

Microbiology in the News

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Objectives

- Identify microorganisms which have caused infectious disease reported in the news
- Discuss the method of transmission/pathogenesis of microorganisms causing infectious disease in the news
- List methods of laboratory diagnosis for microorganisms causing infectious disease in the news

Case 1: May 24, 2021

- May 24, 2021: 4-year old female, Lylah, with symptoms of stomach virus
- Symptoms quickly worsen; admitted to Children's Medical Center Dallas
- Received respiratory support, CT, MRI, multiple treatments, steroids, multiple antibiotics
- Within days, infection spread to brain
- For weeks, patient condition deteriorates
- Infection results in brain damage: can't speak, hold head up, walk
- Blood and brain sample collected; brain sample led to diagnosis of infection
- Moved to a specialty children's rehabilitation hospital in Dallas
- Came home Aug 12, 2021
- Requires physical therapy to learn to talk and walk again



Additional Cases in Outbreak

- **Total of 4 infected people across 4 states between Feb – Oct 2021**
 - **March 2021: adult in Kansas**
 - Died 10 days after being hospitalized; had preexisting health issues including chronic obstructive pulmonary disease and cirrhosis
 - **May 2021: adult in Minnesota (underlying health conditions) and child in Texas (Lylah)**
 - Both hospitalized for extended periods of time; discharged to transitional care facilities
 - **July 2021: Child in Georgia – died**
 - One case had co-infection with SARS-CoV-2
 - Both pediatric cases had severe neurologic involvement
- At least two of cases developed pneumonia; consistent with inhalation as route of exposure

Burkholderia pseudomallei

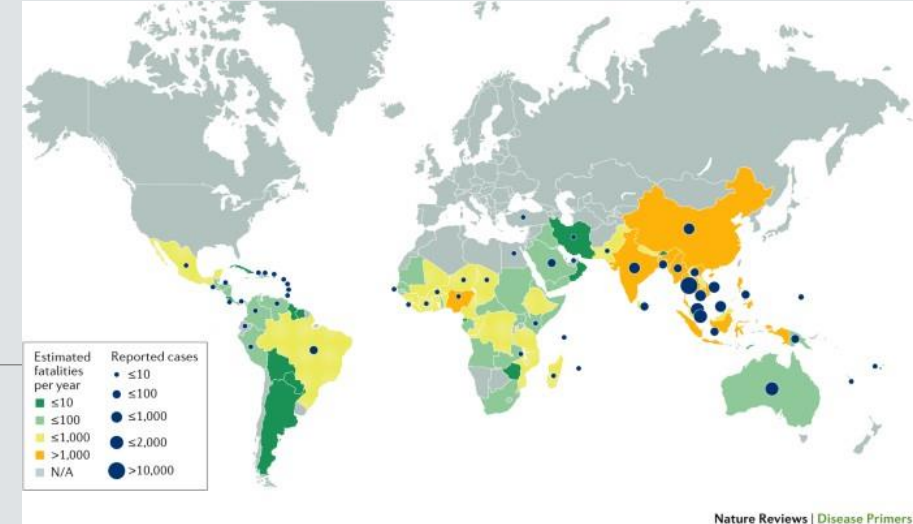


- **Causes Melioidosis**
 - Organism survives within macrophages
 - Disease forms: skin/internal abscesses, sepsis/septic shock, pulmonary disease
 - Serious disease, sometimes fatal; patient may require ICU care
- **Also called Whitmore's disease**
 - First IDed by Whitmore and Krisshnaswami in 1912 in Rangoon, Burma: septic disease
- **Tier 1 select agent**, requires additional security measures
 - Potential to cause mass casualty event after deliberate release

Burkholderia pseudomallei

Epidemiology

- Found in moist environments: soil and contaminated water
 - After heavy rains/winds that bring bacteria to soil surface
- Most common: **Southeast Asia** (Thailand, Malaysia, Singapore), **Northern Australia**, and Mexico
 - Workers in South Asian paddy fields
- Also frequent in: India, Sri Lanka, S. China, Hong Kong, Taiwan, Indonesia, Vietnam, Cambodia, Laos, Burma
- Worldwide frequency: 165,000 cases/year human melioidosis; 89,000 (54%) fatal
 - Under-reported in 45 countries; disease present in 34 countries
- United States: only found naturally in Puerto Rico and U.S. Virgin Islands
 - About 12 cases/year in U.S. (usually travelers/immigrant from endemic areas)



Burkholderia pseudomallei Transmission

- Direct contact with contaminated soil and water
- Inhalation of contaminated dust/water droplets
- Ingestion of contaminated water or soil-contaminated food
- Possibly through tropical freshwater fish

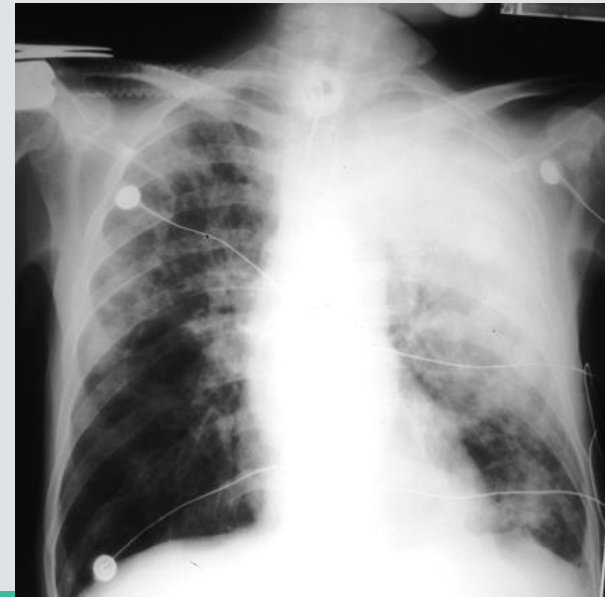
- Not typically spread by person-to-person contact
- Animals susceptible to disease: sheep, goats, swine, horses, cats, dogs, cattle
- **Incubation:** Symptoms occur 1 day to years after exposure; generally occur 2-4 wks after exposure

- **Risk factors:** diabetes, kidney disease, alcohol abuse, liver disease, thalassemia, chronic lung infections (i.e. chronic obstructive pulmonary disease), cancer and other conditions that affect immune system

Burkholderia pseudomallei Symptoms

- **Wide range of symptoms**
- **Overall fatality rate of 10-50% with up to 90% mortality in septic infections if untreated**
 - If survive acute melioidosis, 5–28% develop recurrent infection
- **Pulmonary Infection** – most common presentation
 - Cough and shortness of breath
 - Chest pain
 - High fever
 - Headache
 - Appetite loss/Weight loss
- Ranges from mild bronchitis to severe pneumonia
- Can mimic tuberculosis

Acute pulmonary infection



Burkholderia pseudomallei Symptoms

- **Localized infection:** direct inoculation of skin through skin abrasion
 - **Localized pain/swelling** – such as parotid glands (located below/in front of ear)
 - **Fever**
 - **Skin ulcerations/abscesses** – may start as firm, gray or white nodules that become soft and inflamed; look like wounds caused by flesh-eating bacteria
- Can progress to septicemia, osteomyelitis, meningitis, and brain, liver or spleen abscesses



Neck swelling



Burkholderia pseudomallei Symptoms

- **Bloodstream infection**
 - Fever
 - Headache
 - Respiratory distress
 - Abdominal discomfort
 - Joint pain
 - Disorientation
- Without fast treatment, pulmonary infection can progress to septicemia
- Also known as septic shock; most serious form of melioidosis

Burkholderia pseudomallei Symptoms

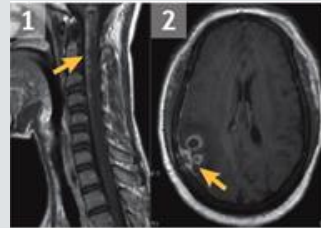
- **Disseminated infection**

- Fever
- Weight loss
- Stomach or chest pain
- Muscle or joint pain
- Central nervous system/brain infection
- Neurological symptoms
(i.e. altered mental status, headache, seizure, extremity weakness)

- **Asymptomatic exposure**

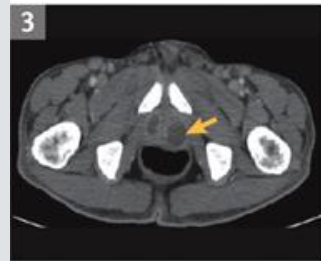
- Asymptomatic seroconversion: antibodies develop in blood but no illness
- <5% of cases, may result in infection becoming apparent many years after exposure

Burkholderia pseudomallei: Clinical Manifestations



Central nervous system (1–5%)

- Encephalomyelitis
- Brain abscess



Cardiovascular system (40–60%)

- Bacteraemia (40–60%; bacteria without evident focus 10%)
- Pericarditis
- Mycotic aneurysm

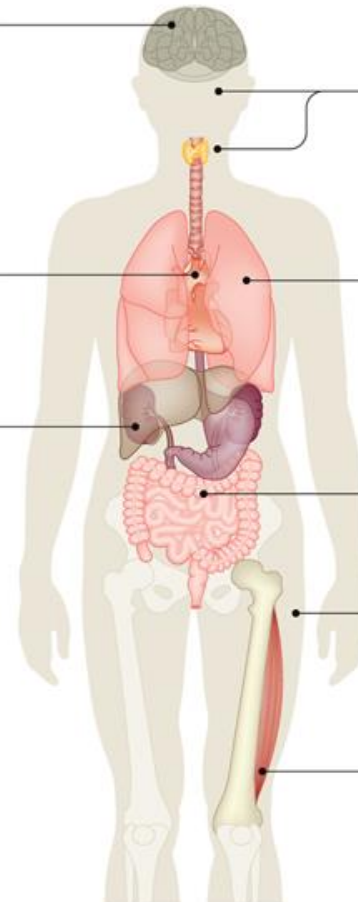


Urinary tract system (14–28%)

- Acute pyelonephritis
- Kidney abscess
- Prostatic abscess (20% of males in Australia)

Other

- Mastitis
- Mediastinal mass
- Corneal ulcer
- Epididymo-orchitis
- Scrotal abscess



Head and neck (0–30%)

- Parotid abscess (30% of children in Thailand)
- Neck abscess
- Lymphadenitis



Respiratory system (40–60%)

- Pneumonia (40–60%)
- Pulmonary abscess
- Pleuritis



Gastrointestinal system (10–33%)

- Liver abscess
- Splenic abscess
- Para-intestinal mass

Skin and soft tissue (13–24%)

- Skin ulcer
- Soft tissue abscess

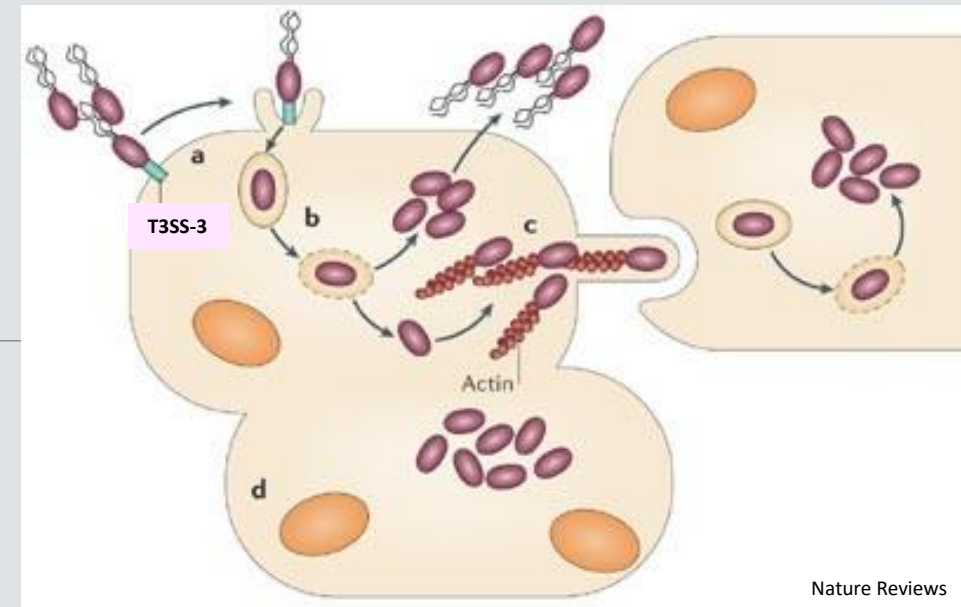


Musculoskeletal system (4–14%)

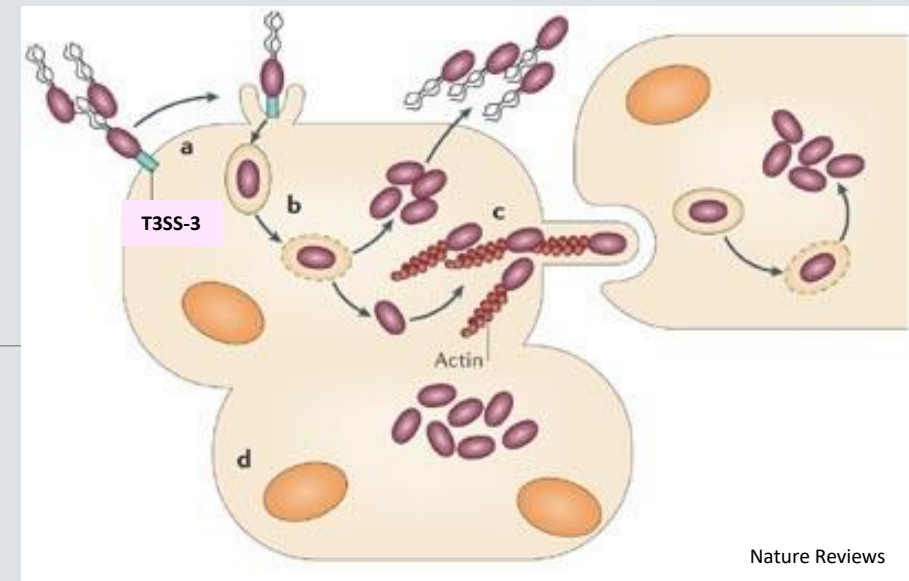
- Septic arthritis
- Myositis
- Osteomyelitis

Burkholderia pseudomallei Pathogenesis

- Infection process: invasion/replication in phagocytic and non-phagocytic cells (epithelial cells)
- Survives/proliferates for prolonged periods within phagocytic cells
- **Steps in infection process:**
 - **a. Epithelial attachment and cell invasion**
 - **Type III secretion system (T3SS-3)** encodes proteins required for invasion
 - Escape from phagosomes → intercellular spread
 - **b. Endosome escape and intracellular proliferation**
 - Escapes from endocytic vacuoles into cytoplasm of infected cells by lysing endosome membrane
 - Resists several host antimicrobial peptides, interferes with intracellular bacterial killing mechanisms
 - Induces apoptosis in both phagocytic and non-phagocytic cells



Burkholderia pseudomallei Pathogenesis



- **Steps in infection process:**

- **c. Cell-to-cell spread**

- Induces formation of actin-based membrane protrusions
- Actin-based motility allows free bacteria in cytoplasm to spread intracellularly & intercellularly
- Cell-to-cell movement of *B. pseudomallei* occurs when neighboring cells phagocytize protrusion
- Bacteria spreads without exposure to Abs/immunoactive molecules

- **d. Cell fusion**

- Induces formation of multinucleated giant cells by cell fusion
- Bacteria spreads from cell to cell to evade immune surveillance

- **Latent or persistent infection**

Burkholderia pseudomallei Treatment

Intravenous antibiotics for a minimum of 2 weeks (up to 8 weeks)

Follow with oral antibiotics for 3–6 months

- Used to prevent common relapses

Intravenous therapy consists of:

- Ceftazidime administered every 6–8 hours

OR

- Meropenem administered every 8 hours

Oral antimicrobial therapy consists of:

- Trimethoprim-sulfamethoxazole (TMP/SMX) taken every 12 hours

OR

- Amoxicillin/clavulanic acid (co-amoxiclav) taken every 8 hours if (TMP/SMX) contraindicated or not tolerated
- If penicillin allergies, need alternate antibiotic



Lab ID: Culture (Gold Standard for Diagnosis)

- **Specimens:**

- Blood, sputum, urine, throat swab, pus from skin/internal abscesses, joint aspirate, and CSF

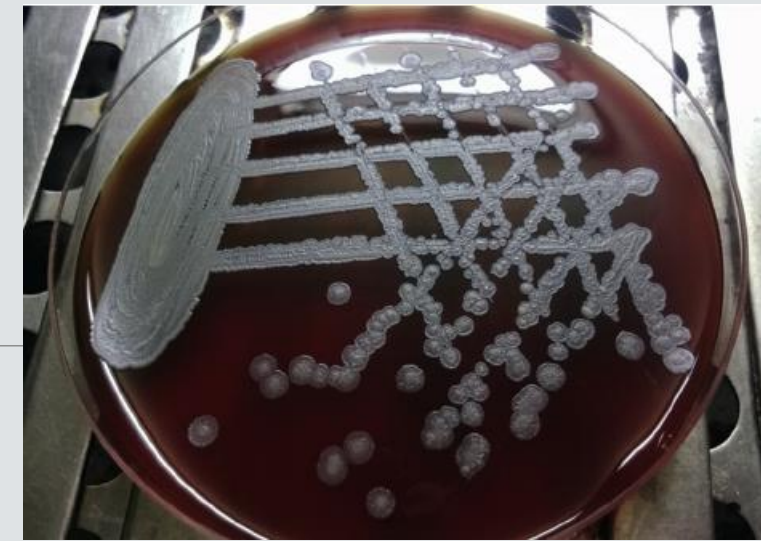
- **Gram stain:** “safety pin”, bipolar Gram-negative bacilli

- **Culture:**

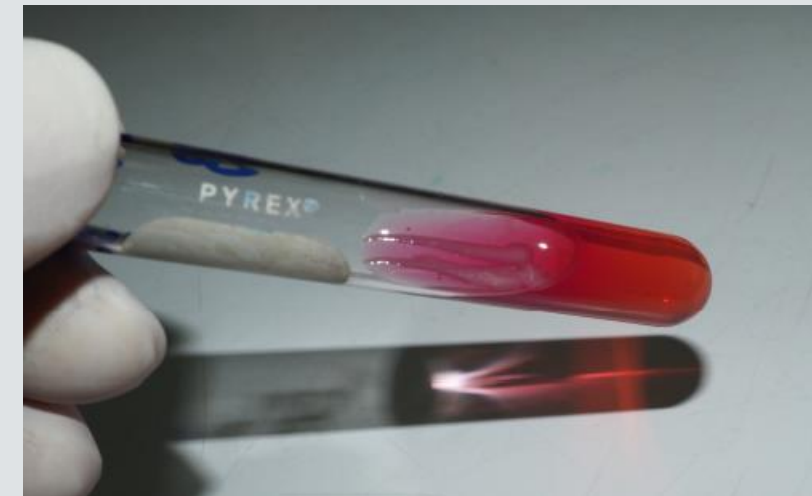
- Blood agar: dry, wrinkled colonies; can have metallic sheen
- MacConkey: most are non-lactose fermenter
- Odor: putrid/earthy – but don't sniff it!!

- **Characteristics**

- Oxidase-pos, catalase-pos
 - Strict, obligate aerobe
- Polar flagella, motile
Non-glucose fermenter

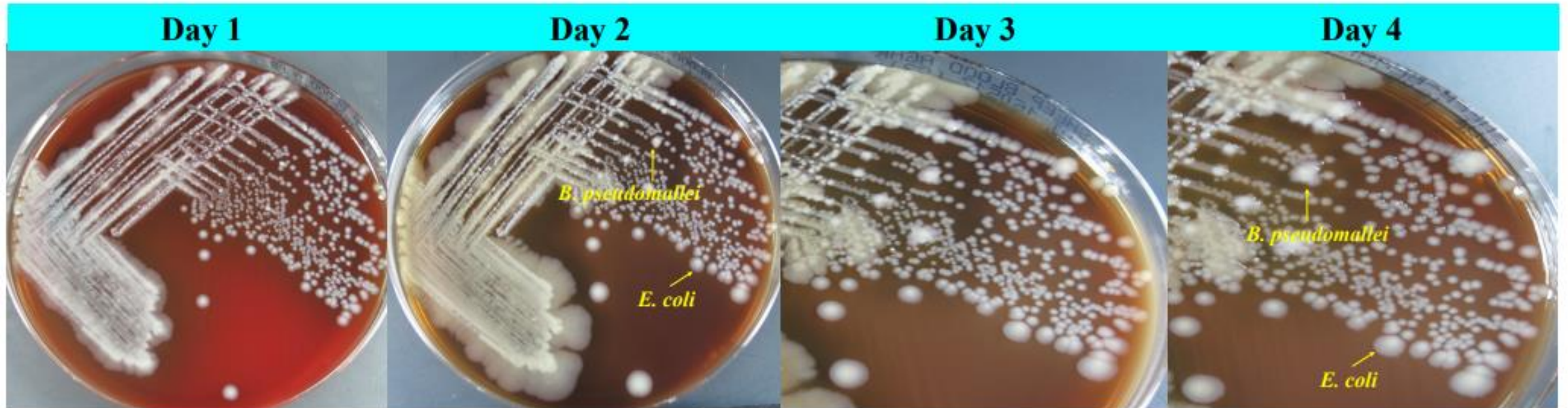


Research gate.net



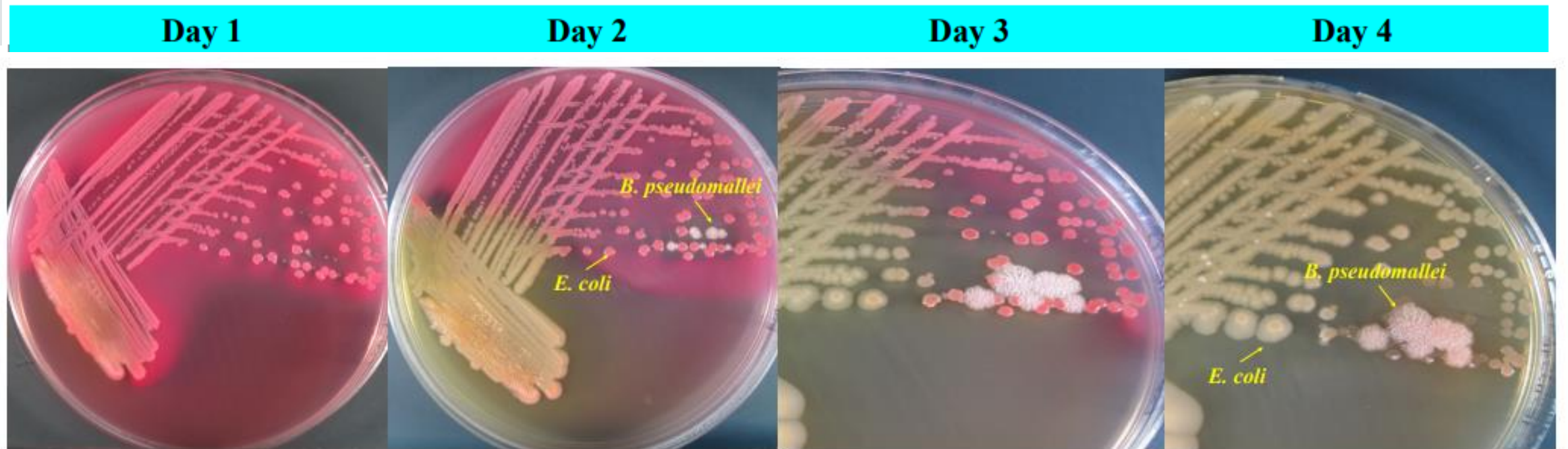
www.melioidosis.info

Blood Agar: *B. pseudomallei* mixed with *E. coli*, non-sterile clinical sample



B. pseudomallei forms creamy colonies which are non-haemolytic and resemble a coliform. Slight metallic sheen. Becoming dry and wrinkled after 2 days of incubation. *E. coli* has similar morphology and tends to overgrow *B. pseudo*.

MacConkey Agar: *B. pseudomallei* mixed with *E. coli*, non-sterile clinical sample



B. pseudomallei resembles a non-lactose fermenting coliform (colourless). Becoming dry and wrinkled after 2 days of incubation. *E. coli* is a lactose fermenter (pink to red colour) with colonies that become colourless on longer incubation.

Lab ID: Ashdown media

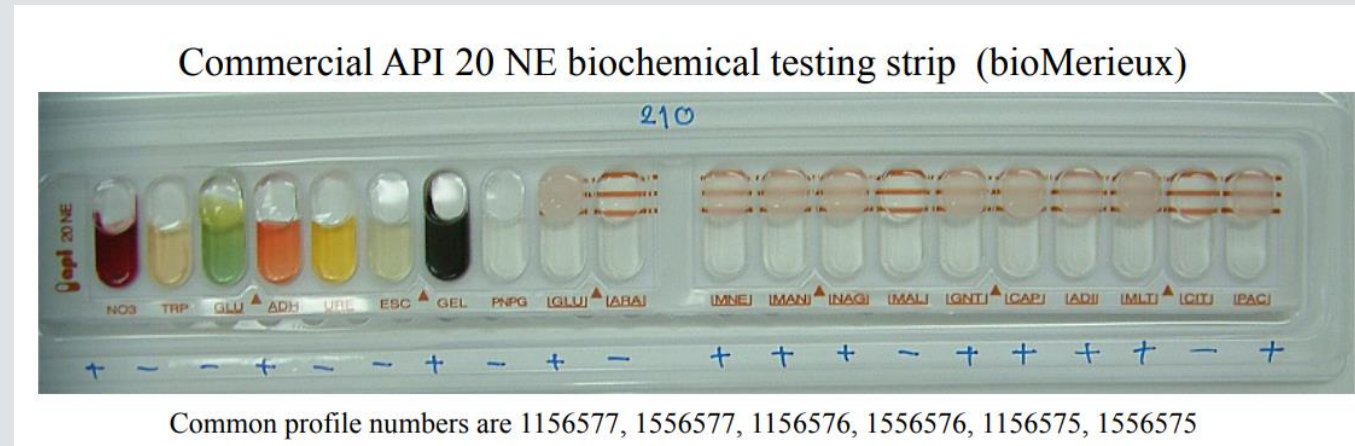
- **Selective media:** culture nonsterile body sites; inhibit normal flora
 - Crystal violet and gentamicin: selective agents
 - 4% glycerol enrichment: required for growth of *B. pseudomallei*
 - Neutral red: *B. pseudomallei* absorb dye
- Requires 96 hours (4 days) of incubation
- **Colony appearance**
 - 24 hours: pinpoint, clear and pale pink
 - 96 hours: **dry, wrinkled**



Lab ID: Commercial Biochemical Test

- Can mis-ID *Burkholderia pseudomallei* as other bacteria

- *Burkholderia thailandensis*
- *Burkholderia cepacia* complex
- *Chromobacterium violaceum*
- *Ochrobactrum anthropic*
- *Pseudomonas*
- *Acinetobacter*
- *Aeromonas*

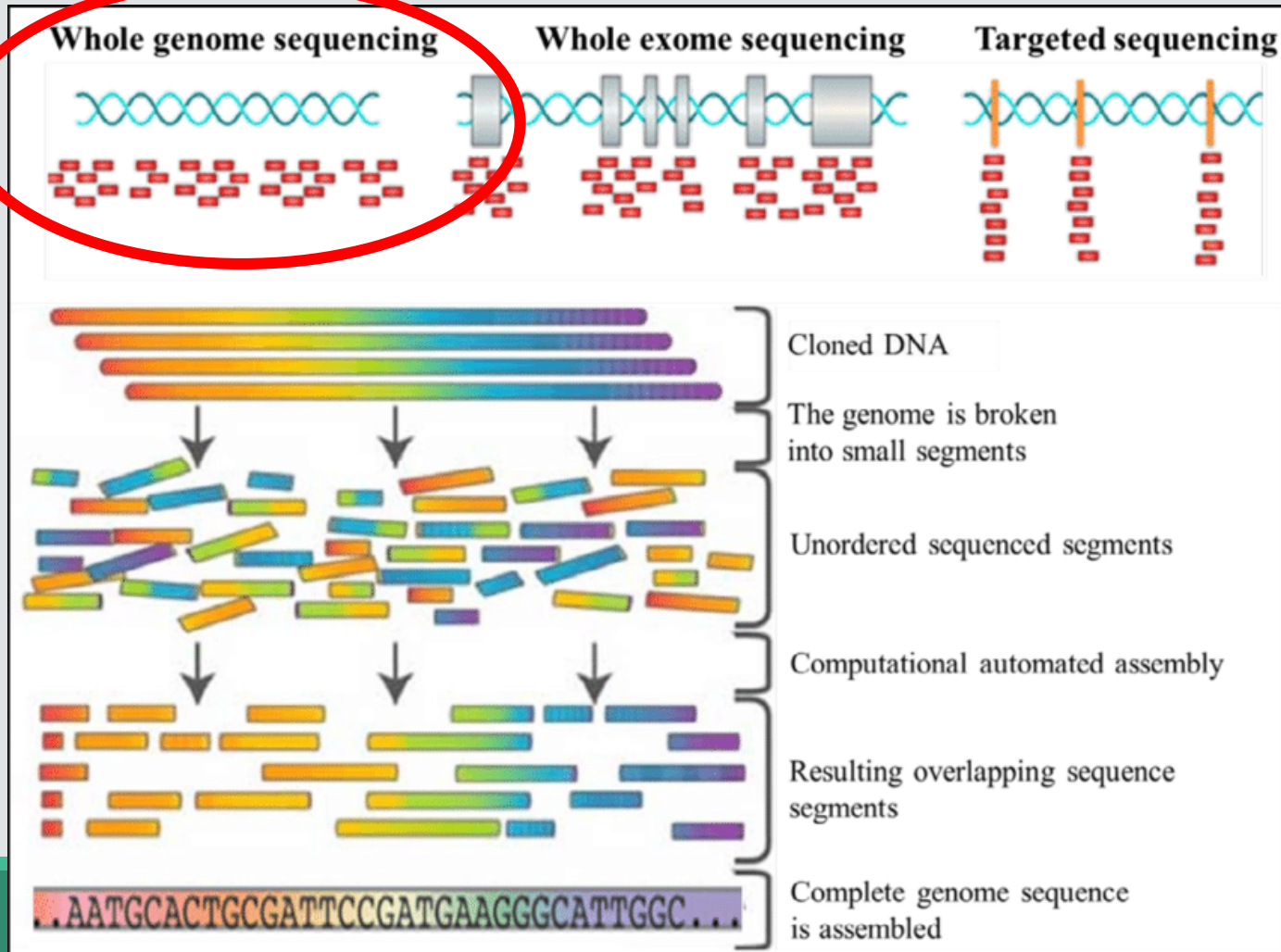


www.melioidosis.info

- Arabinose assimilation (Use of L-arabinose as sole carbon source)

- *Burkholderia pseudomallei* = negative
- *Burkholderia thailandensis* = positive

Lab ID: Genetic Testing



- **Whole Genome Sequencing**
- **Polymerase Chain Reaction (PCR):** detects difference in 16s rRNA
- Possible gene targets: Type III secretion system and Tat domain protein

Laboratory Identification: Whole Genome Sequencing

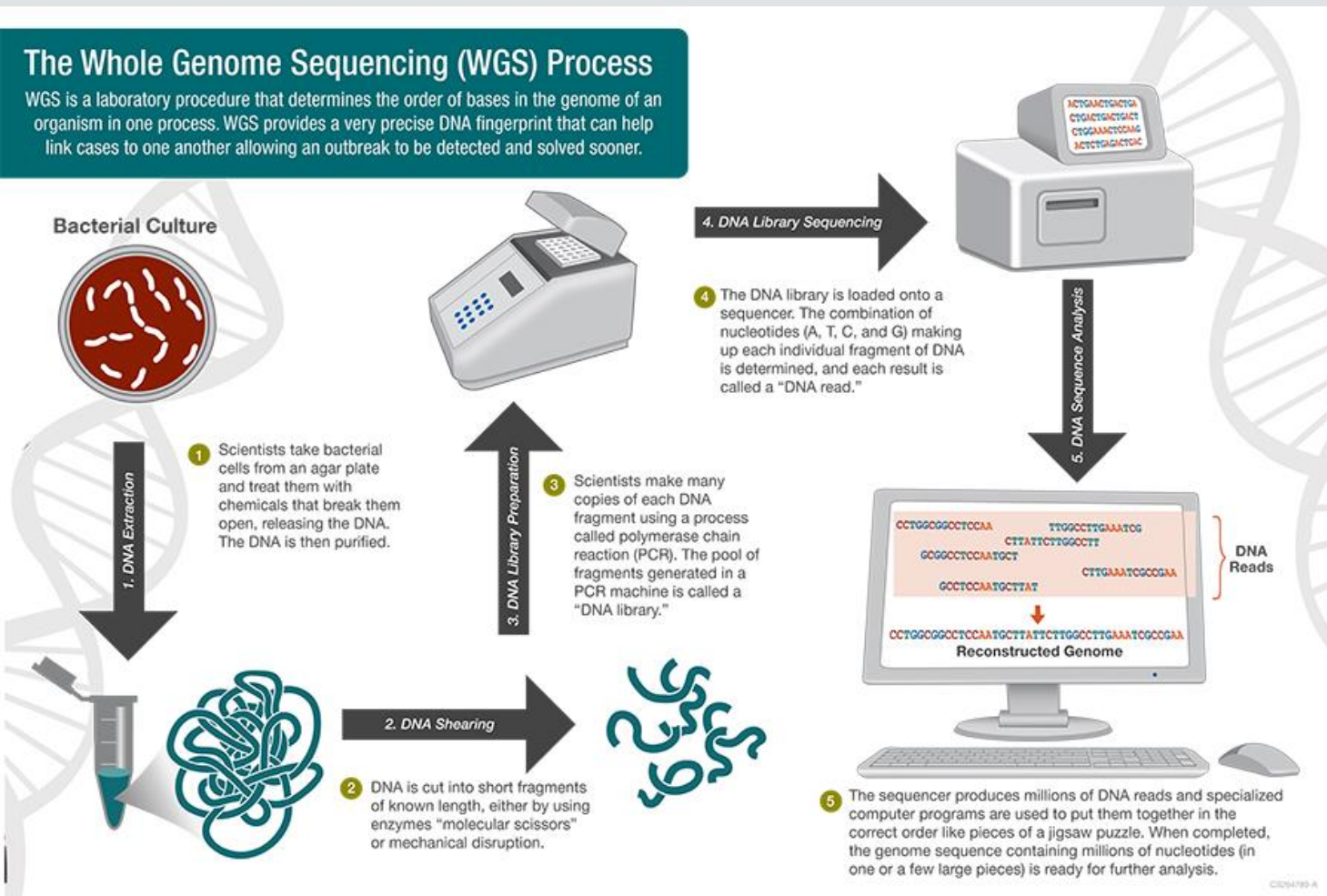
1. **DNA shearing:** DNA is cut into small pieces
2. **DNA bar-coding:** small DNA tags, bar codes, added to identify which piece of sheared DNA belongs to which bacteria

3. Whole genome sequencing:

- Bar-coded DNA from multiple bacteria are combined, put into sequencer
- Sequencer identifies A's, C's, T's, G's (bases)
- Uses bar code ID which bases belong to which bacteria

4. Data analysis: computer analysis tools used to compare bacterial sequences/identify differences

***Number of sequence differences: indicate how closely related the bacteria are, how likely it is that they are from the same outbreak**



Lab ID: Serology

- Used to confirm exposure to *Burkholderia pseudomallei*
- Adjunctive role to culture (gold standard of diagnosis)
 - Screen travelers returning from endemic area with fever
 - Aid in diagnosis if unusual presentation (e.g., chronic disease) or when specimens for culture unavailable (e.g., deep brain abscesses)
 - Supporting information if disease suspected but organism fails to grow
- Advantage: simple, rapid; can ID cases earlier than culture
- Disadvantage: variable sensitivity, specificity

Lab ID: Serology

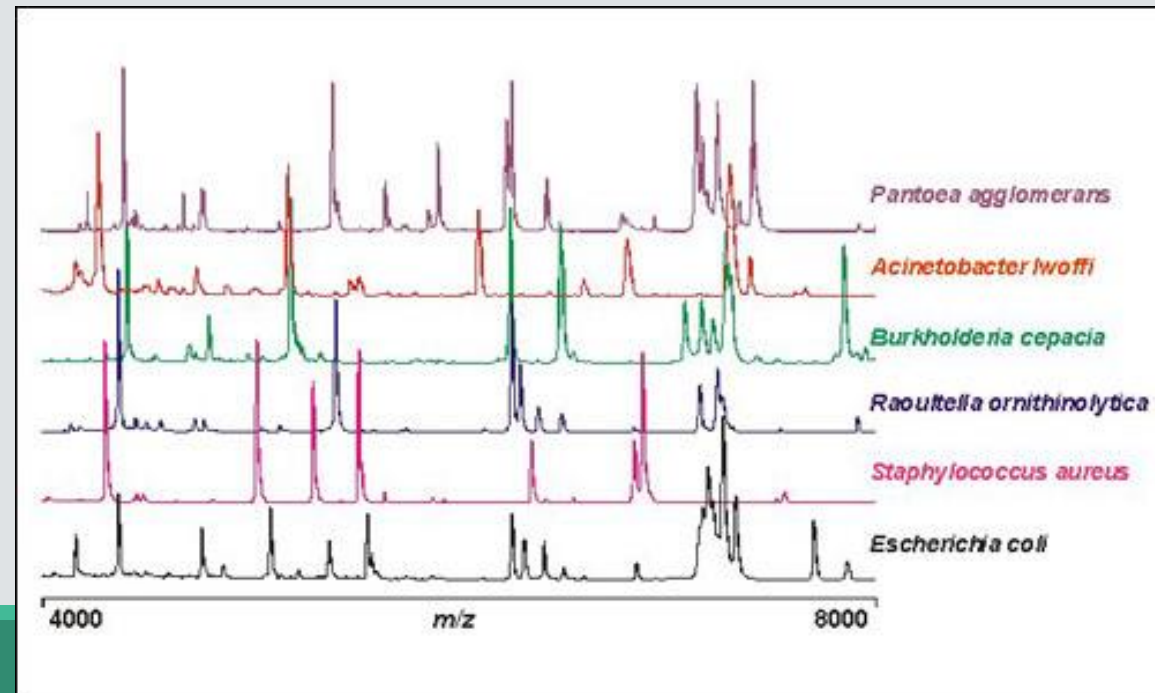
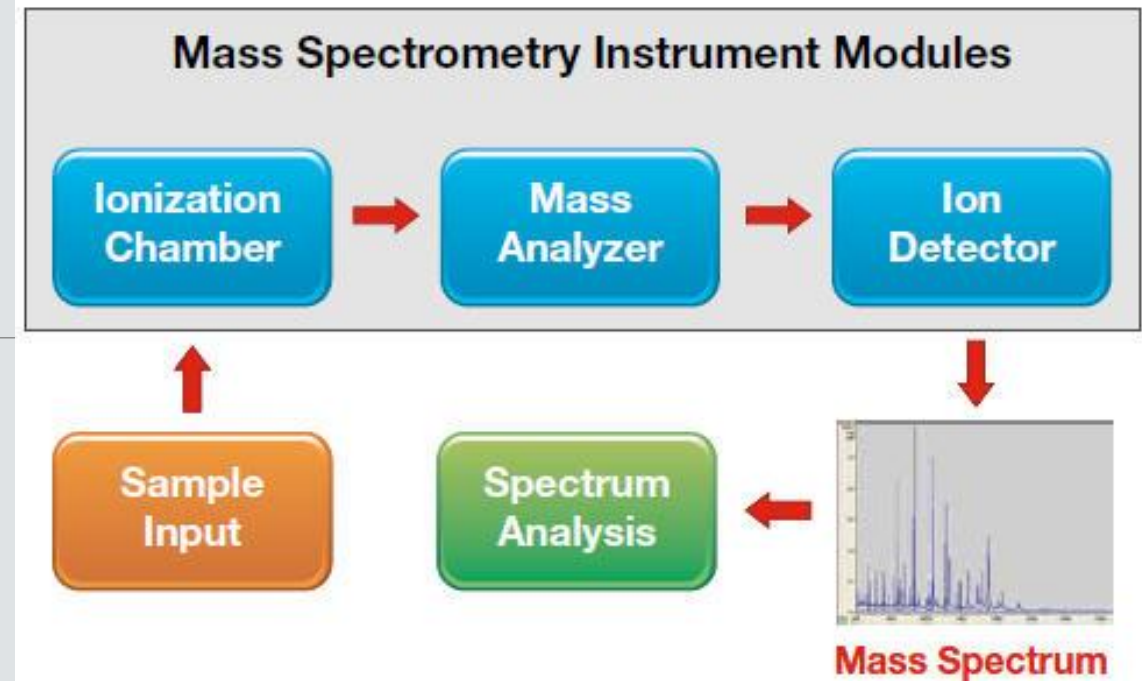
- **Indirect hematagglutination assay (IHA)**
 - Principle = Sensitized RBCs added to serial dilution of heat-inactivated patient sera
 - RBCs sensitized with *B. pseudomallei* polysaccharide/lipopolysaccharide antigens
 - Titer > 1:40 or greater considered reactive in Australia
 - High background rates of seropositivity in endemic areas
 - Reduces utility of test
 - Varied serologic response over time in culture-positive patients
 - Late seroconversion, persistently reactive, persistently nonreactive, & seroreversion
- **Enzyme immunoassay (EIA) detecting IgM/IgG against *B. pseudomallei* plus IHA**
 - Increased specificity/sensitivity in diagnosis of acute disease in endemic areas

Lab ID: MALDI-TOF

MALDI-TOF = Matrix-assisted laser desorption/ionization - Time of Flight

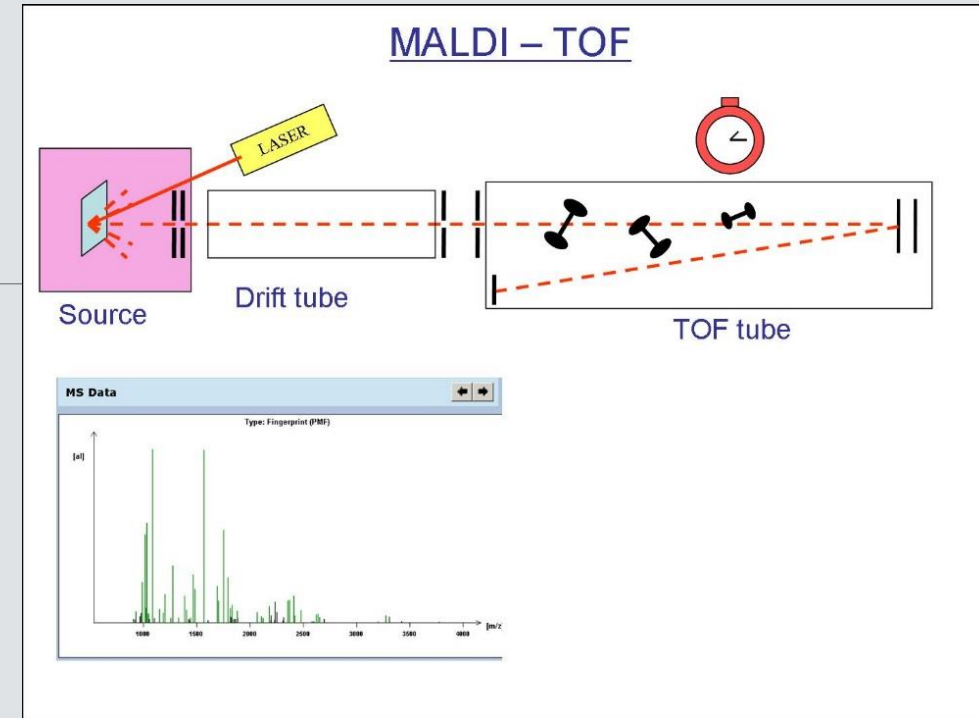
Mass Spectrometer (MS) Principle

- Sample is ionized
- Ions separated in magnetic field based on charge & mass
- Output = Spectrum
 - Mass/charge ratio on x axis
 - Abundance of ion on y axis
- Bacteria is IDed by characteristic spectrum (set of peaks)



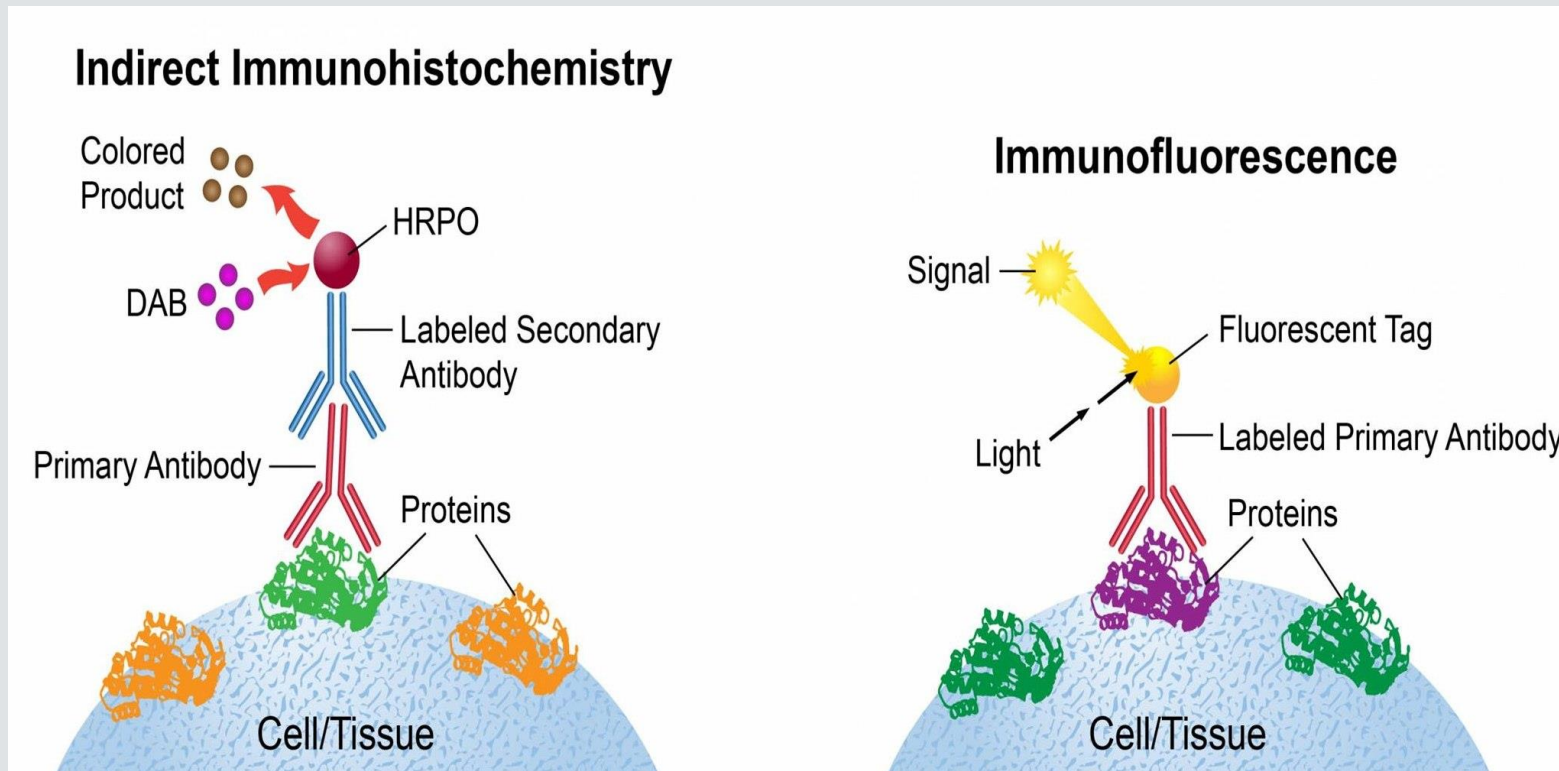
Lab ID: MALDI-TOF

- Sample adhered to sample plate matrix
- Laser pulse fired into surface coated with sample
- Laser ionizes (protonates) sample and release it
 - Positive ions generated
- Ions fly to detector at a speed (time of flight) based on mass/charge ratio
 - Ions separate in drift tube
 - Lighter ions travel faster, reach detector first
- Specimen TOF pattern: compared to reference patterns to ID isolate
- Misidentification possible
 - Texas case was first misIDed as *Burkholderia thailandensis*



Lab ID: Immunohistochemistry

Anti-Burkholderia pseudomallei Ab reacts positively with tissue



The Investigation

- **Genetic analysis of bacteria from first three patients**
 - Matched strain for SE Asia in all 3 cases
 - Strain did not match strains in Americas, not from natural reservoir of bacteria in North America
- **Three cases in Kansas, Minnesota, and Texas were linked**
 - US Centers for Disease Control and Prevention issued health alert June 2021
- **CDC and State health departments investigated imported liquid products as source of exposure**
- **Tested several hundred specimens before source identified**
 - Lotions, soaps, food items, liquid vitamins, laundry detergent, bathroom cleaners, deodorizing sprays, dish soap, hand sanitizer, hand/body wipes, mouthwash, nasal sprays, essential oils, juices, fruit cups, applesauce, garden soil and plants
 - Pet Betta fish (Siamese fighting fish; tropical freshwater fish native to Southeast Asia) Lylah got during winter and died in February
 - Flowers that Lylah helped plant days before feeling ill

The Investigation

- **Genetic test linked Georgia patient to first 3 patients**
- **Contaminated bottle IDed** upon revisiting home of Georgia patient, bottle not previously collected
 - Positive PCR results for *Burkholderia pseudomallei*, resulted in product October 2021 recall
 - Sequence used to confirm that bacterial strain in bottle matched all four patients
 - Same strain IDed in an unopened bottle of same scented product recalled from Walmart store in different state

Source of Infection

- **Product description:** Better Homes and Gardens Essential Oil Infused Aromatherapy Room Spray with Gemstones “Lavender & Chamomile”
 - Other Scents: Lemon & Mandarin, Lavender, Peppermint, & Eucalyptus, Sandalwood & Vanilla
- Sold at about 55 Walmart stores in 18 states and online: Feb- Oct 2021
- Contamination during manufacturing process
 - Manufactured in India
 - Made by Flora Classique Inc., a subsidiary of Gala Group
- Recalled about 3,900 bottles of aromatherapy spray (6 scents) on Oct 22, 2021



Source of Contamination?

- CDC epidemiologist Dr. Jennifer McQuiston (CNN news article)
 - “Healing” gemstones?
 - Bacteria on rocks collected from environment if rocks weren't sterilized
 - Another component was contaminated and rocks made micro-environment in bottle, bacteria grew
 - Significance of rocks uncertain, but unusual to have rocks in fragrance bottle

CDC Definition of Exposure

Exposure is defined as:

- Being in the room while the product is being sprayed
- Having directly “sniffed” or inhaled from the product bottle
- Having direct contact with an item (such as pillowcases or other linens) on which the product has been sprayed

CDC Case Classification: Suspect Case

A case that meets the clinical description, meets one or more of the suspect lab criteria (suspect lab evidence), and meets any of the following:

has known exposure to *B. pseudomallei*

has an epidemiological (incidence/disease distribution) link to a confirmed case

there is no history of travel to a *B. pseudomallei* endemic place

Suspect Lab Evidence:

Evidence of titer result by **indirect hemagglutination (IHA) $\geq 1:160$** on acute-phase serum specimen

Evidence of titer result by IHA $\geq 1:160$ on convalescent-phase serum specimen that is stable/unchanged from acute-phase serum specimen titer result obtained greater than or equal to 2 weeks apart

CDC Case Classification: Probable Case

A case that meets the clinical description, meets one or more of the probable lab criteria (presumptive lab evidence), and meets any of the following:

has known exposure to *B. pseudomallei*

has an epidemiological link to a confirmed case

there is no history of travel to a *B. pseudomallei* endemic place

Probable Cases (Presumptive Lab Evidence):

Evidence of a **four-fold or greater rise** in *B. pseudomallei* antibody **titer by IHA** between acute- and convalescent-phase serum specimens obtained greater than or equal to 2 weeks apart.

Evidence of *B. pseudomallei* **DNA** (e.g., by Laboratory response network (LRN)-validated **polymerase chain reaction**) in a clinical specimen collected from a normally **sterile site** (e.g., blood).

CDC Case Classification: Confirmed Case

Meets either of the following:

A case with or without a known exposure, whose isolate from a clinical specimen is laboratory-confirmed, and WGS and strain analysis link isolate to outbreak strain.

A case with a known exposure, with no travel history to an endemic place, whose isolate from a clinical specimen is laboratory-confirmed with no WGS results.

A case with a known exposure, and immunohistochemical evidence of *B. pseudomallei* in a tissue specimen.

Confirmed Cases (Definitive Lab Evidence):

Isolation and confirmation of *B. pseudomallei* from a clinical specimen of a case of severe febrile illness: **culture** of the organism may be done by blood, sputum, urine, pus, throat swab, swabs from organ abscesses or wounds, or tissue collected post-mortem.

- Whole genome sequencing (**WGS**) and **strain analysis** links isolate to outbreak strain.

Immunohistochemical (IHC) evidence of *B. pseudomallei* in a tissue specimen.

Recommendations for Healthcare Providers

- **Maintain high clinical suspicion** for melioidosis diagnosis if patient has:
 - Illness compatible with melioidosis
 - Exposure history in the past 21 days to Better Homes & Gardens Aromatherapy Room Spray “Lavender & Chamomile” with Gemstones or similar products
- **Culture of *B. pseudomallei*** from any clinical specimen is considered **diagnostic** for melioidosis
- **When ordering** cultures, **advise lab** of *B. pseudomallei* suspicion to ensure proper safety precautions
- Consultation with infectious disease specialists strongly recommended

Burkholderia pseudomallei

Postexposure prophylaxis (PEP)

- Healthcare providers should offer postexposure prophylaxis (Trimethoprim/sulfamethoxazole or Amoxicillin/clavulanic acid) if patient exposed to aromatic spray within last 7 days
- To be cautious, PEP guidance also applies to other five scents
- High risk groups where PEP is highly recommended if exposed include:
 - Any child with no known risk factors who has directly “sniffed” or inhaled product in past 7 days
 - Anyone with known risk factors for melioidosis which include diabetes, excessive alcohol use, chronic liver disease, chronic renal disease, chronic lung disease, cancer, or immune suppressing condition other than HIV
 - Diabetes = most significant risk factor

Recommendations for Consumers

1. Stop using this product immediately. Do not open bottle.
2. Do not attempt to throw away or dispose bottle down drain.
3. Double bag bottle in clean, clear zip-top resealable bags/place in small cardboard box. Return to Walmart.
4. Wash sheets or linens sprayed with product using normal laundry detergent; dry completely in hot dryer, bleach if desired.
5. Wipe down counters and surfaces that might have been exposed to spray with undiluted disinfectant cleaner.
6. Minimize handling of product and wash hands thoroughly after handling bottle or linens. Wash hands thoroughly after removing gloves.
7. If product was used within past 21 days and you develop fever or symptoms, seek medical care; inform physician of exposure.
8. If no symptoms but were exposed to product in last 7 days, physician may recommend antibiotics

Case 2: November 6, 2021

- 12-week old Australian cattle dog puppy, Disco, in Upper West Side New York City
 - Lethargic during day
 - Vomiting violently that night
 - Declined rapidly overnight
 - Admitted to animal hospital in morning
 - Continued to decline for 3 days in hospital
 - Developed acute renal failure
 - Jaundiced
 - Owner declined \$30,000 dialysis treatment
 - <20% chance of recovery according to vet
 - Even with recovery, likely to have life-long organ damage
 - Euthanized



Case 2: Leptospirosis

- **Bacterial spirochete:** *Leptospira interrogans*
- **Incubation:** usually 5-14 days, ranges from 2 days - 4 weeks
- Illness lasts days or weeks
- **Common symptoms**
 - Fever, chills, headache, vomiting, muscle aches, diarrhea
 - Abdominal pain
 - Jaundice
 - Skin Rash
 - Red eyes
 - Can lead to fatal organ damage (kidney, liver, brain, lung, heart)
 - **Kidney/liver failure**
 - Meningitis
 - Respiratory distress

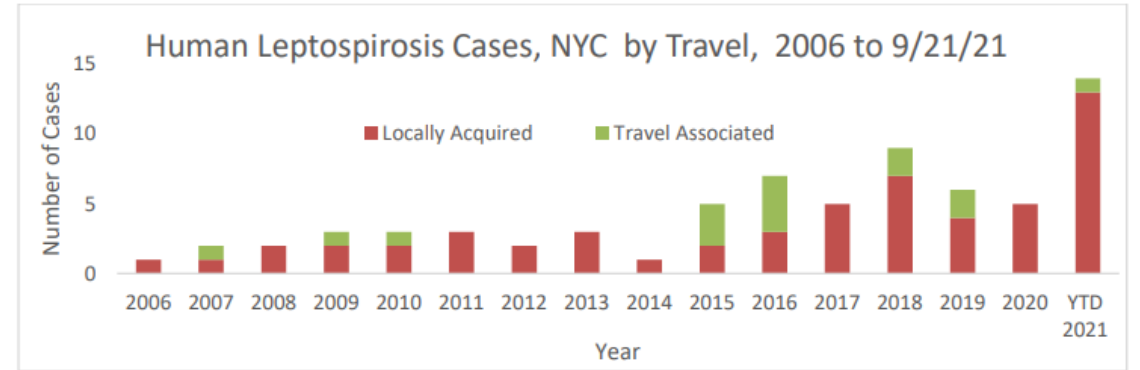
Leptospira Transmission

- **Spread of disease**
 - Infected rats, raccoons, opossums, cattle, swine, dogs, horses, buffaloes, sheep, goats
- **Routes of human infection:** skin abrasions, mucous membranes of oral cavity and conjunctiva
 - Warm, moist environment (rural or urban)
 - Drinking or contact with water (swimming, rafting kayaking) or soil/food contaminated with infected animal's urine
 - Direct exposure to urine/body fluid of infected animal
 - Bacteria enter through wound
 - Person-to-person transmission is rare
- **Resurgence of disease in New York: Exposure to urine of infected rat in water/soil**
 - Rats on uncollected piles of garbage
 - Slowdown for sanitation workers – protesting COVID-19 vaccine mandates

Leptospira Transmission

• Human cases in New York City

- Human cases rarely reported, but have been increasing over time
- 14 cases reported in 2021
 - More than total number reported in NYC in any prior year
- 13 of 14 hospitalized - acute renal/hepatic failure
 - Two with severe pulmonary involvement
 - One died from infection
- 13 of 14 acquired infection locally, the other from travel
- Three cases reported homelessness
- Associated with exposure to rats
- In all boroughs except Staten Island



1. CDC Leptospirosis Fact Sheet for Clinicians <https://www.cdc.gov/leptospirosis/pdf/fs-leptospirosis-clinicians-eng-508.pdf>
2. Up to Date <https://www.uptodate.com/contents/leptospirosis-treatment-and-prevention>

• 57 NYC Cases between 2006-2021

- 44 locally acquired
(Range of 1-7 cases/year)
- 13 associated with international travel

Leptospirosis

Re-emerging, neglected zoonotic disease

- More common in tropical or sub-tropical climates

Causative agent: *Leptospira interrogans*, spirochete

At risk population

- People who work outdoors or with animals
 - Farmers, dairy farmers, fishermen; Workers in mines, sewers, or slaughterhouses
 - Veterinarians/Animal Caretakers; Military Personnel

High-risk activities

- Wading, swimming, or boating in contaminated floodwater or freshwater (rivers, streams, lakes)
 - Prolonged immersion in, submerging head in, or swallowing = increased risk
- Activities that lead to skin abrasions and water/soil exposure (clearing brush, trekking, gardening)



Leptospirosis

- **Incidence**

- Worldwide: 1 million cases occur worldwide annually, including almost 60,000 deaths
- US: approximately 100–150 cases/year
 - Puerto Rico majority, followed by Hawaii

- **Most infections asymptomatic**

- **Clinical illness**

- 90% = nonspecific acute febrile illness
- 10% = severe, potentially fatal illness with multi-organ dysfunction
- May be biphasic
 - Patient briefly recovering from mild illness, but then developing more severe illness
- Can cause fetal death/abortion during pregnancy

- **Fatality rate**

- 5%–15% in patients with severe illness
- Can exceed 50% in patients with severe pulmonary hemorrhagic syndrome

Leptospirosis

Biphasic disease

- **First phase: septicemic stage (acute)**
 - High fever, chills, headache, muscle aches, vomiting, diarrhea
 - Self-limiting, mild flulike illness
 - Eyes become very red on 3rd/4th day (conjunctival suffusion)
 - Resembles conjunctivitis, but redness w/o inflammatory exudates
 - Most characteristic physical finding, < 50% of patients
 - Some cough, occasionally bringing up blood, have chest pain
 - Most recover within 1 week



Leptospirosis

Biphasic disease

- **Second phase: Immune stage (delayed)**
 - Symptoms return a few days later
 - Caused by inflammation in immune system
 - Hallmark: aseptic meningitis (fever, stiff neck, headache)
 - Lungs can be severely damaged

Leptospirosis

Weil's disease (icteric leptospirosis)

- Most severe illness
- Liver, kidney, vascular dysfunction (bleeding)
 - Nosebleeds, coughing up blood, bleeding in tissues, anemia possible
- Jaundice
- Can also severely affect heart/lungs
 - Pulmonary hemorrhage
- High mortality rate
 - 5-10% of patients with jaundice die



A: Icterus

B: Conjunctival suffusion

Leptospira Pathogenesis



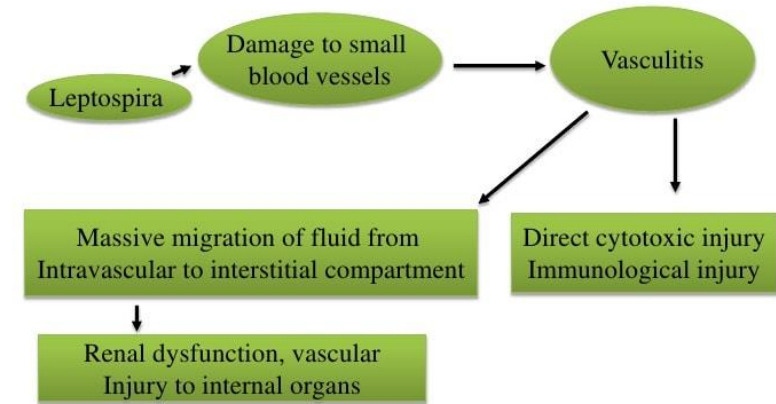
- **Pathogenic leptospire in animals**
 - Widespread in nature
 - Hematogenous/intercellular dissemination to proximal kidney tubules of reservoir hosts
 - Animal discharges urine containing bacteria

Leptospira Pathogenesis

• *Leptospira* in humans

- Animal urine infects accidental human host
 - Chemotaxis mechanism for adhesion/transmembrane passage
- Bacteria may settle in kidney tubules and shed in urine (weeks to months)
- *Leptospira* invade bloodstream, multiply in blood/tissue (leptospiremia)
- Bacteria disseminates hematogenously to organs (brain, liver, lung, heart, kidney)
- Produce hemorrhage/necrosis of tissue, leading to dysfunction/lesions
 - **Generalized vasculitis:** Damage capillary endothelial cells → permeability, hemorrhage, interrupt oxygen supply to tissue
- Hepatocellular dysfunction = jaundice
- Hypoxic tubular damage = renal dysfunction
- Antigen antibody complexes deposit in organs → complications

Pathogenesis of Severe Disease



Lab Testing: *Leptospira*

Leptospirosis is a nationally notifiable disease

Specimen types: Blood, Urine, CSF

Specimen submission guidelines

- Submit as many specimen types as possible
- Acute illness (first week): whole blood and serum
- Convalescent illness (after first week): serum +/- urine

Screening test: IgM serology (i.e. ELISA)

- Antibodies develop 3-10 days after symptom onset
- Acute phase = leptospira in blood (septicemia) for first 4–6 days
 - Neg results in first week do not rule out disease
- Repeat on convalescent sample (7-14 days after first sample)

IgG serology: detected \geq 1 month after infection

- Convalescent sera contains protective antibodies

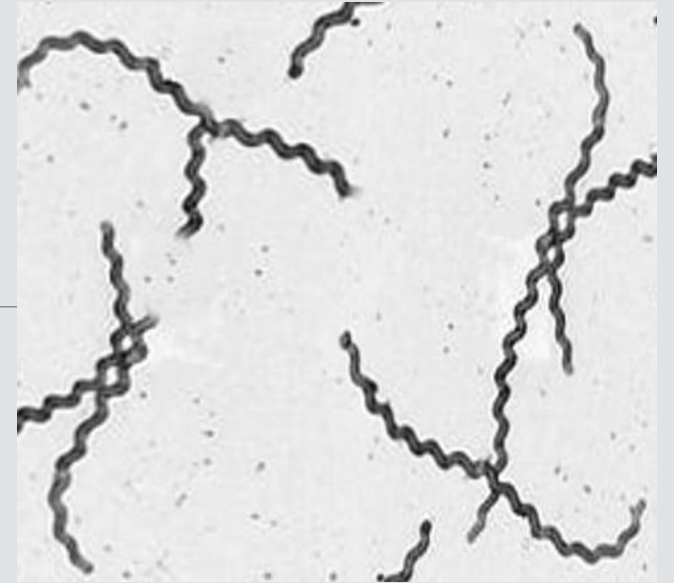
Lab Testing: *Leptospira*

- **Microscopic agglutination test (MAT)** - available at CDC
 - Historical serotype ID
- **PCR (CDC or commercial labs)**
 - Whole blood collected in first week of illness (in first 4 days is ideal)
 - Urine (collected at least 1 week after symptom onset is ideal)
 - Bacteria shed intermittently in urine after first week of illness onset
 - Negative PCR test does not rule out disease
 - CSF (signs of meningitis)
 - Fresh frozen kidney and/or liver (if available from deceased patients) - kidney preferred
- **Pathology (immunohistochemistry)** - available at CDC
 - Formalin-fixed tissues: from the kidney (preferred), liver, lung, heart, or spleen
- **16S rRNA sequencing, MALDI-TOF:** possible species identification

Lab Testing: *Leptospira*

Culture

- Specimen
 - **Blood/CSF** (first few weeks of infection)
 - No citrate anticoagulant
 - **Urine** (weeks to months)
 - Several dilutions of urine used to minimize inhibitory substances
- **Semi-solid Media**
 - Enriched with rabbit serum (Fletcher's or Stuart's) or bovine serum albumin
- **Incubation**
 - Room temperature/ 30°C for up to 6-8 wks in dark
- **Culture Evaluation**
 - Examine growth from top few cm of media weekly
 - Direct wet prep under dark-field microscope (hooked ends, tightly coiled)



Leptospirosis

Treatment

- Mild symptoms: oral antibiotics
- Drug of choice: doxycycline
- Alternatives: azithromycin, ampicillin, amoxicillin
- Severe symptoms: IV antibiotics
- Drug of choice: penicillin
- Alternatives: doxycycline, ceftriaxone, cefotaxime

Prevention

- Vaccinate pets
- Avoid contact with animal urine/body fluids, especially if wound on skin
- Do not swim in, walk in, or swallow water containing animal urine
- Wear protective clothes/footwear near soil that may be contaminated
- Boil/chemically treat potentially contaminated drinking water
- Control rodent populations
- Do not eat food possibly exposed to rats
- Chemoprophylaxis (doxycycline) - considered in high-risk people with short-term exposures



Case 3: September 2019

- **September 6-15, 2019: North Carolina Mountain State Fair**

- Held at Western North Carolina Agricultural Center in Fletcher, NC
 - Rides
 - Competitions including quilt making, photography, extreme garden vegetables, flowers, crafts
 - Home improvement exhibitors
 - Barn/animals
 - Musical entertainment: bluegrass bands and clogging
 - Kenya safari acrobatics, human cannon ball, comedy, magic and a daring motorcycle show

Case 3: September 2019

- 136 cases of illness in individuals attending or working at fair
- **135 (99%)** presented with **pneumonia** (fever, chills, cough, and shortness of breath) **within 2–14 days** after fair attendance
- **1 (1%)** presented with **fever, myalgia, headache, chills, nausea, vomiting or diarrhea within 3 days** after fair attendance

Case Characteristics	
Male	77 (57%)*
Female	58 (43%)*
Median age in years (range)	61 (24-91)
Hospitalizations	96 (71%)*
Deaths	4

<https://epi.dph.ncdhhs.gov/cd/legionellosis/outbreak.html>

Case 3: *Legionella pneumophila*

- **Gram-negative bacilli ubiquitous in environment**
- **Source of infection: Warm/extreme temp water environments (esp 77-107° F)**
- Found naturally in freshwater environments (lakes/streams)
- **Transmission:** aerosol inhalation (mists/sprays) from man-made water systems
 - Showerheads, sink faucets
 - Cooling towers (contain water and fan as part of centralized A/C systems)
 - Hot tubs
 - Decorative fountains/water features
 - Hot water tanks/heaters
 - Large complex, plumbing systems
 - Humidifiers
 - Whirlpools
 - Medical equipment, Nebulizers



Legionella pneumophila



Disease forms

- **Legionnaire's disease**
 - Primary pneumonia
 - Cough, shortness of breath, fever, muscle ache, headache
 - Most healthy people don't get sick
 - At risk: immunocompromised, >60 yrs, heavy smoker, chronic lung condition, kidney/liver disease
 - **90% of people with Legionnaires' disease at fair had underlying risk factor
- Treated with antibiotics
 - Fatality rate = 10%

Legionella pneumophila

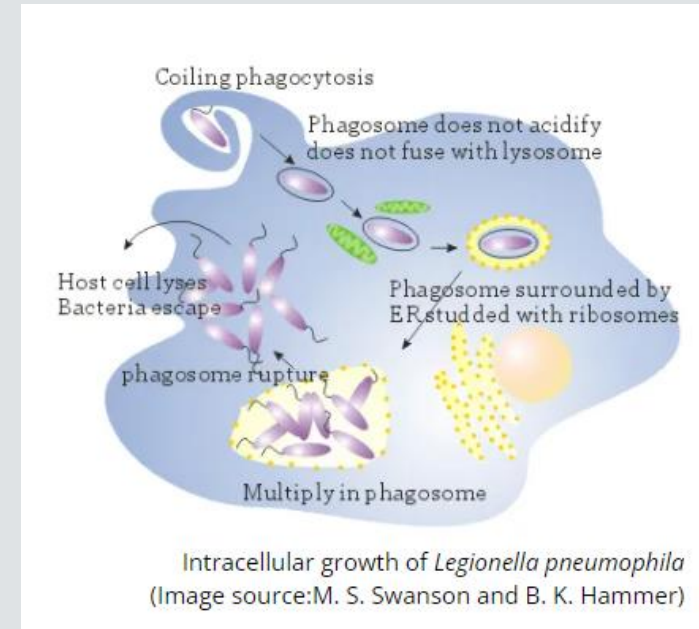


Disease forms

- **Pontiac fever**
 - Mild, self-limited, does not present as pneumonia
 - Non-fatal flu-like respiratory infection
 - Rarely requires hospitalization
 - Resolves without antibiotic treatment
- **Extrapulmonary legionellosis**
 - Endocarditis, wound abscess, encephalitis, joint infection

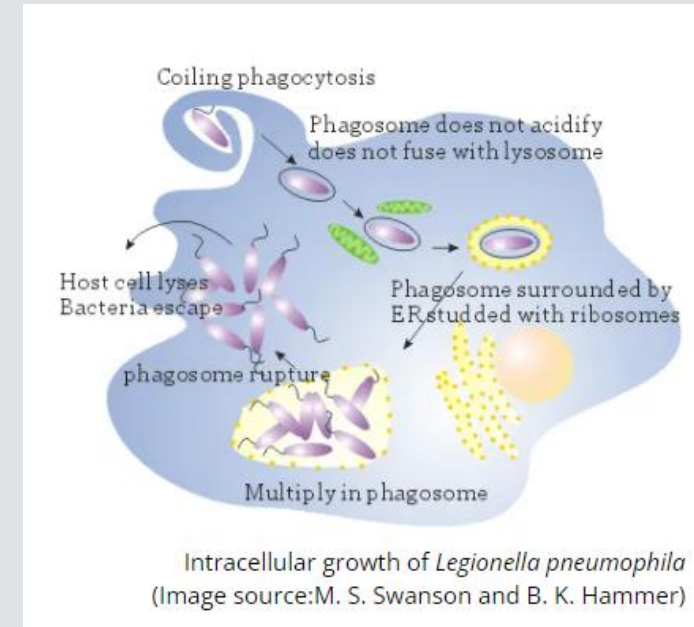
Legionella Pathogenesis

- **Facultative, intracellular pathogen**
 - **Survive/replicate in alveolar macrophage**
 - In water, *Legionella* grows/multiplies in amoeba/ciliate protozoa
 - Alveolar macrophages (lungs) resemble protozoa
 - *Legionella* mistaking alveolar macrophage for natural host, causing disease



Legionella Pathogenesis

- **Facultative, intracellular pathogen**
 - **Steps in *Legionella* infection within human phagocytes**
 - Attachment to complement receptors on phagocyte
 - Entry into cell (coiling phagocytosis)
 - Bacteria in phagosome evades fusion to lysosome
 - Phagosome fuses transiently with mitochondria
 - Remodels into endoplasmic reticulum (ER)-like vesicle
 - Replicates within ribosome studded vacuole
 - Hundreds of bacteria fill vacuole; rupture monocyte/macrophage



Lab Testing: *Legionella pneumophila*

Culture: Confirmatory

- Requires specialized media, slow growing
- Detects all species and serogroups of *Legionella*
- Clinical and environmental isolates can be compared
- Necessary to detect *Legionella* species and serogroups that urinary antigen test does not detect

Urinary Antigen Test for *L. pneumophila* serogroup¹ (Lp1): Confirmatory

- Rapid
- Only detects single serogroup, Lp1 may account for 84% of cases
- Can detect *Legionella* infections in some cases for days to weeks after treatment

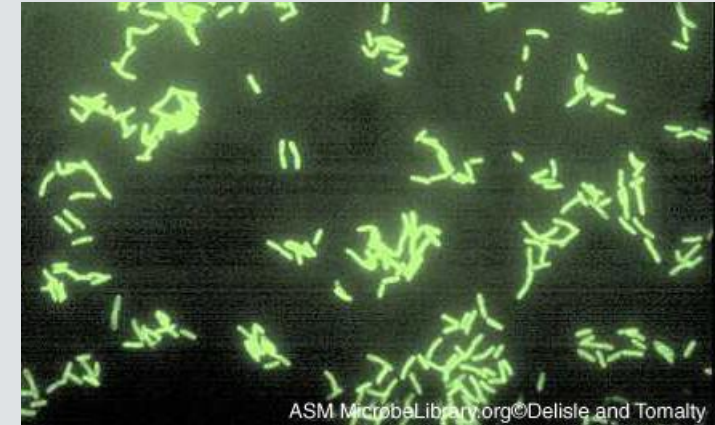
Lab Testing: *Legionella pneumophila*

Polymerase Chain Reaction (PCR): Confirmatory

- Rapid
- Detects species/serogroups other than Lp1

Direct Fluorescent Antibody (DFA) Stain: Supportive

- Detects species/serogroups other than Lp1



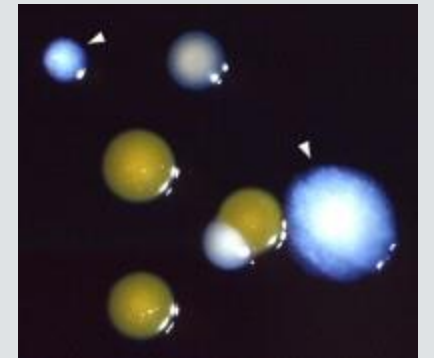
Paired serology: Confirmatory for Lp1; supportive for other type of *Legionella*

- 5-10% of population has titer 1: ≥ 256
- Single acute phase antibody titers of 1: ≥ 256 **not** discriminatory
- Must have paired sera (convalescent sample drawn 3-6 weeks after acute)
 - Four-fold rise in Ab titer to $> 1:128$

Lab Testing: *Legionella pneumophila*

Culture

- Specimen: Respiratory, blood, pleural fluid, tissue/biopsy
- No growth on BAP
- Specialized media: **Buffered charcoal yeast extract (BCYE)**
 - Charcoal detoxifies media, removes CO₂
 - Contains **L-cysteine**, iron, & buffered pH 6.9 required by organism
 - Colonies: Speckled opalescent gray-white to blue-green, catalase-pos
 - Slow growth at 3-5 days
- Non-CO₂ incubation

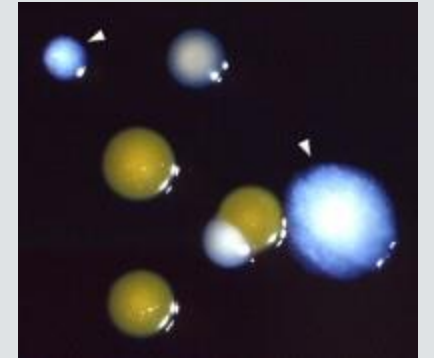


Two *Legionella pneumophila* colonies on BCYE
[photo courtesy of CDC]

Lab Testing: *Legionella pneumophila*

Culture: Clinical consideration

- Ideally, obtain sputum prior to antibiotic administration, but do not delay antibiotic to facilitate this process
- *Do not reject poor quality sputum
- Sputums associated with Legionnaires' disease may not have WBCs
- Contaminating bacteria do not negatively impact isolation of *Legionella* on selective media



Two *Legionella pneumophila* colonies on BCYE
[photo courtesy of CDC]

Laboratory Diagnosis: CDC Criteria

Suspect: A clinically compatible case with supportive laboratory evidence for *Legionella*.

Suspect:

- Fourfold or greater rise in Ab titer to specific species or serogroups of *Legionella* other than *L. pneumophila* serogroup 1 (e.g., *L. micdadei*, *L. pneumophila* serogroup 6)
- Fourfold or greater rise in Ab titer to multiple species of *Legionella* using pooled antigens
- Detection of specific *Legionella* antigen or staining of the organism in lower respiratory secretions, lung tissue, pleural fluid, or extrapulmonary site associated with clinical disease by direct fluorescent antibody (DFA) staining, immunohistochemistry (IHC), or other similar method, using validated reagents

Laboratory Diagnosis: CDC Criteria

Confirmed: A clinically compatible case with confirmatory laboratory evidence for *Legionella*

Confirmed:

- Isolation of any *Legionella* organism from lower respiratory secretions, lung tissue, pleural fluid, or extrapulmonary site
- Detection of any *Legionella* species from lower respiratory secretions, lung tissue, pleural fluid, or extrapulmonary site by a validated nucleic acid amplification test
- Detection of *Legionella pneumophila* serogroup 1 antigen in urine using validated reagents
- Fourfold or greater rise in specific serum antibody titer to *Legionella pneumophila* serogroup 1 using validated reagents

North Carolina Statistics

- Approximately 200 cases of Legionnaires' disease reported each year from 2014–2018
- Number of cases on rise in North Carolina and nationally over past two decades
- Majority of 2021 outbreak case-patients were fair visitors (as opposed to workers)
- Graph showing more cases during second half of fair (Sept 11-15)

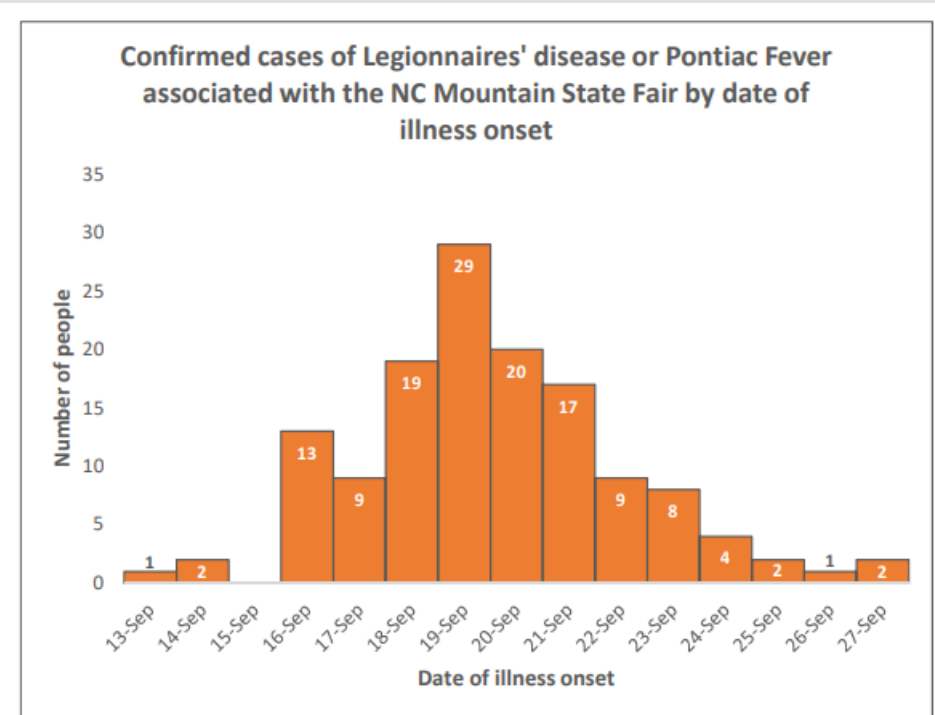


Figure 1. Number of confirmed cases of Legionnaires' disease or Pontiac Fever associated with the NC Mountain State Fair. N=136.

Laboratory Diagnosis: NC Public Health

Clinical Criteria

A. Legionnaire's Disease

Pneumonia (clinical or radiologically confirmed) in an individual who attended or worked at fair with symptom onset within 2 – 14 days after fair attendance - OR -

B. Pontiac Fever

Fever, myalgia, headache, chills, nausea, vomiting or diarrhea within 3 days of attending or working at fair

Laboratory Criteria: Laboratory confirmed *Legionella*

- By culture: isolation of any *Legionella* organism from respiratory secretions, lung tissue, pleural fluid, or other normally sterile site - OR -
- By detection of *Legionella pneumophila* serogroup 1 antigen in urine using validated reagents - OR -
- By seroconversion: fourfold or greater rise in specific serum antibody titer to *L. pneumophila* serogroup 1 using validated reagents on specimens collected 3-6 weeks apart

Confirmed Case: Patient meets clinical criteria A or B **AND** laboratory criteria

The Investigation

- Comprehensive list of aerosolized water sources at fair investigated
 - **Hot tub was suspected as most likely source of infection**
- Other potential sources of aerosolized water
 - Diffusers on display (Davis Event Center)
 - **Evaporative cooling fan (outside of Davis Event Center)**
 - Rides
 - Hoses used to spray ground
- Rides = low risk, none confirmed to have aerosolized water
- Diffuser and hoses = low risk due to small amount of aerosols
- **Case-patient vs. control survey results**
 - When restrooms/café included as part of Davis Event Center, 100% of case-patients reported entering Davis Event Center, in comparison to 72% of controls
 - Compared to controls, cases were 12 times more likely to report entering Davis Event Center, and 10 times more likely to report walking by or spending time near hot tubs

The Investigation

- **27 Environmental samples collected**
 - 6 from Davis Event Center
 - 16 from hot tubs on display
 - 5 from evaporative cooling fan
- **Note:** samples were not collected until 12–22 days after end of fair
 - Hot tubs had been cleaned and dried
 - Did not represent conditions in existence during fair
- **14 sputums collected** (patients with Legionnaires' disease in 5 western NC hospitals)
 - Sputum culture is necessary to compare patient samples to each other and patient samples to environmental samples

The Investigation

- **CDC laboratory testing on samples:** Polymerase chain reaction (PCR), sequence-based typing (SBT), and bacterial cultures
 - **Environmental samples:** All neg except one sample of water from women's restroom in Davis Event Center
 - Positive sample = Sequence types (ST) 7 and ST8
 - **Sputum samples:** 10 of 14 pos; genetic profile consistent with *L. pneumophila* ST224
 - **Conclusion:** Isolates obtained from patients matched each other genetically (ST244) but differed from the one positive environmental sample (ST7 and ST8)

NC Public Health Findings

- **Key case findings**

- Hot tubs on display (in Davis Event Center) - only significant source of aerosolized water
- People who got sick were more likely than other fair attendees to report entering the building where hot tubs were displayed, walking by hot tubs, and visiting fair during the second half
- Infected individuals inhaled bacteria in aerosolized water (mist/vapor)

- **Indications of findings**

- Exposure to Legionella occurred via aerosolized water from hot tubs
- *Legionella* likely present in one or more of hot tubs from beginning of fair and was dispersed throughout Davis Event Center via regular air flow within building
- Amplified over course of fair leading to more exposures as time went on
- Investigation could not determine how Legionella was introduced into hot tubs
- Outbreak highlights importance of properly maintaining equipment that aerosolizes water

Case 4: September 2019

- **Example Case**
 - 40-year old shopkeeper, Lanzhou city, China
 - Crippling joint pain and persistent fever
 - Several people from same neighborhood had same unexplained symptoms
 - Unknowingly exposed to a bacteria about 2 months ago
 - Unexplained symptoms, not diagnosed until January 2020



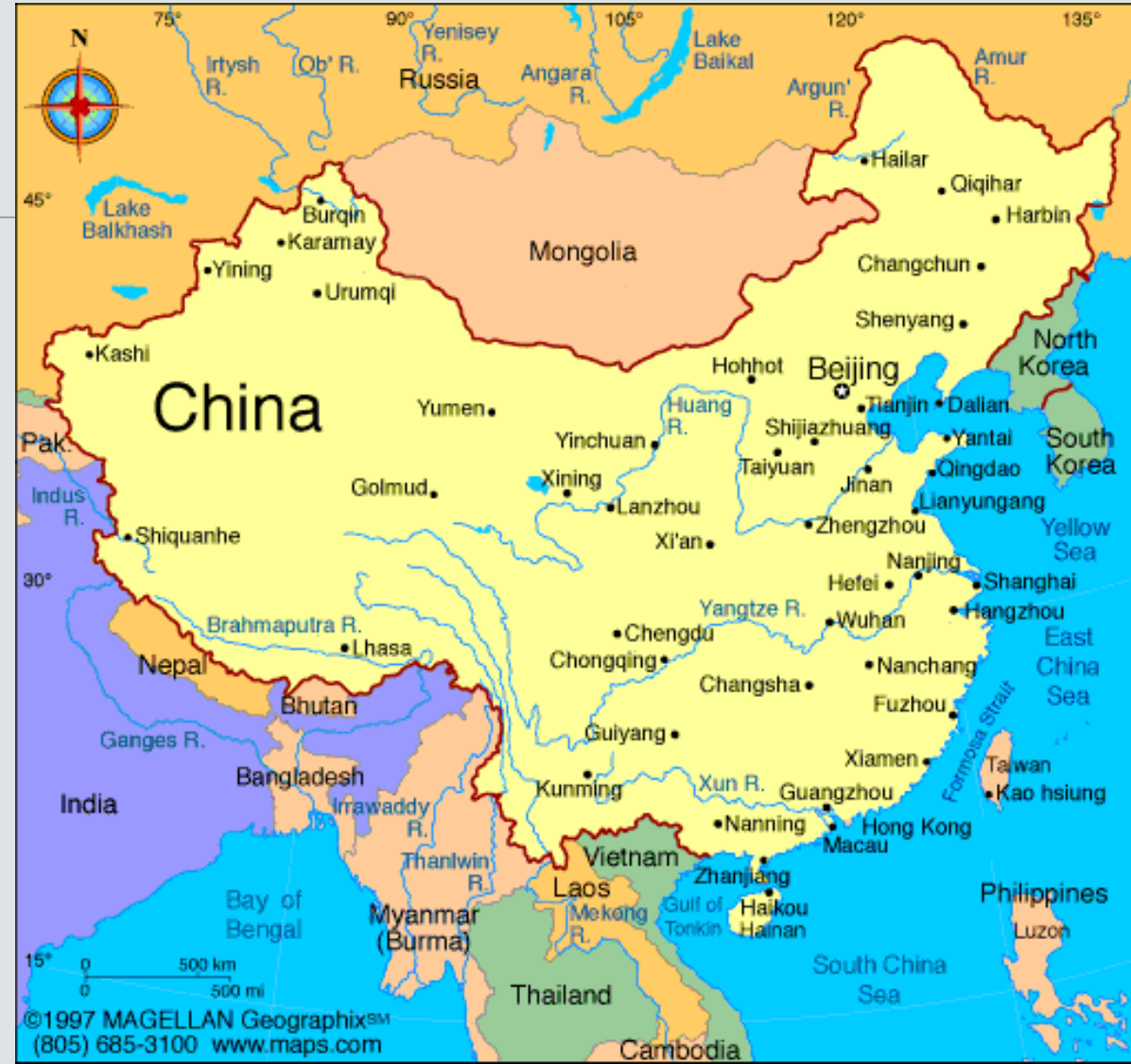
Case 4

- **First cluster of cases: Dec 2019** - Lanzhou Veterinary Research Institute in Gansu province, Western China
- Lab mice infertile, found to be infected
- 4 students = positive serology
- 181 antibody-positive by end of December
- Some had mild physical discomfort, some asymptomatic



Case 4

- **Second cluster of cases: Dec 2019**
Harbin Veterinary Research Institute
(Graduate School)
- 2600 km (1600 miles) NE of Lanzhou
- 13 antibody-positive students
 - Previous short-term research work at Lanzhou Vet Institute in August
 - Had animal contact history



Case 4

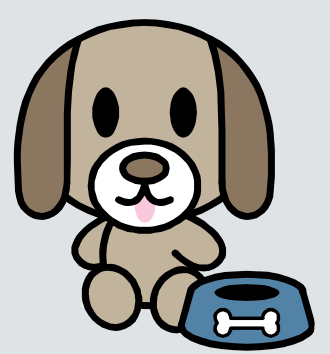
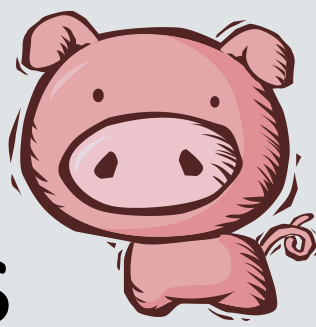
- **Community transmission** to animals and humans continued for at least 1 year
- Spread to farms in Shaanxi province and Inner Mongolia
 - Improper screening of farm animals to diagnose disease
 - Trade of infected animals
- 10,528 people infected after testing 68,571 as of November 30, 2020



Case 4: Brucellosis

- **Outbreak first reported: Nov 2019**
- In late July - late August 2019, Zhongmu Lanzhou Bio-pharmaceutical plant of Gansu province used expired disinfectants/sanitizers when producing *Brucella* vaccines for animal use
 - Not all bacteria was eradicated in waste gas
 - Contaminated waste leaked from plant as aerosol
- **Action: by authorities: Jan 2020**
 - 2019: Lanzhou Vet Research Institute closed facility/halted research while investigating cause of outbreak to prevent more cases
 - Jan 2020: authorities revoked vaccine production licenses for plant; withdrew product approval numbers for its two Brucellosis vaccines

Brucella species



Organism	Animal host
<i>B. abortus</i> *	Cattle
<i>B. melitensis</i>	Sheep or goats
<i>B. suis</i>	Swine
<i>B. canis</i>	dogs

- **RB51**

- Live, attenuated cattle vaccine strain
- Rare, but possible for cattle to shed RB51 in milk
 - Even if vaccine label recommendations followed
- Can cause human infection
- Resistant to rifampin *in vitro* and to penicillin
 - Rifampin is first-line antibiotic used to treat human brucellosis
- Does not stimulate Ab response detectable by commercially available serological assays
 - Missed by testing used for diagnosis

Brucella Epidemiology

- **Incidence:** Found worldwide; infect humans as an incidental host; 500,000 new human infections occur annually
- Rare in U.S. – 80-120 cases annually; most associated with *Brucella* exposure abroad
- 2% of cases are lab-acquired
- Found worldwide
- Brucellosis in humans/livestock uncommon in industrialized nations
 - Pasteurization & state/federal health programs focused on routine screening of domestic livestock and animal vaccination
- **High risk**
 - Mediterranean Basin
 - Mexico, South/Central America
 - Eastern Europe
 - Asia
 - Africa
 - The Caribbean
 - The Middle East

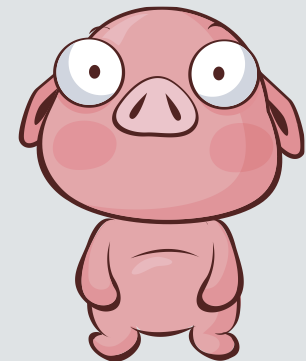
Brucella Transmission

Zoonotic transmission: Infected animal (reservoir) – primarily cattle, sheep, swine

- Ingestion of raw dairy
- Inhalation from contaminated environment
- Through skin wounds/mucous membranes
 - Slaughterhouse workers, Meat-packing plant employees, Veterinarians
 - People who hunt animals (ingestion of undercooked meats, inhalation)
 - Bison, elk, caribou, moose, and wild hogs

Rare human-to-human transmission

- Breastfeeding
- Sexual transmission
- Tissue transplantation or blood transfusion



Brucella Transmission

Transmission in this case: aerosol with some evidence of zoonotic transmission

Key Questions

- How long did aerosol transmission from the pharmaceutical factory occur?
- How long does aerosolized *Brucella* persist in the air?
- What is the viability of bacteria in aerosolized form?
- Can bacteria be re-aerosolized after airborne particles settle in the environment?
- How has the outbreak spread from Gansu to Shenyang province? Was the outbreak initially aerosol spread, followed by widespread human and animal infection, and then zoonotic and some human-to-human spread?

Brucella Symptoms

Brucellosis, also called Malta fever, Mediterranean fever

Incubation: 2-3 weeks up to months

Acute: initial non-specific symptoms

- Fever
- Headache
- Sweating
- Weight loss
- Malaise
- Muscle, joint, or back ache
- Fatigue

Brucella Symptoms

Residual problems & musculoskeletal symptoms, may become chronic

- Undulant fever – fever with daily fluctuations, may continue for more than one year
- **Arthritis (most common complication)**
- Endocarditis
- Neurologic symptoms (up to 5% of cases)
- Inflammation of spleen and liver
- Swelling of testicles/scrotum (epididymo-orchitis)

Treatment: Doxycycline and rifampin, min 6-8 weeks

If chronic: Triple antibiotics (rifampin, doxycycline, streptomycin)

Typical Recovery: few weeks to several months

Fatal in < 2% of cases

Brucella Pathogenesis

Intracellular lifestyle

- Limits exposure to innate and adaptive immune responses
 - Reduces, modifies, or cloaks pathogen-associated molecular patterns
 - Delays recognition by immune system
- Sequesters organism from effects of antibiotics
- Drives clinical disease manifestations and pathology

Three phases of life cycle

- Incubation phase: before clinical symptoms are evident
- Acute phase: pathogen invades and disseminates in host tissue
- Chronic phase: Can result in severe organ damage and death of host organism

Brucella Pathogenesis

Strategies

- Evasion of intracellular destruction
 - Restricts fusion of type IV secretion system-dependent *Brucella*-containing vacuoles with lysosomal compartments
 - T4SS essential for prolonged persistence
- Inhibition of apoptosis of infected mononuclear cells
- Prevention of dendritic cell maturation, reduced antigen presentation, and reduced activation of naive T cells

Once adapted to intramacrophage environment, extends its intracellular persistence indefinitely

- Contributes to systemic metastasis and infection of preferred targeted cells or tissues
 - Placental trophoblasts, fetal lung, male genitalia, skeletal tissues, reticuloendothelial system, and endothelium

Brucella Lab Testing

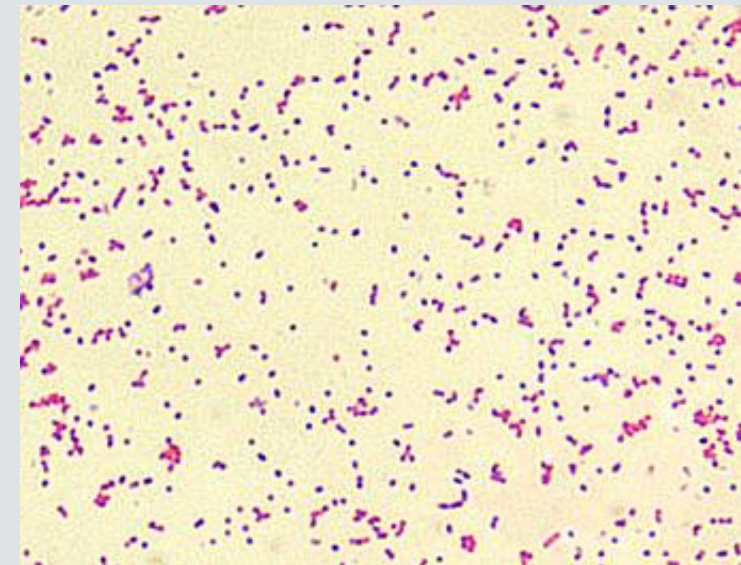


Gram stain = small, nonmotile, Gram-negative bacilli

- Stain poorly with GS method, require extended safranin application

Facultative, **intracellular**

- Replicate/live within neutrophils, monocyte, and macrophage (RES/ phagocytic cells)
- **Biosafety level III** pathogen: Potential bioterrorism agent (aerosol transmission)
- **Class B** – easy to disseminate; moderate morbidity, low mortality



Brucella Lab Testing



Culture

- Specimen of choice: blood or bone marrow
 - 50-80% positive in acute cases
 - 5% positive in chronic cases
- Other specimens: CSF, wounds, purulent discharge, joint fluid
 - Normal blood culture bottles can be used
 - Hold cultures at least 28-30 days or 4-6 weeks
 - Will grow on BAP/CHOC
 - Smooth, translucent, nonhemolytic, slightly yellow
 - Growth after 48 hrs

Biochemicals

- Catalase +
(unsafe to perform)
- Most oxidase +
- Nitrate +
- Urease +
- Most H₂S +

Brucella Lab Testing



Serological test

- *Brucella* microagglutination test (BMAT) for *B. abortus*, *melitensis*, and *suis* antibodies
 - No serological test for *B. canis* or RB51 vaccine exposure
 - Acute/convalescent paired sera
 - Detects Abs to smooth (S) lipopolysaccharide (LPS) of the outer membrane and internal proteins
- ELISA, but SAT preferred

Polymerase chain reaction (PCR) – blood

- Genetic targets
 - *Brucella* gene *BCSP31* (cell surface protein)
 - 16S-23S rRNA operon

Brucellosis Prevention

General Population

- Do not consume:
 - Undercooked meat
 - Unpasteurized dairy (milk, cheese, or ice cream)
- Hunters and animal herdsman should protect self by using:
 - Rubber gloves
 - Goggles
 - Gowns or aprons

Post-exposure prophylaxis if needed

- Routine Brucella species: Doxycycline, rifampin

Brucellosis Prevention

Lab Workers exposed to RB51

- Perform all manipulations in class II biological safety cabinet
- **Follow biosafety level 3 (BSL-3) practices**
 - Standard personal protective equipment must be worn, and respirators might be required
 - Solid-front wraparound gowns, scrub suits or coveralls are often required
 - Access hands-free sink and eyewash are available near the exit
- Sustained directional airflow to draw air into lab from clean areas towards potentially contaminated areas (exhaust air cannot be re-circulated)
- A self closing set of locking doors with access away from general building corridors
 - Access to BSL-3 laboratory is restricted and controlled at all times
- **Post-Exposure prophylaxis** if needed
 - **RB51:** Doxycycline, in addition to trimethoprim-sulfamethoxazole or other suitable antimicrobial, 21 days

Brucella abortus RB51 in U.S., 2017-2019

- **Three confirmed cases**
 - Consumption of domestically acquired unpasteurized (raw) milk
- **Eight probable cases:** clinically compatible illness epidemiologically linked to shared contaminated source
- Hundreds of people, from dozens of states, potentially exposed to raw milk products
- 75% of U.S. states have laws allowing raw milk sales

Brucella abortus RB51 in U.S.

- **2017: raw milk from K-Bar dairy in Paradise, Texas**
 - Raw milk only sold on site at dairy
 - Over 800 households affected
 - People who drank K-Bar milk and are symptomatic in Alabama, Arkansas, California, Ohio, North Dakota, Tennessee, and Texas
 - CDC recommendation
 - If consumed milk or milk products for K-Bar June 1 – Aug 7, 2017, get antibiotic treatment to avoid lifelong chronic infections

Brucella abortus RB51 in U.S.

- **2018: raw milk from Miller's Biodiversity Farm in Quarryville, Pennsylvania**
 - 19 states Alabama, California, Connecticut, Florida, Georgia, Iowa, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Virginia
 - Nov 2018: One confirmed case of RB51 infection (New York)
 - Cow positive for RB51 removed from milking herd
 - People within 6 months of date of last consumption at increased risk for brucellosis
 - Should receive antibiotics to prevent infection
 - Monitor health for symptoms for 6 months
 - People who drank raw milk > 6 months ago and have symptoms, but have not been treated
 - May need antibiotics to prevent long-term health problems