Haemophilus and Other Fastidious Gram-Negative Bacilli

PART III

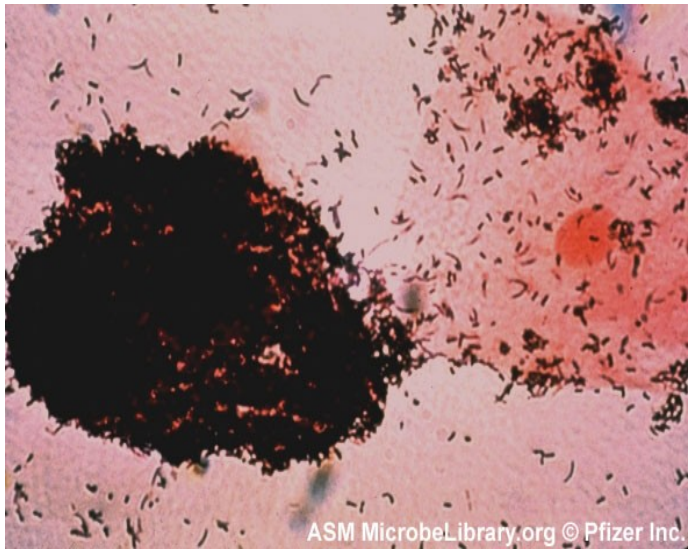
Other fastidious organisms

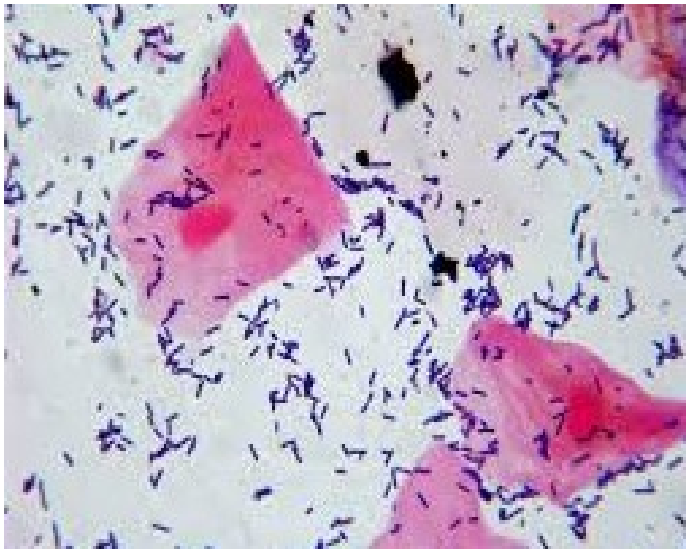
- Gardnerella
- Brucella
- Francisella
- Pasteurella
- Legionella
- Bordetella
- Bartonella
- Capnocytophaga

Gardnerella vaginalis

- Part of normal vaginal flora
- Major cause of bacterial vaginosis
- Can cause diseases
  - Bacterial vaginosis
  - UTI
  - Amniotic infections
  - Postpartum sepsis
  - Preterm labor/birth and PROM
  - Septicemia
  - Umbilical and scalp monitor infections
  - Post-surgical gynecologic infections

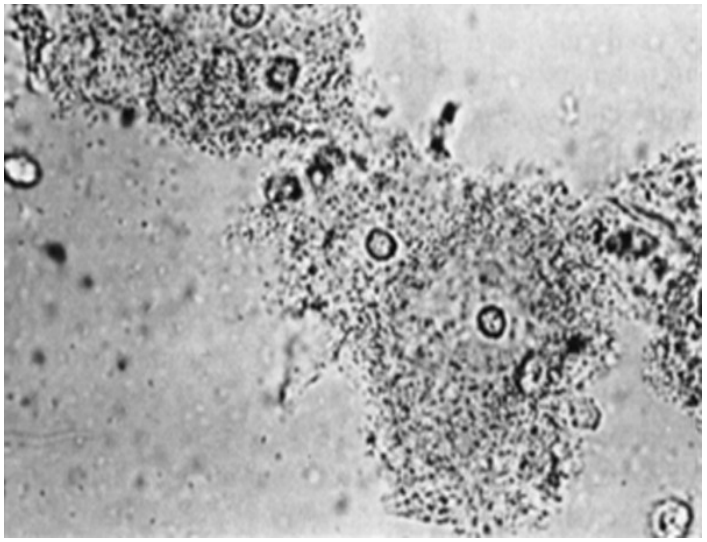
Gardnerella vaginalis





- Formerly Haemophilis vaginalis then changed to Corynebacter vaginalis
- Gram positive/variable rod
- Culture is not recommended for BV
  - Isolated from 40% of women without BV
- Disrupted balance of NF

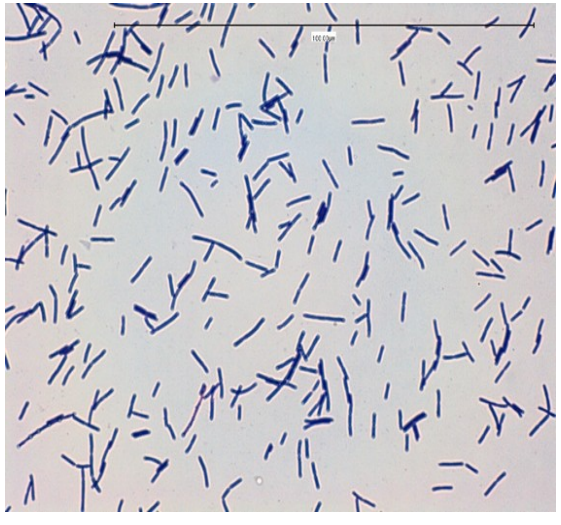
Gardnerella vaginalis

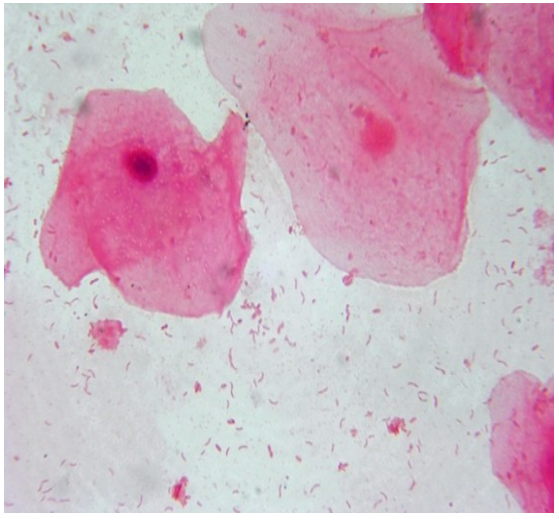


- Diagnosis of Bacterial Vaginosis (BV)
  - Must meet 3 of the 4 of the following criteria:
    - Vaginal discharge that lacks WBC's
    - Increased pH of >4.5
    - Positive Whiff test
      - Vaginal discharge mixed with KOH gives fishy odor
    - Clue cells seen on low power
      - Bacteria covering or "sticking to" squamous epithelial cells

BV Confirmation

- Perform Nugent Scoring of BV grams
  - Normal score indicates:
    - High numbers of Lactobacillus sp.
  - BV score indicates:
    - Low numbers of Lactobacillus sp.
    - High numbers of Gardnerella and/or Mobiluncus sp.
    - High numbers of other anaerobes





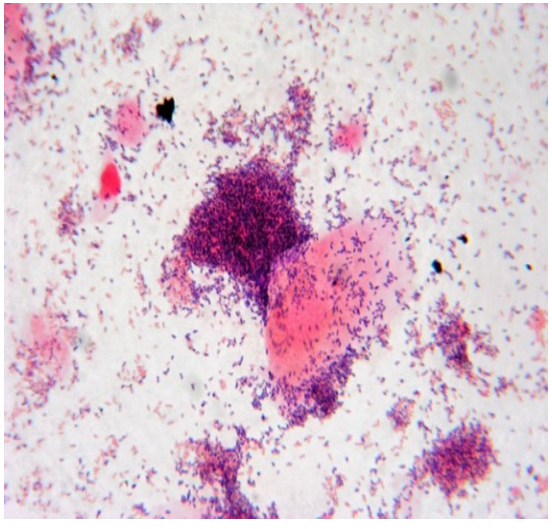


FIGURE 1. Diagnostic tests for bacterial vaginosis.

1. Amsel criteria: Three or more of these clinical conditions correspond to a diagnosis of bacterial vaginosis.

- A homogenous, white, noninflammatory milky discharge that adheres to the vaginal walls
- The presence of clue cells upon microscopic examination
- Vaginal pH greater than 4.5
- Positive "whiff" test upon the addition of 10% KOH to a specimen (i.e. olfactory detection of the "sharp" smell of amines).

2. Nugent criteria: A total score of 7 or more indicates a case of Bacterial Vaginosis; a score of 4 to 6 is considered intermediate, and a score of 0 to 3 is normal.

Nugent Scoring System for Gram Stained Vaginal Smears

Score	Lactobacillus types	Gardnerella types	Mobiluncus types
0	4+	0	0
1	3+	1+	1+ - 2+
2	2+	2+	3+ - 4+
3	1+	3+	
4	0	4+	

Deborah B. Nelson, and George Macones Epidemiol Rev 2002;24:102-108

epidemiologic reviews

- Affirm VP
  - Direct vaginal specimens
  - Nucleic acid hybridizes with DNA on the bead
  - Color development probe hybridizes
  - Enzyme conjugate binds
  - Substrate is added

Gardnerella vaginalis

- Identification
  - Very tiny colonies
  - Gram stain – gram variable rods
  - Catalase negative
  - Sensitive to SPS (sodium polyanethol sulfonate)
  - Sensitive to a streak of alpha hemolytic streptococcus

Brucellosis

- Zoonotic infection* causing spontaneous abortion in animals secondary to bacteremia
  - Dairy farmers
  - Livestock handlers
  - Slaughterhouse handlers
  - Veterinarians
  - Lab personnel (2% of all cases)

Brucella Routes of Infection

- Inhalation of aerosols
- Penetration of ocular and oral mucosa
- Direct inoculation into bloodstream through abrasions
- Ingestion of unpasteurized and contaminated milk or cheese (B. abortus and B. melitensis)
- Rare person-to-person contact

Stages of Brucellosis (Undulant fever)

- Acute (within 8 weeks of exposure)
  - Fever, malaise, headache, myalgia
- Subchronic (within 1 year of exposure)
  - Chronic fever that disappears and reappears, arthritis, epididymoorchitis (in males)
  - “ Undulant fever”
- Chronic (1 year after exposure)
  - Depression, arthritis, chronic fatigue syndrome

Brucella

- Category A Select Biological Agent by CDC*
  - Agent of bioterrorism that is easy to disseminate and causes moderate morbidity but low mortality
  - Identification to species level should be done by a reference lab

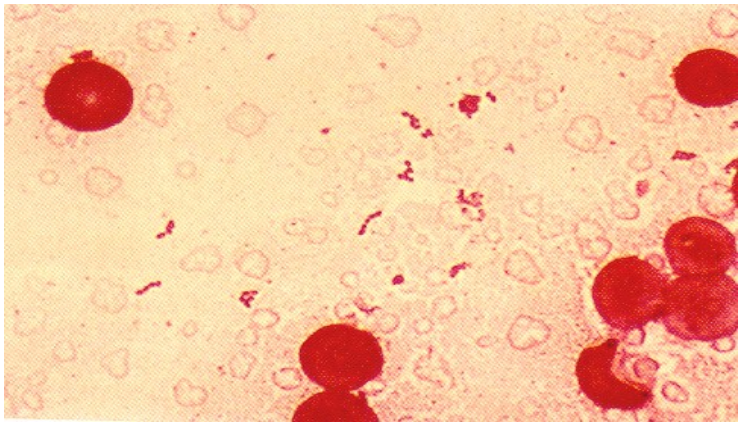
Culture of Brucella Species

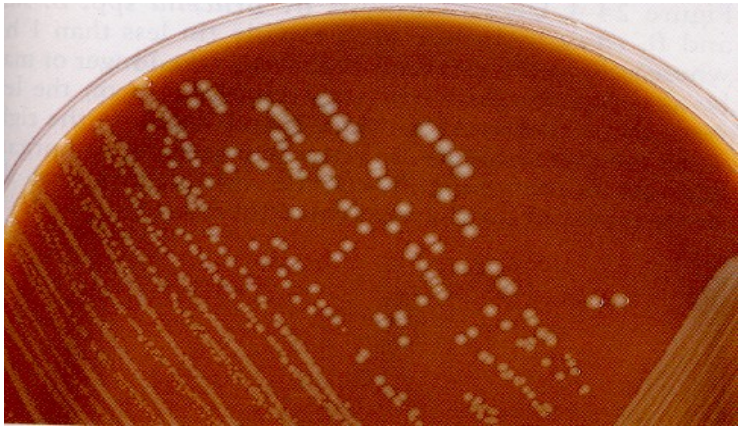
- Biolevel 3 pathogen – Use level 2 biosafety cabinet
- Fastidious
- Specimens include blood, bone marrow, tissues
- Send to reference lab for confirmation

Brucella

- Diagnosis using serology
  - Difficult to diagnose through direct exam of clinical specimens
  - Serologic assays are most commonly relied upon test for diagnosis of brucellosis
  - Patient history is helpful

Brucella Species

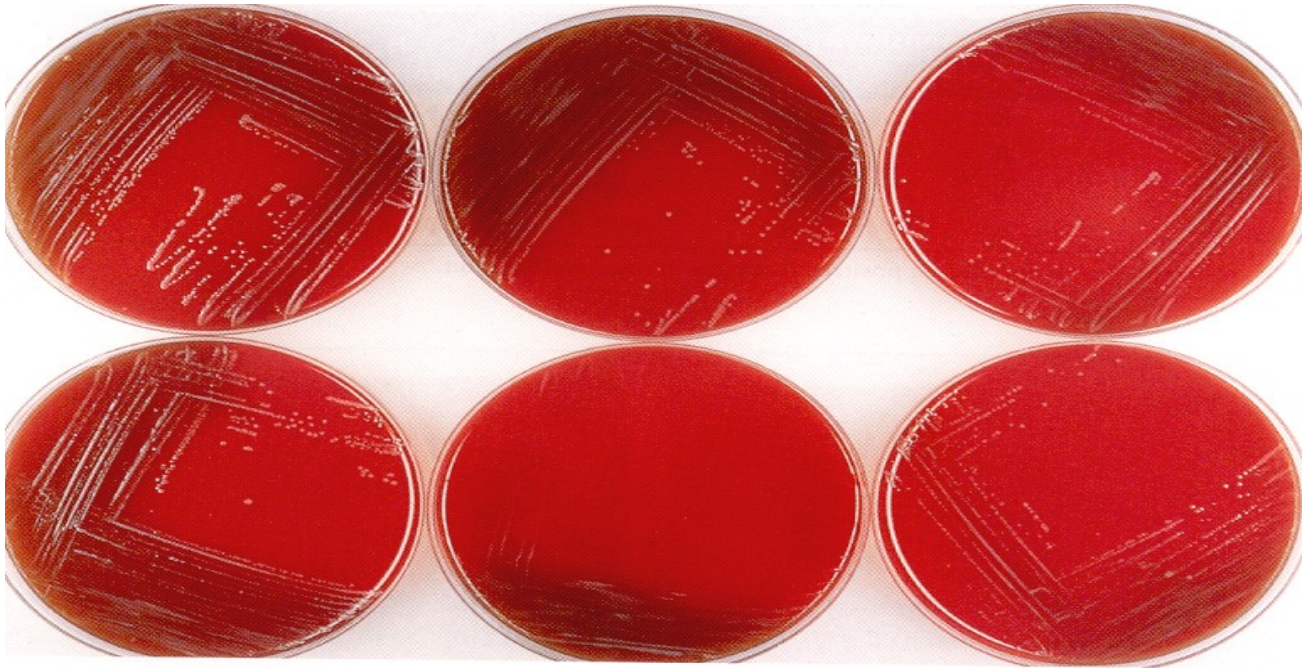




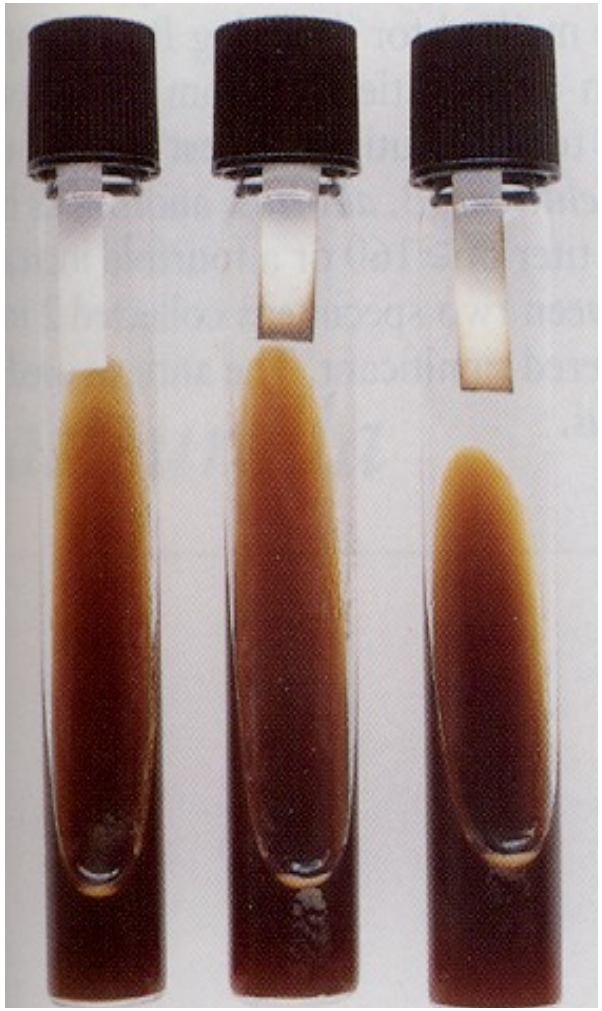
- Obligate aerobes
- Gram negative coccobacilli
- Nonmotile
- Oxidase positive*
- Catalase positive
- Facultative intracellular parasites

Brucella species

- CO_2 requirement
  - B. abortus (middle plates) grows better in the presence of 5-10% CO_2 (top row)



Brucella species



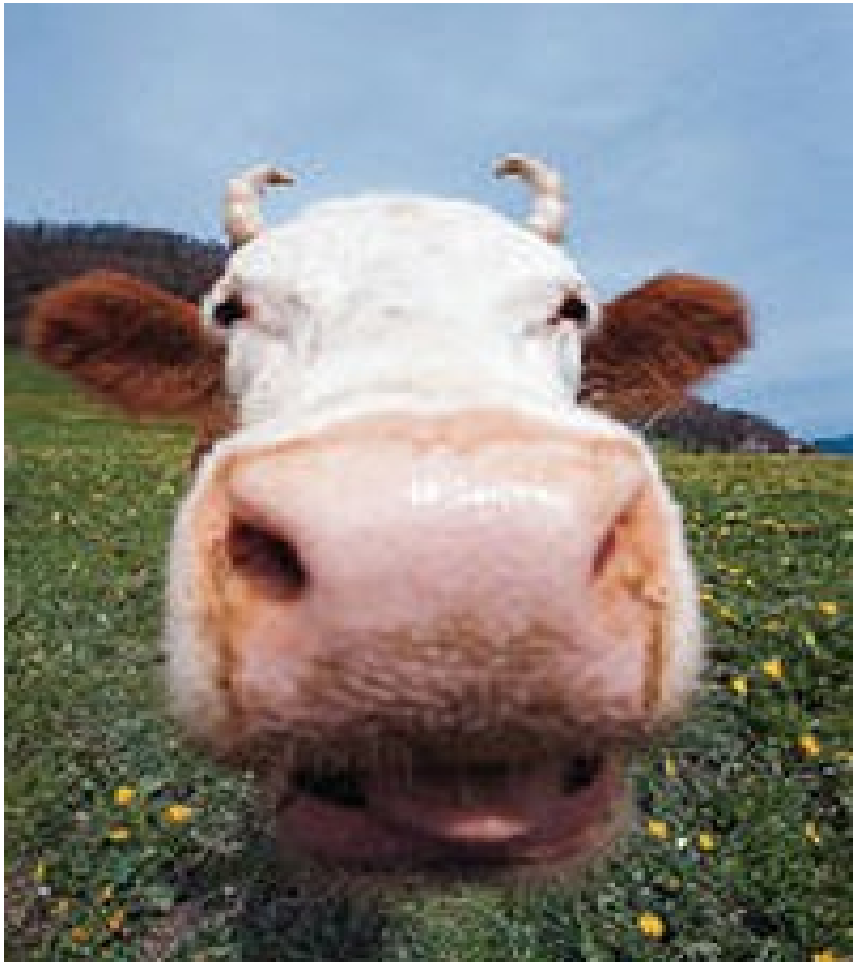
- H2S*
  - Inoculate a Brucella slant
  - Hang a lead acetate strip in the tube.
  - B. abortus (middle) and B. suis (right) are positive.
  - B. melitensis (left) is negative.

Brucella

- Urea*
  - The three tubes on the left were incubated 1 hour.
  - The three tubes on the right were incubated for 24 hours
  - B. melitensis, B. abortus, B. suis

	Animal Host	Increased CO_2	Urea	H_2S
B. abortus	Cattle	**+**	2h	**+**
B. suis	Swine	**-**	15min	**+**
B. melitensis	Sheep or goats	**-**	V	**-**
B. canis	Dog	**-**	15min	**-**

Brucella Treatment



- Vaccine for cattle has eradicated Brucella abortus in the US
- Intracellular parasite
  - Treat for 4-6 weeks

Francisella tularensis

- Causes Tularemia or Rabbit Fever
  - Zoonotic infection*
  - Direct contact with animals & environments
    - Direct infection - cut, bite, ingestion or inhalation from infected animal, water, plants and soil.
      - Trappers and Hunters
    - Indirect infection – insect feeds on infected animal and passes it to humans
      - Biting insects

Francisella tularensis

- Four subspecies:
  1. Francisella tularensis subsp. Tularensis
     - (Most severe disease)
  2. Francisella tularensis subsp. Holarctica
  3. Francisella tularensis subsp. Mediasiatica
  4. Francisella tularensis subsp. Novicida
     - (opportunistic pathogen)

Francisella tularensis

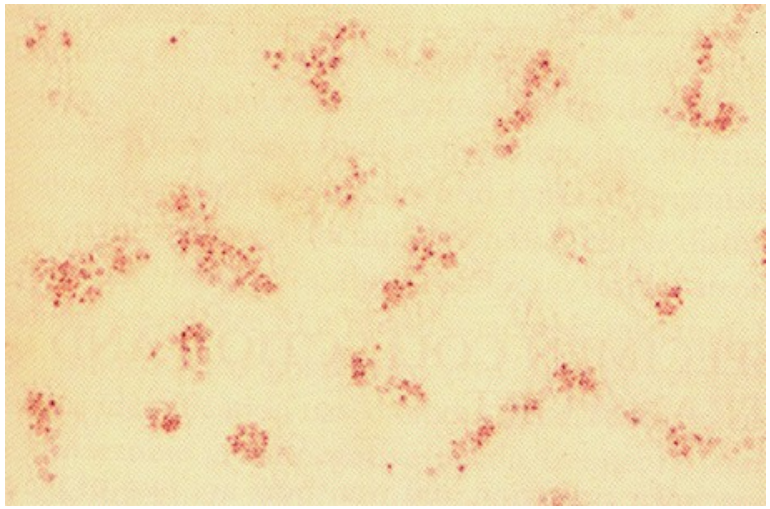
- Forms of disease:
  - Ulceroglandular (80%)
    - Ulcer with enlargement of regional lymph nodes.
  - Oculargland ular
  - Systemic or Typhoidal
    - Untreated cases have a 30-60% mortality.
  - Pneumonic
    - Untreated cases have a 30-60% mortality.
  - Oropharyngeal
  - Intestinal

Francisella tularensis

Francisella Culture

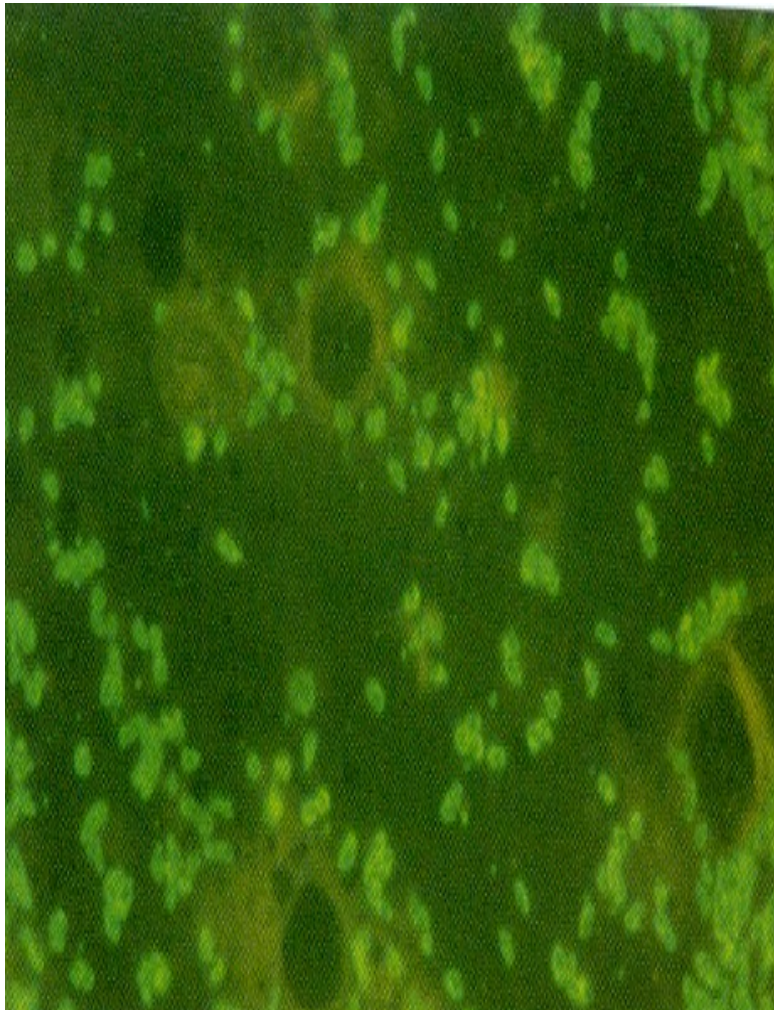
- Biolevel 3 pathogen
  - Highly infectious (50 organisms), virulent, pathogenic
  - Laboratory personnel are at risk when manipulating cultures
  - Survives for weeks in mud, water and decaying animals
  - Passes through intact skin
- Use cysteine glucose blood agar
  - Chocolate agar, BCYE or modified Thayer Martin can also be used
  - Cysteine dependent*

Francisella tularensis



- Small pleomorphic GNCB
- Oxidase negative
- Catalase weakly positive
- B lactamase weakly positive
- Urea negative
- Nonmotile
- Facultative intracellular parasite*
- Martin Lewis plate @2-5 days

Francisella tularensis



- DFA (direct fluorescent assay) using a polyclonal rabbit antibody

Francisella tularensis

- Diagnosis using serology
  - Single positive titer of ≥1:160 in patient with no history of tularemia vaccination
  - Fourfold rise in titer between acute and convalescent serum
  - Patient history is helpful

Francisella tularensis

- Category A Select Biological Agent by CDC
  - Risk to national security
  - Spread through person-to-person contact
  - Easily disseminated
  - High mortality
  - Low infectious dose (10 bacteria)
  - Notify CDC of suspected cases

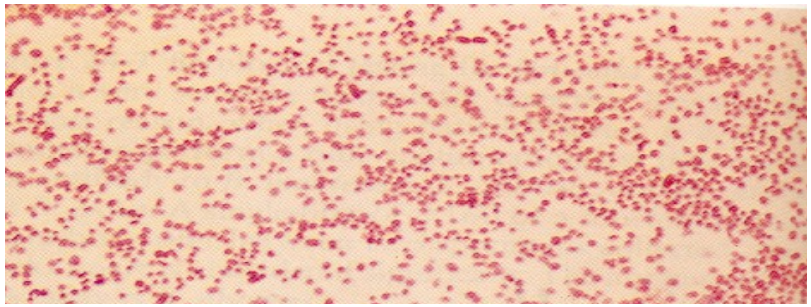
Pasteurella

- *Pasteurella multocida*
  - Subspecies multocida, septica and gallicida
- *Pasteurella canis*
  - Associated with dogs
- *Pasteurella stomatis* and *Pasteurella dagmatis*
  - Associated with dogs and cats

Pasteurella multocida

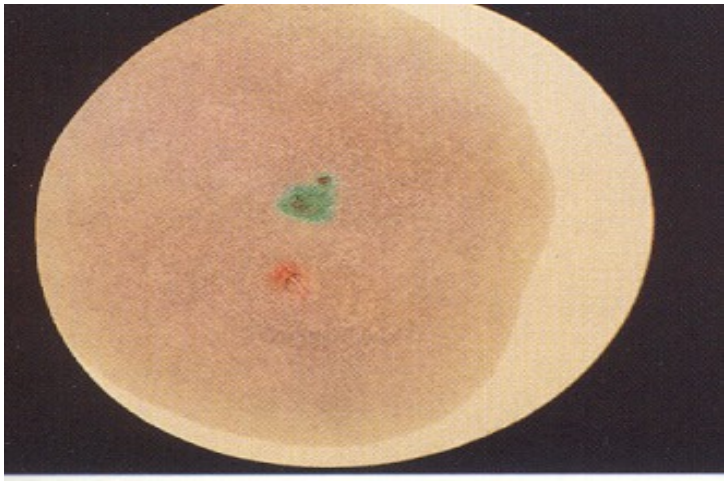
- Respiratory flora of birds and mammals
- Zoonotic infections* in humans
  - Bite wounds- most common
    - Cats and dogs
  - Systemic
    - Septicemia
    - Arthritis
    - Endocarditis
    - Osteomyelitis
    - Meningitis
  - Pneumonic

Pasteurella multocida



- Identification
  - Nonhemolytic
  - GNR with bipolar staining
  - Oxidase positive*
  - Indole Positive
  - Catalase positive
  - No growth on MAC or EMB
  - Musty smell
  - Nonmotile
  - May be mucoid
  - Green/brown halo around the colony

Pasteurella multocida



- Identification
  - Indole positive
  - Sensitive to penicillin
- This may resemble haemophilus in colony morphology and gram stain
  - Haemophilus quad plate can be used to differentiate between the two

Legionella Pneumophilia

- Respiratory disease
  - Inhalation of aerosols
  - Legionnaires disease
- Wound infections
- Specimens
  - Respiratory secretions (sputum, bronchoalveolar lavage, bronchial washing) pleural fluid, blood, lung biopsy, etc

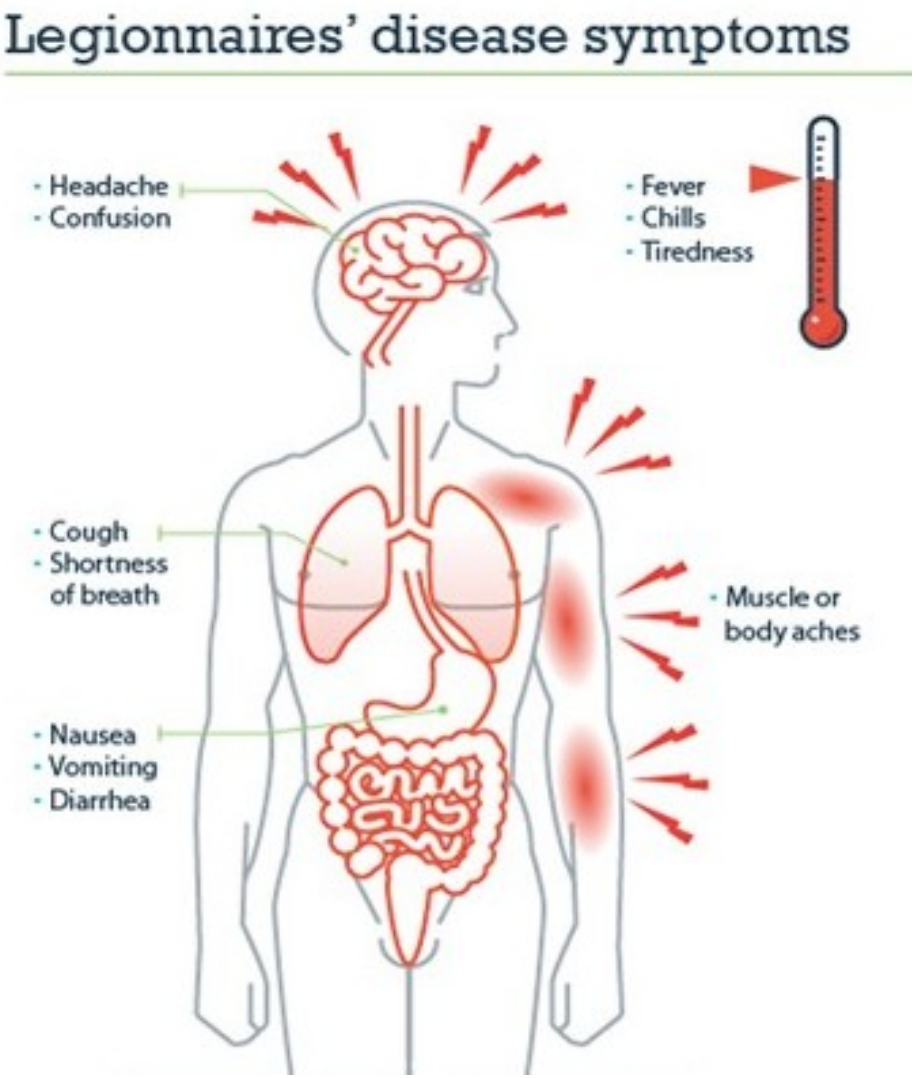
Legionella pneumophilia

- Environmental organism
  - Potable and non-potable water
- Tolerates chlorination
- Multiplies over large temp. range (20-43°)
- Adheres to pipes
- Survives and multiplies in free-living protozoa

Pontiac Fever

- Caused by legionella spp. (most probable is pneumophilia)
  - Febrile disease without pulmonary involvement
  - Fever, headache, myalgia that spontaneously subsides
  - 2 day incubation period

Legionnaire's Disease



- Febrile disease with pneumonia
- 2-15% of Community acquired pneumonia
- 15-30% Mortality
- Non-productive Cough, fever, headache, pulmonary infiltrates, chills
- May disseminate

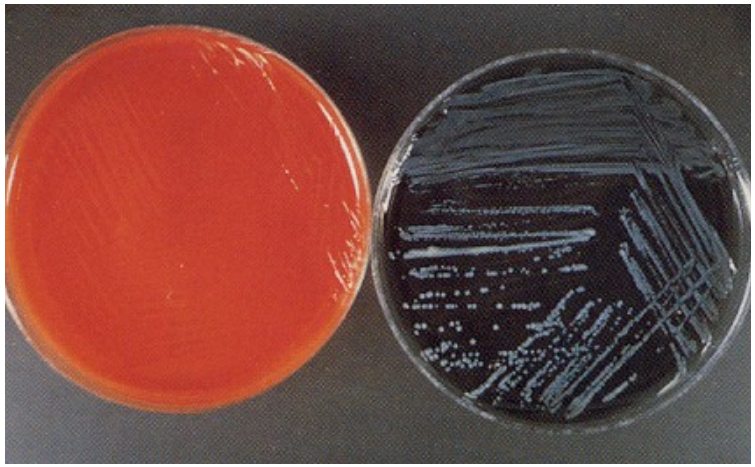
Legionella sp.

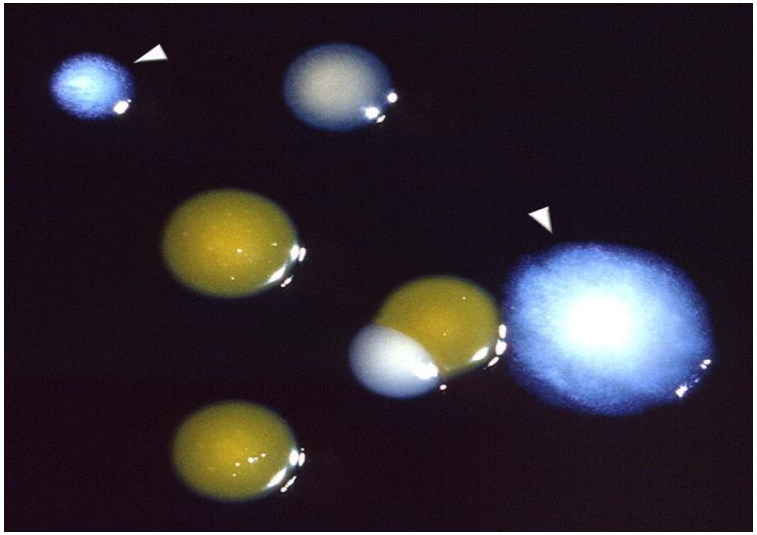
- There are 42 species of Legionella
- Legionella pneumophila is responsible for 80-90% of Legionella infection
- Within the species of L. pneumophila, the most common serogroups to cause human infection is 1, 4, and 6
- L. pneumophila serogroup 1 is responsible for about 70% of all legionellosis

Culture media for Legionella

- Require media supplemented with **L-cysteine**
- Buffered Charcoal Yeast Extract agar (BCYE)
  - With PAC (polymyxin B, anisomycin, cefamandole)
  - With PAV (polymyxin B, anisomycin, vancomycin)
- Growth Conditions
  - 35-37°C
  - Increased humidity
  - Dilute specimen with 1:10 0.2N KCl-HCl for 5 minutes

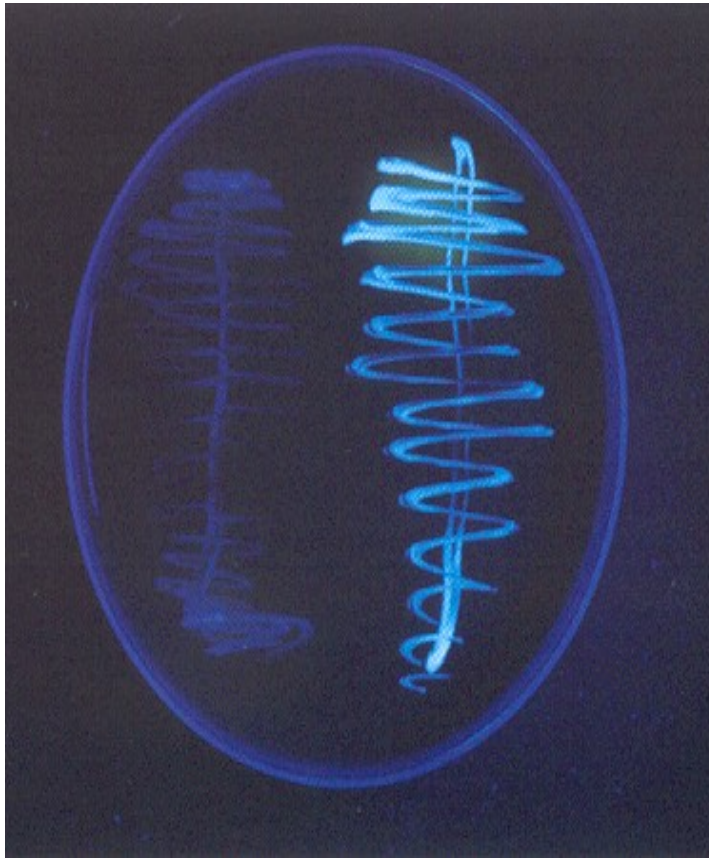
Identification of Legionella





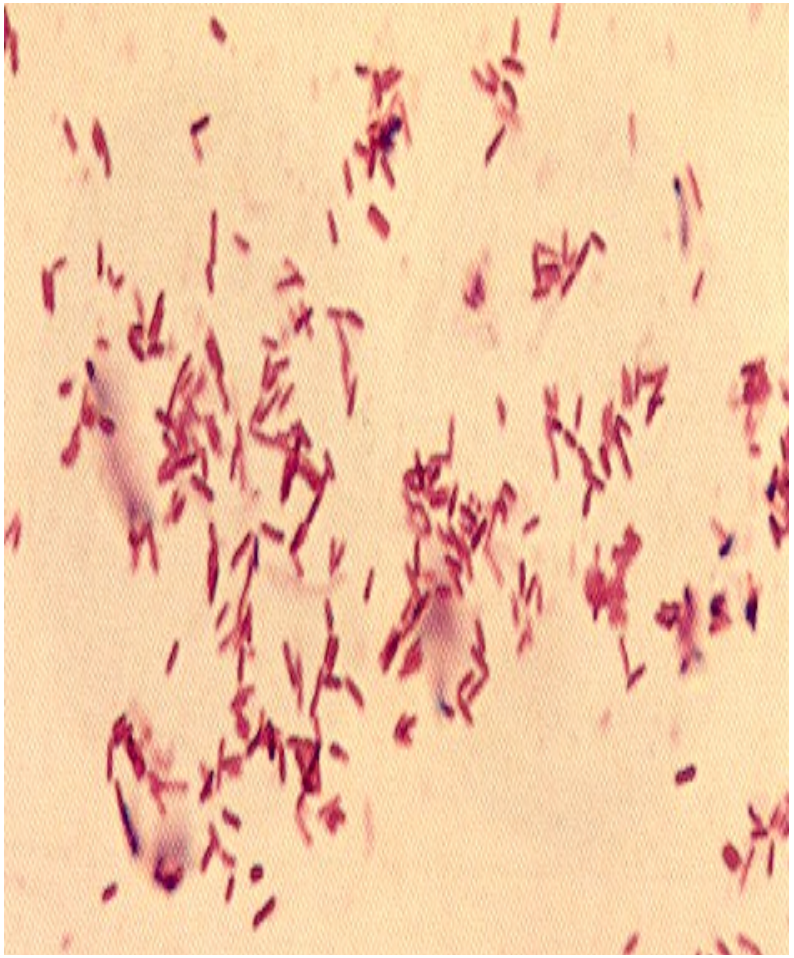
- Gray-white colonies with ground-glass appearance on BCYE in 3-4 days
- No growth on blood agar

Identification of Legionella



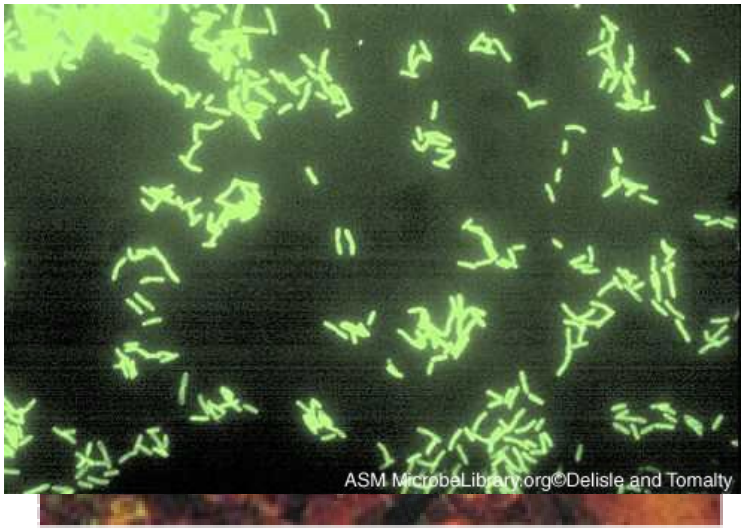
- L. pneumophlia does not fluoresce under UV light
- Other legionella spp will.

Identification of Legionella



- GNRS
  - Weakly staining
  - Extend safranin counterstain for 10 min
  - Pleomorphic
- Weakly catalase positive
- Weakly oxidase positive
- β lactamase positive

Identification of Legionella



- DFA from colony or direct specimen
- FITC conjugates
  - “Fluorescein isothiocyanate” = fluorochrome

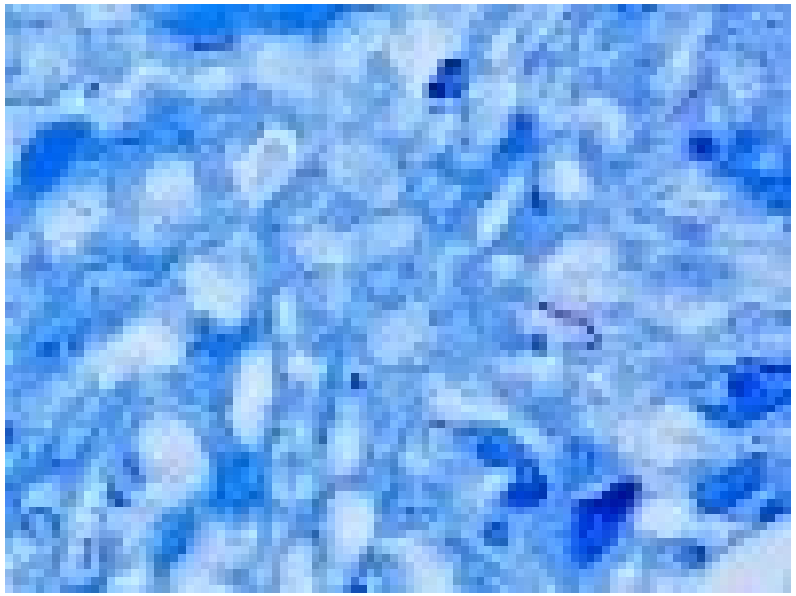
Serology for Legionella

- Legionella pneumophila antigen detection from urine
  - Detects serogroup 1 only
  - 85% of cases are caused by serogroup 1
  - Detects antigen as early as 3 days after infection and up to 1 year

Legionella Assays

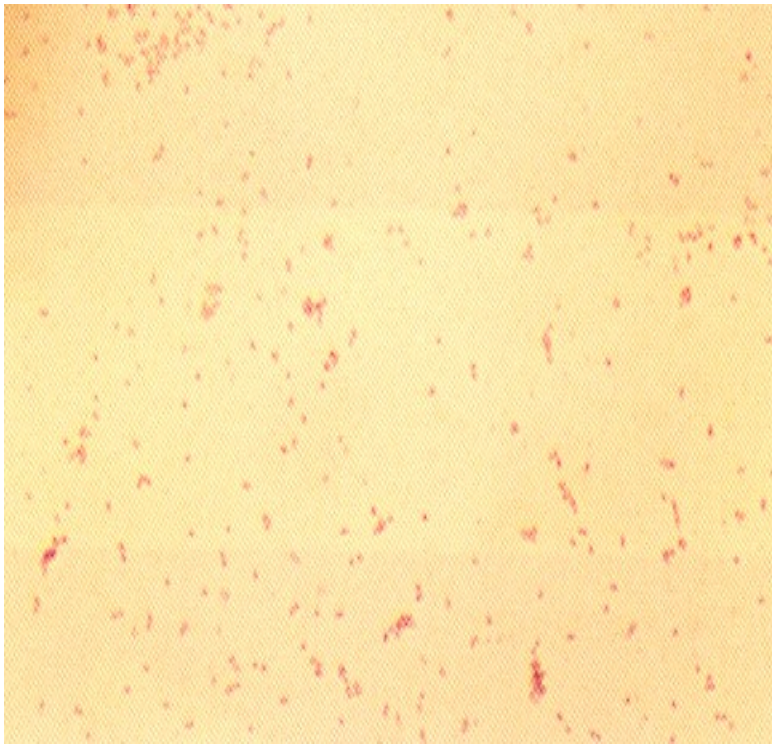
- Probe-Tec ET Legionella assay
  - amplified DNA
  - results in minutes

Legionella micdadei



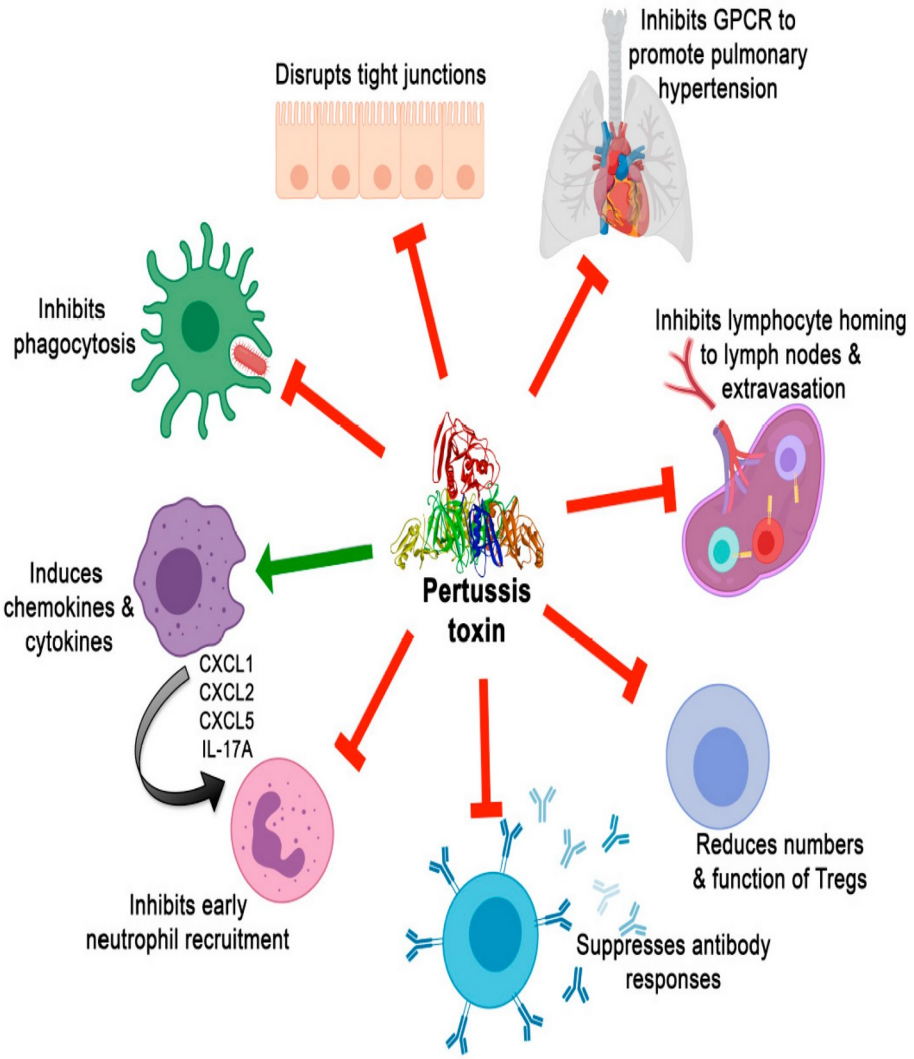
- Weakly acid fast (modified Kinyoun)
  - AFB stain red
- Causes Pittsburgh pneumonia

Bordetella species



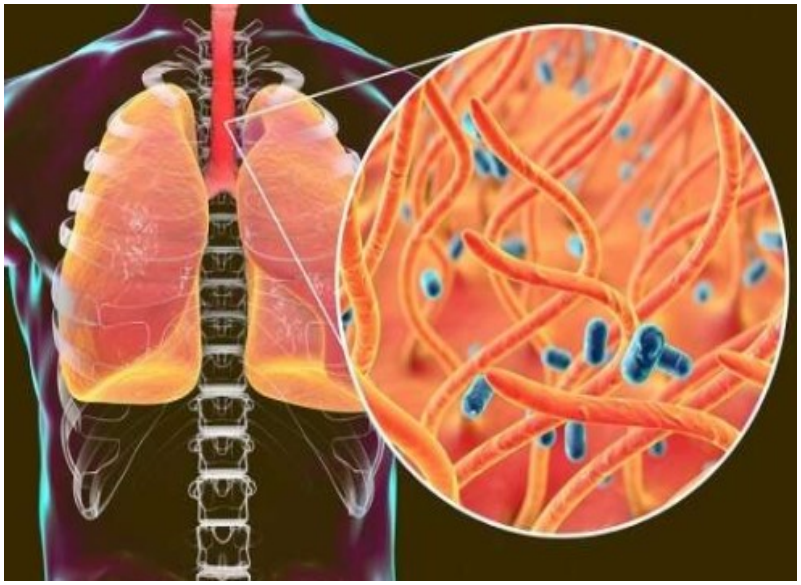
- Small gram negative coccobacilli
- Obligate aerobes
  - Will not grow anaerobically

B. pertussis and B. parapertussis



- Infect the human respiratory tract through respiratory droplets
- Adhere to and replicate on ciliated respiratory epithelial cells
- Cause Pertussis or Whooping Cough

B. pertussis and B. parapertussis

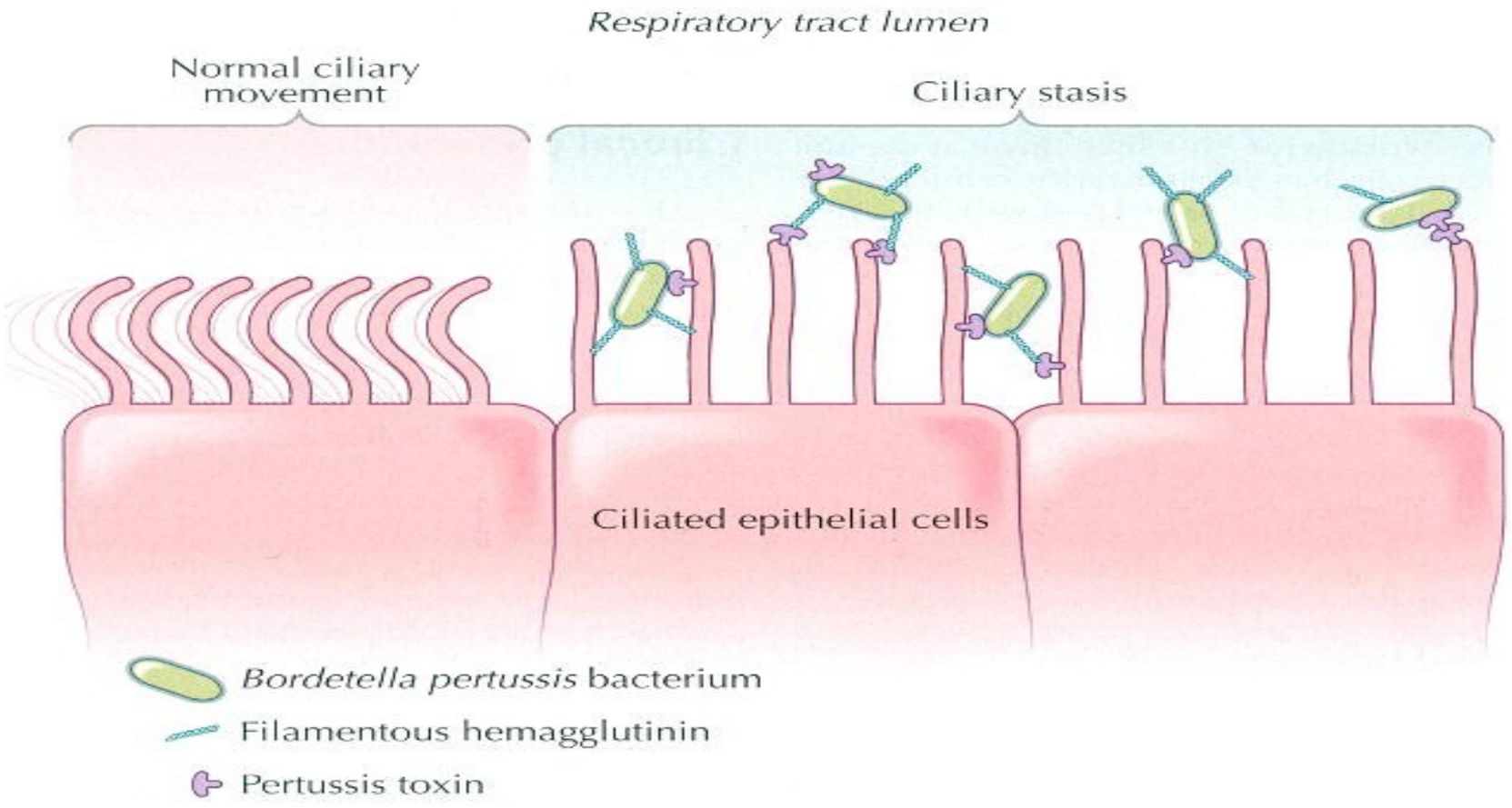


- B. pertussis
  - Fastidious
  - Main cause of Pertussis
- B. parapertussis
  - Causes a milder form of Pertussis
  - Somewhat fastidious

B. pertussis

- Highly contagious
- Virulence factors
  - Filamentous hemaglutinin (FHA) – attachment to ciliated epithelial cells
  - Pertussis toxin (PT) – endotoxin
  - Tracheal cytotoxin- ciliostasis, inhibits DNA synthesis

B. Pertussis toxin



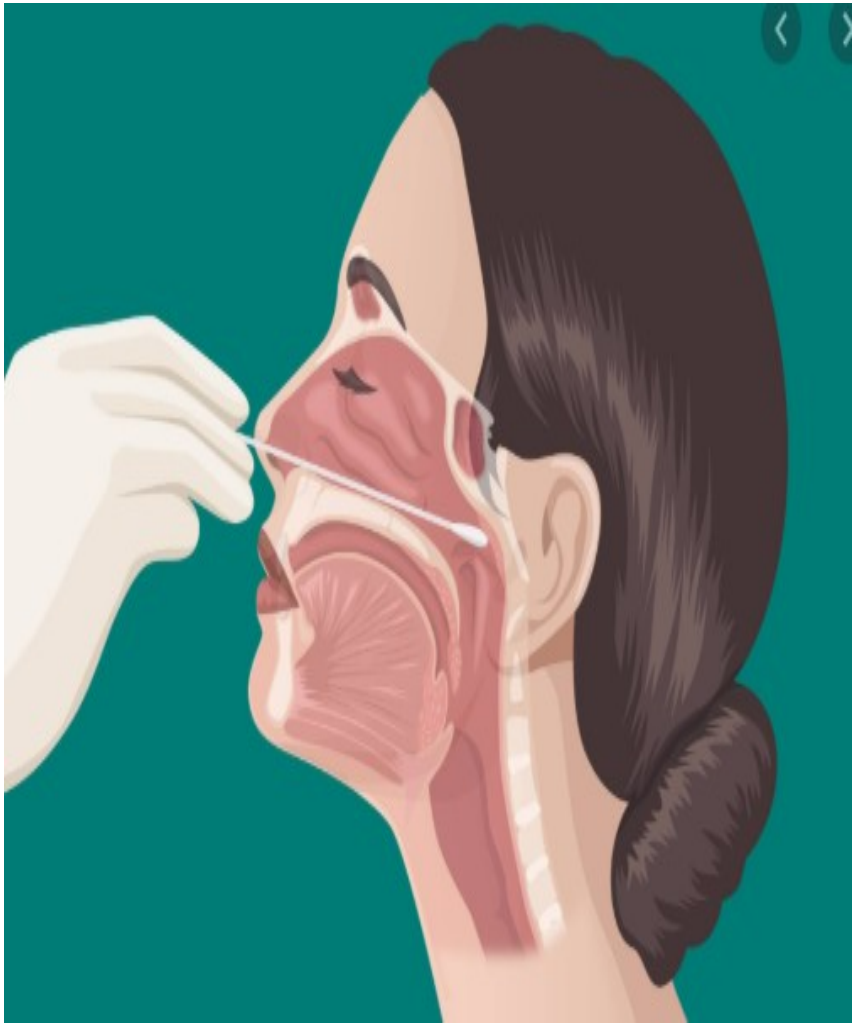
Stages of Pertussis

- 1-3 week incubation period
- Catarrhal phase
  - “Common cold “ or “flu”, nonspecific
- Paroxysmal
  - Violent repetitive cough ending in “whoop”
- Convalescent
  - Decrease in symptoms within 4 weeks of onset
  - May require weeks to months for complete recovery

Bordetella

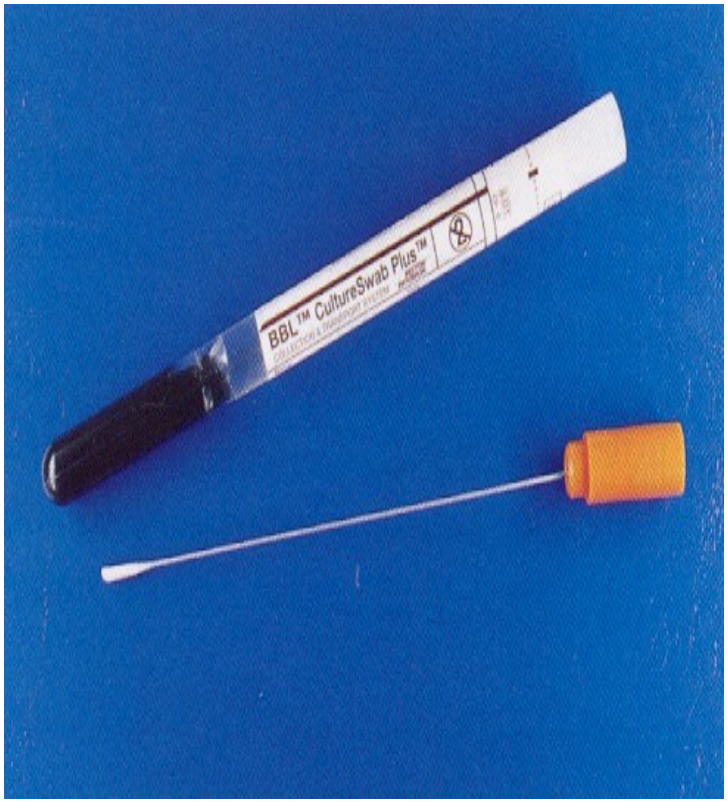
- High mortality rate in US prior to vaccine
- Immunizations common in the US, but outbreaks still occur
  - Decreases in immunization rate
  - Immunity is short lived
  - Adults are transiently colonized
  - Infants too young to be immunized

Bordetella Culture



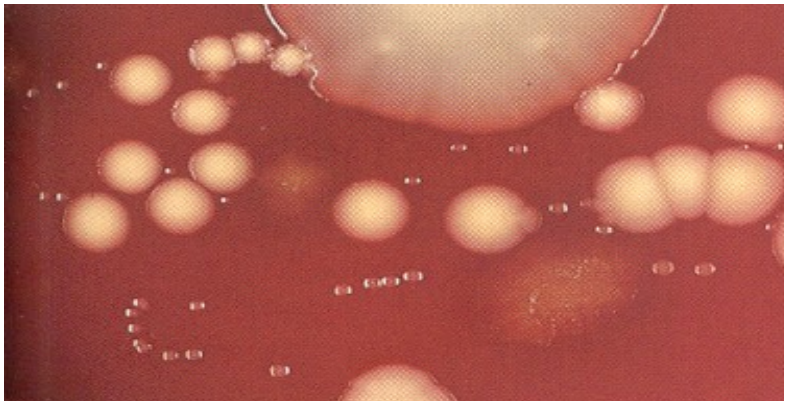
- Specimens
  - Use calcium alginate or Dacron aluminum shafted swab to collect
  - NP aspirate or swab
  - Discourage throat specimens
  - Plate at the bedside and transport to the lab immediately or use an approved transport system

Bordetella culture



- Amies transport with charcoal
- 1% casein hydrolysate broth
- Regan-Lowe transport medium

Bordetella Media



- Bordet-Gengou Potato infusion agar
  - Glycerol
  - Sheep blood
  - Penicillin, methicillin, or cephalexin can be added
  - Beta hemolytic

Bordetella Media

- Regan-Lowe
  - 10% horse blood
  - 40mg/L Cephalexin
  - Charcoal
  - Incubate at 35-37°C in ambient air for 7 days

Bordetella

- B. bronchiseptica will grow on blood and MacConkey as a nonfermenter
- B. pertussis will not grow

Bordetella

- Urea slants are inoculated and incubated for 4 hours
  - B. pertussis and B. parapertussis are negative
  - B. bronchiseptica is positive
- After 24 hours incubation
  - B. pertussis remains negative
  - B. bronchiseptica and B. parapertussis are positive

Characteristic	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
Catalase	+	+	+
Oxidase	+	-	+
Motility	-	-	+
Urease	-	+ (24 h)	+ (4h)
Growth on:			
Regan-Lowe	3-5 days Mercury droplets	2-3 days	1-2 days
Blood Agar	-	+	+
MacConkey	-	+/-	+

Bordetella DFA

- Performed in conjunction with culture
  - Only 60-70% sensitive
- Two slides are collected at the same time the culture is collected
- Stained with FITC conjugated antibodies
  - One slide against B. pertussis
  - One slide against B. parapertussis

Bordetella Testing

- PCR
  - DNA probes bind to complementary sequences of Bordetella spp.

Bordetella bronchiseptica

- B. bronchiseptica
  - Not fastidious
  - Causes respiratory and wound infections
  - “Kennel cough”
  - Respiratory pathogen of birds and wild animals

Bartonella

- There are currently 18 species with main human pathogens:
  - B. henselae: cat scratch fever
  - B. Quintana: trench fever
  - B. bacilliformis: Carrions disease
- Approximately 50% of these species can cause disease in humans
- Found in animals and their vectors

Bartonella species

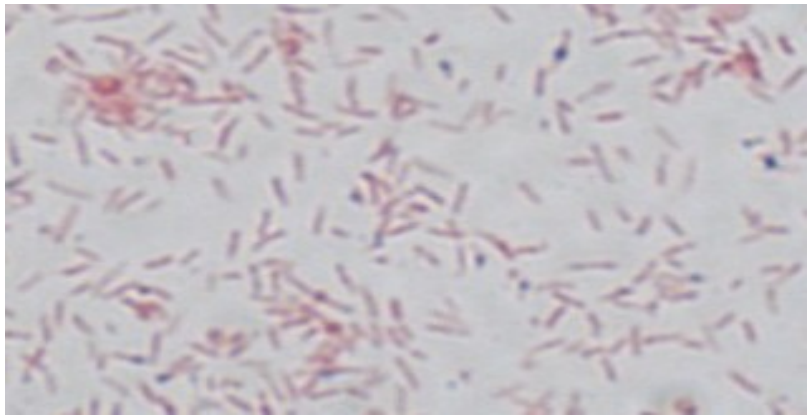
- Bartonella henselae: cat, flea
- Bartonella quintana: louse
- Bartonella clarridgeiae: cat, flea
- Bartonella vinsonii ssp. vinsonii: voles, ear mites
- Bartonella vinsonii ssp. arupensis: ticks

Bartonella henselae

- Common forms of bartonellosis
- Causes Cat Scratch Disease
- From cat bite, scratch, or fleas
- Presents as a pustule followed by regional lymphadenopathy associated fatique, fever, malaise
- Can resolve spontaneously after weeks to months
- Can also progress to bacteremia and endocarditis

Bartonella

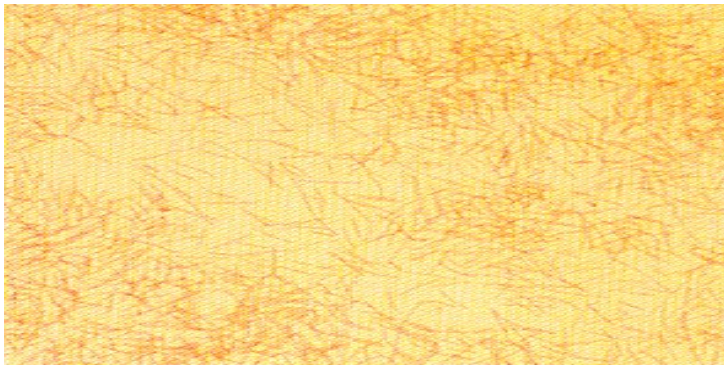
- **Short facultative, intracellular, pleomorphic, gram negative rod**
- Usually recovered from tissue (cutaneous lesions or lymph nodes) or blood
- Isolate from blood using lysis centrifugation and plating the concentrated blood on media containing 5% horse or rabbit blood. Incubate for at least 3 weeks in elevated CO2
- Serology and molecular methods for detection are preferred

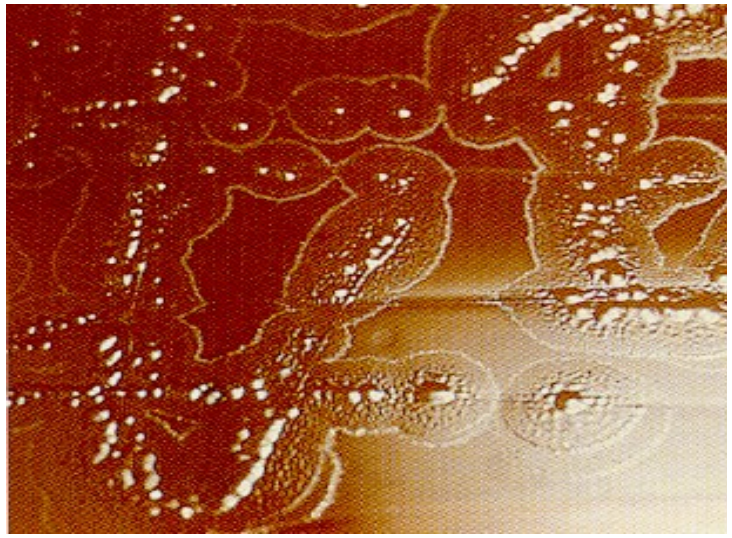


Capnocytophaga

- Normal oral flora
- Name comes from “Capno” for its dependence on increased CO2 and “cytophaga” for its gliding motility
- Diseases
  - Septicemia (neutropenic patinets)
  - Endocarditis
  - Oral ulcers
  - Peridontal disease

Capnocytophaga





- Identification
  - Fusiform GNR*
  - Facultative ANO2
  - Pitting agar surface (some)
  - Gliding motility on agar surface (some)*
  - Yellow-orange pigment
  - Catalase negative
  - Oxidase negative
  - Grows on blood and chocolate, but not on MacConkey
- Facultative Anaerobe

Capnocytophaga canimorsus

- Oral flora in dogs and cats
- Oxidase positive; other capnocytophagia spp are negative
- Catalase positive
- Life threatening infections following bites
  - Asplenic patients and alcoholics are more susceptible to infection