V factors are released following lysis of red blood cells**
- **5% CO_2 enhances growth**



Satellite Phenomenon

H. influenzae



Public Health Aspects-
H. influenzae

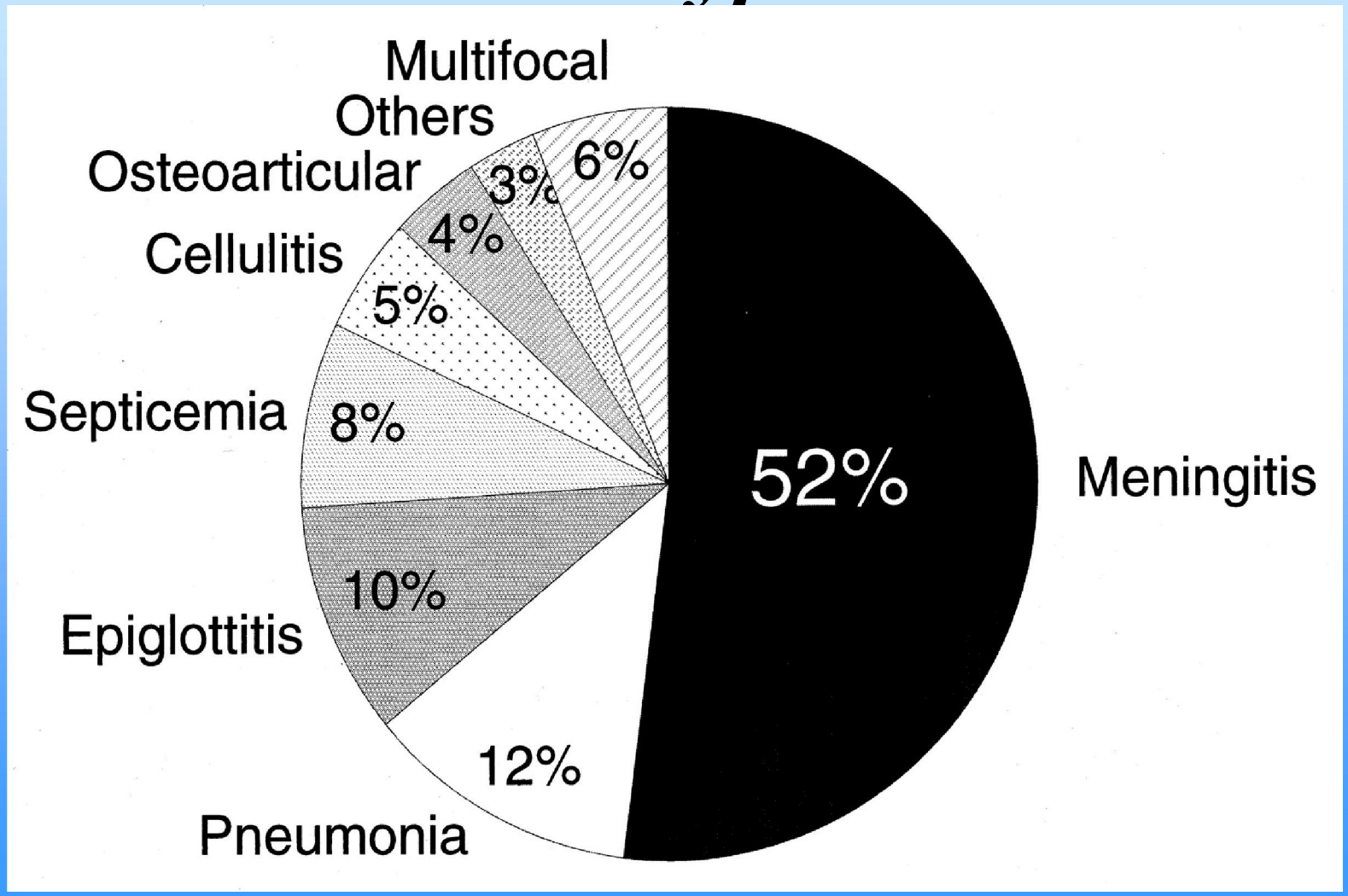
- **Typing based on capsule polysaccharide a → f**
- **Polyribose-ribitol phosphate (PRP) capsule (type b)**
- **Nonencapsulated (nontypeable) organisms are part of normal flora of the respiratory tract**
- **95% of invasive disease caused by type b**

Public Health Aspects

- ***H. influenzae*** **type b incidence has fallen 99% post-vaccine**
- **Pre-immunization**
  - **Serotype b was the most common invasive species**

- **Post-immunization**
  - **Most cases in unvaccinated or incompletely vaccinated children.**
  - **Non-encapsulated and serotype f are the most common**
  - **Children - Pneumonia and meningitis less common**
  - **Most infections (~2/3) are currently attributed to nontypeable strains.**

Disease caused by *H. influenzae* *Serotype b*



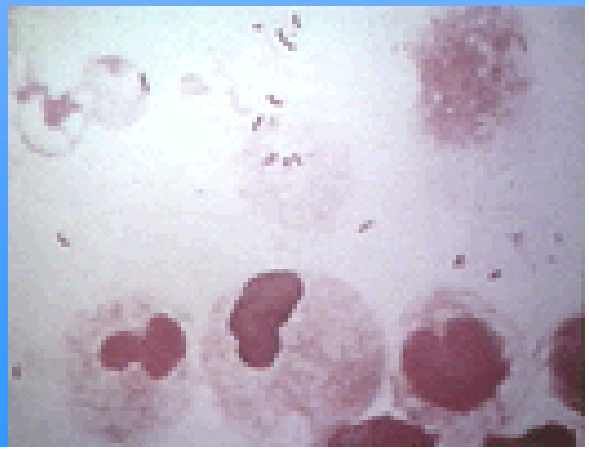
Clinical Microbiology Reviews, April 2000, p. 302-317, Vol. 13, No. 2

Invasive Diseases post-immunization

- **Septic arthritis**
- **Osteomyelitis**
- **Cellulitis**
- **Pericarditis**
- **Pneumonia - most frequent is serotype f**
- **Otitis media**
  - ***Streptococcus pneumoniae*** **and then non-typeable Hi**

Pathogenic Mechanisms

- ***H. influenzae***
  - **Antiphagocytic polysaccharide capsule is the major pathogenesis factor**
  - **Lipopolysaccharide lipid A component from the cell wall (major role in non capsule strains)**
  - **All virulent strains produce neuraminidase and an IgA protease**
  - **No exotoxins**



Pathogenesis – Host Factors

- **Hib conjugate vaccine (PRP capsule)**
- **The Hib conjugate vaccine does not protect against nontypeable strains.**
- **Persons at risk for invasive *H influenzae* disease**
  - **Asplenia**
  - **Immunocompromised**

Public Health Aspect of other *Haemophilus* strains

- ***H. ducreyi***
  - **Sexually transmitted disease - chancroid**
- ***H. influenzae* biogroup *aegyptius***
  - **Brazilian Purpuric Fever**
- ***H. aegyptius***
  - **“pink eye” (purulent conjunctivitis)**
- ***H. aphrophilus***
  - **pneumonia**
  - **Infective endocarditis**

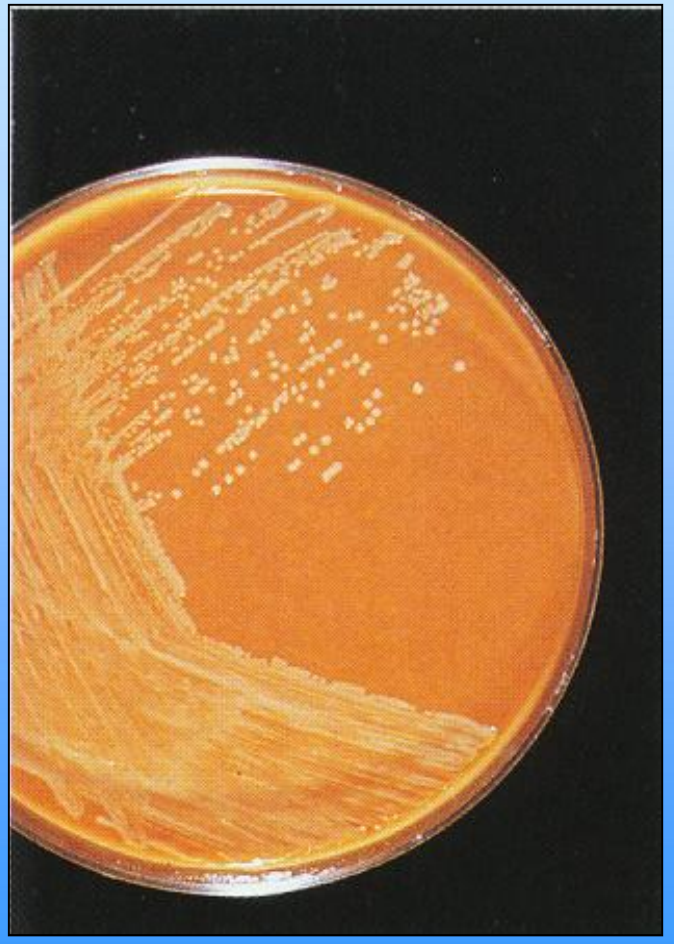
Haemophilus ducreyi- chancroid

- **~5,000 cases per year in the US**

Haemophilus ducreyi

- **Occurs in strands**

- **Grows of chocolate agar requires factors X (hemin) but not factor V (NAD)**



Haemophilus influenzae biogroup *aegyptius*

- **Brazilian purpuric fever in children**
  - **High fever**
  - **Death within 48 hours**

Case Study

- History
  - 13 year old white male
  - fully vaccinated
  - cold-like symptoms and persistent cough- 10 days duration
  - 2 weeks later
    - progressive coughing spells with inspiratory whoop
    - posttussive vomiting

Case Study

- Tests
  - Nasopharyngeal swabs
    - Bordet-Gengou medium
  - Blood samples for serology
    - positive IgM and IgA antibodies
  - Treatment
    - azithromycin

Case Study

- History
  - 4 month old white female
  - 1 day history
    - 103^0 fever, lethargy, irritability, stiff neck
- Tests
  - Cerebral spinal fluid culture
    - IsoVitaleX-enriched chocolate agar