

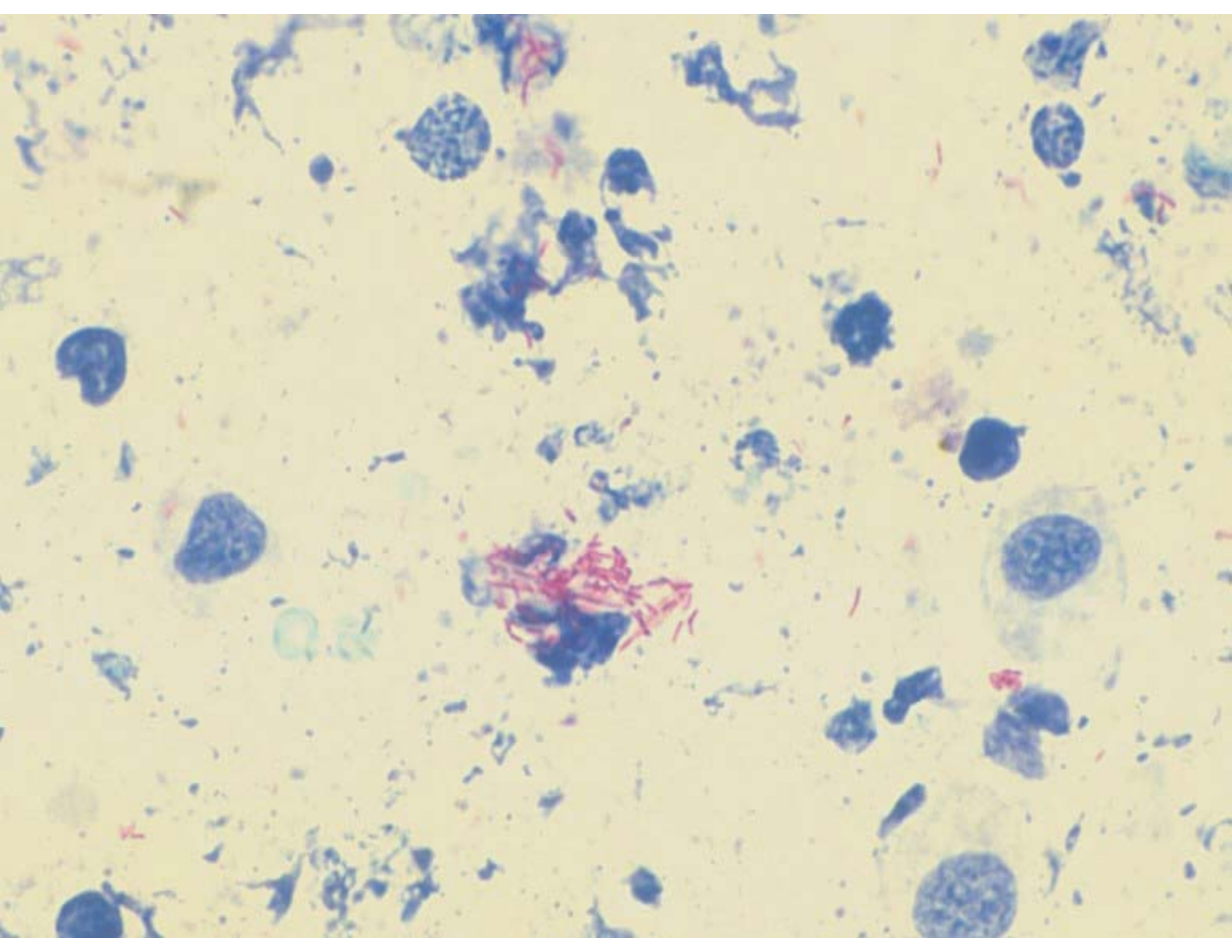
Bovine tuberculosis

Presenter in disguise

20 April 2007

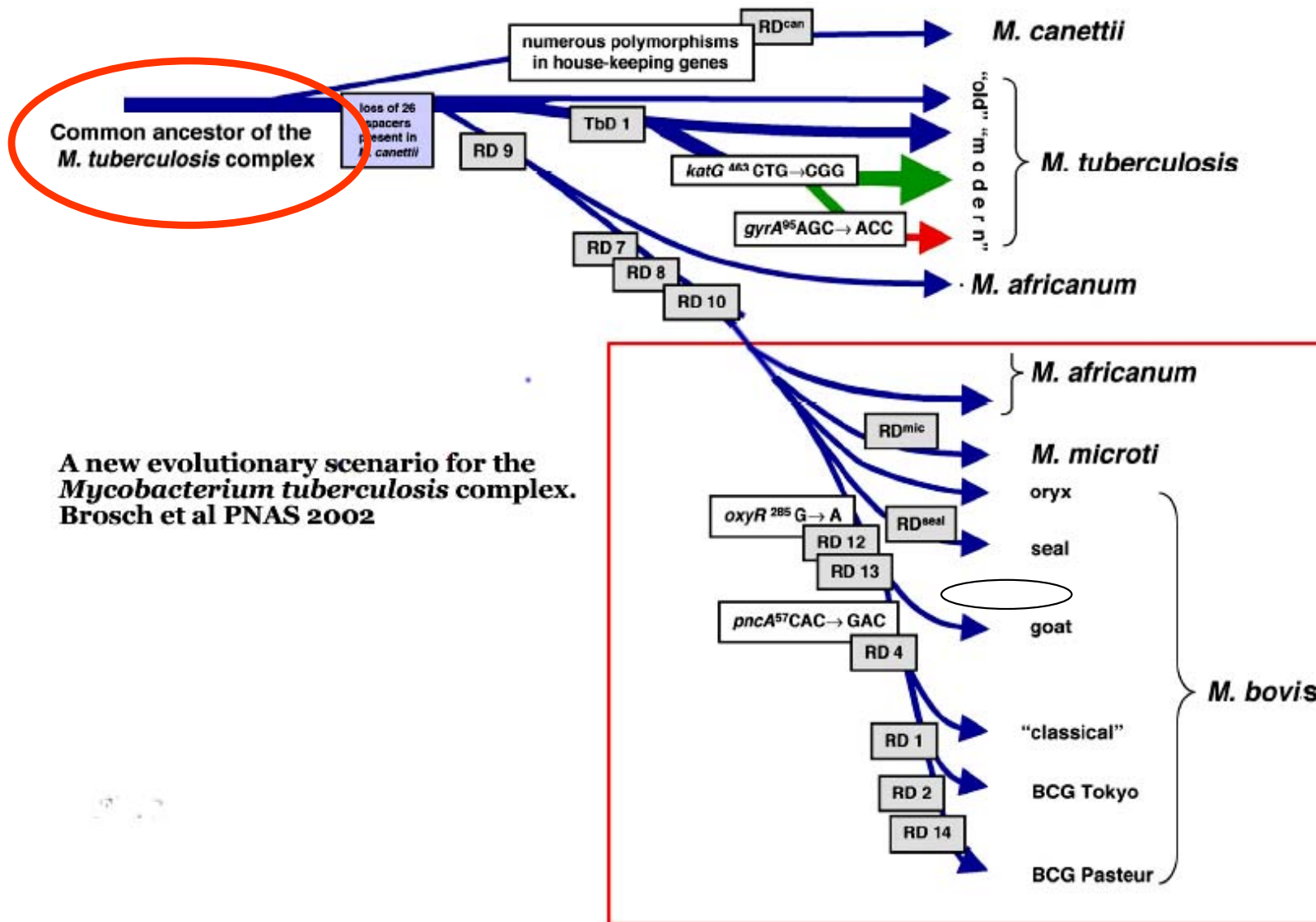
Skukuza Network Meeting





Aetiological agent

- Genus *Mycobacterium* comprises about 85 species
- Many species of this genus occur in the environment and are rarely associated with disease in humans or animals
- However, a number of species of mycobacteria are important pathogens of animals or human beings
- *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium africanum* together with *Mycobacterium microti* (associated with infection of rodents) + *Mycobacterium canettii* form a very closely related phylogenetic group
- Referred to collectively as the *M. tuberculosis* complex (MTBC)



- *M. bovis* is a series of host-adapted 'clones' or 'ecotypes'
- Wide host range

Context: *Mycobacterium bovis*

- **Ubiquitous multi-host bacterium**
- **Humans, livestock, and wildlife**
- **Mode of transmission assumed**
- **Epidemiology unclear**
- **Suspected to be sustained by wildlife sources**

Context: diagnostics

- **Intra-dermal skin tests (comparative)**
- **Sero-diagnosis**
 - Interferon-gamma
 - ELISA
 - Other newer tests
- **Culture**
- **Macroscopic and histological examination**
- **Inter- and intra-species variation**

Context: diagnostics

- **No test that is 100% sensitive and specific**
- **Thus, group tests**
- **Tests not validated for most wildlife species**
- **Varying efficacy of tests depending on stage of the disease**
- **Interplay between cell-mediated and humoral immune responses**

Context: Control

- **Detect and slaughter-out policy for eradication in livestock**
- **In animals:**
 - No effective treatment
 - No effective vaccines
 - Control policies not effective when there is another maintenance host in the environment
- **Focus in Kruger**
 - Improved vaccine
 - Control mechanisms
 - Understanding the epidemiology
 - Level of infection

ELISA with MPB83



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Introduction

- **Different serological tests**
 - FPA (Fluorescent Polarization Assay)**
 - ELISA (Enzyme-Linked Immuno-sorbent Assay)**
 - MAPIA (Multi-antigen Print Immunoassay)**
 - Rapid tests**

Introduction

ELISA: search for the optimal antigen

Research has been done using many antigens, including:

- ESAT6, CFP10, PE13, PE5, MPB70, TB10.4 and TB27.4 (Aagaard *et al.*, 2006)
- MPB83, MPB70, MPB59, Acr1, ESAT-6 and CFP10 (Waters *et al.*, 2005)
- Fusion protein: rM70-83-E6 (recombination of MPB70, MPB83 and ESAT-6) (Siguo Liu *et al.*, 2006)
- MPB70 (work by Gineke Hoogeveen)
- MPB83 in this study

Introduction

MPB83

- Rec antigen that is highly expressed in *M. bovis*
- Exported lipoprotein associated with the bacterial surface (Wiker *et al.*, 1998)
- Specific for *M. bovis*
- Early antigen

Results

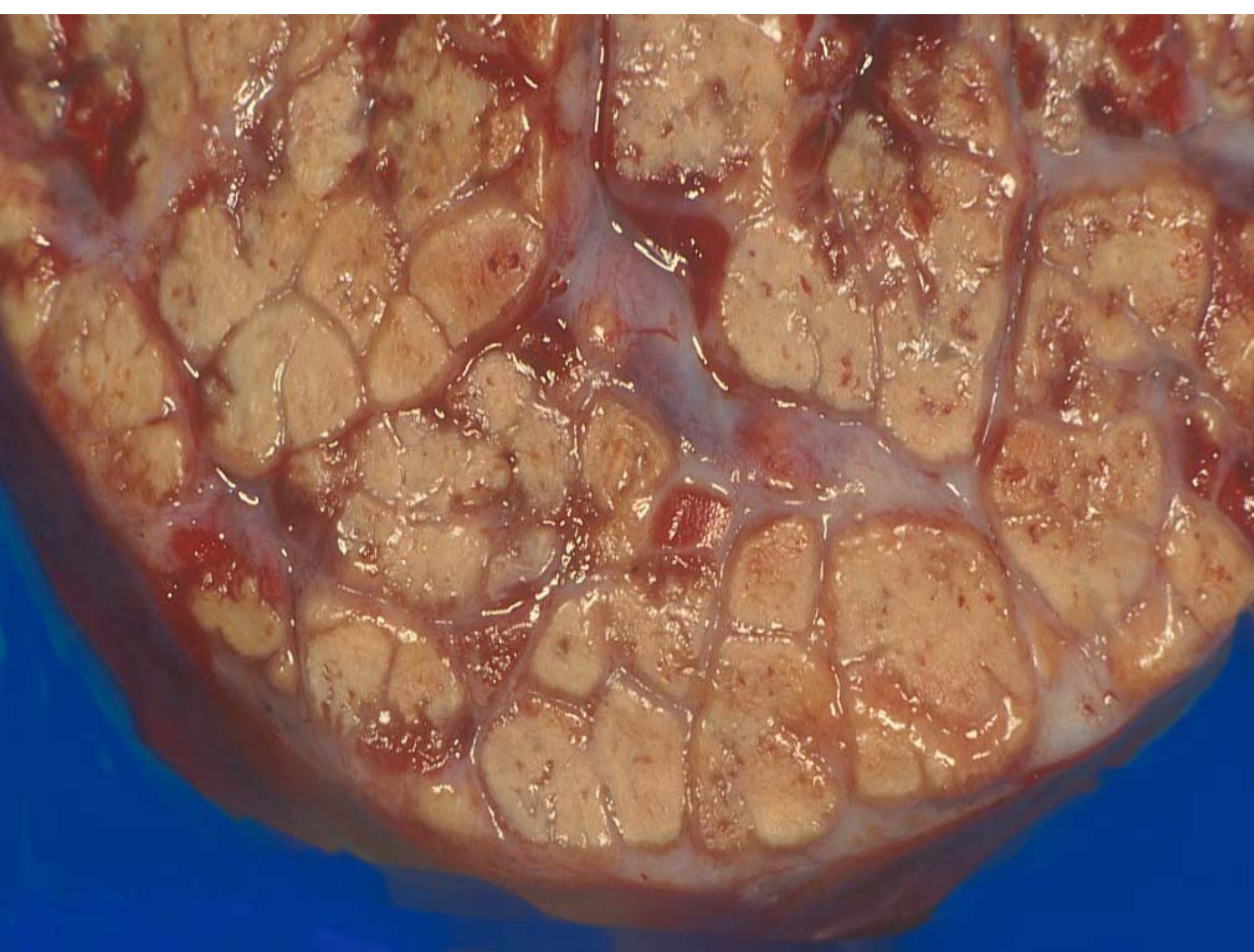
Sera	True positive	True negative	Totals
MPB83-positive	11	1	12
MPB83-negative	87	233	320
Totals	98	234	332
Sensitivity: 11.2%		Specificity: 99.6%	

Discussion

- **OIE advises to test 300 pos and 1000 negative sera**
 - In this study only 98 pos and 234 negative sera were tested
- **Sensitivity of ELISA is low, among others because animals in the early phase of the disease are not detected**

Application

- **Is there a correlation between antibody titers, disease progression and shedding of mycobacteria?**
- **It is anticipated that animals with progressive disease will be detected with the ELISA – these are expected to be shedders**
- **If so, low sensitivity does not matter, only take super-shedders out of the herds (control) to reduce pathogen load**



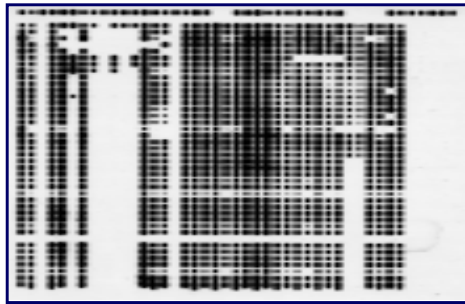
Evaluation of the Epidemiological Relevance of Variable-Number Tandem-Repeat Genotyping of *Mycobacterium bovis* and Comparison of the Method with IS6110 Restriction Fragment Length Polymorphism Analysis and Spoligotyping†

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M. bovis strain typing methods

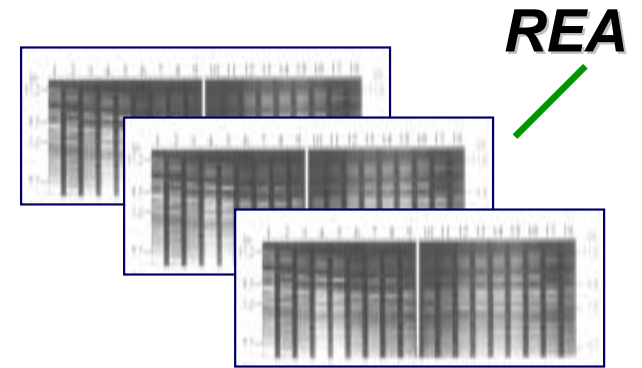


Spoligotyping

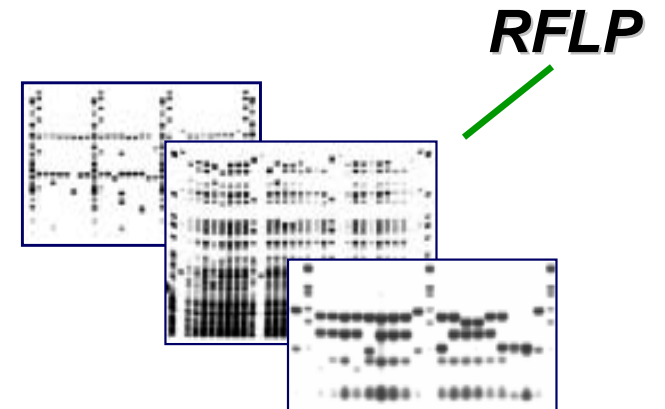
- *Discrimination*
- *Reproducibility*
- *Convenience*
- *Throughput*



VNTR



REA



RFLP

Molecular typing

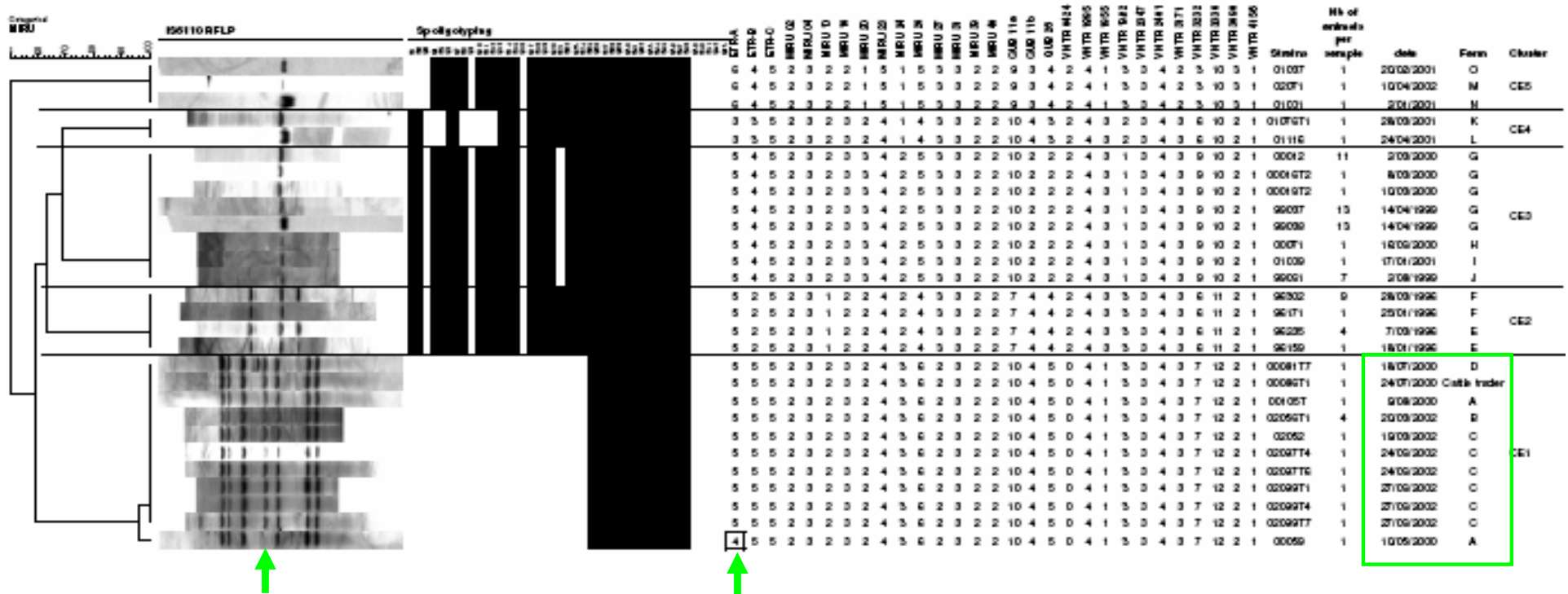
- **Insufficient tests**

- RFLP – limited discrimination between *M. bovis* isolates
- PCR-based spoligotyping – same problem

- **MIRU-VNTR – new possibilities**

- Mycobacterial interspersed repetitive units (MIRU)
- Variable number tandem repeats (VNTR)

Longitudinal stability in isolates from different epi-linked farms



> 2 year outbreak
With 5 herds

Conclusions

- **MIRU-VNTR typing: better resolution than IS6110-RFLP and spoligotyping**
- **Longitudinal stability in almost all 83 epi-linked isolates:**
 - Reliable exclusion method, i.e. even small differences
 - Interpretable as absence of link
- **In low-incidence countries, resolution may be sufficient to trace transmission**
- **Application to be tested in KNP isolates to clarify epidemiology and transmission dynamics**

Two techniques

- **ELISA – selection of ‘super shedders’**
- **MIRU-VNTR – fine-scale molecular epidemiology that will allow better explanation of transmission and expansion of the disease in a complex system**