PNS Lab

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Dr. Sulaiman Mahmoud Bani Abdel-Rahman

MBBS, Mutah university

MSC Medical Microbiology – University of Manchester

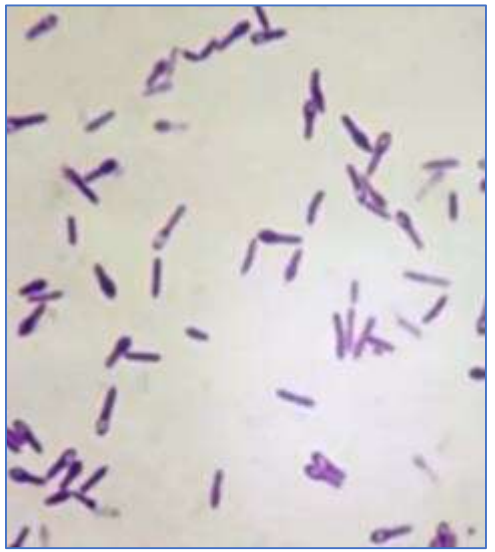
PhD Medical Virology - University of Manchester

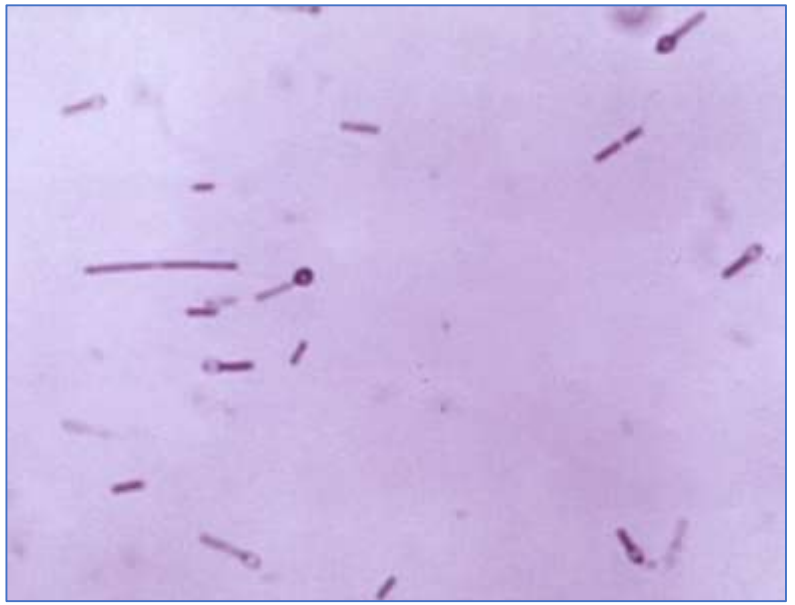
Topics included

- Tetanus
- Botulism
- Leprosy
- Rabies

Tetanus

- **Diagnosis**
  - Tetanus is a clinical diagnosis
- **Laboratory Diagnosis**
  - **Specimen**
    - Wound swab
    - Exudate or tissue from the wound.
  - **Gram Staining**
    - Gram +ve with terminal and round spores (drum stick appearance).

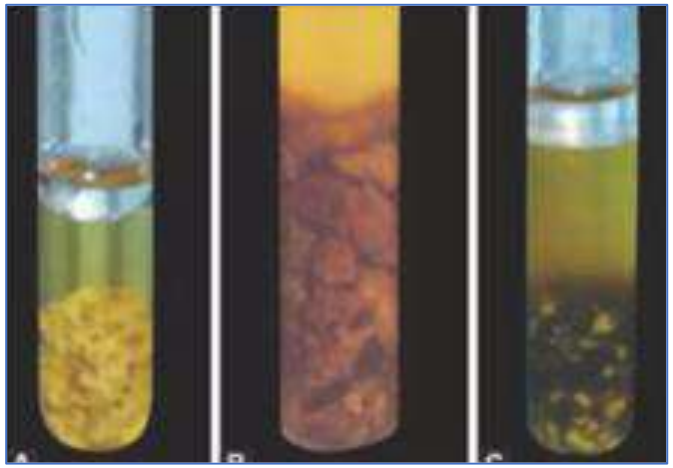




Tetanus (cont.)

Culture

- RCM (Reinforced Clostridial Medium)
  - When using meat-based anaerobic media to cultivate Clostridium species, the change in the meat particles' appearance serves as a diagnostic tool:
    - **Blackening:** Proteolysis (protein breakdown). Example: *C. tetani*.
    - **Reddening:** Saccharolytic (sugar fermentation). Example: *C. perfringens*.
- Blood agar with polymyxin B Swarming growth



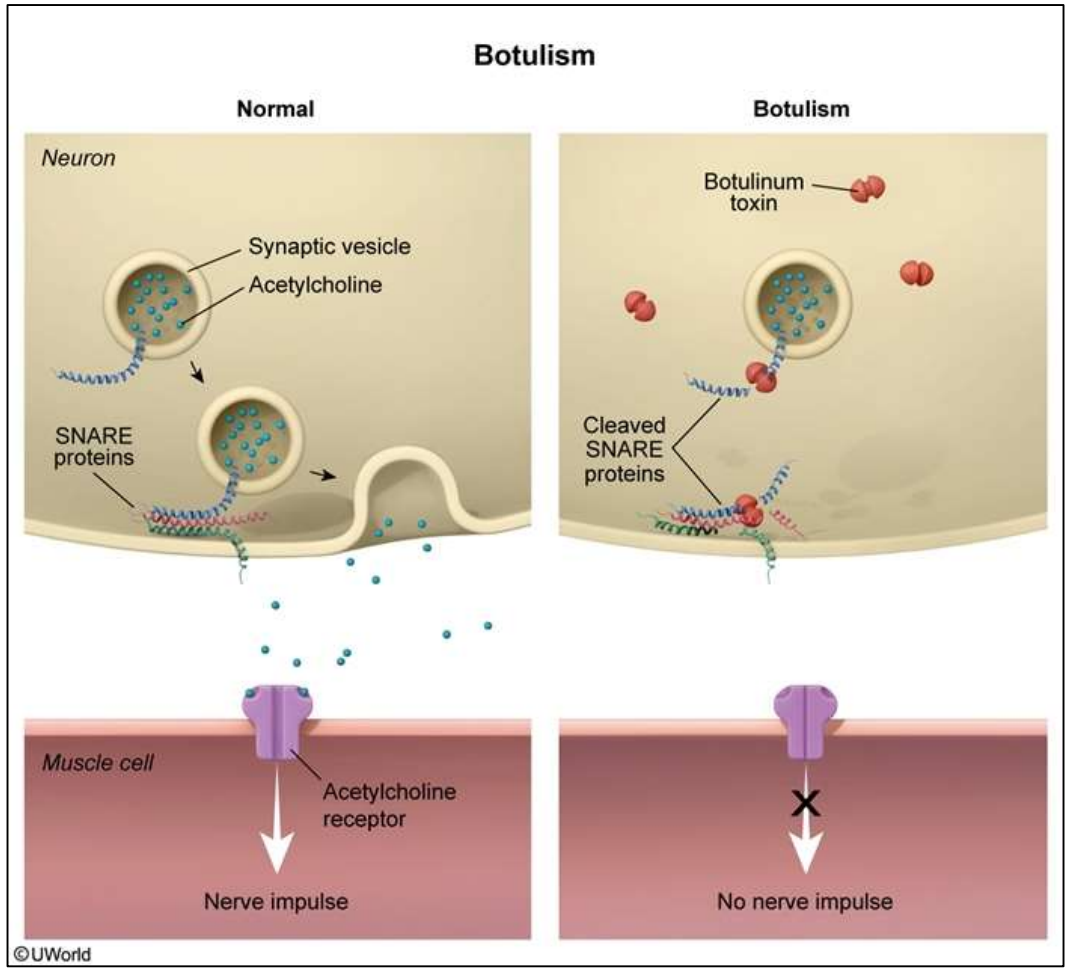
Clostridium botulinum

Normal vs. Botulism:

- **Normal:** Ca^{2+} influx → SNARE protein fusion → ACh vesicle release
- **Botulism:** Toxin cleaves SNARE proteins → blocks vesicle fusion

Clinical Result:

- **Complete blockade** of acetylcholine release
- **Affects both:** Nicotinic (skeletal muscle) and muscarinic (autonomic) receptors



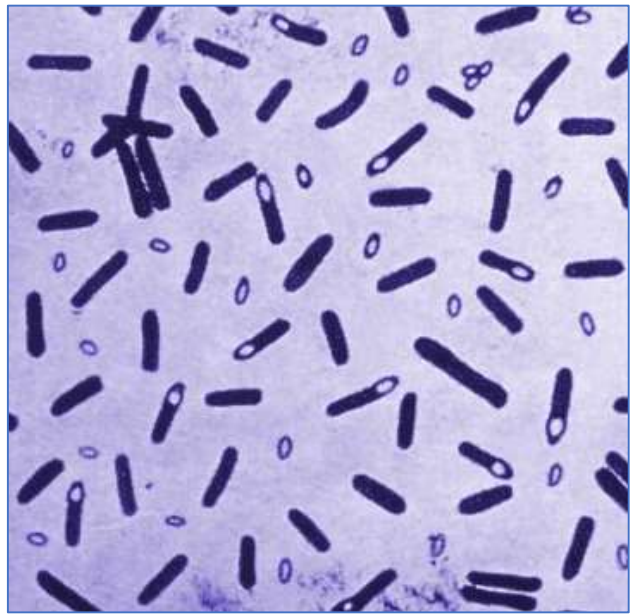
Clostridium botulinum (cont.)

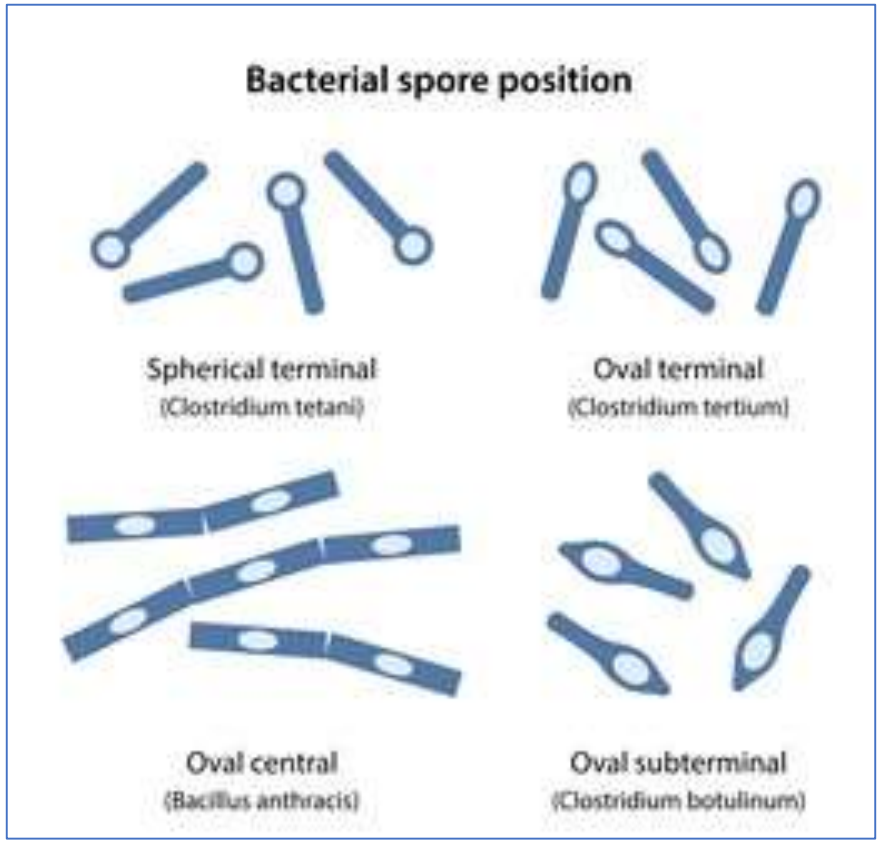
Diagnosis

- Botulism is primarily a clinical and symmetrical diagnosis (descending paralysis)

Laboratory Diagnosis

- Specimen
  - Serum (blood), Stool or Gastric aspirate, Suspected food samples
- Toxin Detection (Gold Standard)
  - Mouse Bioassay: To detect and typed botulinum toxin.
  - ELISA: For rapid toxin identification.
- Gram Staining
  - Gram +ve with subterminal and oval spores (looks like a "tennis racket").





Feature	*Clostridium tetani*	*Clostridium botulinum*
Spore Location	**Terminal** (at the tip)	**Subterminal** (near the tip)
Cell Shape	Drumstick / Tennis racket	Oval / Club-shaped

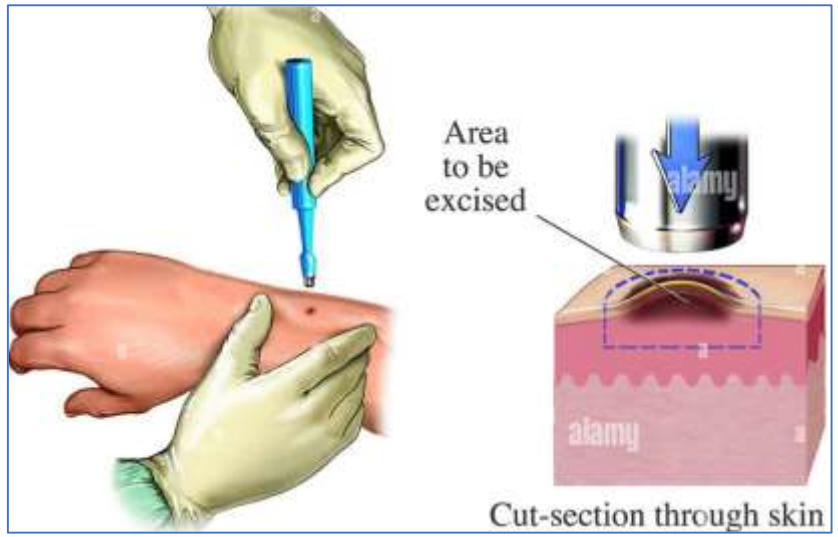
Leprosy

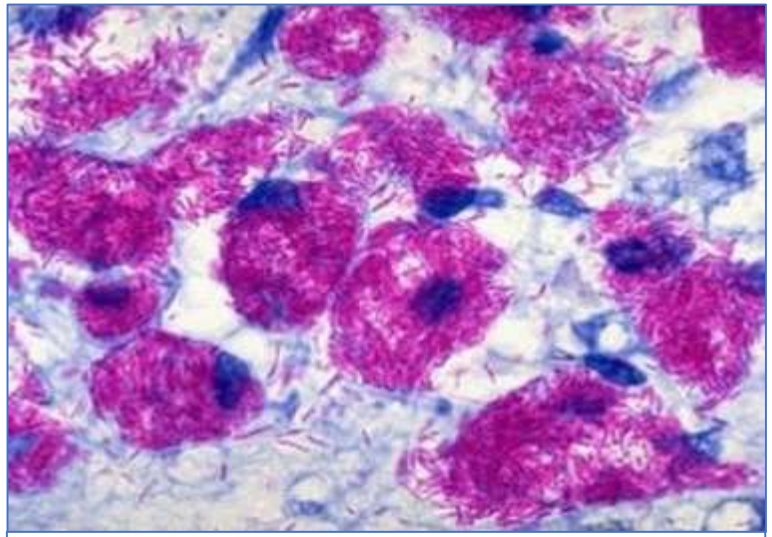
Diagnosis of Leprosy

- Specimen collection:
    1. Nasal mucosa.
    2. Skin: active edges of the patches
    3. Nerve biopsy: from thickened nerves

Procedure

- Lesion is cleaned with spirit
- 5mm long incision made by scalpel deep enough at edge of the lesion
- Scalpel blade is rotated transversely to get tissue pulp from below epidermis.
- Tissue pulp is used to produce smear on slide
- Stained with modified ZN stain.





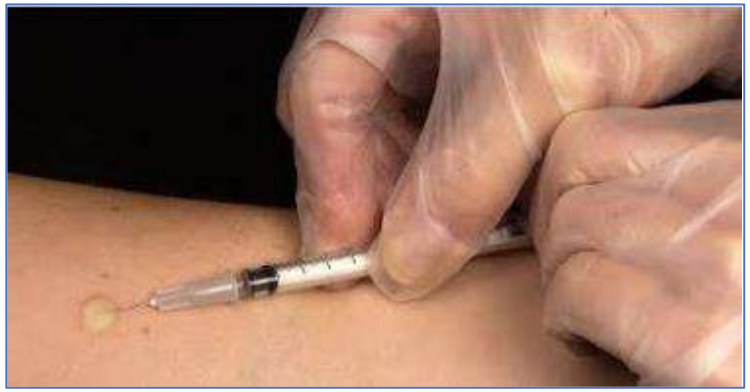
The bacilli are present inside the foamy macrophages called Virchow's lepra cells or foamy cells.

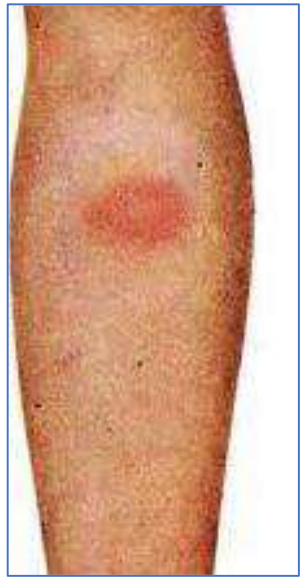
Leprosy (cont.)

- Lepromin test
  - The lepromin test is used to study host immunity to *M.leprae*.
  - The test is an intradermal skin test performed by using lepromin antigen, which is a suspension of killed *M.leprae* obtained from infected human or armadillo tissue.
  - The lepromin test is not used to confirm the diagnosis of leprosy.
  - It is not useful to indicate prior contact of the person with leprae bacilli.
  - Principle: Delayed hypersensitivity reaction of leprosy Ag.
  - Procedure: 0.1 ml of lepromin Ag is injected I/D in forearm. Lepromin Ag is obtained from killed *M. leprae*.

Leprosy (cont.)

- Assessment for induration over 24-72 hours:
  - **Positive:** Induration (≥5 mm) indicates robust CMI response (lower risk).
  - **Negative:** Lack of induration indicates poor CMI with inability to form granulomas (higher risk).
- **Interpretation.**
  - **Positive**
    - Tuberculoid leprosy (CMI intact): good prognosis
    - After BCG vaccination
  - **Negative**
    - Lepromatous leprosy (Bad prognosis).
    - Healthy



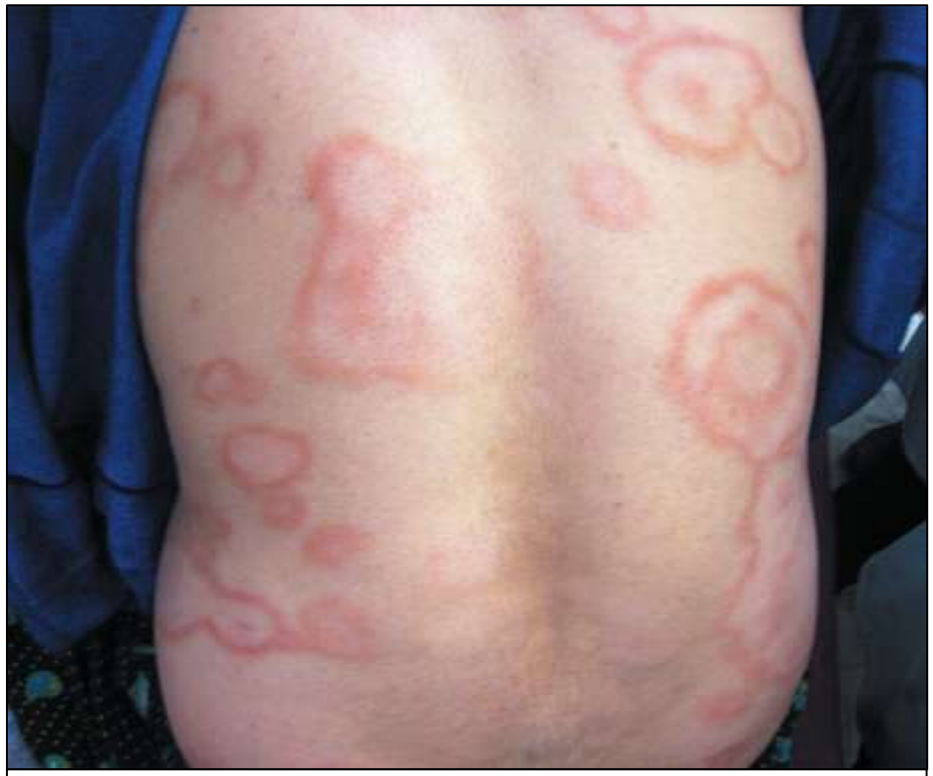


Leprosy (cont.)

Diagnostic Tests

Test	Method	TT Result	LL Result
Slit-Skin Smear (SSS)	Scraping from earlobe/active lesion → Modified ZN stain	**Negative** (paucibacillary)	**Positive**
Skin/Nerve Biopsy	Full-thickness; from **leading edge** of lesion → modified ZN stain	Epithelioid granulomas, Langhans cells, many lymphocytes, **NO bacilli**	Foamy histiocytes (lepra cells), **numerous AFB**
PCR	*M. leprae*-specific gene sequences; from biopsy/smear/blood	Definitive — detects 40-50% of cases missed histologically	Definitive
Lepromin Test	Intradermal killed M. leprae	**Positive** (≥5mm induration)	**Negative**
Serology (PGL-1, LAM)	Antibody detection	30% sensitivity	**95% sensitivity**

Mycobacterium leprae
Leprosy main forms

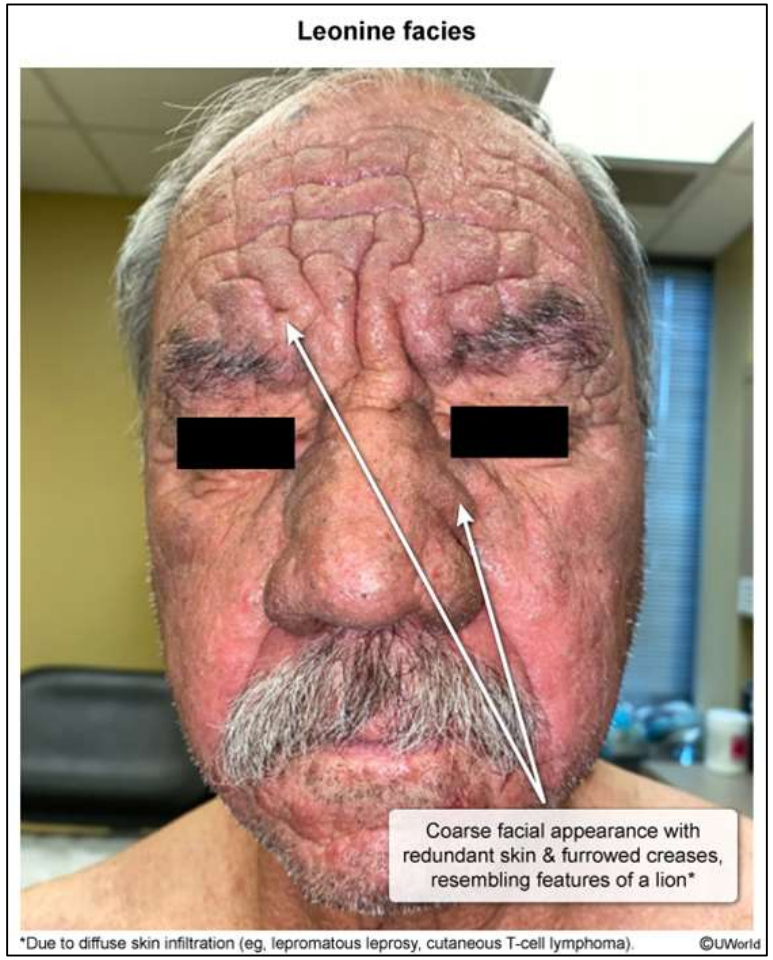


Tuberculoid leprosy

- Patients with an **effective** Th1 CMI response develop tuberculoid leprosy
- Low bacterial burden (paucibacillary) due to successful immune control.

Lepromatous leprosy (Leonine facies)

- Patients with an **ineffective** Th1 CMI response develop lepromatous leprosy, a more severe and diffuse form
- High bacterial burden (multibacillary) due to poor immune control


Leonine facies
Coarse facial appearance with redundant skin & furrowed creases, resembling features of a lion*
*Due to diffuse skin infiltration (eg, lepromatous leprosy, cutaneous T-cell lymphoma).
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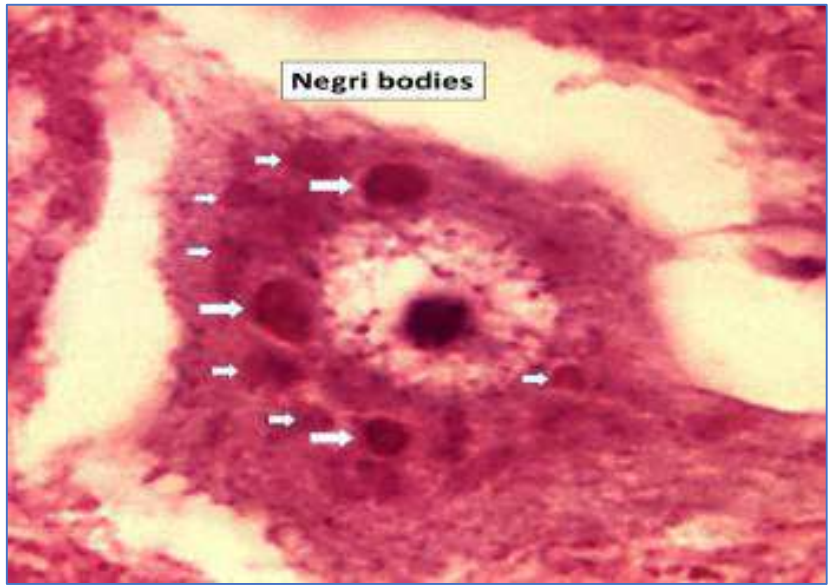
Rabies



Antemortem diagnosis

- Antibody titers
- PCR to detect viral RNA
- Isolation of virus (viral culture)
- Immunofluorescent staining of biopsy specimens for viral antigen

Postmortem diagnosis

Postmortem brain tissue autopsy

- Immunofluorescent staining of viral antigen in infected CNS tissue
- Histopathological findings: **Negri bodies** (eosinophilic cytoplasmic inclusion bodies typically found in the cerebellum and hippocampus)

وفقكم الله

وكل عام وأنتم وأهاليكم بألف خير

محبيكم:

د. سليمان محمود بني عبدالرحمن

د. حلا محمود الطراونة