CURRENT METHODS FOR LABORATORY DIAGNOSIS OF *BORDETELLA PERTUSSIS*

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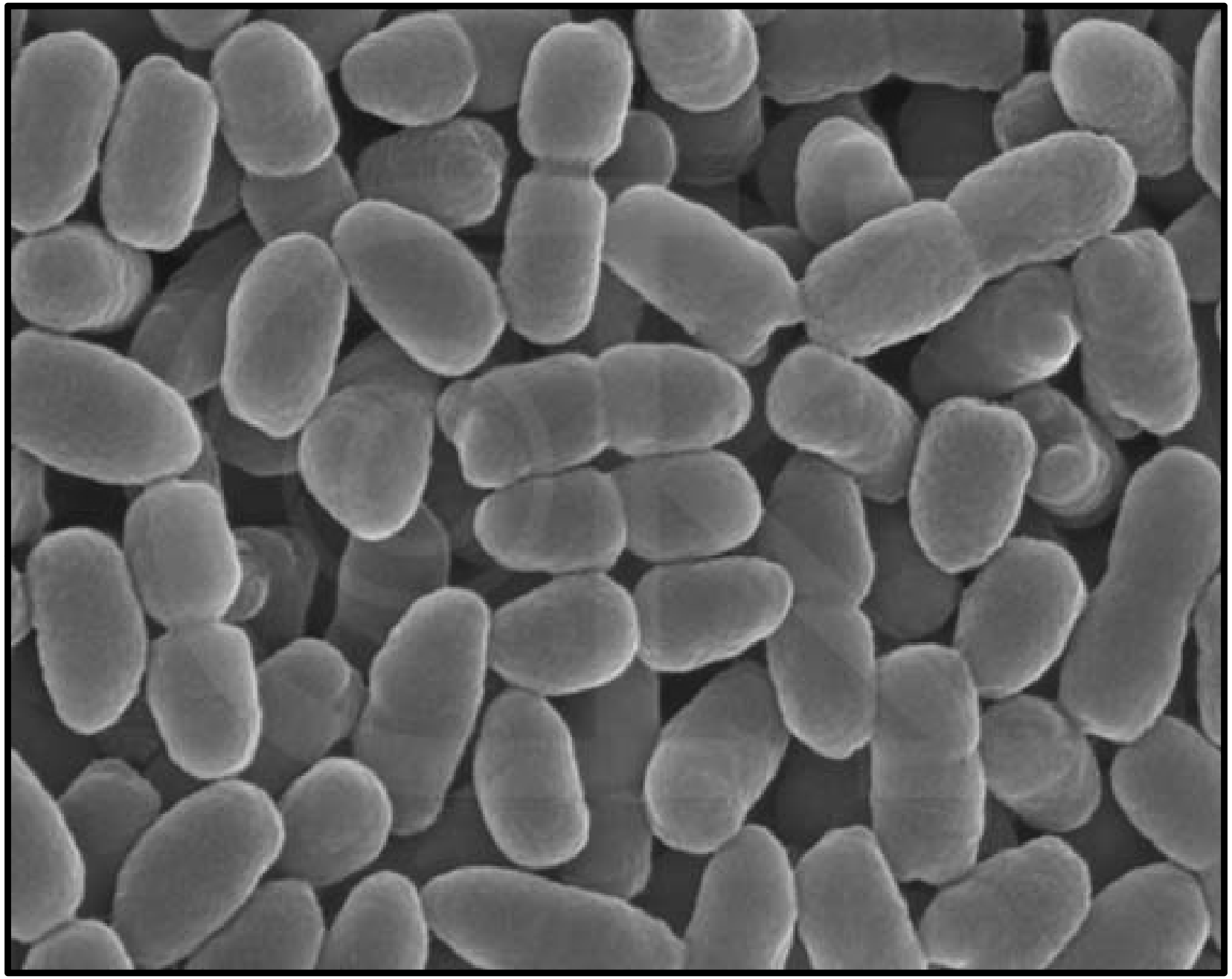
What this talk will cover (and what it will not)…



SOME BACKGROUND

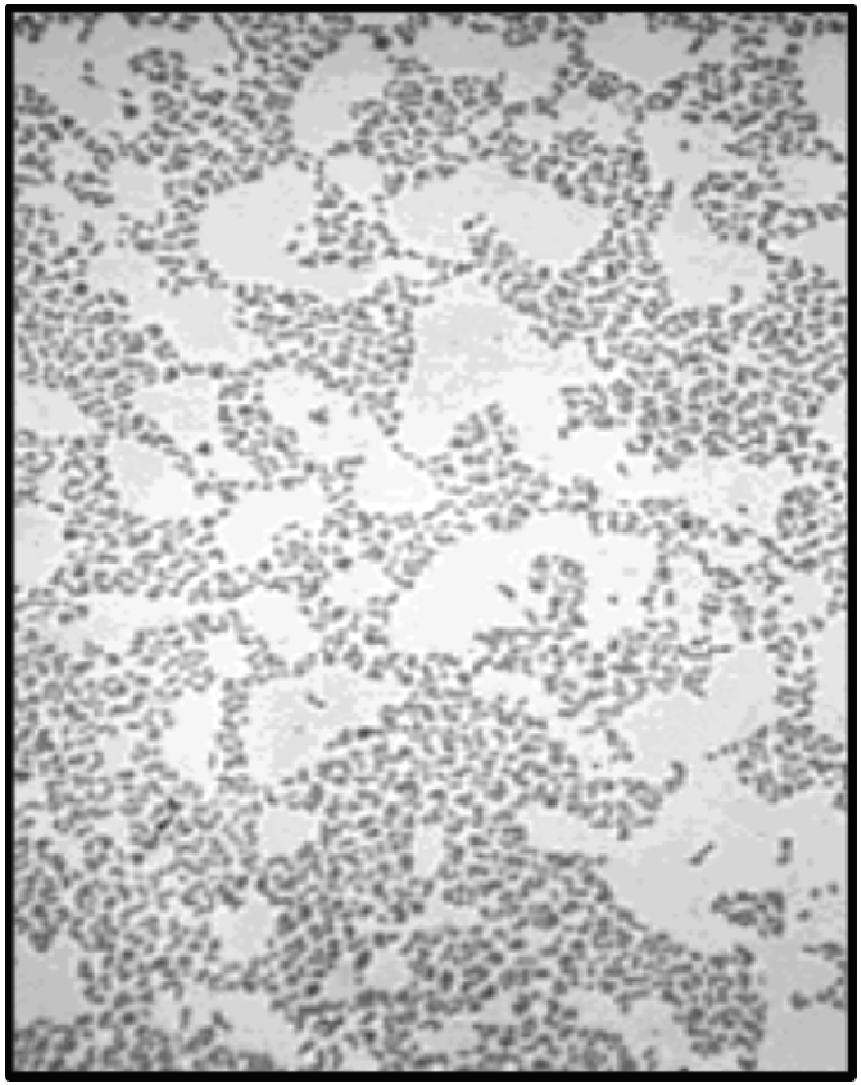
- After the introduction of routine pertussis vaccination more than 50 years ago, the belief was that *Bordetella pertussis* infections would diminish greatly.
- Pertussis did decrease initially
- Incidence of newly diagnosed cases has been increasing over the last decade
- In 2004, more than 25,000 cases were reported in the USA – the highest number of cases since 1959
- Increased prevalence has led to the realization that the immunity afforded by childhood vaccination may wane significantly with time
- Important to know clinical manifestations, diagnostic methods for pertussis

More Background

- Severe, debilitating cough illness (“100 day cough”)
- Highest morbidity/mortality rates in infants
- Despite high vaccine coverage, remains a public health problem
- Clinical diagnosis and laboratory diagnosis can be challenging
- Outbreaks regularly occur

Bordetella Basics

- Aerobic, Gram negative coccobacillus
- *Alcaligenaceae* Family
- Colonizes the respiratory tract



Bordetella pertussis (1)

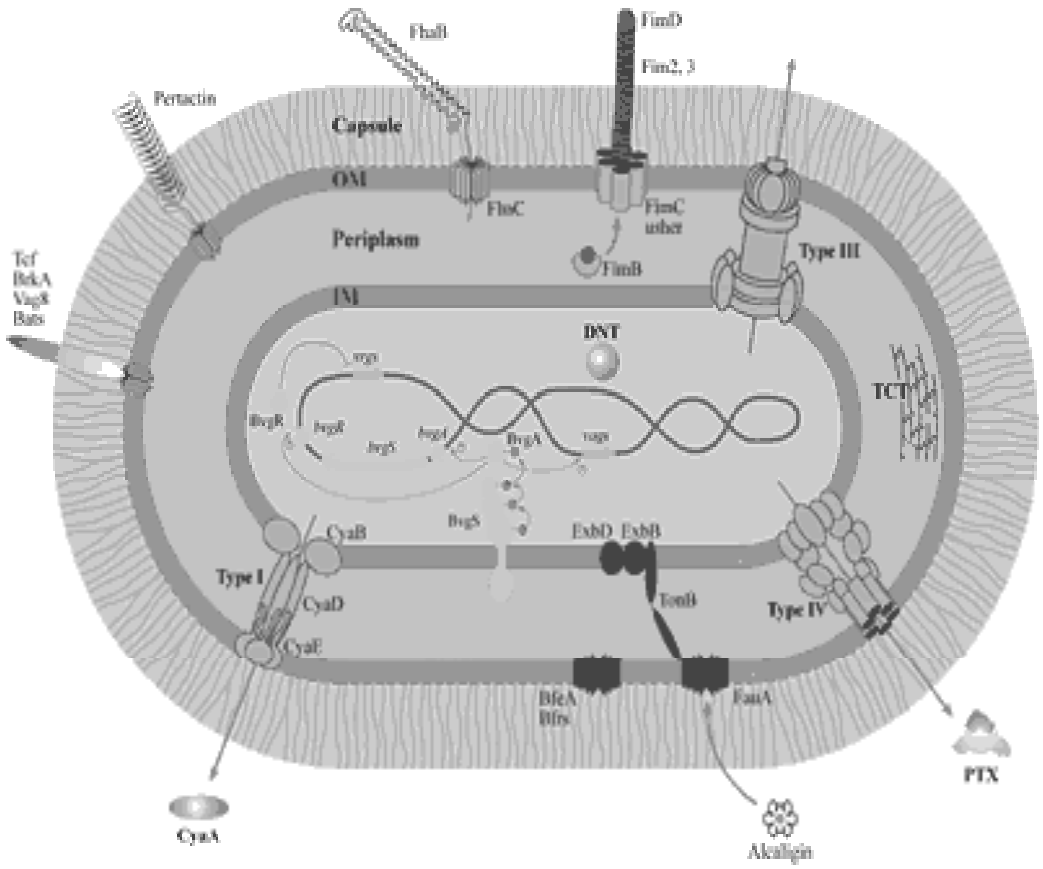
- *B. pertussis* – most important because it infects only humans & causes the most severe symptoms
- Nonmotile organism – transferred host to host through aerosolized droplets by coughing
- Destroys airway tissue through a 4-step process of attachment, avoidance of host defense, cellular destruction, and systemic effects

Bordetella pertussis (2)

- Produces a number of toxins and biologically active substances that influence its pathogenicity
- Pertussis toxin is most virulent factor due to mitogenic activity
- Cell surface contains hemagglutinin, pertactin, and fimbriae – aids adherence to epithelial cells in respiratory tract
- Several other toxins: adenylate cyclase toxin – impairs host immune cell function; tracheal cytotoxin – causes respiratory epithelial damage

TOXINS

- Pertussis Toxin
- Adenylate Cyclase Toxin
- Tracheal cytotoxin
- Dermonecrotic toxin
- Heat-labile toxin

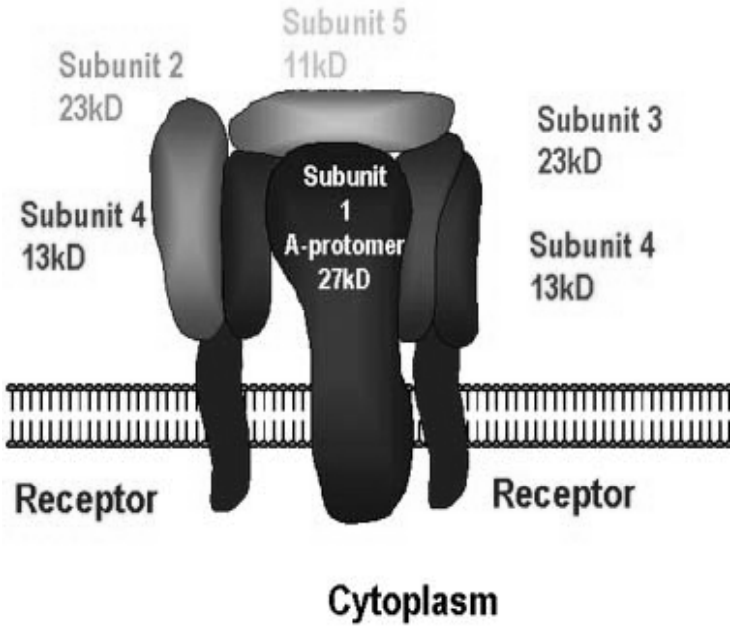


PERTUSSIS TOXIN

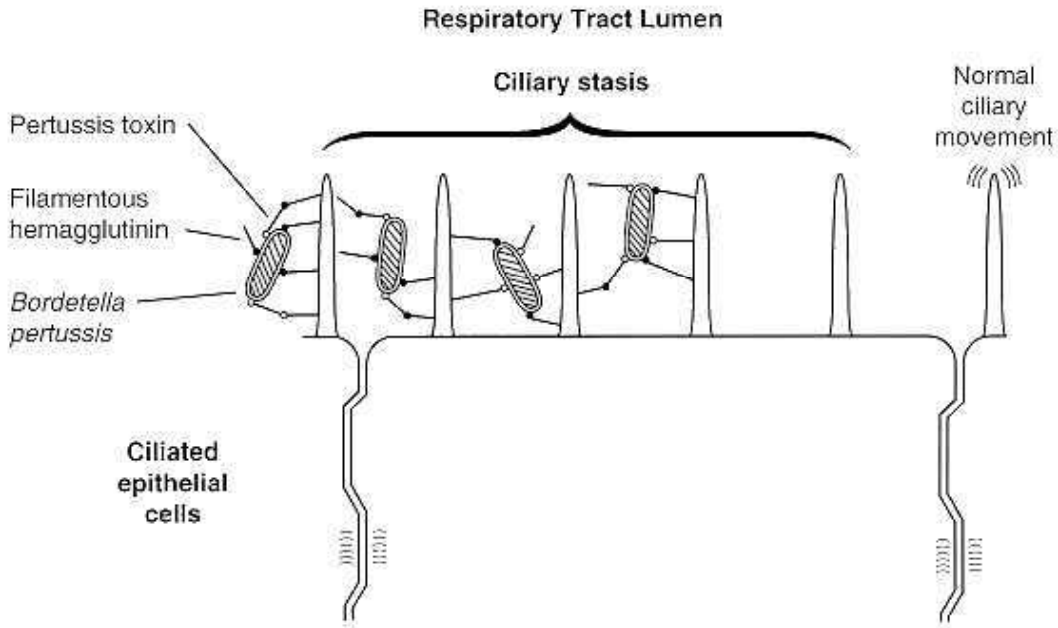
- Pertussis toxin is most virulent factor due to mitogenic activity
- Affects lymphocyte circulation
- Serves as adhesion site for binding to respiratory ciliated cells
- Role in the pathogenesis not fully understood – shown to cause lymphocytosis, hyperinsulinemia, and encephalopathy

PERTUSSIS TOXIN

- Colonizing factor and endotoxin
- Cell bound and extracellular

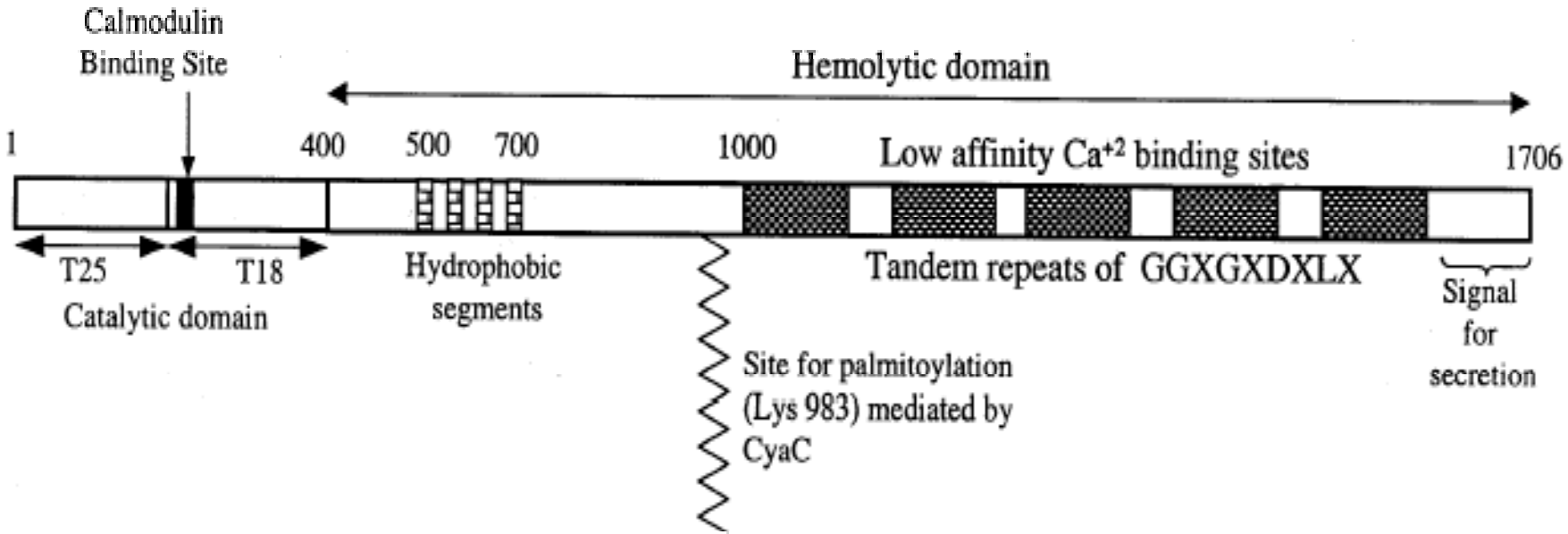


www.med.sc.edu:85/ ghaffar/pertussis.jpg



ADENYLATE CYCLASE TOXIN

- Invasive toxin
- Activated by host cell calmodulin
- Impairment of immune effector cells



Bvg LOCUS

- Controls expression of virulence factors
- Encodes BvgA, BvgS and BvgR
  - BvgA-BvgS signal transduction system

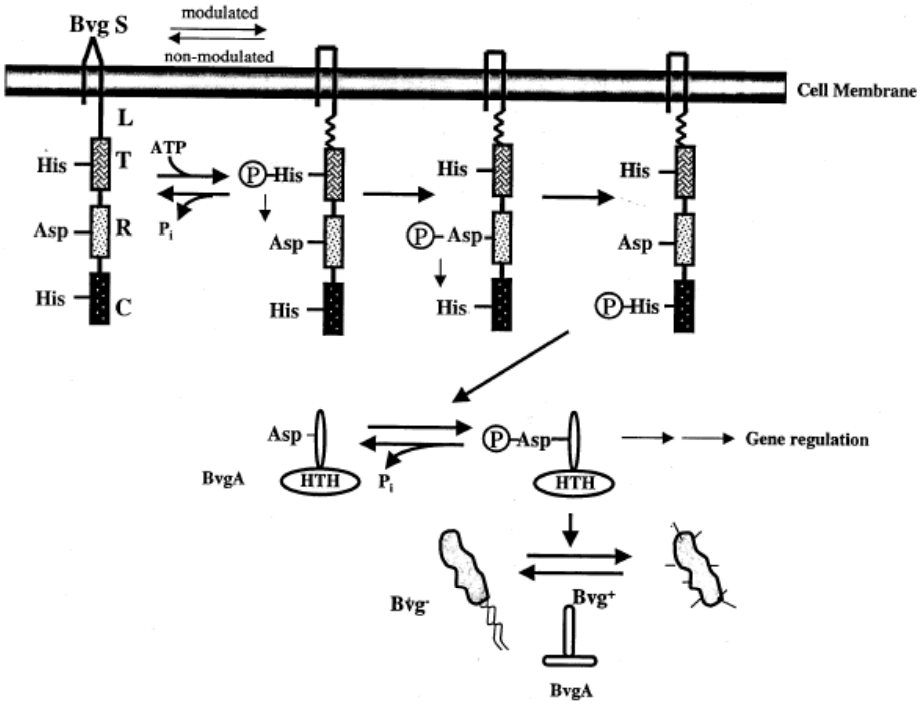
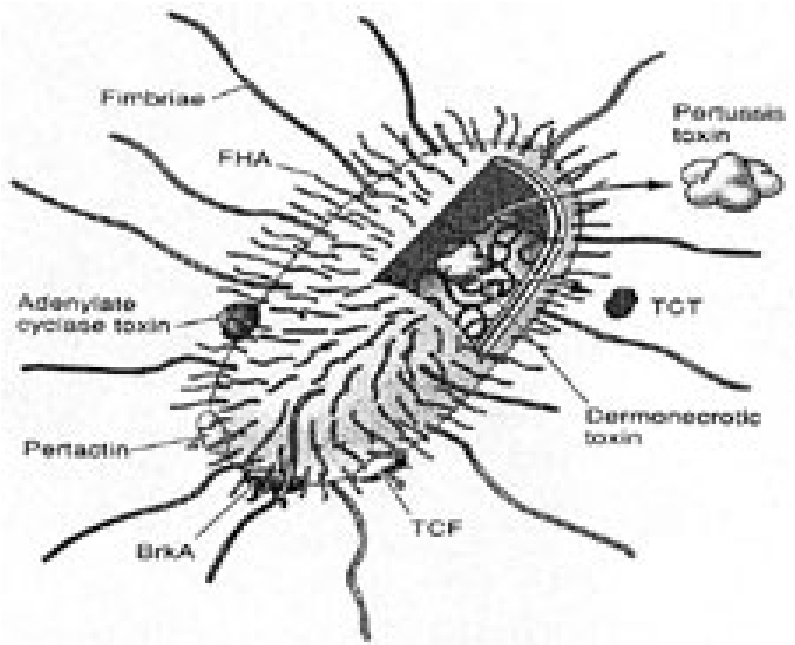


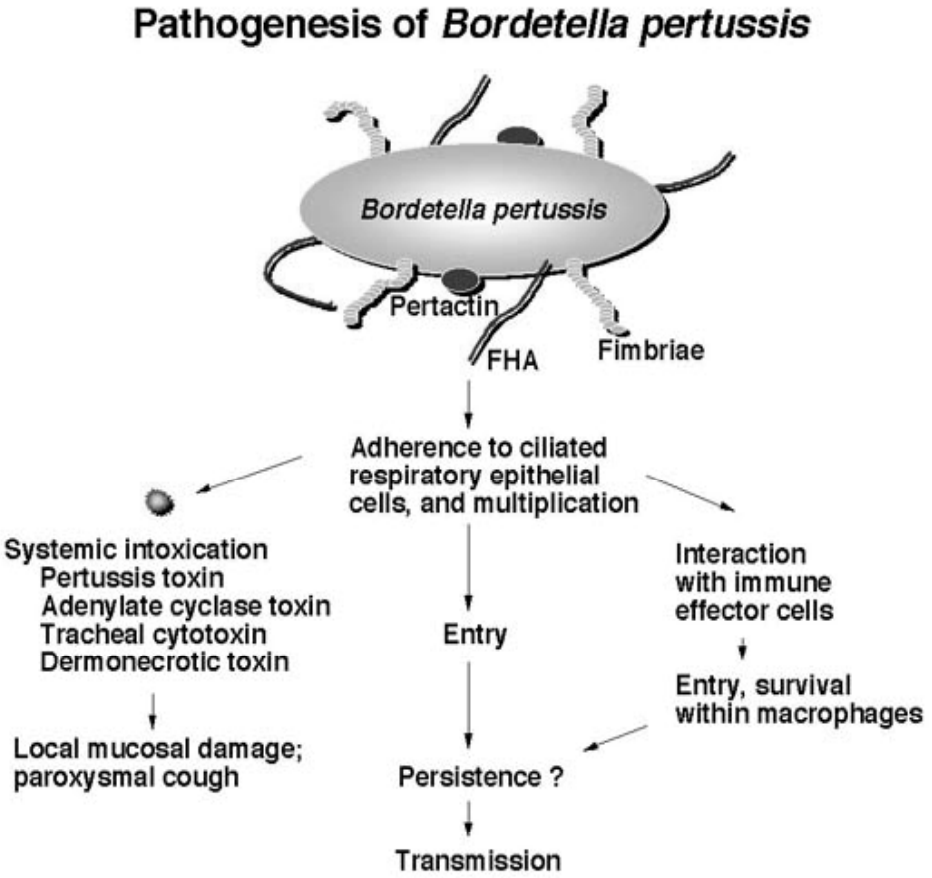
Figure 3. Model for the two-component signal transduction system by the BvgAS proteins. L, Linker; T, Transmitter; R, Receiver and HTH, Helix-turn-helix. Conserved histidine (H) and aspartic acid (D) residues to which phosphotransfer occurs are shown.

Babu et al., 2001

ADHESIONS

- Filamentous hemagglutinin
- Pertactin
- Fimbriae





STRAIN VARIATION

- *B. pertussis* has changed since vaccine introduction
  - Adaptation to vaccine
  - Antigenic divergence
  - Mismatch between vaccine strains and circulating strains played role in reemergence
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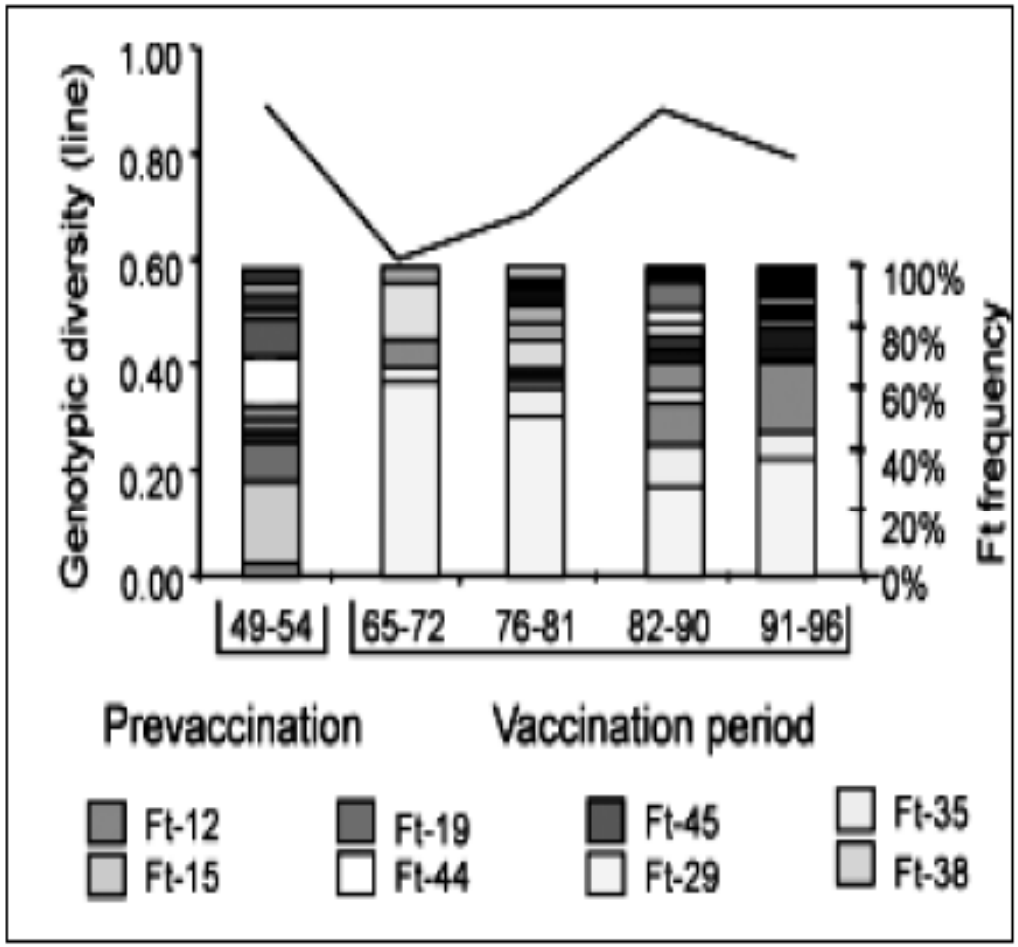


Figure 2. Changes in the population structure of *B. pertussis* in The Netherlands as determined by IS*1002*-based DNA fingerprinting.

CLINICAL RECOGNITION

- Clinical diagnosis is complicated by heterogeneity of disease expression
- Pertussis commonly misdiagnosed as *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* infection, laryngitis, upper respiratory tract infections, bronchitis, sinusitis, asthma, or chronic bronchitis
- Vaccination can modify disease severity
- Mixed infections can complicate diagnosis

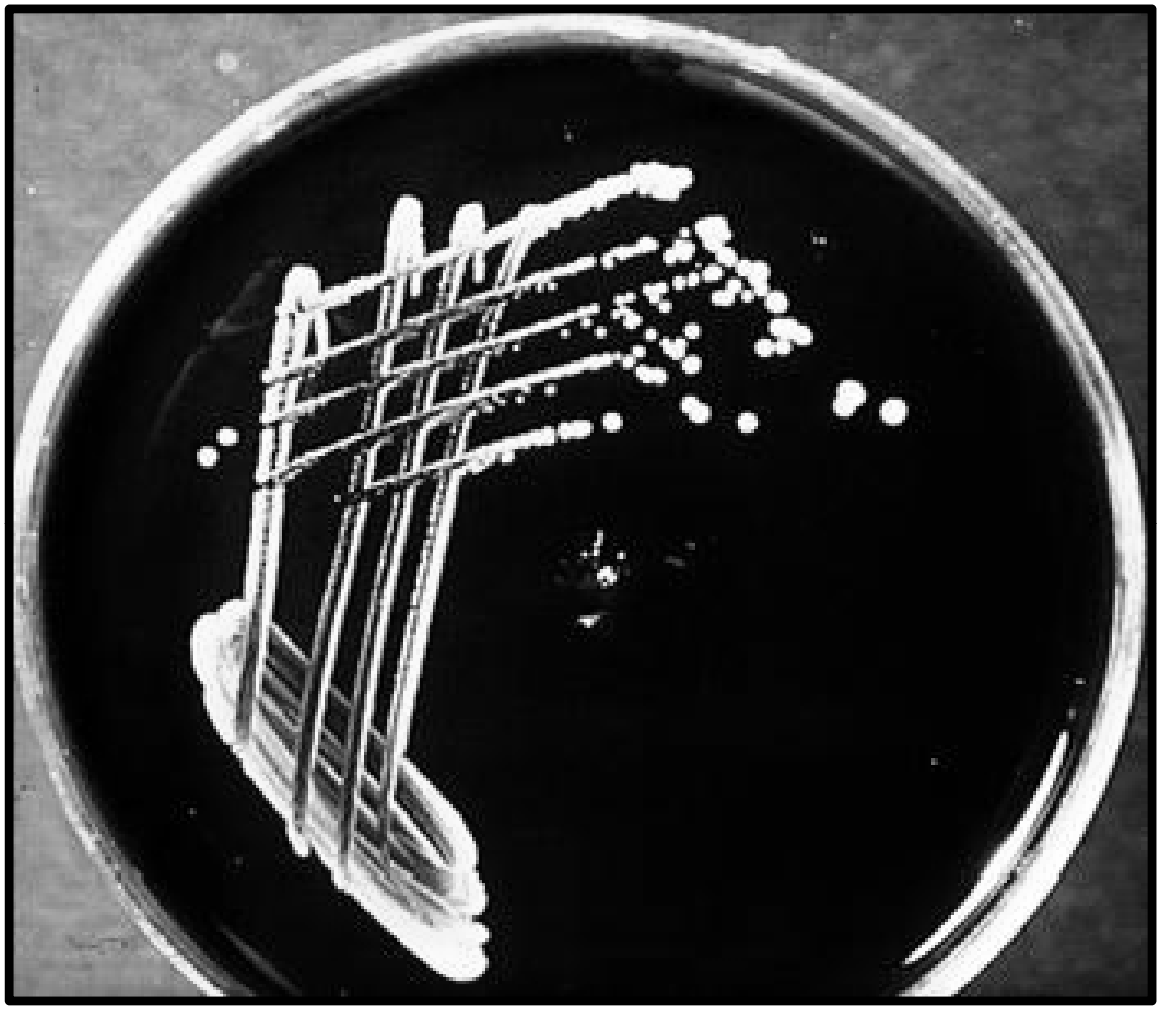
CLINICAL RECOGNITION

- Diagnosis of pertussis must occur early in the course of the disease in order to reduce severity and prevent the spread of infection
- Identification based on clinical criteria alone has varying sensitivity and specificity depending on previous exposure, age, antibiotic treatment, immunization status, and concomitant infections

Laboratory Methods

Lab Diagnosis: Overview

- Culture
- PCR
- DFA
- Serology



SPECIMEN COLLECTION (1)

- Specimen type will impact ability to isolate bacterium
- Nasopharyngeal (NP) aspirates yield similar or higher rates of recovery than NP swabs (rayon or polyester)
- Throat and anterior nasal swabs yield unacceptably low rates of recovery

SPECIMEN COLLECTION (2)

- Cultures most often positive if the nasopharyngeal swab is obtained within the first week of cough onset
- Beyond the first 3 weeks of illness the organism is recovered less often
- Demonstration video of NP swab technique available on the broadcast updates and resources webpage

http://www.cdc.gov/vaccines/ed/surv07/surv07-resources.htm

SPECIMEN COLLECTION (3)

- Plate immediately or place into Regan-Lowe transport medium
- Dispensing & plating should be completed within 24 hours of specimen collection
- Specimen can be used for culture & PCR

CULTURE (1)

- “Gold Standard” – 100% specific, but low sensitivity
- Most sensitive within 2 weeks after cough onset
- Highest yield in young patients, unvaccinated patients, patients early in cough illness prior to antimicrobials
- Incubation time 4-10 days
- Specific collection methods, transport, media and growth conditions are needed

CULTURE (2)

- Regan-Lowe or Bordet-Gengou media
- Inoculate with and without antibiotics
- 35-36°C incubation with high humidity
- Ensure plates do not dry out
  - Plastic bags
  - Canisters
  - Pan of water
- Check plates every day

CULTURE (3)

- Bordet-Gengou (BG)
  - Small colony size
  - Appearance similar to mercury droplets
  - Colonies appear hemolytic
- Regan-Lowe (RL)
  - Small colony size
  - Glistening, cut glass appearance

CULTURE (4)

- *B. parapertussis*
  - Colonies typically appear within two-three days
  - On RL agar the colonies will appear greyish
  - On BG agar colonies have a brown pigmentation
- *B. holmesii*
  - Colonies look similar to *B. pertussis*
  - Growth is inhibited by cephalexin
- *B. bronchiseptica*
  - Large colonies
  - Appear after one day
  - On RL agar colonies have a slight brown coloration

SEROLOGY

- Significant variation (4-fold increase) in IgG or IgA titers against virulence factors in acute and convalescent phases. Samples in both phases required
- Post-infection, increases in serum IgA, IgG, and IgM occur
- Best specificity by ELISA for IgG & IgA to pertussis toxin
- Factors that can alter results include history of previous immunologic priming by vaccination or prior infection
  - In patients with reinfections, a rapid increase in antibody occurs, often resulting in the titer having already peaked by the time the acute phase sample is obtained, making serologic diagnosis nearly impossible.
- Serology is difficult to use clinically due to variability in results and the lack of standardized reagents

DIRECT FLUORESCENT ANTIBODY

- Direct fluorescent antibody (DFA) testing has been used for ~ 40 years
- Inexpensive, rapid, positive results when cultures are negative due to antibiotic use
- Lacks sensitivity and specificity because of cross-reactivity with normal flora
- No longer recommended

Polymerase Chain Reaction

- PCR assays are widely available
- Rapid, sensitive, and specific
- Some PCR assays have not been completely reliable
- Cultures should continue to be performed even if PCR tests are used

REAL-TIME PCR ASSAY: IS*481*

- Present in three *Bordetella* species
  - 50 to >200 copies in *B. pertussis*
  - 8 to 10 copies in *B. holmesii*
  - 1 copy in *B. bronchiseptica*
- High Ct value could indicate
  - Positive test result
  - False positive
  - Positive result of a *Bordetella* species other than *B. pertussis*

MULTI-TARGET PCR APPROACH

- Multiplex real-time PCR utilizes 3 targets:
  - IS*481*
  - hIS*1001: B. holmesii* (3-5 copies/cell)
  - pIS*1001: B. parapertussis* (20-23 copies/cell)
- *ptxS*1 targets gene of S*1* subunit of pertussis toxin
  - 1 copy in *B. pertussis* and *B. parapertussis*

MULTI-TARGETS: SPECIATION!

		Multiplex		
Species	ptxS1	IS481	hIS1001	pIS1001
B. pertussis	+	+	-	-
B. parapertussis	+	-	-	+
B. pertussis & *B. parapertussis*	+	+	-	+
B. holmesii	-	+	+	-

CDC ALGORITHM

	IS481+	IS481+	IS481-
	(Ct<35)	(35≤Ct<40)	(Ct≥40)
ptxS1+	*B. pertussis*	*B. pertussis*	*B. parapertussis* (1)
(Ct<40)			
ptxS1-	*B. holmesii* (2)	*Indeterminate*	Negative
(Ct≥40)			
(1) Confirmed by pIS1001 target			
(2) Confirmed by hIS1001 target			

CONCLUSIONS & RECOMMENDATIONS

Rules for Lab Confirmation

- Isolation of *Bordetella pertussis* from a clinical specimen
- Positive real-time PCR assay
- Direct fluorescent antibody (DFA) testing should NOT be used (low sensitivity and variable specificity)
- Serology can be useful but lacks standardization

RECOMMENDATIONS

- PCR is more sensitive than culture
- Important to try culture the organism:
  - Strain variation (PCR can miss)
  - Emergence of antibiotic resistance
  - Phenotypic and genotypic characterization that would not be identified if only PCRused
- Combining culture, PCR, serology may increase diagnostic sensitivity

CONCLUSIONS

- Major problem is the lack of access to diagnostic laboratory methods
- Many routine laboratories are not equipped for the diagnosis of *B. pertussis* infection
- General misconception that *B. pertussis* infection is uncommon – it is a re-emerging infection globally!

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