



Tuberculosis in Free-Ranging African Wildlife

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ZAHP Webinar 2024

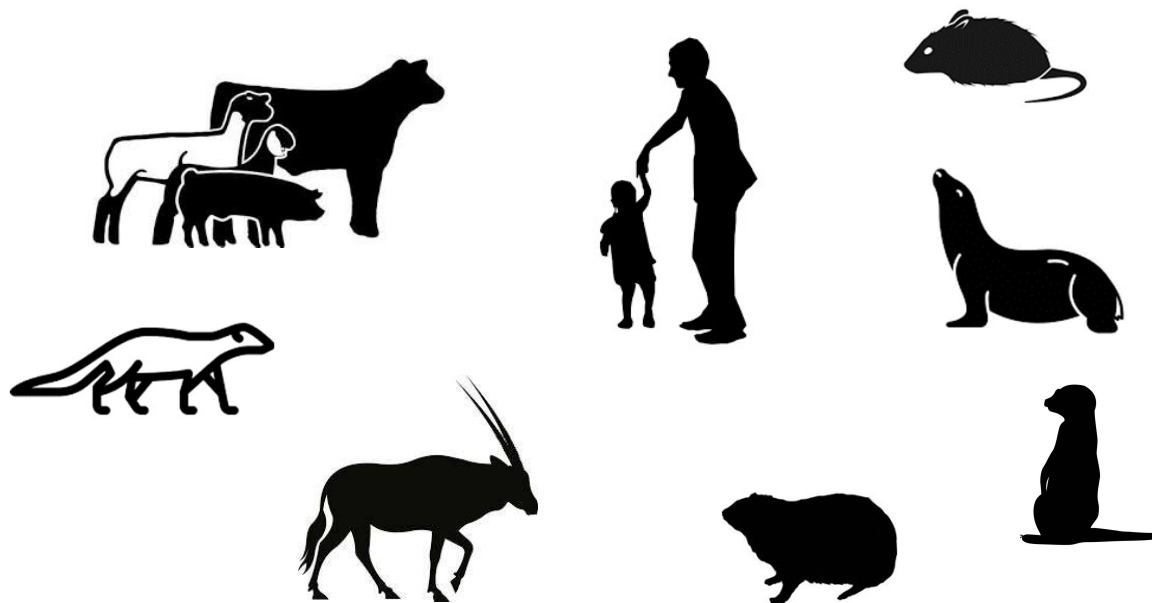
Overview

- Etiologies
- Susceptible mammalian species
- Epidemiology
- Clinical presentation
- Gross pathology
- Diagnostic approaches



Tuberculosis - A Global Health Threat To Humans and Animals

Mycobacterium tuberculosis complex (MTBC) bacteria infect a wide range of species, including humans, domestic animals & wildlife



M. tuberculosis

M. africanum

M. bovis

M. microti

M. pinnipedii

M. caprae

M. orygis

Dassie bacillus

M. mungi

M. suricattae

Chimp bacillus

Animal-
adapted
strains

Animal Tuberculosis

- Animal tuberculosis is chronic progressive bacterial infection
 - Usually affects the lungs (pneumonia) but can be found in other sites
- Most often caused by “cattle strain” of TB - *Mycobacterium bovis*
- Animals can be infected with *M. tuberculosis* (human strain)

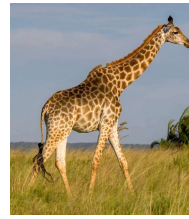


**Areas in
South Africa
With
Reported
Cases of *M.
bovis*
Infection in
Wildlife**



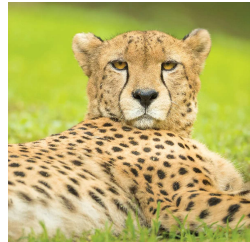
Dr. L-M
deKlerk-
Lorist

TB (*M. bovis*) in 25 Wildlife Species in South Africa



1928

2023

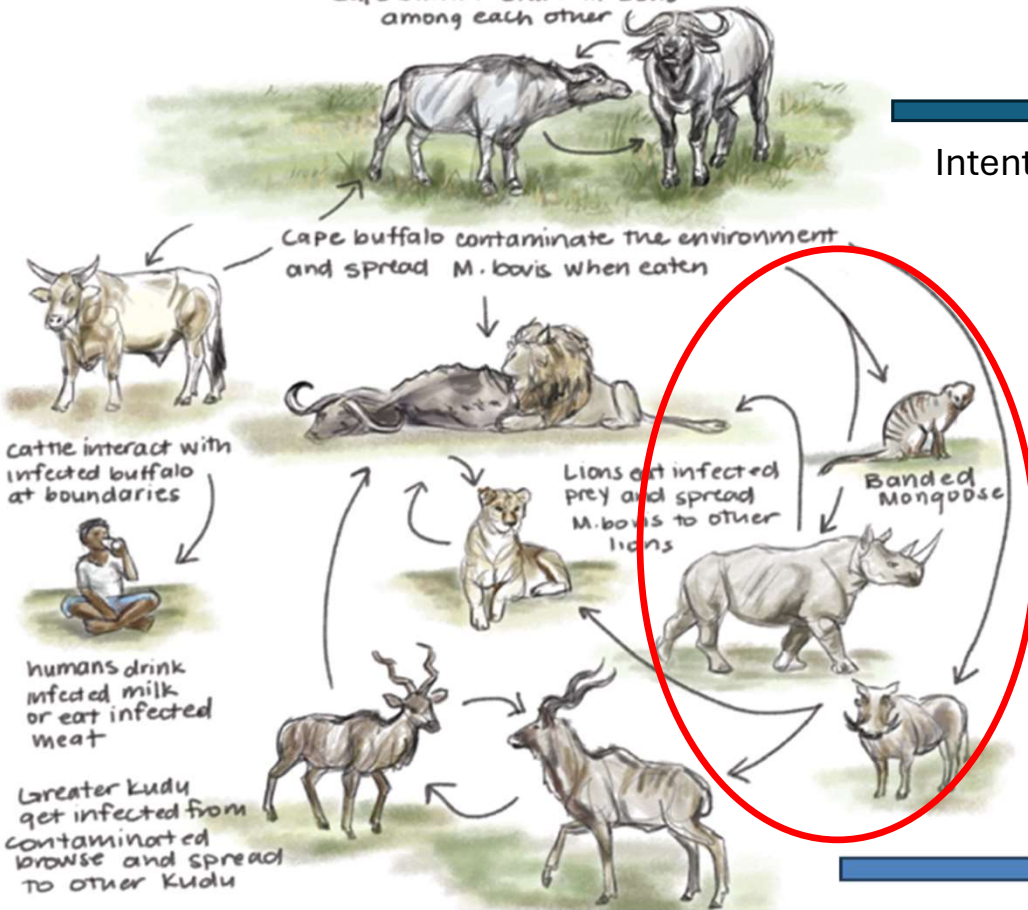


How is TB
transmitted in
South African
wildlife?



Hypothesized transmission of Mycobacterium bovis at African animal interfaces

Cape buffalo share M. bovis among each other



African Tuberculosis Transmission

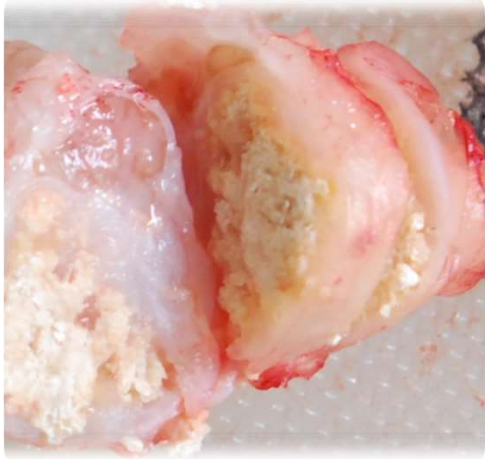
Intentional movement

Unintentional movement





Clinical Signs in Chronic Infection



Gross Pathological TB Lesions in Wildlife

- Mineralized granulomas --- suspicion of TB
- Lesions vary by species
 - Ungulates – similar to cattle
 - caseation, mineralization, necrosis, fibrous encapsulation
 - Carnivores
 - cavitary, without mineralization, +/- proliferative
 - Non-human primates
 - mimics human TB lesions
- **TB should be a DDX for any animal with signs of chronic disease, wasting, respiratory signs, chronic lameness***

Pathological Lesions



Warthog



Vervet monkey



Lion



Buffalo



Kudu

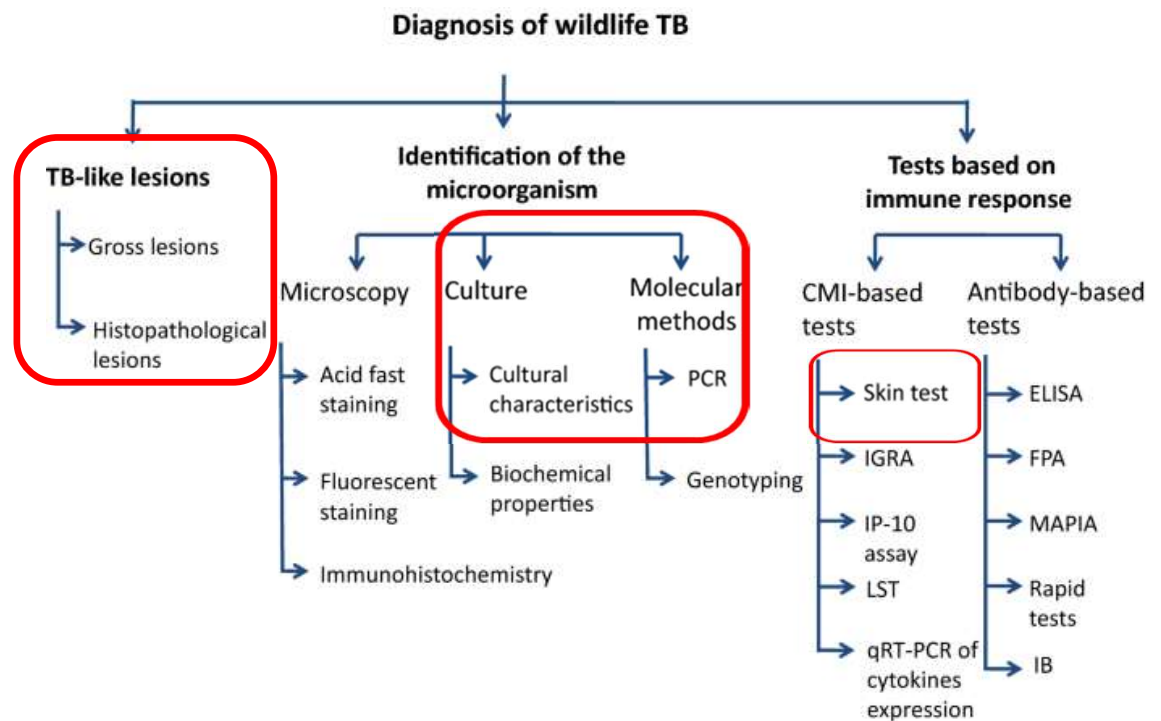


Elephant

Overview of Methods for Diagnosis of Wildlife TB

- Diagnostic approach based on humans and domestic animals
- Often only available as research tools, limited species, limited geographic regions

**Check with veterinary and medical schools, research institutions, veterinary laboratories for availability.*



Thomas, Balseiro, Gortazar, Rialde 2021

Veterinary Research

Direct Detection of MTBC Organisms



- Presence of mycobacterial organisms in samples (living or dead animal)
 - Provides definitive diagnosis
- Gross and histopathological lesions
 - Provide presumptive diagnosis
- Detection methods:
 - Microscopy
 - acid-fast stain, immunohistochemistry
 - Culture
 - takes up to 8 weeks
 - difficult to grow (false negative results)
 - PCR
 - detects bacterial DNA
 - can't determine if bacteria are viable



Cepheid GeneXpert MTB/RIF Ultra qPCR Assay

- Rapid screening test for human TB (results < 2 hr)
- Designed for sputum but can use variety of sample types including tissue homogenate or swabs
- Sensitive detection of MTBC DNA
 - Cannot differentiate *M. tb* and *M. bovis*
 - Cartridge based; easy to use



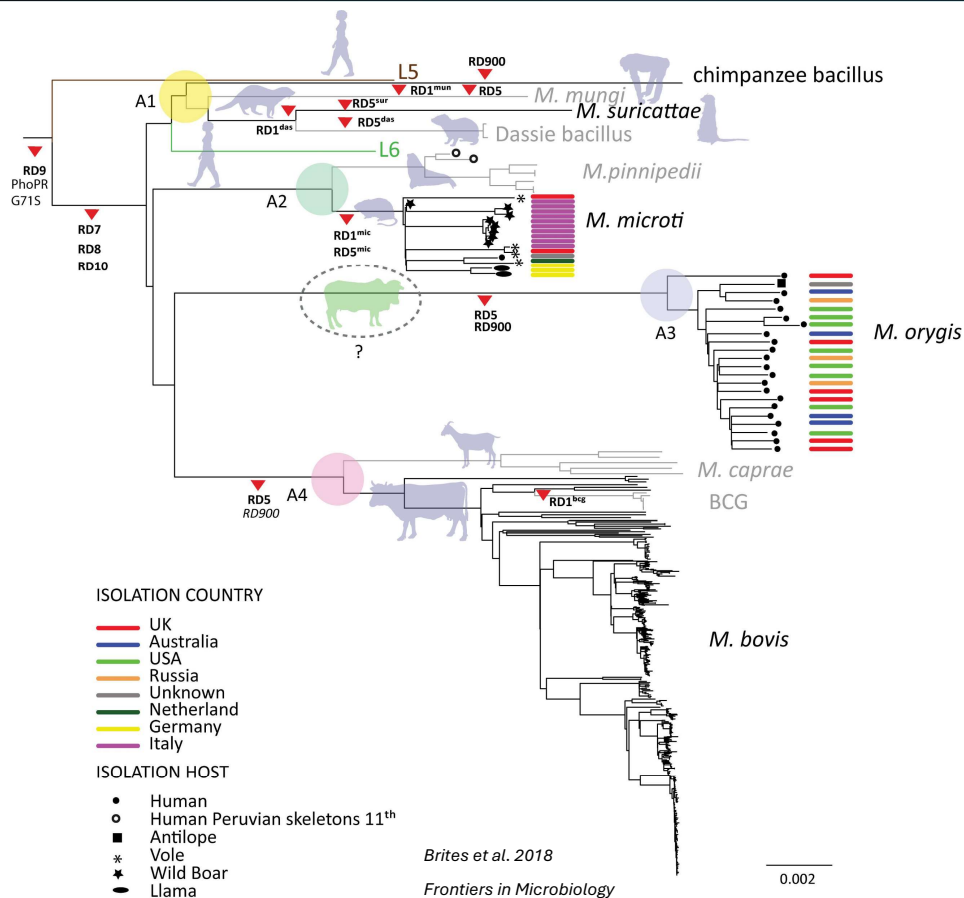
Direct Detection - Pitfalls

False negative culture results common

- Difficult to obtain samples containing mycobacteria especially in early infection
 - Sporadic shedding
 - Paucibacillary lesions (ex. elephants)
 - Harsh decontamination process prior to culture
- **Requires laboratory that specializes in mycobacterial culture and identification**

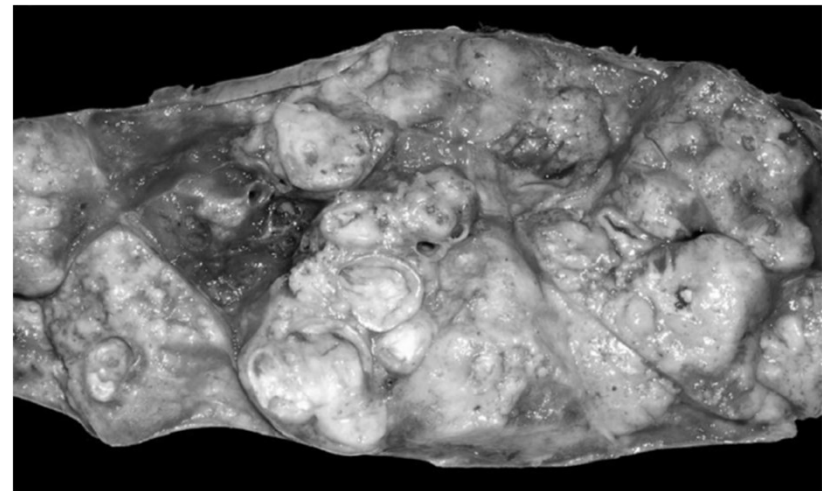


Direct Detection - Pitfalls



Positive mycobacterial culture result is NOT sufficient to diagnose TB

Requires further analyses to differentiate NTM, MTBC & species

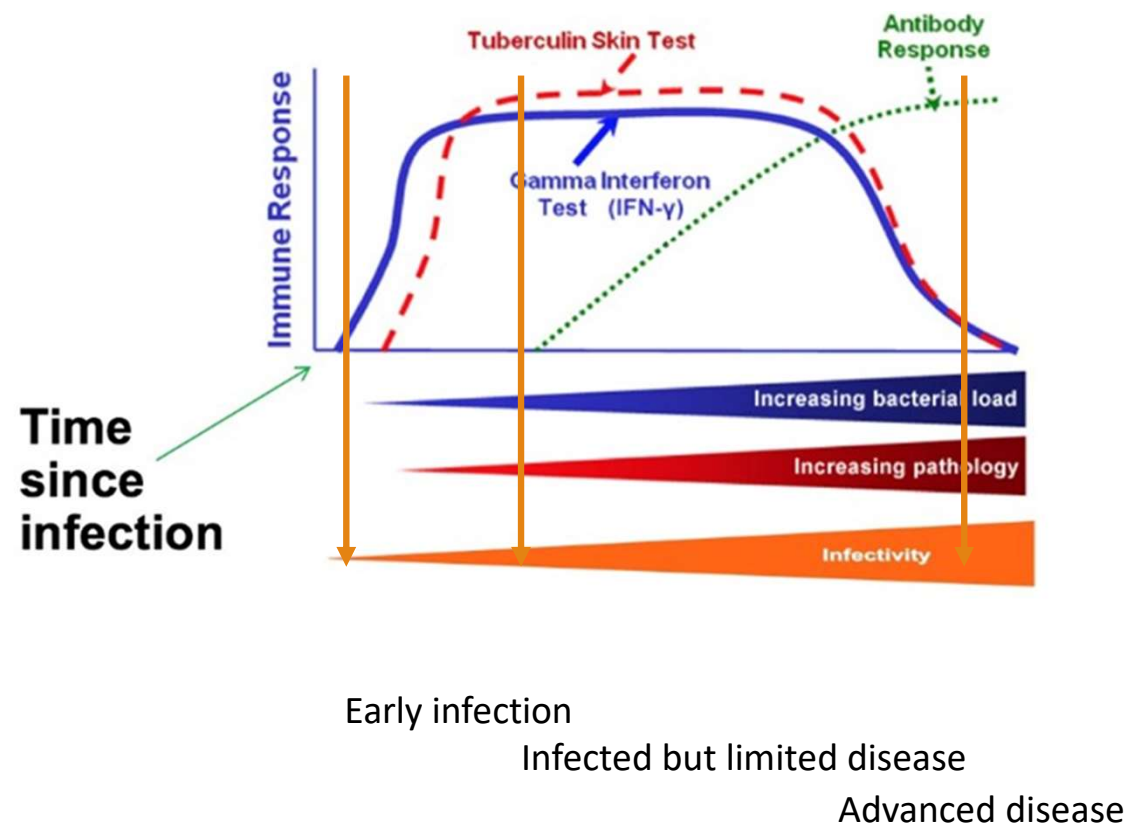


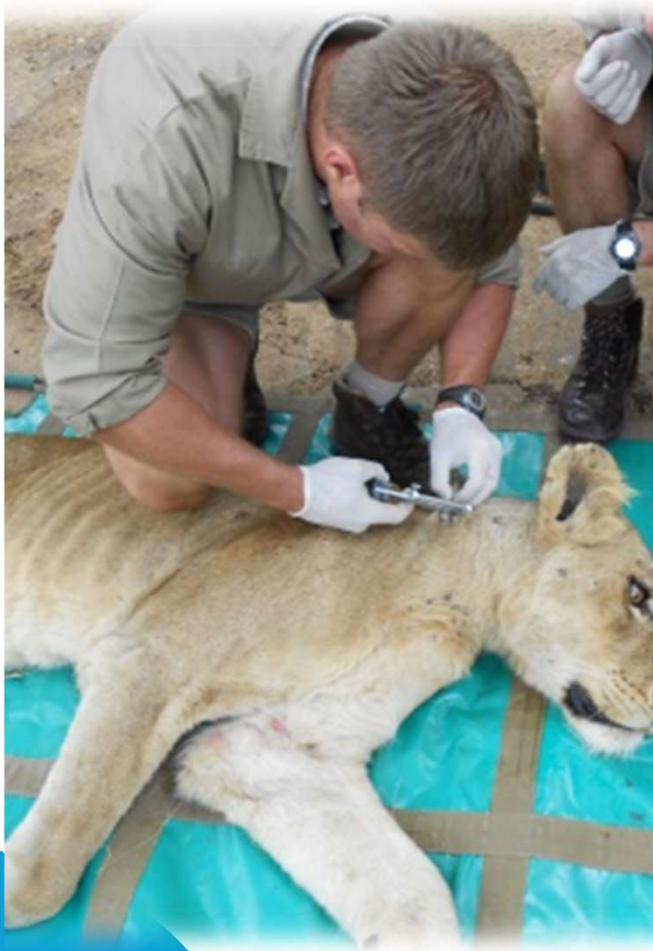
Granulomatous pneumonia in elephant infected with *M. szulgai*

Detection of TB in Animals

Indirect detection

- Based on adaptive immune responses to mycobacteria
 - Cell-mediated immune responses (T cells)
 - Humoral immune responses (antibodies)
- Provides presumptive diagnosis
 - Infected
 - Early infection – below detection
 - Advanced disease - anergy
 - Cleared infection
 - Waning response may be detected





Tuberculin Skin Test

- Can be used in a wide variety of species
- Compare responses to purified protein derivatives (PPDs) of environmental mycobacteria (*M. avium*) and *M. bovis*
- Requires 48-72 hrs to develop DTH response
- Swelling measured with calipers and compared to cut-off value (if available)
 - Also assess necrosis, heat, edema

Tuberculin Skin Test - Pitfalls

- Requires multiple (2) immobilizations
- Not validated in most wildlife species
 - Dose, site, cut-off value
- Thick- and thin-skinned animals
- Species differences in responses
- Cross-reactivity with environmental mycobacteria
- Advanced disease may cause anergy (false negative)
- Technical issues - batches of PPDs, operator experience, type of syringe/needle used, caliper pressure



Cytokine Release Assays

IFN- γ assay (Bovigam, Qiagen IGRA)

- Heparinized whole blood incubated with mycobacterial antigens
- Gamma interferon measured after 20-24 hours
- Based on “memory response” to specific mycobacterial antigens

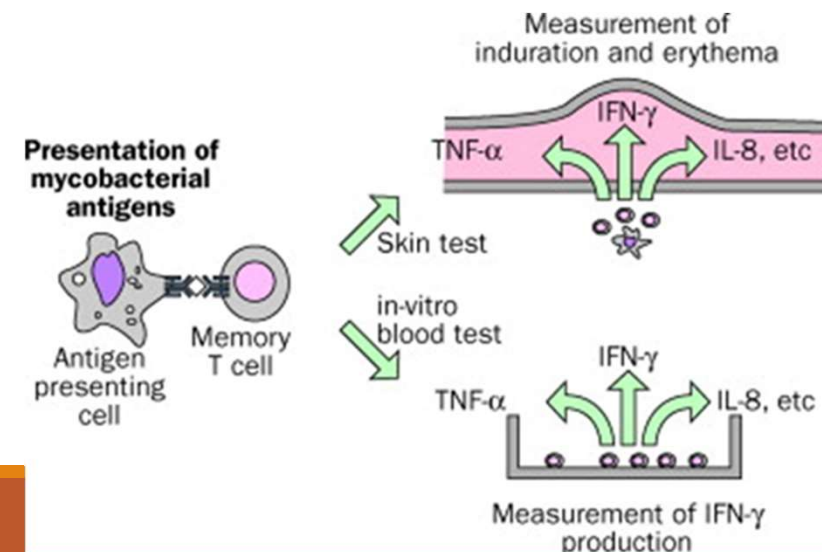
Need for species-specific reagents

- Bovigam assay - some wild bovids and antelope
- Usually requires reagents for taxonomically related domestic animal species
- Primagam (nonhuman primates), Cervigam (cervids) commercially developed assays for wildlife
- Other cytokine assays research tools
 - Wild dogs, lions/leopards/cheetahs, warthog, white/black rhinoceros, meerkats



BOVIGAM TB Kit

OIE registered BOVIGAM™ kit is a widely used diagnostic kit for fast and highly reproducible diagnosis of bovine tuberculosis (bTB) in cattle, sheep and goats



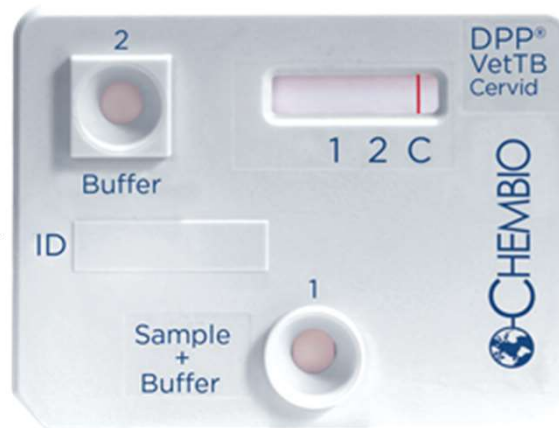
Humoral Responses in TB

Lateral flow chromatographic test

- Use any fluid containing antibodies
- Detects antibodies to immunodominant antigens
- IgG antibody detection by species non-specific reagent
- “Animal side” test

ELISAs

- Usually require species-specific reagents



Reports of DPP use in a wide range of species



Summary

Important to collect appropriate samples if suspect mycobacterial infection

- Living animals
 - Trunk secretions, oral swabs, tracheal washes, gastric washes, feces, biopsies of tissue
- Dead animals – tissue in formalin and frozen
 - Lymph nodes, lung and any suspicious lesions in other tissues
 - Impression smears of any lesions
 - Take photos when possible





Summary

Tuberculin skin tests

- Commonly used but may not be validated
- Requires 2 immobilizations

Interferon-gamma and other cytokine assays*

- Wild ungulates and some carnivores, primates
- Requires blood to arrive at lab same day
- Need species-specific detection assays and validation
- Gene expression assay – research tool

Antibody assays*

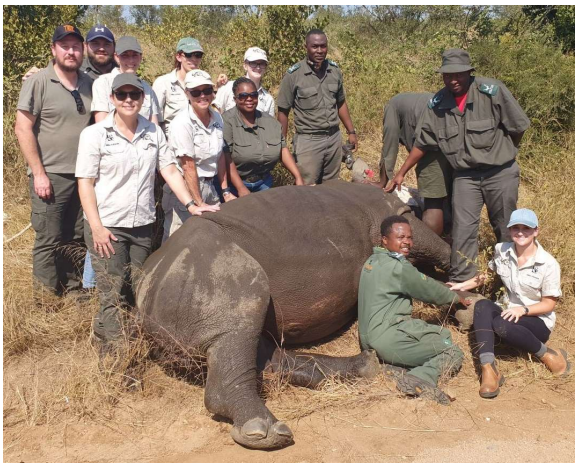
- DPP can be used in most species
- ELISAs often require species-specific reagents
- Useful in suids, elephants, marine mammals, cervids
- Can perform retrospective studies

Mycobacterial culture and PCR

- Direct PCR can provide rapid definitive results
- Mycobacterial culture and speciation remains gold standard for diagnosis

*Blood-based assays

Acknowledgements



➤ Stellenbosch University
Animal TB Research Group



National
Research
Foundation



QUESTIONS?



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