

MOLECULAR STUDIES ON ZONOTIC TUBERCULOSIS IN MOZAMBIQUE

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Introduction

- *M. tuberculosis* is a most common cause of TB in humans. However, a number of other cases of human TB are caused by *M. bovis* (COSIVI et al.1998)
- While *M. tuberculosis* is responsible for the disease almost exclusively in humans, *M. bovis* has a wide range of hosts where it can cause disease; humans and animals

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Introduction

- Human TB is one of the most widespread infectious diseases and a leading cause of death, particularly in developing countries and especially in Africa.
- Tuberculosis affects animals and humans and is usually a chronic debilitating disease caused by bacteria of the *Mycobacterium tuberculosis* complex (MTC) which includes: *Mycobacterium bovis*, *M. tuberculosis*, *M.africanum*, *M. caprae*, *M. microti* *M. canetti* and *M.pinnipedii*
- All these species showing a very close genetic proximity.

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TRANSMISSION

- *Mycobacterium spp.* can be transmitted through
 - contaminated aerosols
 - milk,
 - faeces,
 - urine,
 - genital fluids,
 - feed and water

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TRANSMISSION

Infected Bov \Rightarrow Agricultural Workers \Rightarrow Typical TB
Couth Spray Inhaling

Such patients may infect cattle, but evidence for human-to-human transmission is limited in immune-competent people (Gutierrez *et al.*, 1997; Cosivi, 1998)

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EPIDEMIOLOGY- MOZ

Table 1 – Epidemiological situation of Human TB in Mozambique

Population	19 424 000
Incidence	460/100 000 pop
Annual prevalence	635/100 000 pop
Annual mortality	129/100 000 pop
HIV incidence in adult TB patients	48%
MDR - TB	3,3%

Mozambique is one of the 22 countries classified by WHO as “High Burden Countries (HBCs)”

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WHO, 2006

EPIDEMIOLOGY

- **9 Million cases** of TB were reported in 2004 and of those, around **2 Million people died** due to the disease (WHO, 2006)
- The situation in Africa has tended to worsen, with HIV playing a key role on the situation increasing number of people getting infected.
- More than 80% of TB infected people live in sub-Saharan Africa and in Asia.

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ZOONOTIC TB IN HUMANS

- *M. Bovis* is responsible for 5-10% of human TB in Latin America, (Haddad *et al.*, 2004).
- These rates are quite similar in Africa (Cosivi *et al.*, 1998)
- Recent studies have reported *M. bovis* as being responsible for 1-6% of human TB in Africa (Cadmus *et al.*, 2005 and Kazwala *et al.*, 2001).

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RISK FACTORS FOR ZONOTIC TB

1. The **close physical contact** between humans and potentially infected animals
(very common in many rural areas in Mozambique)
2. **Infection by HIV**
3. **Poor food hygiene practices**
(Contaminated milk)

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Distinction of the two strains

- distinction between TB by *M. tuberculosis* and that caused by *M. bovis* is not possible with use of current routine diagnostic techniques (Amanfu, 2006).
 - similar clinical signs
 - similar bacteriological characteristics in culture media
 - biochemical differential techniques also can not often reveal the difference



Molecular Biology techniques

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RELEVANCE OF THE PROBLEM

- Mozambique is a country where bovine tuberculosis is present in all regions of the country
- Screenings for bovine tuberculosis have revealed prevalence rates varying from **less than 1% to over 17%** (DINAP,2005).

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RELEVANCE OF THE PROBLEM

- On other hand, there has been an increase in the incidence of human TB and HIV
- The risk factors for zoonotic TB are present in Mozambique, particularly in the rural areas.
- The impact of *M.bovis* in Human TB is Unknown

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➤ This work has been developed to fill the gap, allowing us to learn the real situation of pulmonary tuberculosis by *M.bovis* in the community in general and in HIV seropositive patients in particular, looking back to the epidemiological role and importance of animals as sources of the disease.

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OBJECTIVES

- **General Objective**
Identify the involvement of *M. bovis* in human pulmonary TB
- **Specific Objectives**
 - Study the biodiversity of MTC isolates from cases of TB in two rural areas (Buzi and Manhiça) in Central and Southern Mozambique;
 - Identify risk factors important in zoonotic TB in the two rural areas

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Material and Methods

- **2 Districts**
 1. Buzi (Central Mozambique)
 2. Manhiça (Southern Mozambique)

(areas where both bovine & human TB coexist)

	População	Efectivo bovino	Bovine TB
Buzi	179 000Hab	4000	8,5% ¹
Manhiça	192 638Hab	12318 (75% family sector)	0,7% ²

¹DINAP, 2005

²DDADRM

- **Samples**
 - Human sputum isolates



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Material and Methods

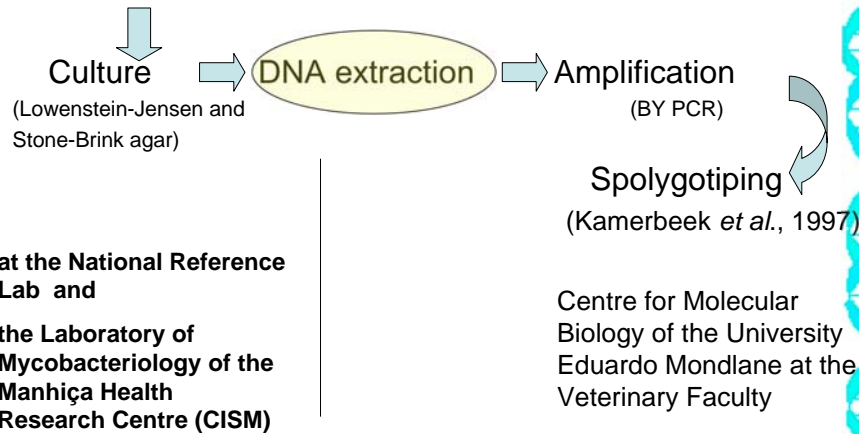
- **Criteria for inclusion in the study**
- **Sex:** Both
- **Age:** Older than 14
- **Health:** Pulmonary TB patient with BK+
- **Acceptance of involvement in the study**
- **HIV Test** (if agreeing to undertake)
 - 2 rapid tests (UniGold™ and Determine™)

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Method

Smear Microscopy (ZN)



at the National Reference Lab and the Laboratory of Mycobacteriology of the Manhica Health Research Centre (CISM)

Centre for Molecular Biology of the University Eduardo Mondlane at the Veterinary Faculty

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Spolygotiping

- A PCR-based method to simultaneously detect and type the close related Mycobacterium was used for typification in this study (Kamerbeek *et al.*, 1997)
- The spolygotiping (*spacer oligonucleotide typing*) method is based on the presence or absence of any of the 43 spacing sequences (“spacers”) located at the direct repetition (DR) region of CMT Mycobacterias.

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Spolygotiping

- The method allows quick screening (48H)
- The results obtained are of easy interpretation and
- can be easily shared in international databases of genotypes of Mycobacterium

The differential characteristic of *M. bovis* is lack of the spacers 39 and 43

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Hybridization pattern of the isolates



(Kamerbeek *et al.*, 1997)

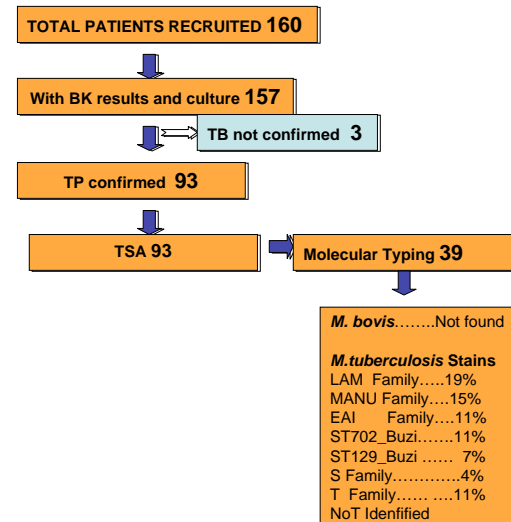
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RESULTS

- A dendrogram showing the patterns of hybridization of the different samples was developed. [SpoligoType.xls](#)
- The spoligotyping results were compared to the World Spoligotyping Database of the Pasteur de Guadeloupe Institute (SPoIDB4) <http://www.pasteur-guadeloupe.fr/tb/spoldb4>

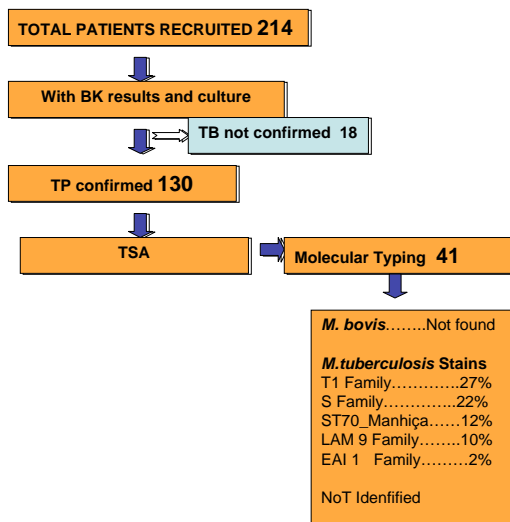
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Results Diagram - BUZI



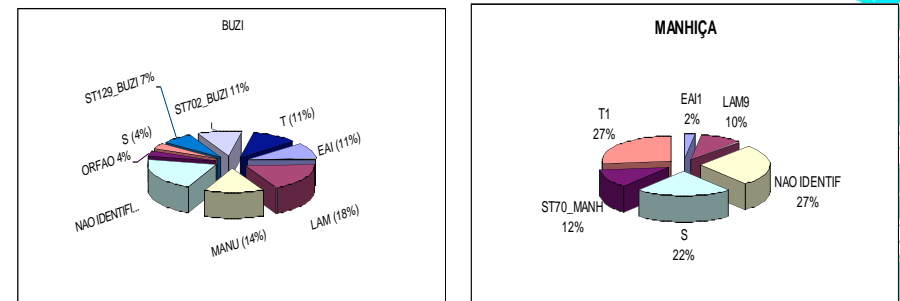
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Results Diagram - Manhiça



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The following diagram shows the distribution of encountered genotypes



These genotypes are similar to some reported in neighbour countries including (Brudey, 2006; Nicol et al. 2005)

Remarks

- Contrary to expected results, no *M. bovis* was identified in the isolates submitted to genotyping

Sample size n=80

Study by Nunes E.(2004) in Maputo n=232 HIV positive patients with pulmonary TB, no *M. bovis* was detected

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Remarks

- The Genotypes founded are the same reported in neighbour countries
- Strains isolated from Buzi showed a wide diversity while in Manhiça they are limited
 - Related with more migratory movements ???
 - Low diversity of genotypes is a indication that the source of infection are the same

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Study Constraints

1. The culture of Mycobacterium under routine techniques - with **glycerol in the culture media** – negatively influenced the growth of *M. bovis*. Culture media enriched with Pyruvate (more specific and favouring growth) are recommended for cultivation of *M. bovis*.
1. Practicing the method was a long process.
2. we experienced **several power breaks at freezing unit** negatively contributed, as several samples were lost.
3. **DNA samples extracted from clinical cases** (sputum) had poor quality and resulted in deprived hybridization pattern.

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Recommendations

- Undertake more epidemiological studies in geographical areas where bovine TB is highly prevalent.
- Specific risk populations should be the main targets in future studies (cattle carers, slaughter house workers and veterinarians)
- Studies on extra-pulmonary TB (intestinal and ganglionic) which may be related to consumption of contaminated raw milk should be undertaken
- Use pyruvate enriched media to boost growth of *M. bovis* in culture media.

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THANK YOU