**Mycobacterium**

- Gram-positive
- Aerobic
- Non-motile
- Non-spore forming
- Grow slowly (pathogenic species usually grow slowly)
  - *M. tuberculosis* & *M. bovis*: 3–8 weeks
  - *M. avium* complex: 2–6 weeks
  - *M. avium* ssp. *paratuberculosis*: ~16 weeks
- Resistant to disinfectants (chlorine and iodine...)
- Susceptible to heat treatment

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**Mycobacterial cell wall**

- Gram-positive, BUT the high lipid and mycolic acid content of cell walls prevent the uptake of the Gram staining dye → poorly stained

**OM**

**P**

**IM**

Althought mycobacteria are phylogenetically classified as Gram-positive bacteria, their wall is more similar to that of Gram-negative bacteria

Hoffmann C et al. PNAS 2008;105:3963-3967
Acid fastness

- Mycobacterium is poorly stained with Gram staining dyes
- Ziehl-Neelsen (ZN) staining: the cell wall lipids bind the red dye "carbol fuchsin" used in ZN staining
  → remove the dye with a weak acid solution (3% HCl + EtOH)
  → bacteria resistant to acid decolorization are called "acid–fast" or "ZN-positive"

Pathogenicity

- Usually cause chronic infections
- Facultative intracellular parasites
  → Pathogenic mycobacteria efficiently survive within phagocytic cells (mostly macrophages)
  → mycobacteria free in the cytoplasm of the macrophage
  → rupture the macrophage
  → infect adjoining cells
  → leads to the formation of granuloma called a tubercle
  → lesions are often localized in the lungs or liver, but may form throughout the body

Pathogenicity

- Phagosome and lysosome fusion

  - Prevent the acidification of phagosome, and Mycobacterium multiplies in it
  - Prevent the fusion of phagosome and lysosome
**M. tuberculosis**

- **General**
  - The principal cause of tuberculosis in humans and primates occasionally in dogs and cattle.
  - Most humans are relatively resistant to the development of progressive tuberculosis (less than 10% of infected people develop disease).
  - The disease does not develop rapidly.

- **Disease**
  - Tuberculosis is often pulmonary.
  - Large lesions are developed in the lungs → compromises large blood vessels → death.
  - Can infect any parts of the body (lungs, bones, liver, spleen, brain, and gastrointestinal tract).

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**M. tuberculosis: Diagnosis**

- **Acid-fast staining of sputum**
  - A useful and inexpensive test.
  - Detection of acid-fast rods is "not" a definitive diagnosis of TB because other *Mycobacterium* spp. can be stained.

- **TB skin test**
  - Intradermal injection of tuberculin (extracts of Mycobacteria).
  - If a person has or has had TB
    - Stimulate CD4+ T helper cells
    - Recruit monocytes, macrophages
    - Local inflammation + phagocyte migration
    - Redness & swelling, hardening the area.
  - Takes about 4 weeks to get "+" status.
  - False positive: BCG vaccination gives positive results.
**M. tuberculosis**

- **Treatment**
  - Most commonly used antibiotics (e.g. penicillin, cephalosporin, tetracycline) are not effective
  - Rifampin + isoniazid (first-line anti-TB medication)
  - Standard anti-TB therapy: taking 1/3 kg of a mixture of anti-TB drugs daily for six months
  - Extensive drug resistant tuberculosis (XDR-TB) is surging
  - Nonhuman primates: treated with drugs used in humans, but with limited success
  - Dogs and cats: euthanized to prevent transmission to humans
  - Cattle: eradicated

- **Vaccination**
  - Attenuated *M. bovis* BCG is the most widely used in humans
  - Vaccination causes false positive reaction in the TB skin test

**M. bovis: General**

- Share >99% genetic identity with *M. tuberculosis*
- A major cause of tuberculosis in all species of cattle
  - cf. *M. tuberculosis*: primarily a human pathogen
- Causes tuberculosis in most warm-blooded vertebrates, including humans, pigs, cats, horses, primates, dogs, sheep, and goats, but not in birds
- Most animals usually clear the infection

**M. bovis: Transmission**

- Silent transmission: the organism can spread without any obvious signs of disease
- Transmission is mainly via the respiratory route
  - Inhalation of infectious aerosols from coughing or sneezing animals with tuberculosis, or dust particles
  - Aerosol transmission is effective over short distance (1–2m)
- Cattle density is a significant factor
- Nose-to-nose transmission
- The organism can survive for several months in the environment
- Wildlife reservoirs (eg. deer) are a source of infection for grazing cattle

**M. bovis: Pathogenesis**

- Inhalation of *M. bovis*
  - *M. bovis* is ingested by pulmonary macrophages
  - Grows in the macrophage
  - Kills the macrophage
  - Forms tubercles
  - May disseminate via the lymph
  - Forms tubercles in the lymph nodes, the liver and spleen
- In some cases, the infection lies dormant for many years
- Stress (e.g. overstocking) and host immune system (e.g. age) are important
**M. bovis: consequences of infection**

- Inhalation of *M. bovis* (by aerosol)
- Bacteria reach lungs
- Bacteria enter macrophages and reproduce in them
- Granuloma formation
- Spread to blood, organs (generalized tuberculosis)
- *M. bovis* present in mucous, feces, urine, milk
- Bacteria cease to grow; lesion calcifies
- Reactivation
- Effective immunity
- *M. bovis* swallowed
- Aerosol shedding of *M. bovis*
- Fecal shedding of *M. bovis*
- Immune suppression
- Reactivation
- Immune suppression
- Reactivation

**M. bovis**

- **Immunity**
  - BCG vaccine can be used in cattle
  - Not sufficiently efficient
  - React positively in a tuberculin skin test
- **Diagnosis**
  - Tuberculin test
- **Treatment**
  - Cattle: all infected animals are eradicated

**M. avium-intracellularare Complex (MAC)**

- **MAC**
  - *M. avium*-intracellulare
  - *M. avium* ssp. *avium* (*M. avium*)
    - ssp. *silvaticum*
    - ssp. *hominisuis*
    - ssp. *paratuberculosis* (*M. paratuberculosis*)
    - *M. paratuberculosis* has not been found in any cases of avian tuberculosis and is often excluded from MAC

- **General**
  - MAC is ubiquitous in distribution; various wild and farm animals, fresh and salt water, soil, etc
  - Responsible for the majority of tuberculosis in birds and pigs
  - They are highly resistant to many of the commonly used anti-mycobacterial drugs → thus, difficult to treat
  - Humans: generally resistant, but affects immunocompromised individuals

**Serotypes of MAC and their susceptibility to various spp. of birds and mammals**

<table>
<thead>
<tr>
<th>Species</th>
<th>MAC serotypes</th>
<th>Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>1, 3, 4</td>
<td>High</td>
</tr>
<tr>
<td>Turkey</td>
<td>1, 3, 4</td>
<td>High</td>
</tr>
<tr>
<td>Pheasants</td>
<td>1, 3, 4</td>
<td>High</td>
</tr>
<tr>
<td>Wild birds</td>
<td>1, 3, 4</td>
<td>High</td>
</tr>
<tr>
<td>Cattle</td>
<td>1, 3, 4</td>
<td>Moderate</td>
</tr>
<tr>
<td>Swine</td>
<td>1, 3, 4</td>
<td>High</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1, 3, 4</td>
<td>High</td>
</tr>
<tr>
<td>Man</td>
<td>11 to 20, 23, 25</td>
<td>Low (in healthy individuals); High (in immunocompromised)</td>
</tr>
</tbody>
</table>

*Source: Dhama et al. 2011*
**MAC: Disease**

- **Poultry**
  - Cause: avian tuberculosis
  - \( M. \) avium is the most significant cause of avian tuberculosis
  - Source of infection: infected birds, contaminated water, soil, or feed
  - Pathogenesis:
    - The lesions primarily develop in "the intestinal tract" and liver can be disseminated to other organs (the lungs, bones, spleen, etc)
    - Lesions in the intestinal tract may appear as nodules
    -开放和排泄到肠道
    - release large numbers of organisms in the feces
    - but eventually show anorexia, weakness, lameness, decreased egg production, sometimes death
  - Economic losses
  - Diagnosis: tuberculin test (intradermal injection in the wattle, 48 hrs)
  - Treatment: depopulation
  - Vaccination: No vaccines are available for use in birds

**M. avium Complex (MAC)**

- **Swine (continued)**
  - Diagnosis: skin test (often on the ear)
  - Vaccination: not available
  - Depopulate infected herds
  - Prevention is recommended
  - Do not mix swine and poultry production
    - protect swine from birds!

**Swine**

- Pigs are susceptible to \( M. \) tuberculosis, \( M. \) bovis, and \( M. \) avium
  - \( M. \) avium is the major cause of swine mycobacteriosis
  - Transmission: infected poultry or pigs, sawdust, soil
  - Swine mycobacteriosis
    - Usually "no clinical signs"
    - not possible to diagnose based on clinical signs
    - Lesions are commonly found in cervical and mesenteric lymph nodes at slaughter
    - affected parts (head, intestines, or whole body) should be removed
    - loss of carcasses
    - economic losses

**Hosts of mycobacteria**

<table>
<thead>
<tr>
<th></th>
<th>( M. ) tuberculosis</th>
<th>( M. ) bovis</th>
<th>( M. ) avium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>+</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Pig</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sheep</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Horse</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Dog</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chicken</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

Positive skin test

Positive for TB test

You chicken, Get away!
Other mycobacteria of clinical importance

- **M. leprae**
  - Cause of leprosy in humans and armadillos
  - Humans: the organism is confined to the skin and peripheral nerves
  - Armadillos: the organism is found systemically

- **M. lepraemurium**
  - Rats and cats: infrequently produce an leprosy-like disease

- **M. kansasii**
  - Cattle, deer, and swine: lymph node lesions infections are difficult to distinguish from *M. bovis* in cattle
  - Humans: respiratory infections and lymphadenitis in people with immunosuppression

Other mycobacteria of clinical importance

- **M. marinum**
  - Humans: “swimming pool granuloma” on the arms and legs
  - Exposure to water containing the organism
    - enter a break in the skin
    - develop reddish nodule
  - Progress slowly (signs are seen 2~3 weeks after infection)

  - Fish: cause fish tuberculosis
    - loss of scales, loss of color, granulomatous lesions in any internal organs
    - once infected, it is really difficult to cure

  - Poikilotherms: cause fatal systemic infections