

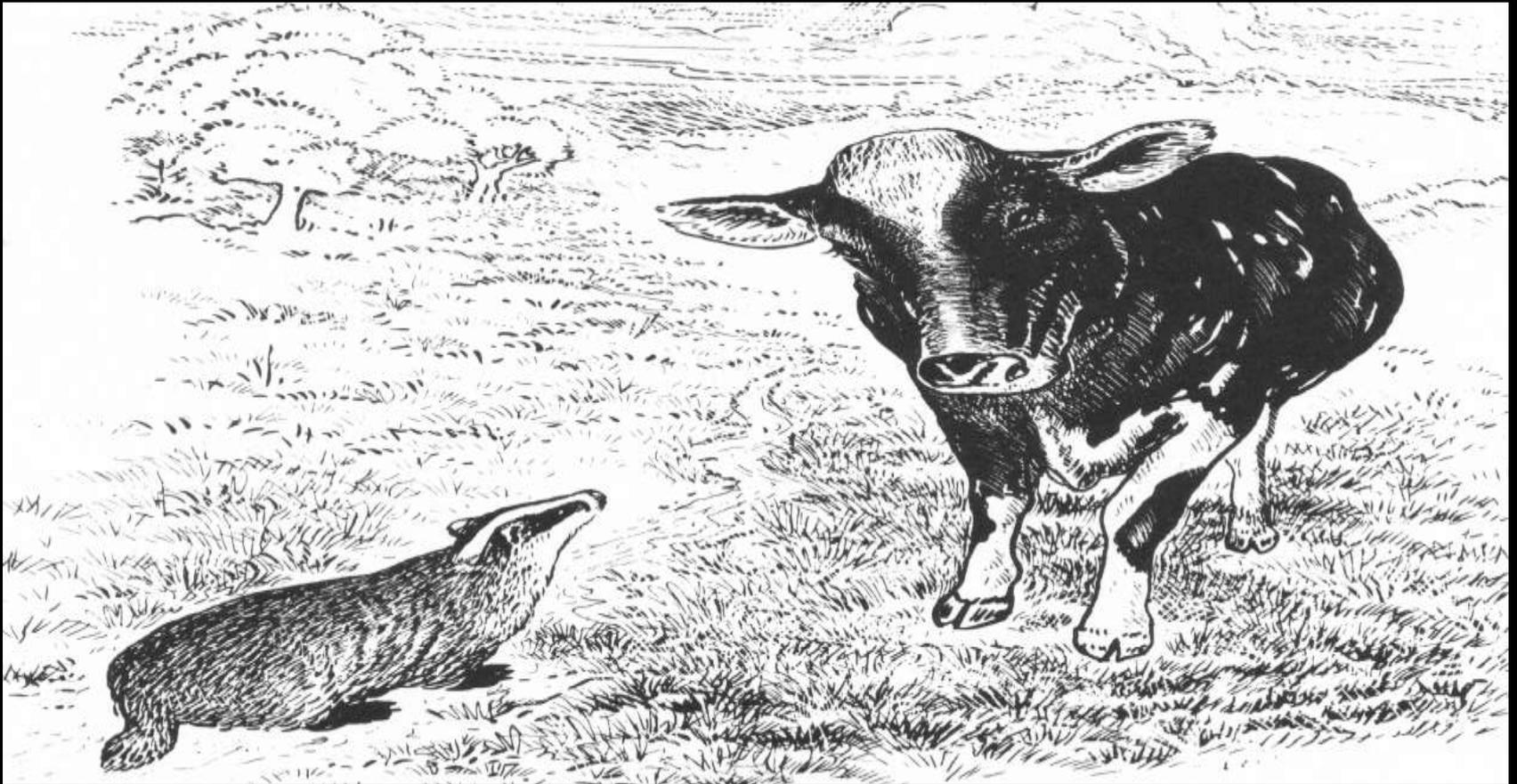


DISEASES CHARACTERIZED BY FORMING GRANULOMATOUS LESION AND LYMPH NODE INVOLVEMENT



Tuberculosis- Nocardiosis- -
Actinobacillosis- Actinomycosis-
Caseous lymphadenitis

Tuberculosis



Tuberculosis

- Tuberculosis (TB) is an infectious, granulomatous disease caused by **acid-fast bacilli** of the genus *Mycobacterium*. Although commonly defined as a chronic, debilitating disease, TB occasionally assumes an acute, rapidly progressive course. It is a major zoonosis.

Who is susceptible?

- The natural host range of *M. bovis* is extensive. It has the potential to infect all warm-blooded vertebrates.
- Before control measures were adopted, was a major disease of humans and domestic animals.



Distribution

- Bovine TB is still a significant zoonosis in many parts of the world.
 - During the early 1900s in the U.S., it is estimated that tuberculosis caused more death losses in farm animals than all other infectious diseases combined.
- 



Etiology

The main types of *M. tuberculosis* complex (mammalian tubercle bacilli) recognized are

M. tuberculosis

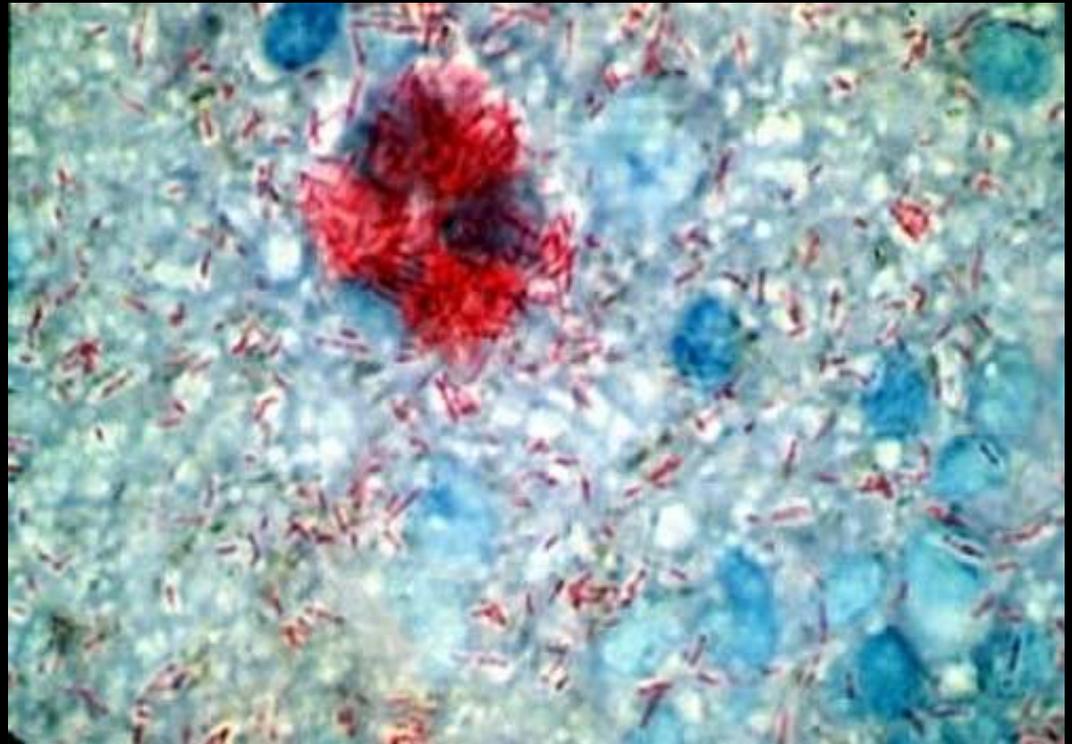
M. bovis

M. caprae

M. pinnipedii

M. microti

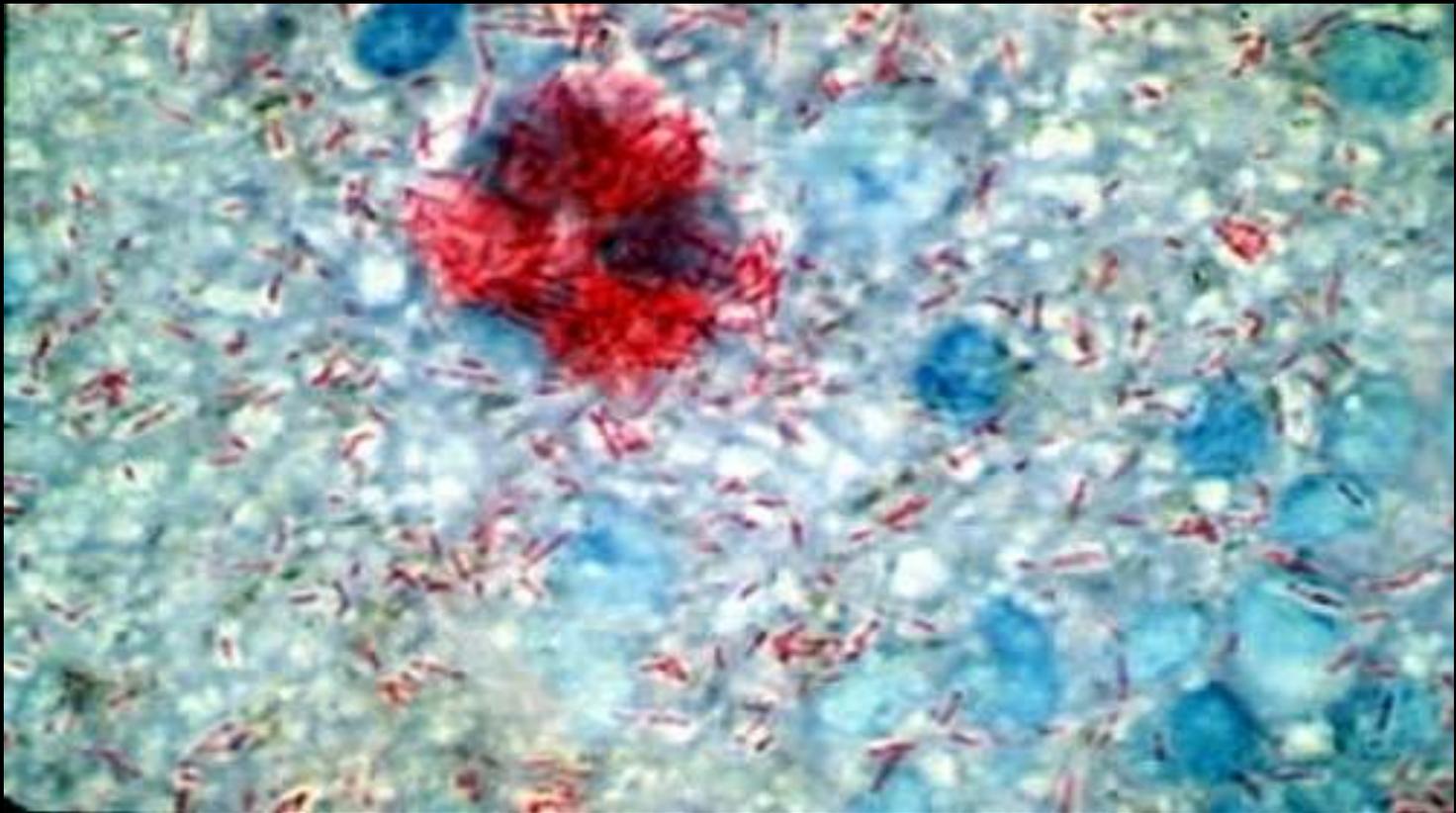
M. africanum



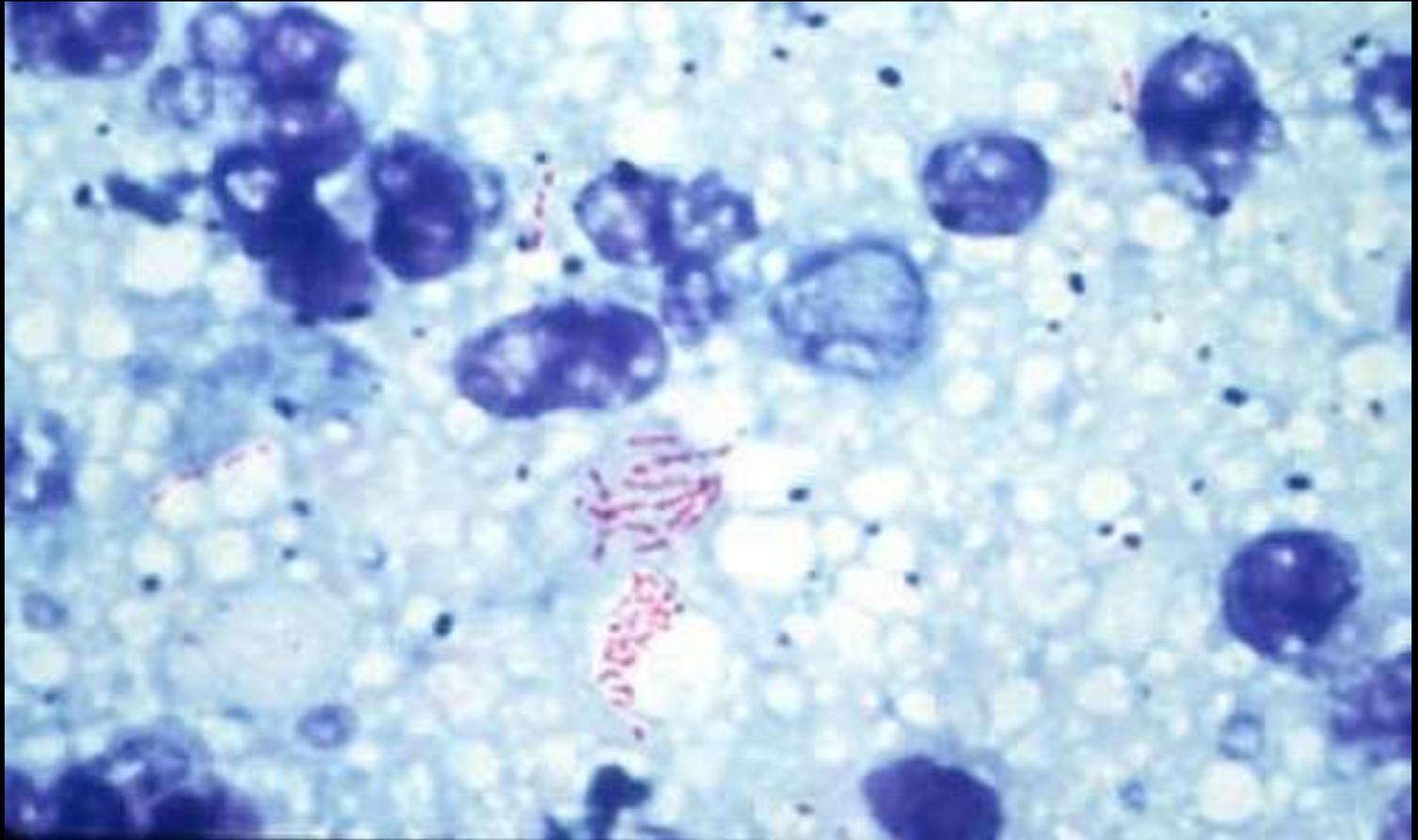
- There are three distinct strains of bacteria that cause tuberculosis in animals and people:
- *Mycobacterium bovis*-- affects cattle and many other species, including people and wildlife.
- *Mycobacterium avium* -- affects mostly poultry, but can cross-infect cattle.
- *Mycobacterium tuberculosis* -- affects humans only. Every year, there are cases of human tuberculosis reported in South Dakota (an average of 15 cases per year from 2006-2010).



Mycobacterium bovis, tissue smear, acid-fast stain, high power. Note the clusters of red acid-fast stain typical of mycobacterium.



Mycobacterium tuberculosis, tissue smear, Note the red acid-fast stain typical of mycobacterium





All types may produce infection in host species other than their own.

- *M tuberculosis* is most specific; it rarely produces progressive disease in animals other than people and nonhuman primates, occasionally in dogs and pigs, and rarely birds.
 - *M bovis* can cause progressive disease in most warm-blooded vertebrates, including humans.
 - *M caprae* has been isolated from cattle and several other species in Europe.
- 

Transmission

1. Inhalation of infected droplets expelled from the lungs is the usual route of infection.
2. Ingestion, particularly via contaminated milk, also occurs.
3. Intrauterine and coital methods of infection are recognized less commonly.

4. Intramammary by using contaminated teat siphons and cups.

Survival of acid fast bacteria in the environment depends on; temperature, moisture, exposure to dessication, ultraviolet light. Survival in soil is measured in weeks rather than months.



Shedding of bacteria

- In respiratory cases bacilli are shed into the air by coughing.
- They are also shed in the faeces in advanced respiratory cases, after swallowing of infected sputum or due to active tuberculous intestinal lesions.

- 
- In generalized cases and in alimentary cases. When the kidneys or udder are involved, bacilli may also be shed in the urine and milk. Ingestion of infected milk can be an important source of infection for calves.
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Distribution

- Bovine TB is widespread throughout the world. It is subject to control programs in a number of countries.
 - In the absence of control measures it tends to be more common in colder climates, because housing of animals favors spread.

Pathogenesis

TB spread by 2 stages;

1. Primary complex.

2. Post primary
dissemination

Pathogenesis- inhalation

Inhaled bacilli → phagocytosed
by alveolar macrophages →
either →

1. Clear the infection.

Or 2. Allow mycobacterial proliferation → primary focus formation- within 8 days at site of entry → pass to respiratory tract (90-95% of cases in cattle) → regional lymph nodes → producing similar lesions .

In alimentary forms of disease, the primary focus may be found in

1. the pharynx and pharyngeal lymph nodes.
2. mesenteric lymph nodes .
3. less commonly, in the tonsils or intestines.

Hepatic lesions are major post primary spread.



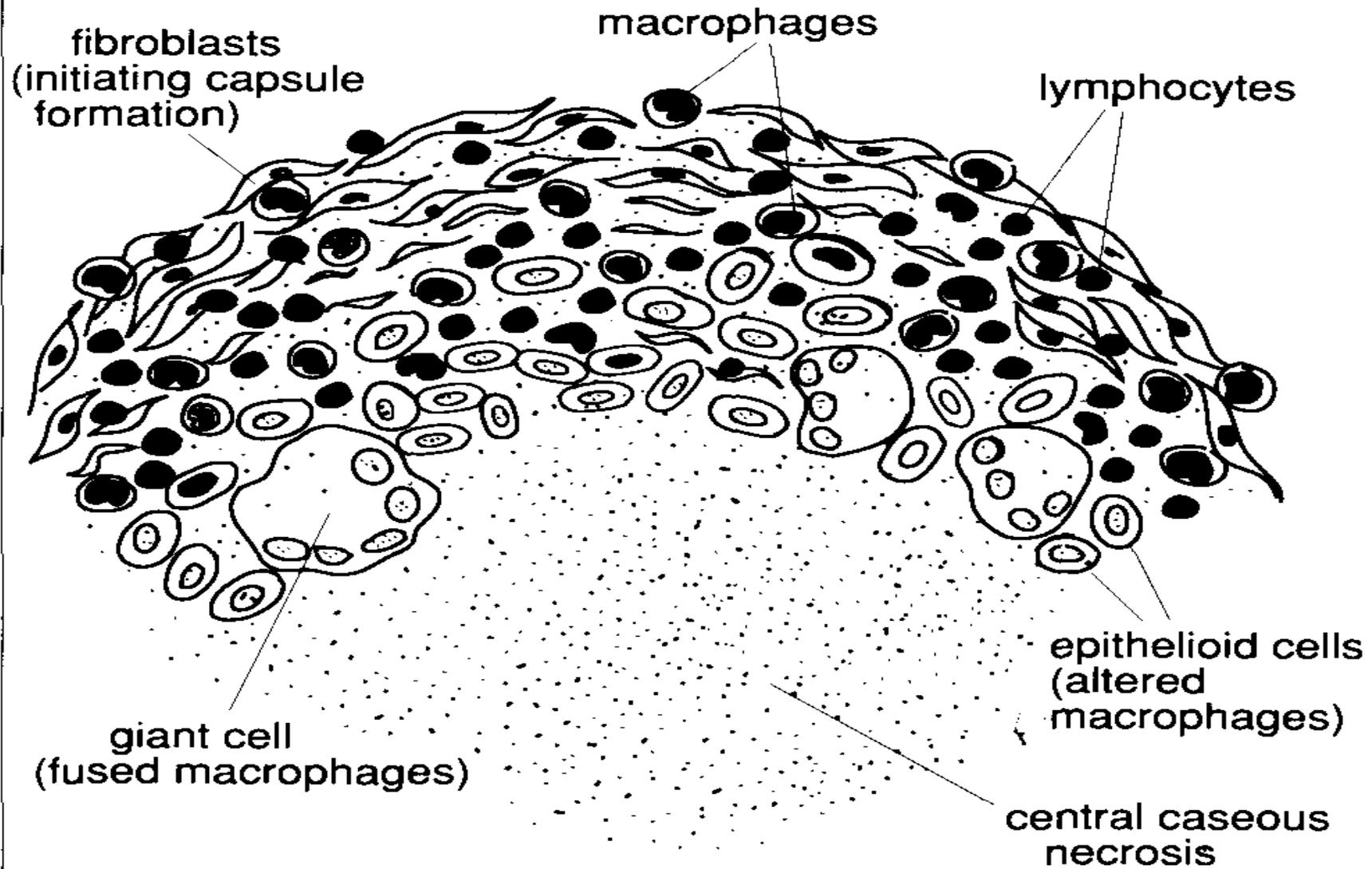
→ The primary focus plus similar lesions formed in the regional lymph node is known as the
“primary complex.”

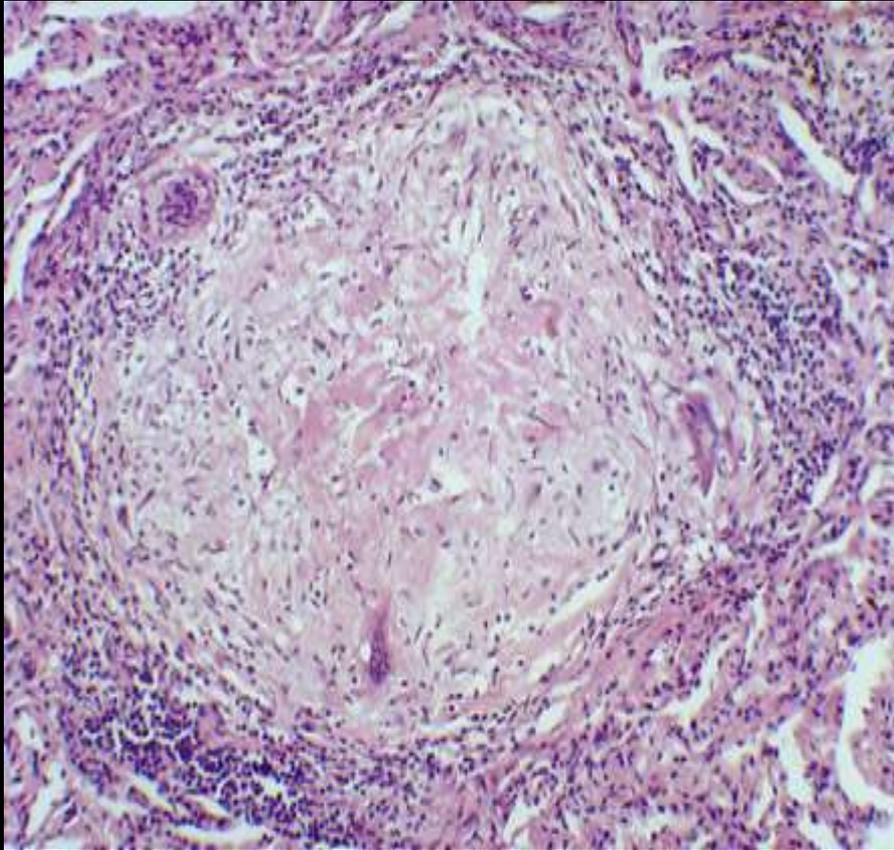
The purulent to caseous, necrotic center may calcify (after 2 weeks), and the lesion may become surrounded by granulation tissue and a fibrous capsule to form the classic “tubercle.”

- **Granulomatous lesion** In pathology, granulomatous lesion is used to describe chronic inflammation in which the predominant inflammatory cell is the macrophage with variable amount of lymphocytes.

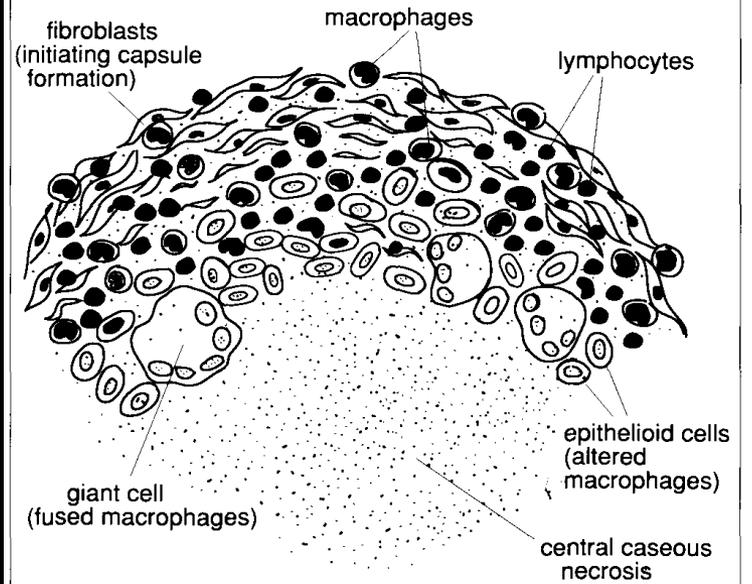
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- The macrophages in such circumstances may aggregate to form a circumscribed mass (granuloma).
 - In addition, the macrophages may fuse together to form multinucleate giant cells. Granulomatous lesion typically occurs when the usual acute inflammatory reaction involving neutrophils could not remove the inciting agent.
 - The second line of defense involving macrophages then take over

Microscopic appearance of a bovine tuberculous lesion





Microscopic appearance of a bovine tuberculous lesion



Primary focus

cytokines- mediated lesion consists
of dead and degenerated
macrophages surrounded by
epithelioid cells, granulocytes,
lymphocytes, and later,
multinucleated giant cells.

Post primary dissemination

Bacteria from primary complex 

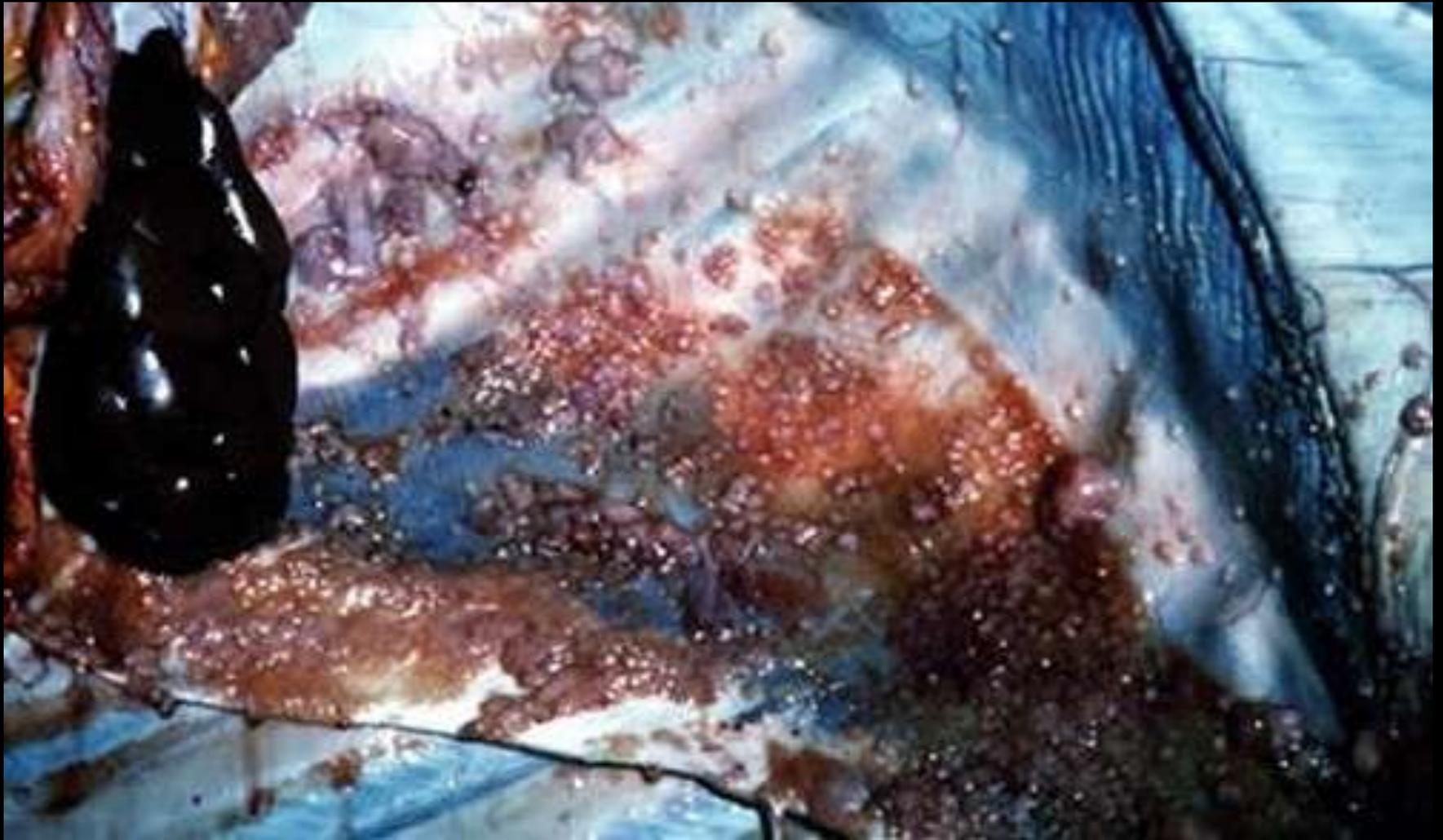
blood and lymph 

various organs  producing

a. Miliary TB- discrete nodular lesions.

b. Chronic organ TB.

Mycobacterium bovis granulomas,
medial surface of rib cage, cow.



Mycobacterium tuberculosis, granulomatous lesions, cow lung



Tuberculosis lesions in the lung of a deer harvested in Michigan. TB due to *M. bovis* is now largely eliminated in the US, but it still circulates in wildlife reservoirs





Clinical signs

- Bovine tuberculosis is a chronic and slowly progressive disease, with an incubation time (time from exposure to onset of clinical signs) ranging from months to years.
- 

Clinical signs Vary according to:

- extent of lesion and
- sites of localization.

Since it is a progressive disease;

There is a constant toxæmia

associated with;

Weakness, debility and death.

Generalized signs include :

- progressive emaciation.
- lethargy and weakness.
- Anorexia.
- A low-grade, fluctuating fever.



The bronchopneumonia of the respiratory form

- Chronic, intermittent, moist cough
- with later signs of dyspnea and tachypnea, and or nasal discharge.
- The destructive lesions of the granulomatous bronchopneumonia may be detected on auscultation and percussion.

- Superficial lymph node enlargement.
- Affected deeper lymph nodes cannot always be palpated, but they may cause obstruction of the airways, pharynx, and gut, leading to dyspnea, dysphagia, noisy breathing, pharyngeal obstruction and ruminal tympany.

Tubercular lymphadenitis of the head of a kudu



- Tubercular lymphadenitis of a parotid lymph node



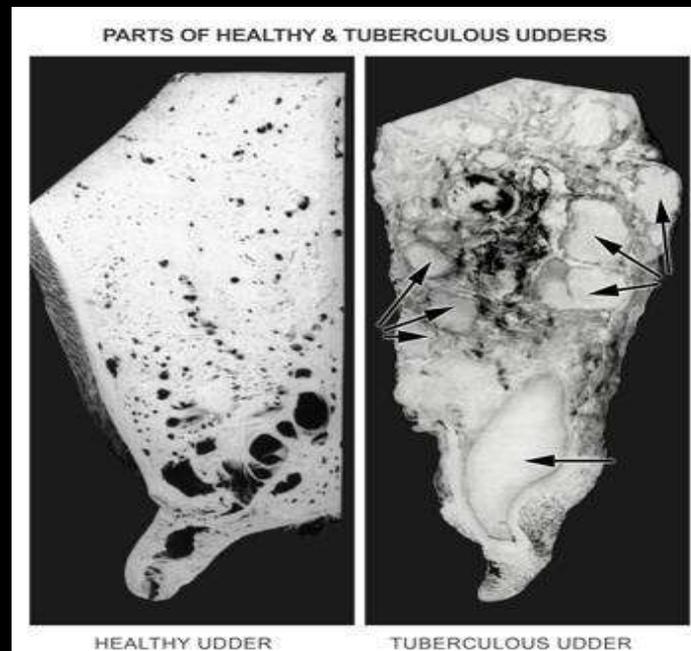
A cow with subcutaneous lesions caused by MOTT

- MOTT: Mycobacteria other than tuberculosis



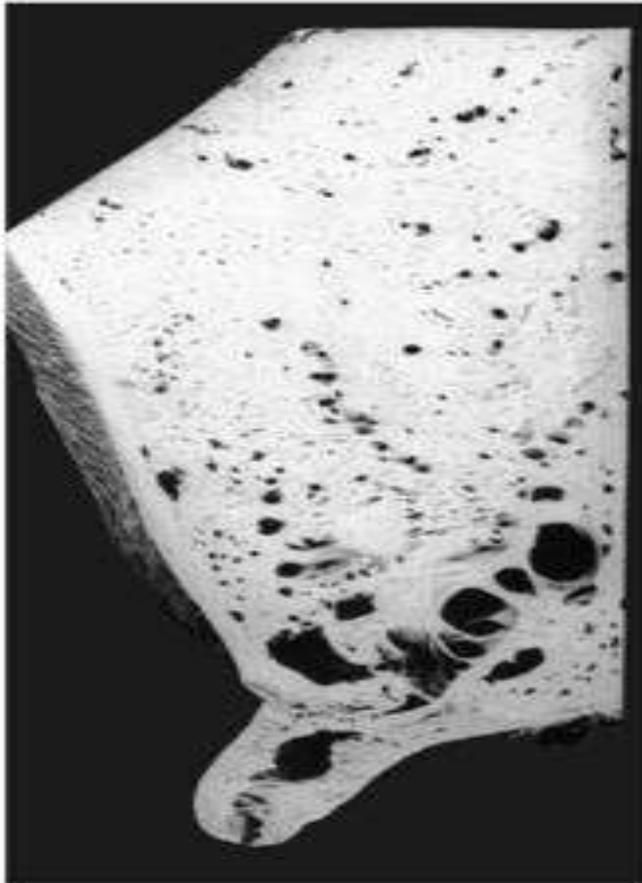
- Tuberculous mastitis in dairy cattle

Characterized by marked induration and hypertrophy especially in the upper part of rear quarter of the udder. Palpation of supramammary lymph node is essential ; usually it is enlarged.

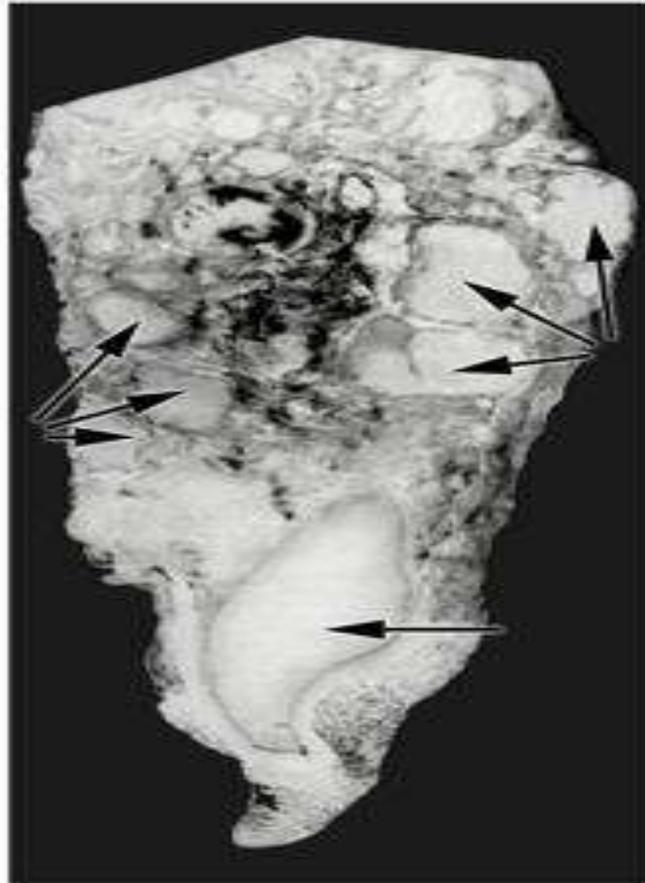




PARTS OF HEALTHY & TUBERCULOUS UDDERS



HEALTHY UDDER



TUBERCULOUS UDDER

- 
- In early stages milk is normal macroscopically, later very fine floccules appear that settles when milk stands leaving a clear amber supernatant fluid.
- 



Rarely tuberculous ulcers of
small intestine may cause
diarrhea



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- Because infected cattle will infrequently show signs of illness, cases of tuberculosis are often diagnosed on the basis of lesions found on slaughter inspection.
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In sheep and goats:

Lesions caused by *M bovis* and *M. Capri* in the lungs and lymph nodes of sheep and goats are similar to those seen in cattle, and the organism may sometimes disseminate to other organs. Sheep and goats are quite resistant to *M. tuberculosis* infection.

Horses:

Are relatively resistant to TB caused by *M. tuberculosis* complex. When TB does develop, tuberculous, noncalcified lesions are often found in the liver, mesenteric lymph nodes, lungs, and other sites. Tuberculin test results are rather erratic.



Dogs:

Dogs may be infected with

M. tuberculosis, *M bovis*, *M .avium* complex or *M. fortuitum*, commonly from a human or bovine source.

Tuberculous lesions are usually found in the lungs, liver, kidney, pleura, and peritoneum; they have a gray appearance, usually with a noncalcified, necrotic center. Lesions are often exudative and can produce a large quantity of straw-colored fluid in the thorax.

False-negative tuberculin tests are common in dogs



Diagnosis

1. Intradermal tuberculin test; it is the single most important field diagnostic test for TB using the purified protein derivatives (PPD) prepared from the culture filtrate of *M. bovis* or *M. avium* can be used.

Test sites vary in sensitivity and between countries and include:

- the neck region,
- caudal fold at the tail base,
- and vulval lip.

One disadvantage of the *M. bovis* SID test is that cross-reactions occur in animals infected with *M. avium avium*, *M tuberculosis*, or *M. avium Paratuberculosis*, *Nocardia asteroides*.

a. The single intradermal (SID) test.

- Involves inoculation of PPD. In a reactor, the antigen stimulates a local infiltrate of inflammatory cells and causes skin swelling that can be detected by palpation and measured by calipers.

- 
- Skin fold thickness should be measured in the area of inoculation before inoculating the tuberculin.



The reaction is read at 48–72 hr for maximum sensitivity and at 96 hr for maximum specificity. Measuring skin fold thickness after 72 hours of injecting tuberculin



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- In the single intradermal test (which requires a single injection of bovine tuberculin), the reaction is commonly considered to be
- 



1. Negative if only limited swelling is observed, with an

*increase of no more than 2 mm.

* and without clinical signs, such as
diffuse or extensive oedema,
exudation, necrosis, pain or
inflammation of the lymphatic
ducts in that region or of the
lymph nodes.





2. Inconclusive (Suspected) reaction is considered to be if* none of these clinical signs is observed and* if the increase in skin-fold thickness is more than 2 mm and less than 4 mm.

3. Positive reaction is considered to be

* if clinical signs, as mentioned above, are observed or

* if there is an increase of 4 mm or more in skin-fold thickness.

- Moreover, in *M.-bovis*-infected herds, any palpable or visible swelling should be considered to be positive.

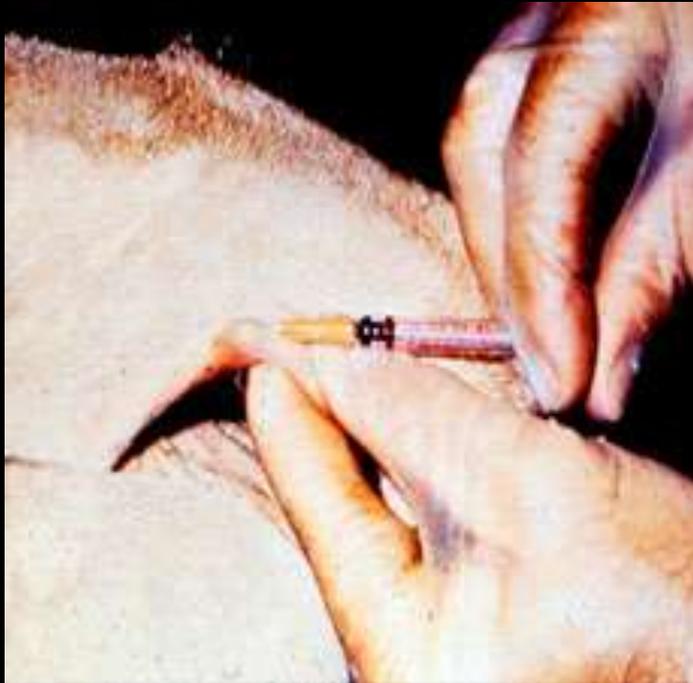
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- Animals that are inconclusive by the single intradermal test should be subjected to another test after an interval of 42 days to allow desensitisation to wane (in some areas 60 days for cattle and 120 days for deer are used).
 - Animals that are positive to the single intradermal test may be subjected to a comparative intradermal test or blood test.

The caudal fold test (CFT). A small dose of tuberculin is injected into the skin of the caudal tail fold..... The injection site is then palpated 72 hours later..... Any amount of swelling is considered a reaction

An animal reacting as such is then subjected to comparative testing to determine if the reaction is due to infection with *M. bovis* or another strain.

A low level of “false positive” reactions (1-3%) are expected with the CFT.

- Caudal Fold Injection Caudal Fold Response



2.comparative tuberculin skin test

- In areas with a high incidence of either avian TB, atypical mycobacteriosis, or paratuberculosis,
- it can be used, with biologically balanced *M bovis* and *M avium* PPD tuberculins
- inoculated simultaneously but at separate sites in the neck.



-Mammalian and avian tuberculin's
are injected one above the other,
12 cm apart.

-Dose 0.1-0.2 ml introduced
intradermally.

Reaction is measured 72 hours later.



The agent causing sensitization
provokes the greater skin
reaction.

1. Positive reaction is usually considered if the increase in skin thickness at the bovine site of injection is more than 4 mm greater than the reaction shown at the site of the avian injection.
2. Inconclusive (suspected) reaction if the increase in skin thickness at the bovine site of injection is from 1 to 4 mm greater than the avian reaction.
3. Negative reaction is considered if the increase in skin thickness at the bovine site of injection is Less than or equal to the increase in the skin reaction at the avian site of injection.

Inoculating tuberculin in the skin of neck



Post tuberculin injection skin thickness measurement



Skin caliber



Applying tuberculin in man

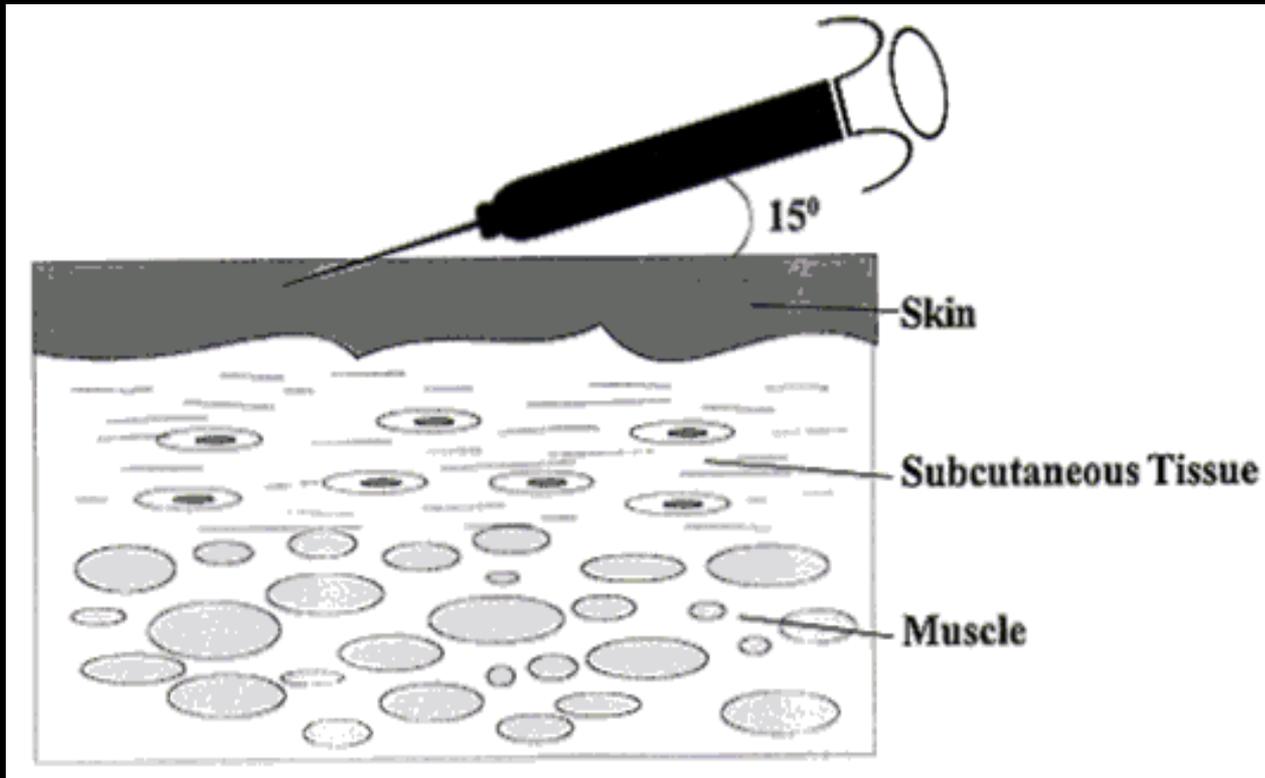


Measuring skin reaction- diameter of redness and oedema





Injecting PPD in the skin- Intradermally



Reading the results







- 3. The thermal tuberculin test, which may detect a pyrexia peak (104°F [$>40^{\circ}\text{C}$]) at 6–8 hr after SC inoculation with tuberculin.

4. The Stormont tuberculin test

Uses an intradermal inoculation of PPD followed by a second inoculum at the same site 7 days later. The test is read for swelling 24 hr later

Stormont test

- An increase in thickness of more than 5mm 24 hours after the 2nd injection is considered positive. It is more accurate than the SIT, but it needs three visits to the farm.

False negative tuberculin tests

- Animals with poor immunity such as those in the early (until 6 weeks after infection) or late stages of infection,
- Cows desensitized by previous tuberculin (After 8-60 day may return to sensitization)

False negative reaction

- Anergic cases in advanced disease,
- Old animals.
- Cows immediately before and after calving- may give negative results due to general immuno-hyporeactivity (post parturient desensitization) associated with parturition. -Returning to normal 4-6 weeks later.
- Low potency tuberculin



No gross lesion positive tuberculin
(False positive tuberculin reaction)

- Animals sensitized to avian TB ,
Johne,s disease, non pathogenic
Mycobacterial sp., Nocardia
asteroids infection (Bovine farcy).
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- 2. Diagnosis based on clinical signs alone is very difficult, even in advanced cases.



3. Radiography is useful in nonhuman primates and small animals.



4. Necropsy findings of the classic
“Tuberculous” granulomas are often
very suggestive of the disease.

Tuberculosis lesions in the lungs of a two-year-
old heifer



- Tuberculous granuloma in the Mediastinal lymph nodes. *M. bovis* was isolated



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- Tuberculous granuloma in the lymph nodes of the head, intestine and carcass. These have usually a well defined capsule enclosing a caseous mass with a calcified centre. They are usually yellow in colour in cattle, white in buffaloes and grayish white in other animals.
- 

- Lesion of tuberculosis in the lungs



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- Active lesions may have a reddened periphery and caseous mass in the centre of a lymph node.
 - Inactive lesions may be calcified and encapsulated
 - Firmer and enlarged udder, particularly rear quarters.

Pyogranuloma of tuberculous mastitis







The lesions may be different shapes and sizes .



- *Tuberculosis-infected deer may have multiple pea-sized tan or yellow lumps on the inside of the ribcage (below)...*

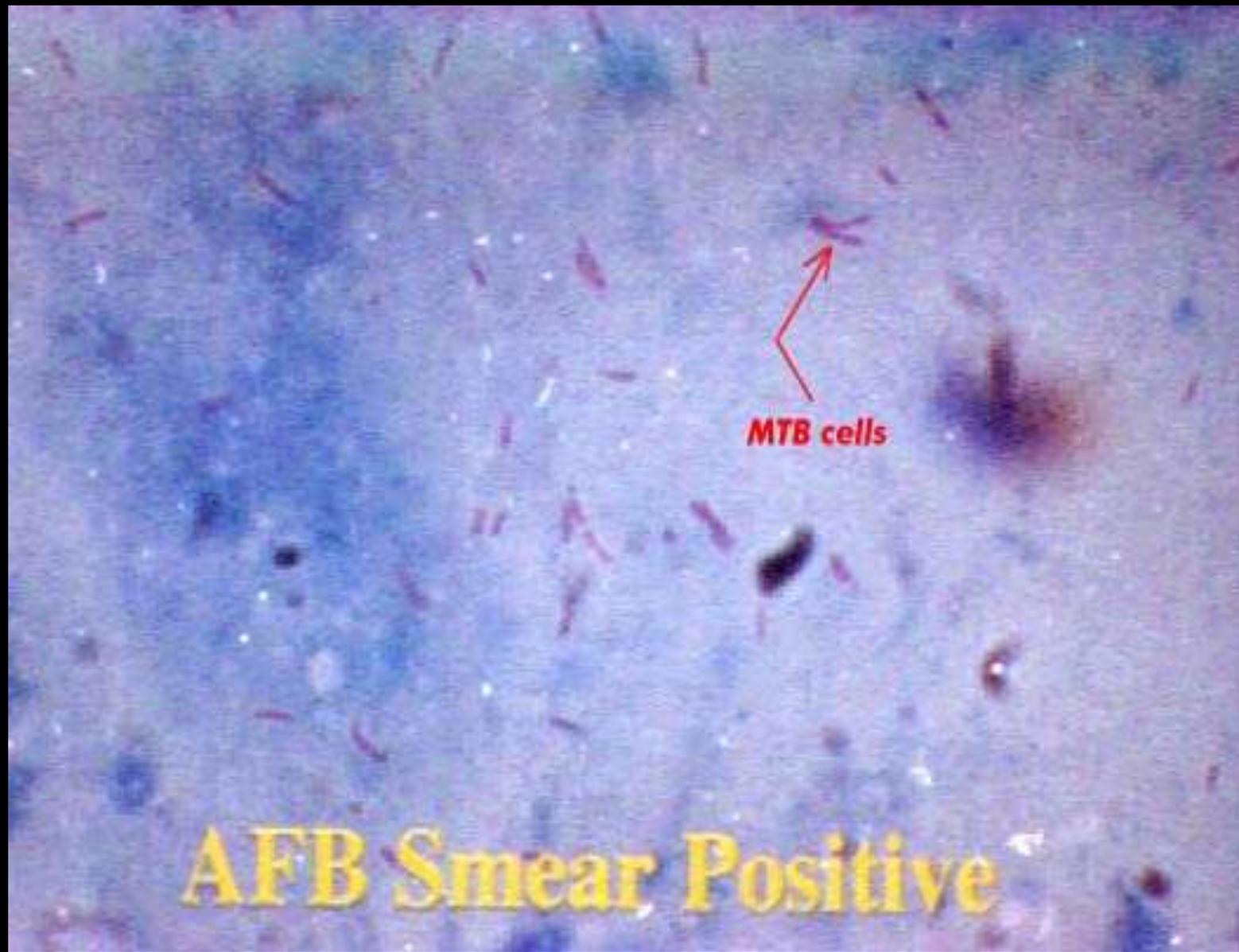


Tubercle lesions in the liver- *M. bovis*





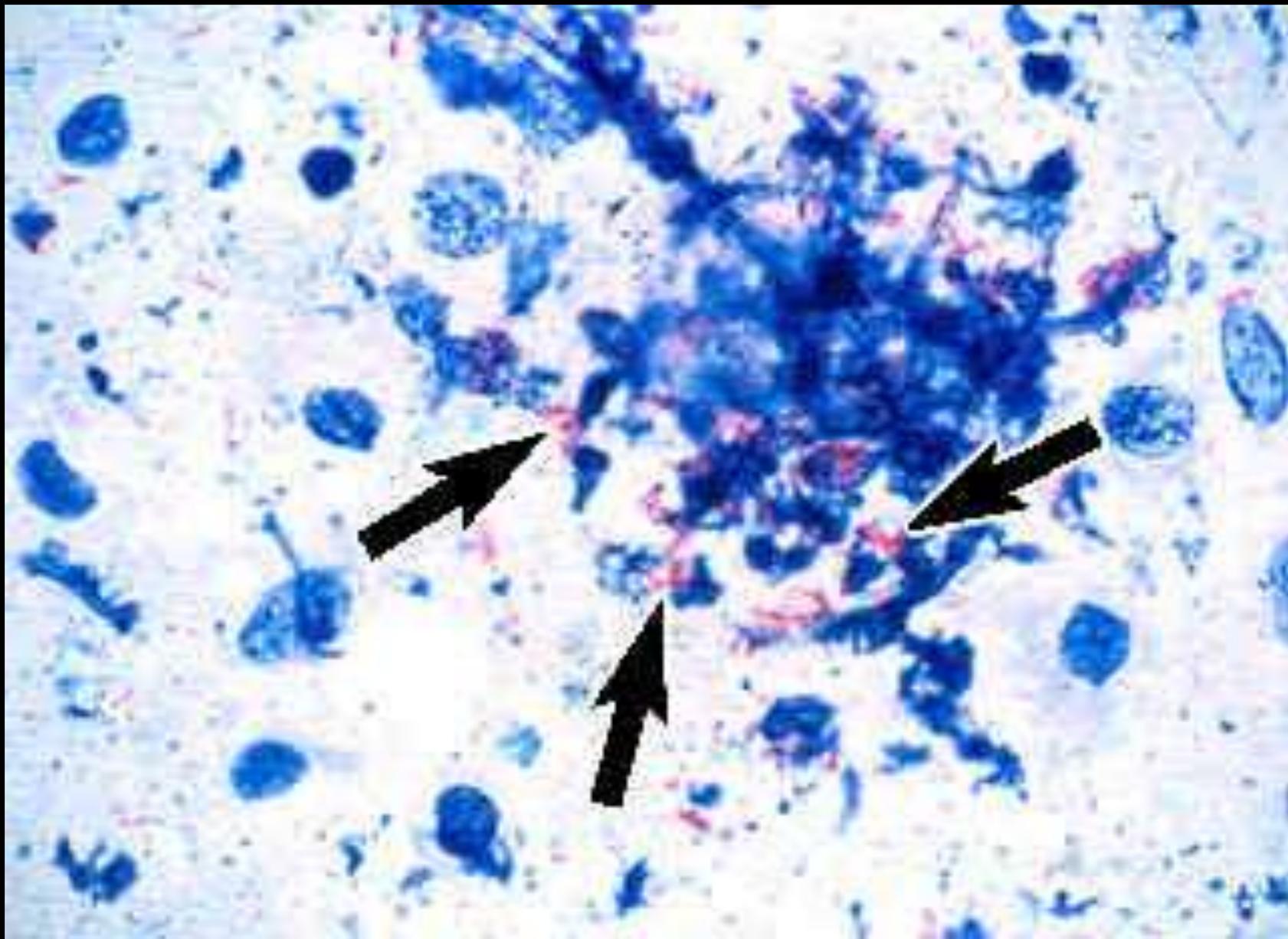
5. Microscopic examination of sputum and impression smears prepared from tubercles or other discharges is mostly used.
Remove fat-----Crushing of nodules in mortars with sand-----making homogenous suspension----- make smear--- stain with acid fast stain---look for small thin curved red acid fast bacilli.



MTB cells

AFB Smear Positive

Acid fast bacilli in tissue



6. Confirmation of diagnosis is by:
1. isolation and identification of the organism, with culture usually taking 4–8 wk.

Mycobacterial colonies on TB-Medium Base according to Löwenstein-Jensen







Culture of *Mycobacteria*

Media for Mycobacteria: The egg based **Lowenstein- Jensen and stone brinks media** are most commonly used in veterinary bacteriology. Lowenstein-Jensen medium can be obtained commercially. An agar-based medium such as middle brook may also be used.

The media are prepared as solid slants in screw-capped bottles.

- 
- **Middlebrook 7H₁₀ Agar** is a solid growth medium specially used for culture of Mycobacterium.
 - **Middlebrook 7H₉ Broth** is a liquid growth medium specially used for culture of Mycobacterium.

- 
- The media can be made more selective by the addition of cycloheximide (400µg/ml), lincomycin (2µg/ml) and nalidixic acid (35µg/ml).
 - Each new batch of culture medium should be inoculated with the stock strains of *Mycobacteria* to ensure that the medium supports satisfactory growth.

- 
- Malachite green dye (0.025g/100ml) is commonly used as selective agent. *Mycobacterium tuberculosis*, *Mycobacterium avium* and many of the atypical mycobacteria require glycerol for growth.
 - However, glycerol is inhibitory to *Mycobacterium bovis* while sodium pyruvate (0.4%) enhances its growth. Thus, the media with glycerol and without glycerol (but with sodium pyruvate) should be inoculated.

©



Mycobacterium tuberculosis

Ham. sN.



2. Molecular techniques as PCR,
which requires only a few days.



8. serology

- Serological methods like the gamma interferon test or ELISA are also available.
- 

Control

The 3 principal approaches to the control of TB are;

- a. test and slaughter,
- b. test and segregation,
- c. chemotherapy.
- d. Vaccination.
- e. Good management and hygiene.



a. The test and slaughter policy is the only one assured of eradicating TB and relies on the slaughter of reactors to the tuberculin test.

- In an affected herd, testing every 3 mo is recommended to rid the herd of individuals that can disseminate infection.
- 



b. In most European countries, where test and slaughter is applied, varying forms of **test and segregation** have been used, with test and slaughter used only in the final stages of eradication.



c. Chemotherapy

- Treatment of TB infections in elephants and nonhuman primates has been attempted using drugs that have had success in humans, e.g., isoniazid, ethambutol, and Rifampin

Chemotherapy

- Efficacy is limited, and there are arguments against therapy.

Based on the removal of infected animals, zoonotic risks, and the danger of encouraging drug resistance.

Treatment is illegal in some countries.

d. Vaccination

The BCG (bacille -Calmette-Guérin) vaccine, sometimes used to control TB in humans.

Proved to provide little protection in most animal species, and inoculation often provokes a severe local granulomatous reaction



d. Routine hygienic measures aimed at cleaning and disinfecting contaminated food, water troughs, etc, are also useful.

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- Effective disinfectants include 5% phenol, iodine solutions with a high concentration of available iodine, glutaraldehyde and formaldehyde.
 - In environments with low concentrations of organic material, 1% sodium hypochlorite with a long contact time is also effective.

Differential diagnosis

- Contagious bovine pleuropneumonia.
- Bacterial pneumonia caused by *Pasteurella* or *Corynebacterium pyogenes*
- Inhalation pneumonia (Drenching pneumonia).
- Traumatic pericarditis.
- Chronic aberrant liver fluke infestation.