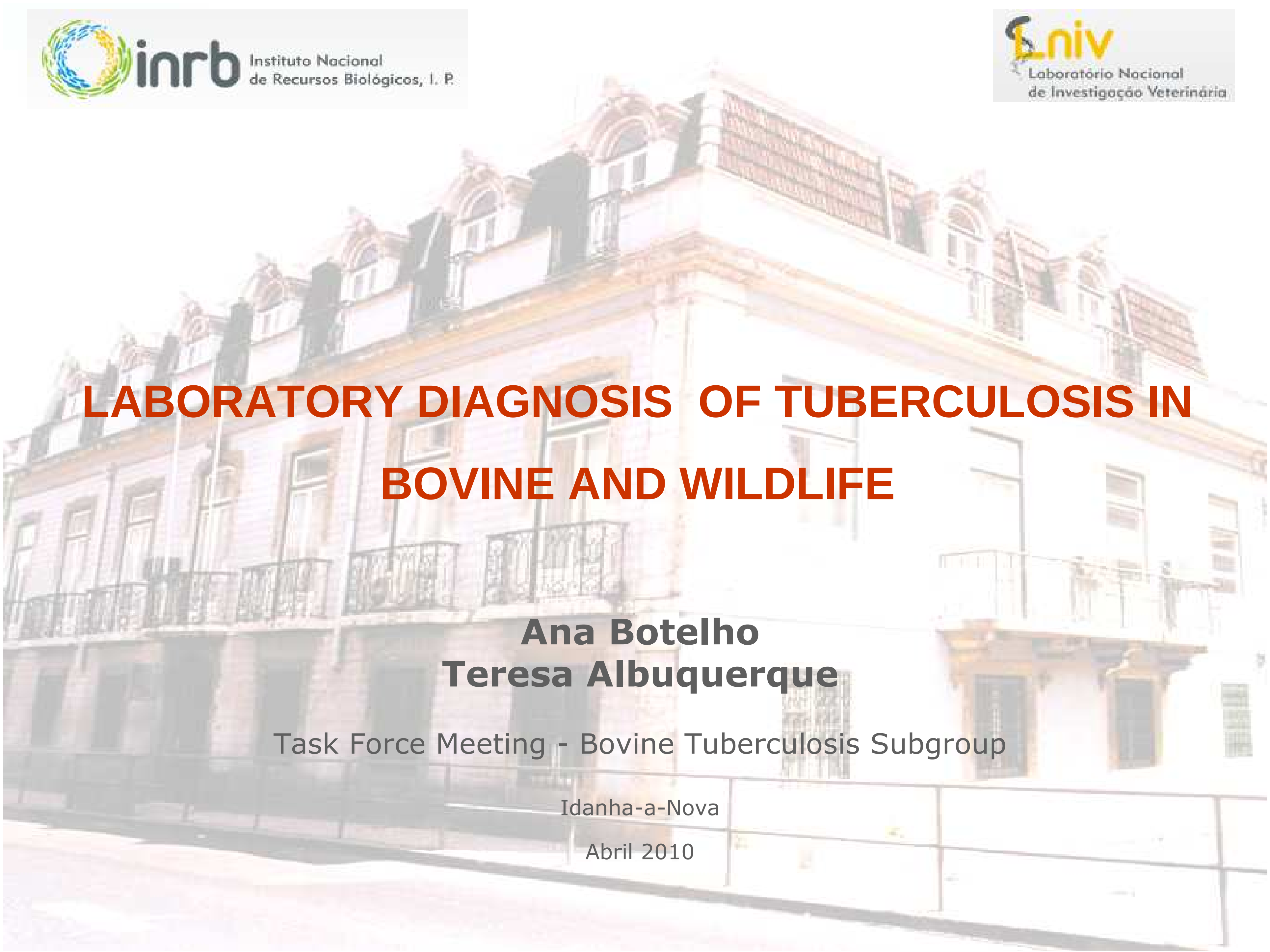


inrb Instituto Nacional de Recursos Biológicos, I. P.

LNIV Laboratório Nacional de Investigação Veterinária

LABORATORY DIAGNOSIS OF TUBERCULOSIS IN BOVINE AND WILDLIFE

Ana Botelho
Teresa Albuquerque

Task Force Meeting - Bovine Tuberculosis Subgroup

Idanha-a-Nova

Abril 2010

ANTE MORTEM DIAGNOSTIC TOOLS FOR TB
Detection of *M. bovis*-infected cattle

Tests based on cellular immunity

Official test

In vivo

- Intradermal Tuberculin test (IDTB) (TB/TA)

Complementary test

In vitro

- Interferon-gamma test (Bovigam)

POST MORTEM OBSERVATION OF SUSPECTED LESIONS

Samples colection at the abattoir from seropositive animals

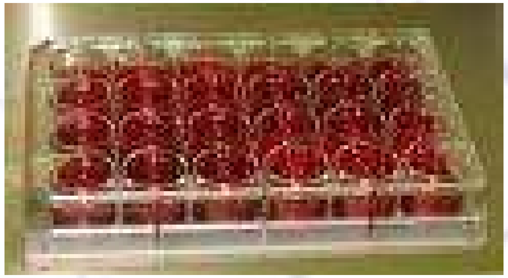
Samples colection at the abattoir from unsuspected animals with tuberculous lesions

LABORATORY APPROACHES TOWARDS A DIAGNOSIS

Ante mortem

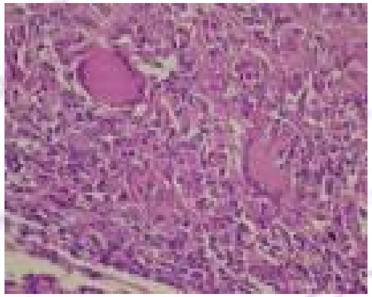
Serological

γ - IFN test

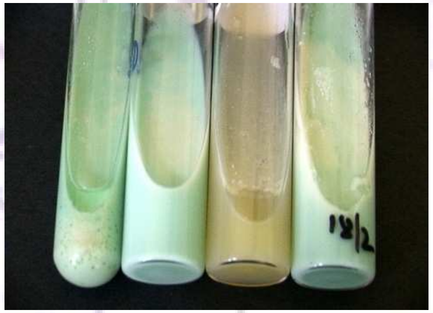


Post mortem

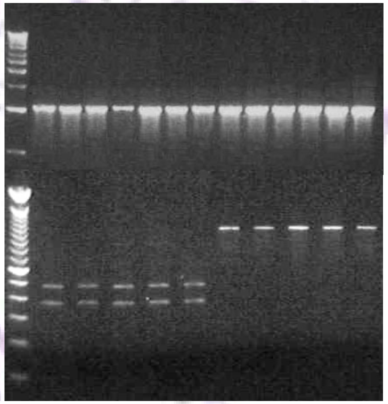
Histological



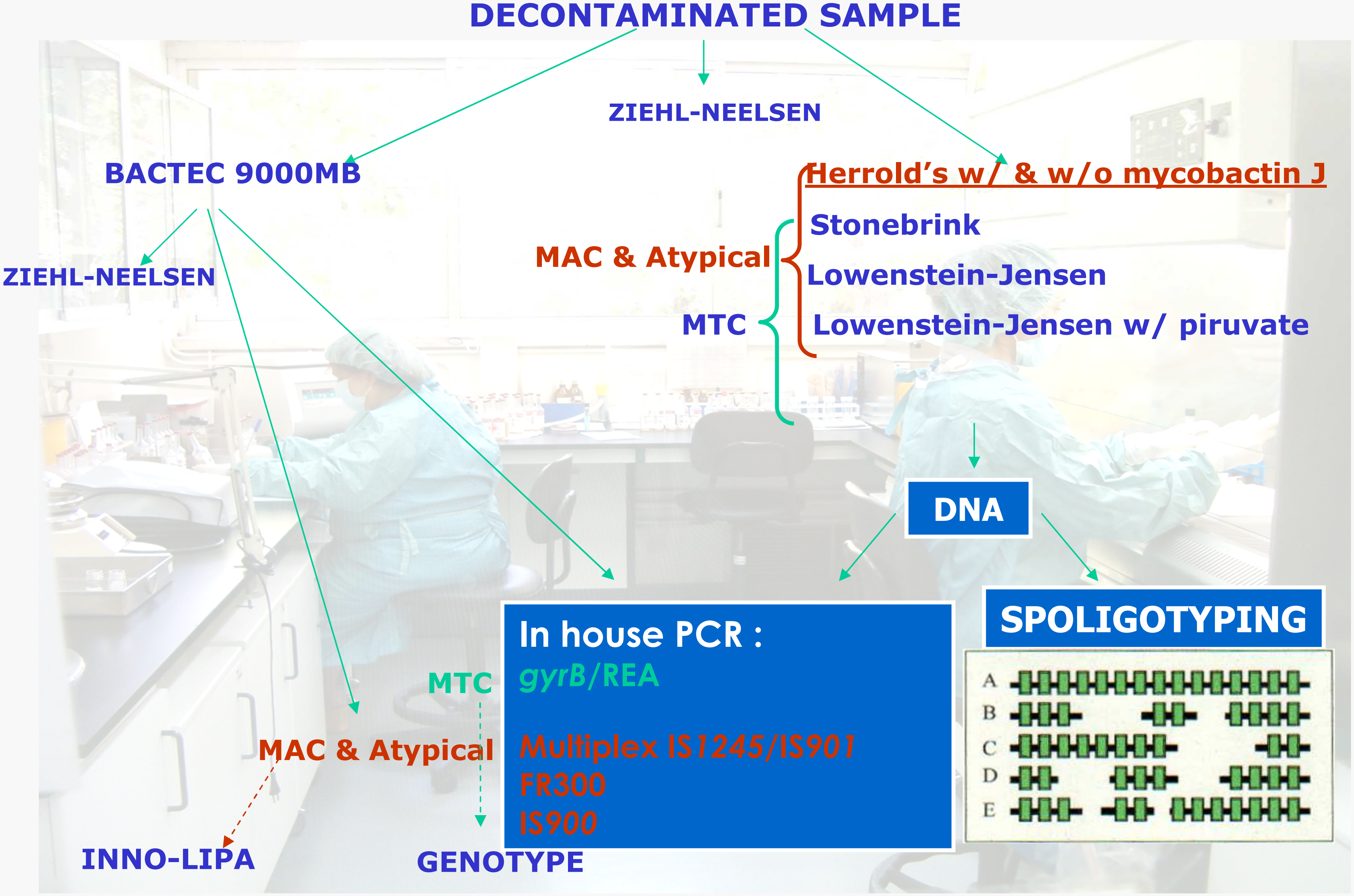
Bacteriological



Molecular



LABORATORY APPROACHES TOWARDS A DIAGNOSIS



DECONTAMINATED SAMPLE

ZIEHL-NEELSEN

BACTEC 9000MB

ZIEHL-NEELSEN

Herrold's w/ & w/o mycobactin J

Stonebrink

Lowenstein-Jensen

Lowenstein-Jensen w/ piruvate

MAC & Atypical

MTC

DNA

In house PCR :

gyrB/REA

Multiplex IS1245/IS901

FR300

IS900

SPOLIGOTYPING

MTC

MAC & Atypical

INNO-LIPA

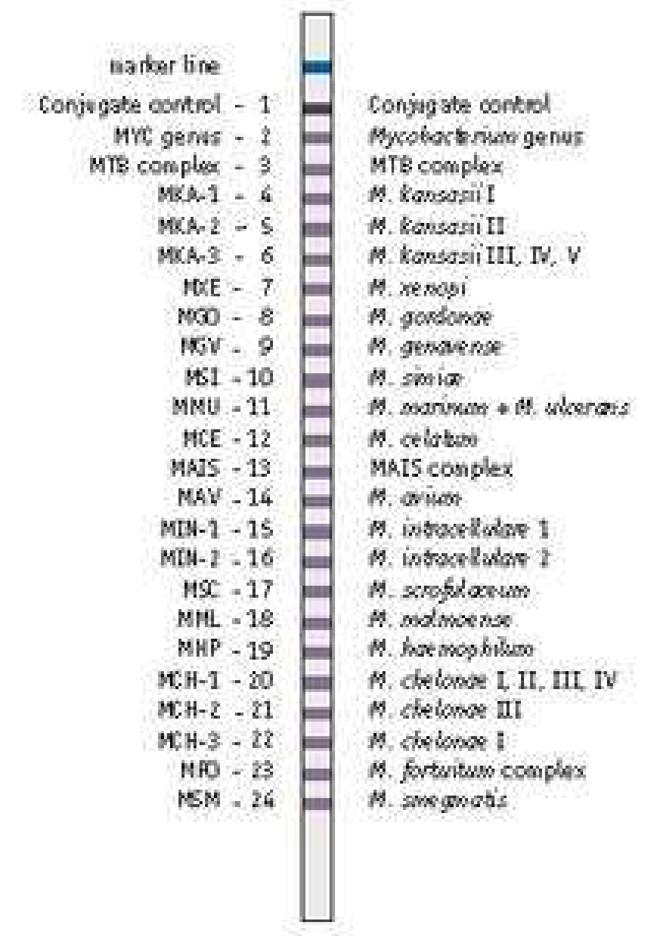
GENOTYPE

LABORATORY APPROACHES TOWARDS A DIAGNOSIS

Molecular identification

INNO-LIPA Mycobacteria (Innogenetics):

ITS- Internal Transcriber Spacer (16S-23S) : 16 species (including MTC)



Genotype MTBC (Hain Lifescience), multiplex PCR:

23S, RD1, *gyrB* : differentiation of MTC species

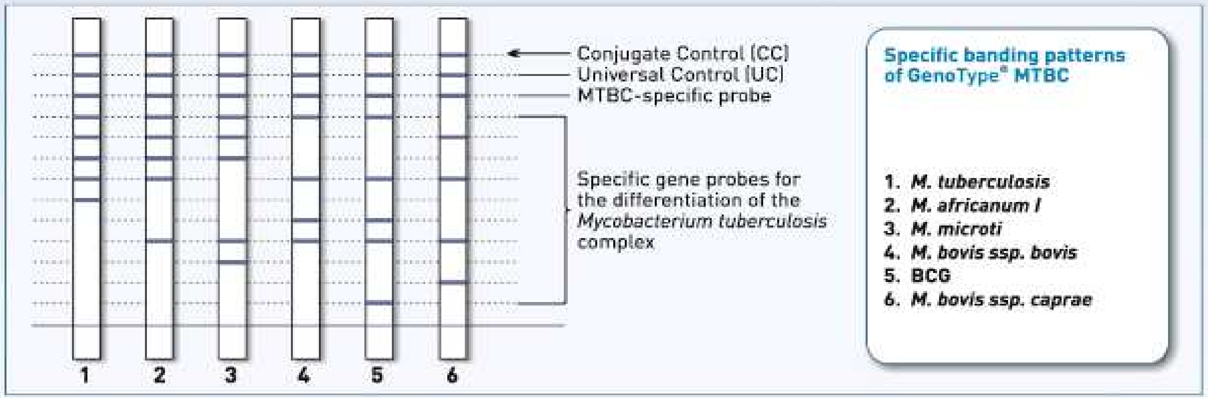


Fig. 1: Reaction zones of the **GenoType® MTBC**

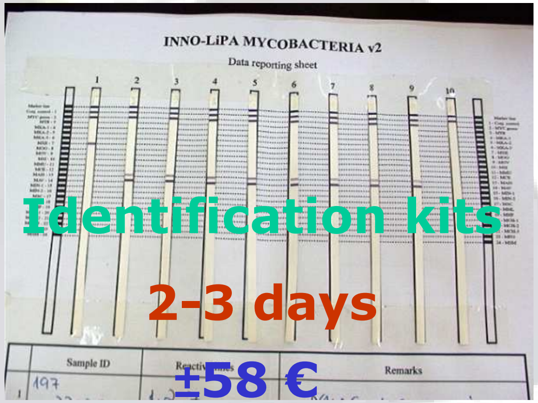
LABORATORY APPROACHES TOWARDS A DIAGNOSIS

TIME & COSTS

± 21 days

±20 €

21 – 45 days


INNO-LiPA MYCOBACTERIA v2

Data reporting sheet

Sample ID

Reactivity

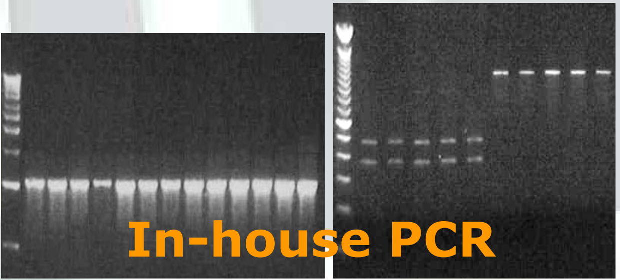
Remarks

197

Identification kits

2-3 days

±58 €


In-house PCR

2 – 3 days

±25 €

TOTAL

24 – 48 days

± 45 €

TOTAL

± 24 days

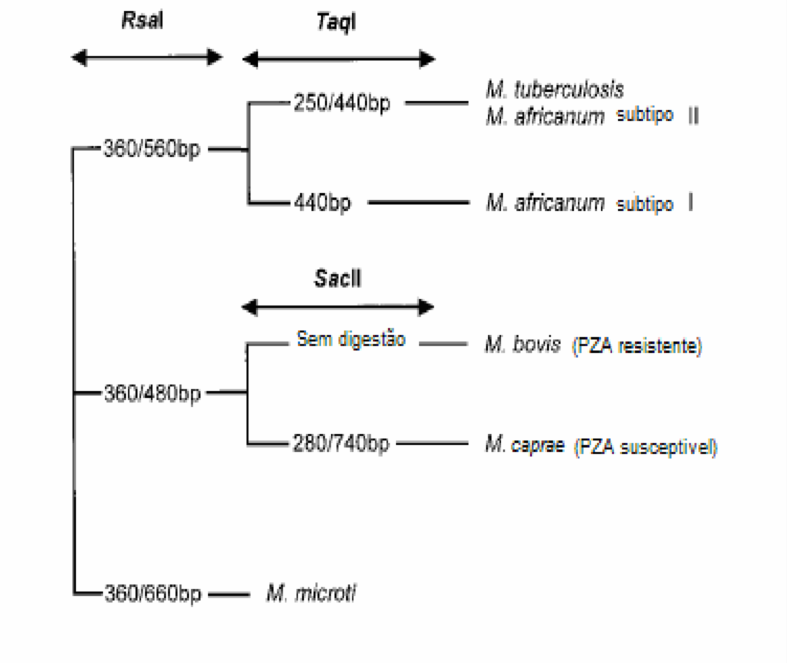
± 78 €

LABORATORY APPROACHES TOWARDS A DIAGNOSIS

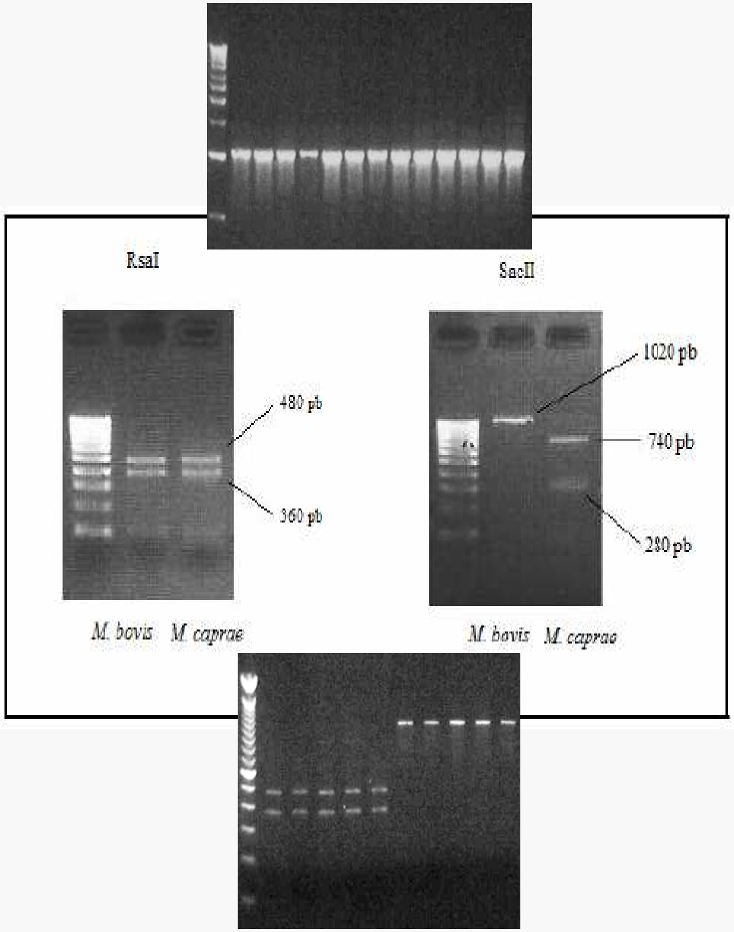
Molecular identification by *in-house* PCR

***PCR gyrB*/REA** for *Mycobacterium tuberculosis* complex (MTC)

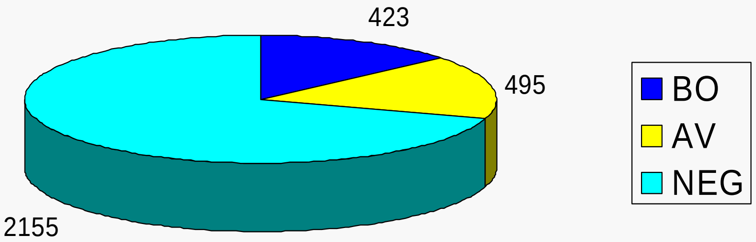
Based on *gyrB* gene polimorphisms

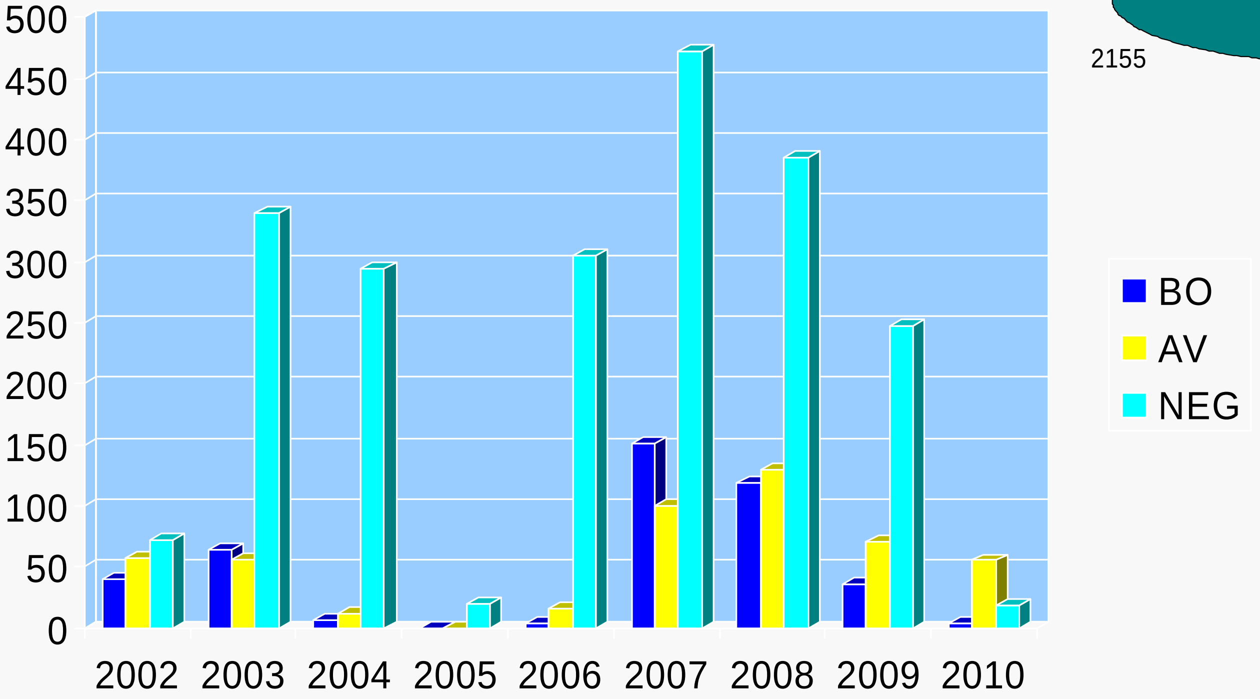


Niemann *et al*., 2000

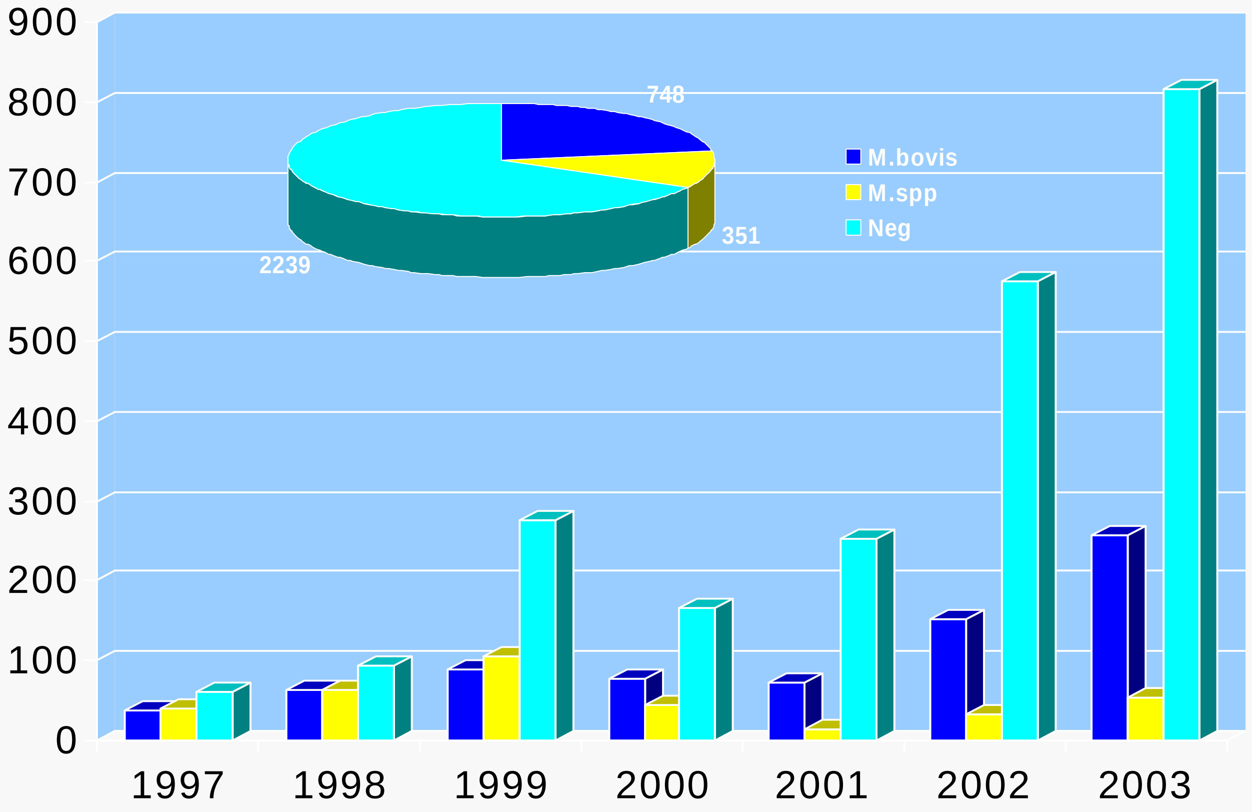


IFN- gamma assays 2002-2010

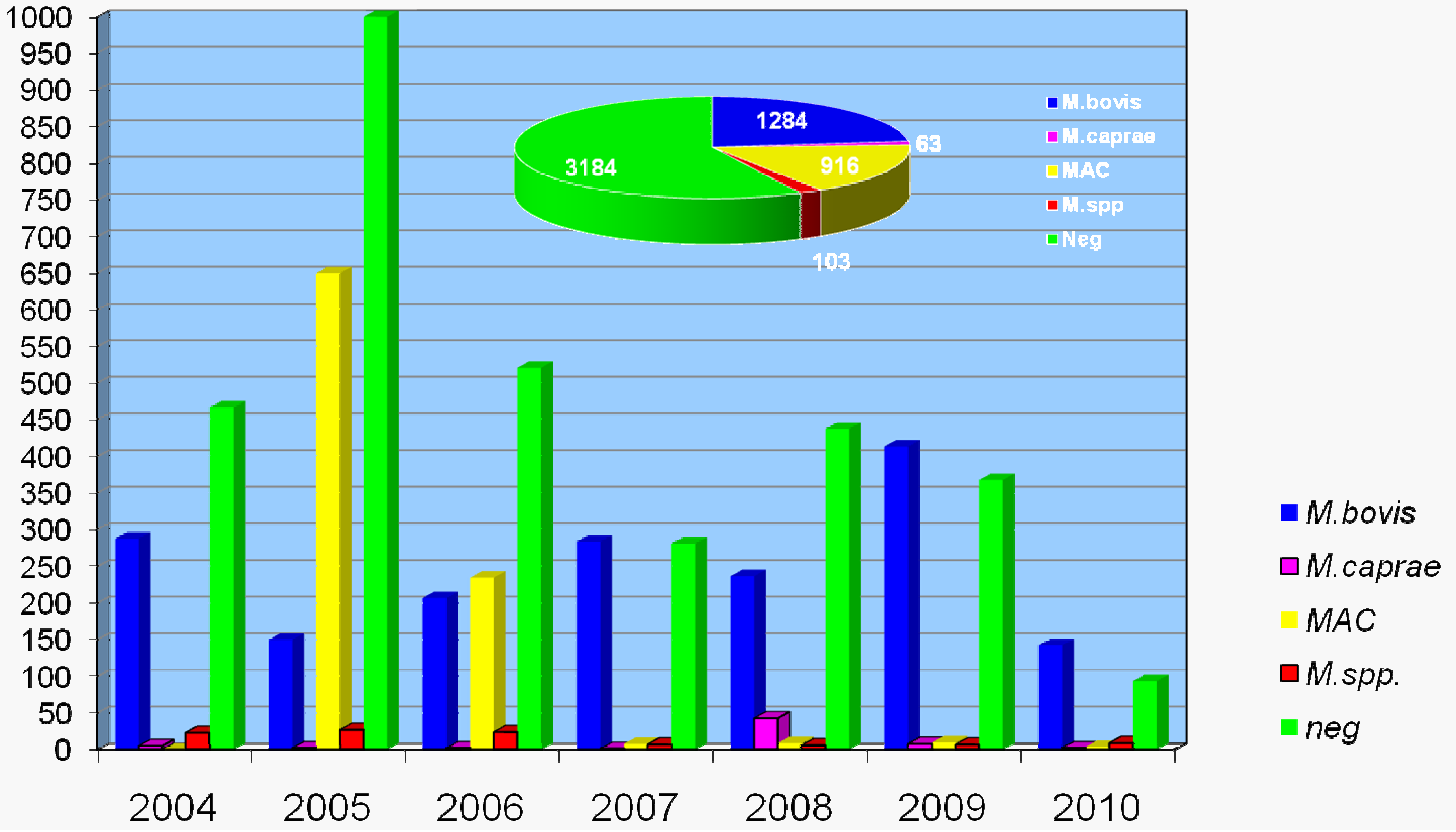




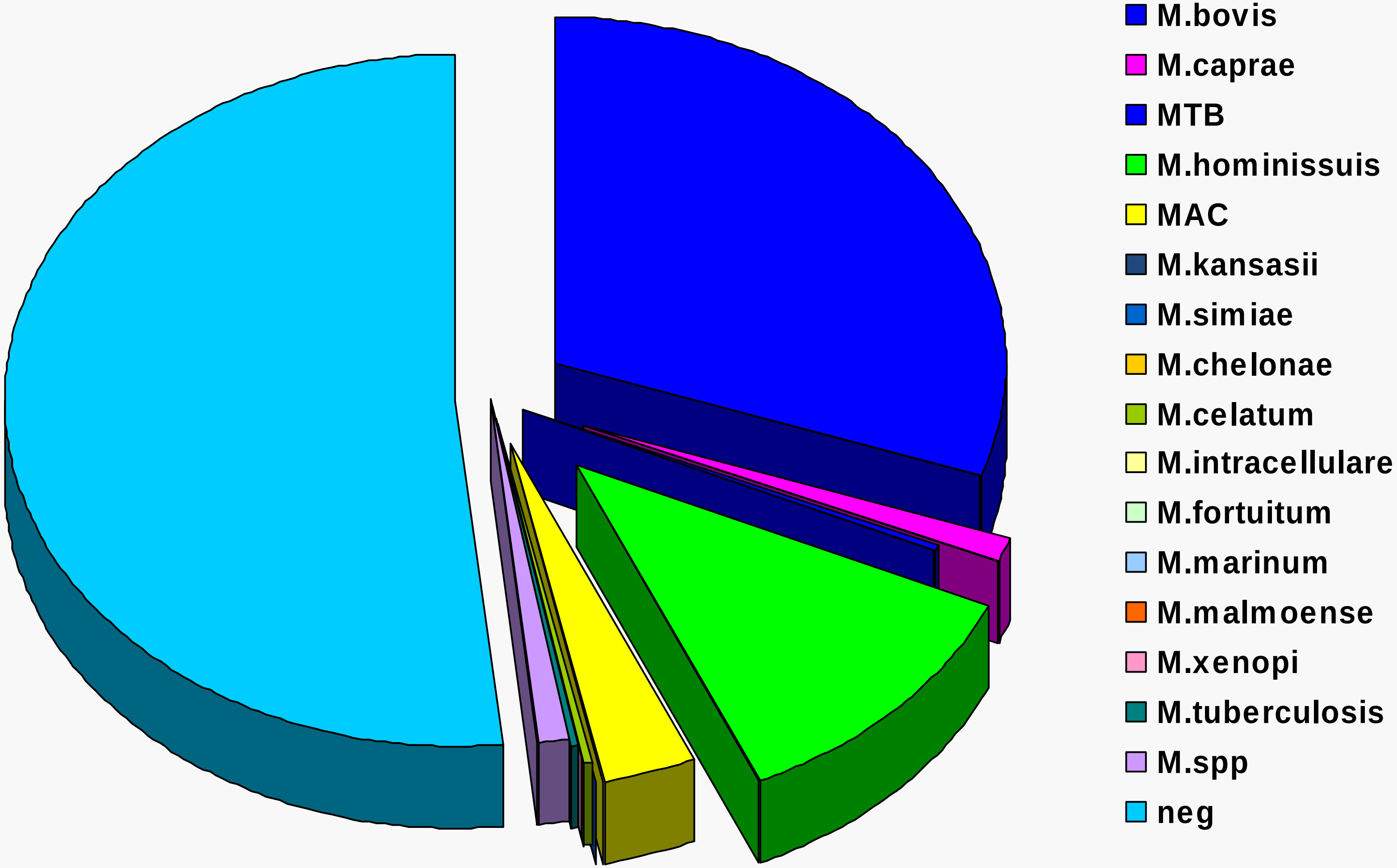
Bacteriological isolation and identification (1997-2003)



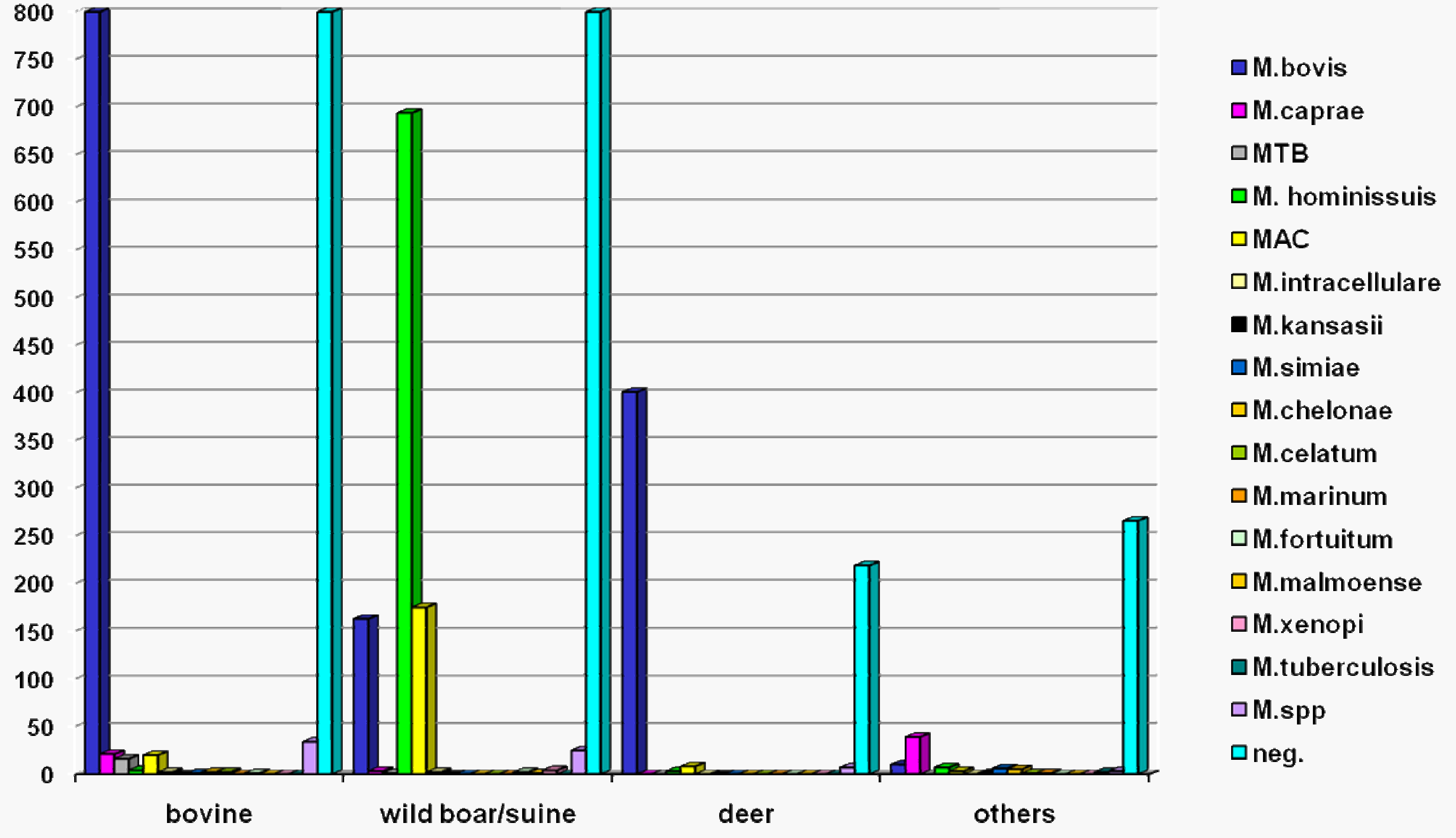
Bacteriological isolation and identification of Mycobacteria in domestic and wild life (2004-2010)



Bacteriological isolation and identification of Mycobacteria in domestic and wild life (2004-2010)



Bacteriological isolation and identification in domestic and wild life (2004-2010)



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Molecular Typing of *M. bovis* and *M. caprae* isolates

- Compare the Portuguese genotypes with other identified in Europe (www.Mbovis.org)

- Determine the frequency and temporal and geographic distribution of *M. bovis* and *M. caprae* genotypes (structure of *M. bovis* and *M. caprae* population in Portugal)

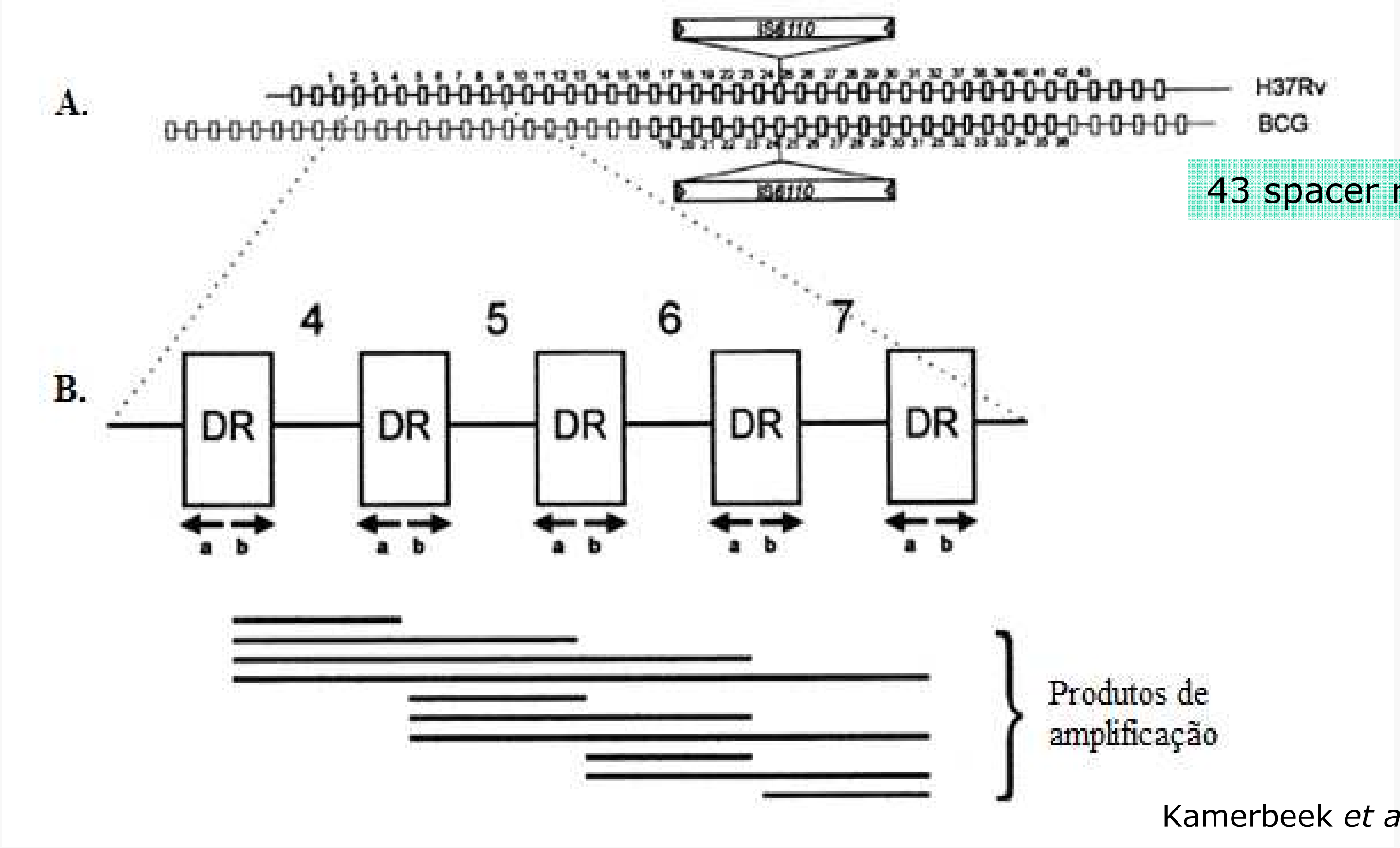
- Evaluate the epidemiological link between domestic and wild life isolates

- Evaluate the existence and type of bTB reservoirs

- Assess the source of infection and routes of transmission of bTB

SPOLIGOTYPING *(Spacer Oligonucleotide Typing)*

Polimorphisms in *locus* DR (*Direct Repeat*), of MTC species.

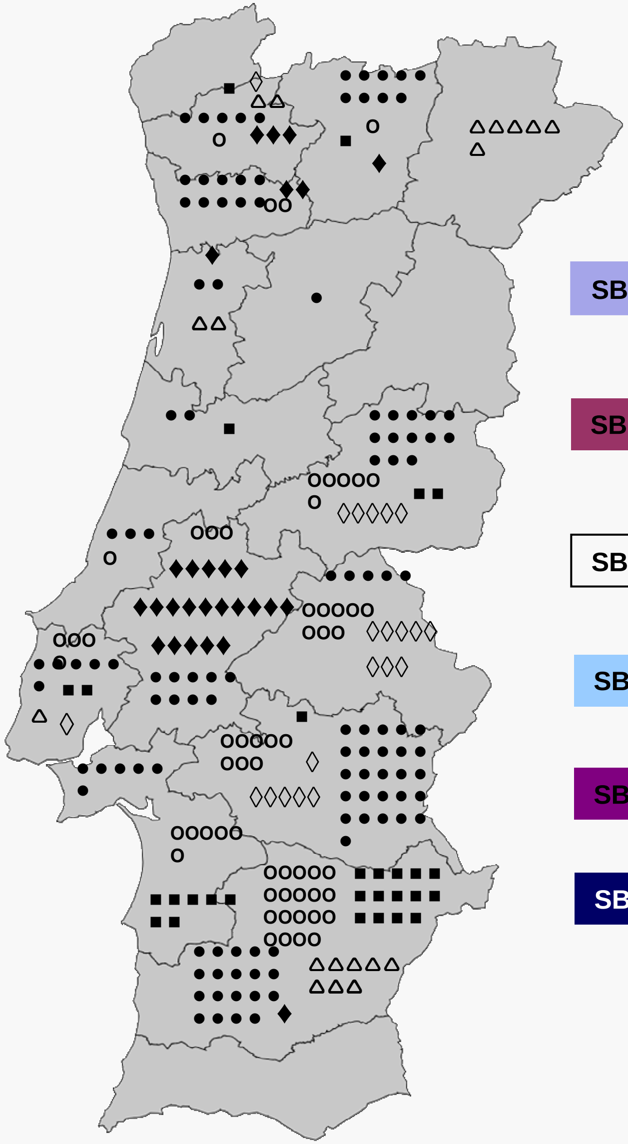


43 spacer regions

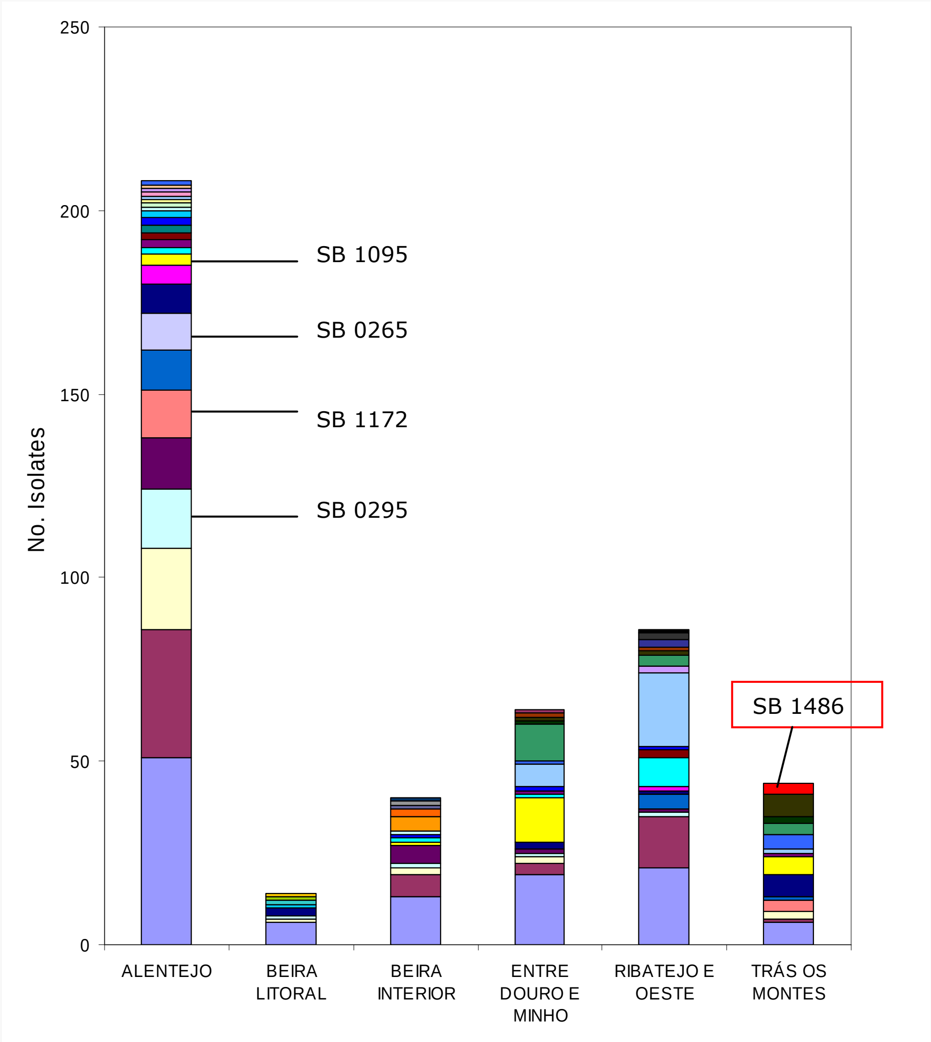
Kamerbeek *et al.*, 1997

M. BOVIS DIVERSITY REVEALED BY SPOLIGOTYPING

Geographic distribution of Spoligotypes in Portugal (domestic animals)



SB0121	●
SB0119	o
SB0140	■
SB0886	♦
SB1090	△
SB0157	◊



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M. BOVIS/M. CAPRAE DIVERSITY REVEALED BY SPOLIGOTYPING

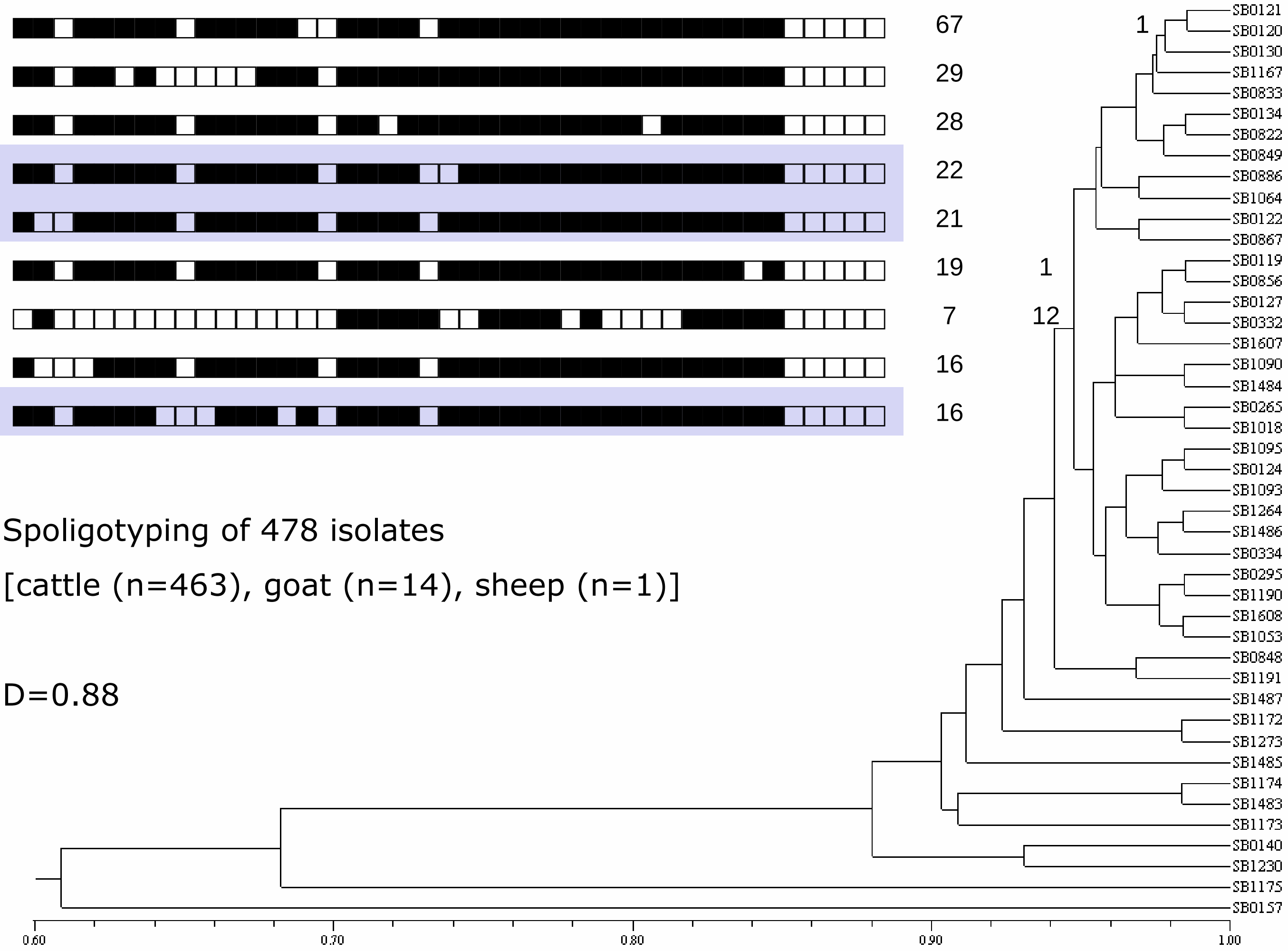
TOP 10 Spoligotypes in domestic animals

Spoligotypes	Pattern	C	G	S	Number of isolates (%)
SB0121	■■□■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	124			124 (25,94)
SB0119	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	67		1	68 (14,23)
SB0140	■■□■■□■□□□□□■■■□■■■■■■■■■■■■■■■■■■■■■■□□□□□	29			29 (6,07)
SB0886	■■□■■■■■□■■■■■■□■■□■■■■■■■■■■■■□■■■■■■□□□□□	28			28 (5,86)
SB1090	■■□■■■■■□■■■■■■□■■■■□□■■■■■■■■■■■■■■■■□□□□□	22			22 (4,60)
SB1095	■□□■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	21			21 (4,39)
SB0295	■■□■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■□■□□□□□	19	1		20 (4,18)
SB0157	□■□□□□□□□□□□□□□□■■■■■□□■■■■□■□□□□■■■■■□□□□□	7	12		19 (3,97)
SB0124	■□□□■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	16			16 (3,35)
SB1172	■■□■■■■□□□■■■□■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	16			16 (3,35)

Spoligotyping of 478 isolates

[cattle (n=463), goat (n=14), sheep (n=1)]

D=0.88



M. BOVIS DIVERSITY REVEALED BY SPOLIGOTYPING

TOP 10 Spoligotypes in wild life

Spoligotyping of 110 isolates from wildlife [deer (n=74), wild boar (n=36)]

D=0.93

Spoligotypes	Pattern	D	B	Number of isolates (%)
SB0121	■■□■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	10	4	14 (12,73)
SB1264	■□□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	8	6	14 (12,73)
SB0119	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	6	4	10 (9,09)
SB0122	□■□■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	10		10 (9,09)
SB0265	■■□■■□■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	6	3	9 (8,18)
SB1195	■■□■□■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	4	4	8 (7,27)
SB1266	□■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	4	3	7 (6,36)
SB0120	■■□■■■■■□■■■■■■□■■■■■■■■■■■■■■■■■■■■■■□□□□□	5	1	6 (5,45)
SB0856	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□□	3	1	4 (3,64)
SB1174	■■□□■■■■□■■■□■■□■■■■■■■■■■■■■■■■■■□□■■□□□□□	3	1	4 (3,64)

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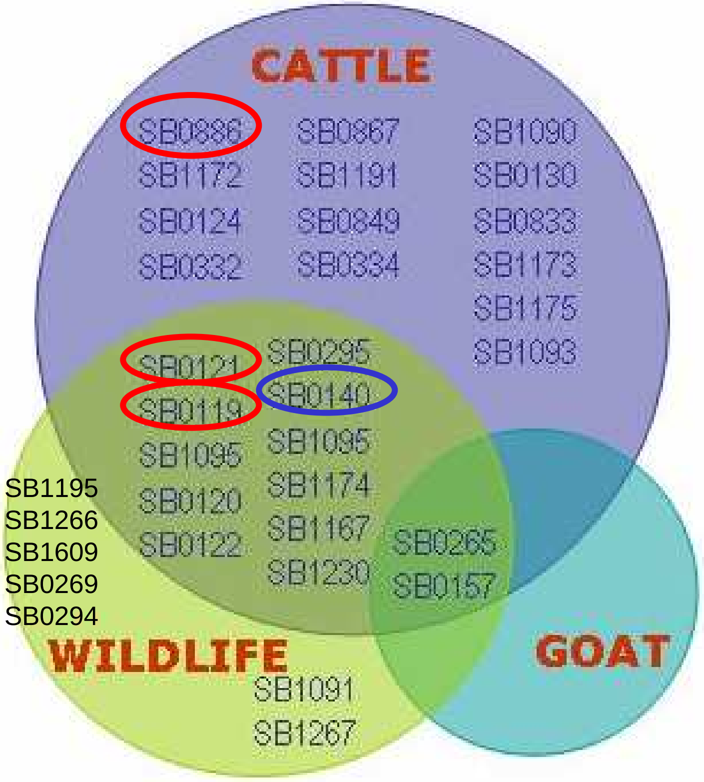
M. BOVIS DIVERSITY REVEALED BY SPOLIGOTYPING

Host distribution of Spoligotypes



CATTLE

SB0886 SB0867 SB1090
SB1172 SB1191 SB0130
SB0124 SB0849 SB0833
SB0332 SB0334 SB1173
SB1175
SB1093

SB0121 SB0295
SB0119 SB0140
SB1095 SB1095
SB0120 SB1174
SB0122 SB1167
SB1230

SB0265
SB0157

SB1195
SB1266
SB1609
SB0269
SB0294

WILDLIFE

SB1091
SB1267

GOAT

- High diversity of spoligotype patterns: 50 different in 588 isolates
- 8.4% of herds presented more than one pattern

Available online at www.sciencedirect.com

ScienceDirect

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veterinary
microbiology

Short communication

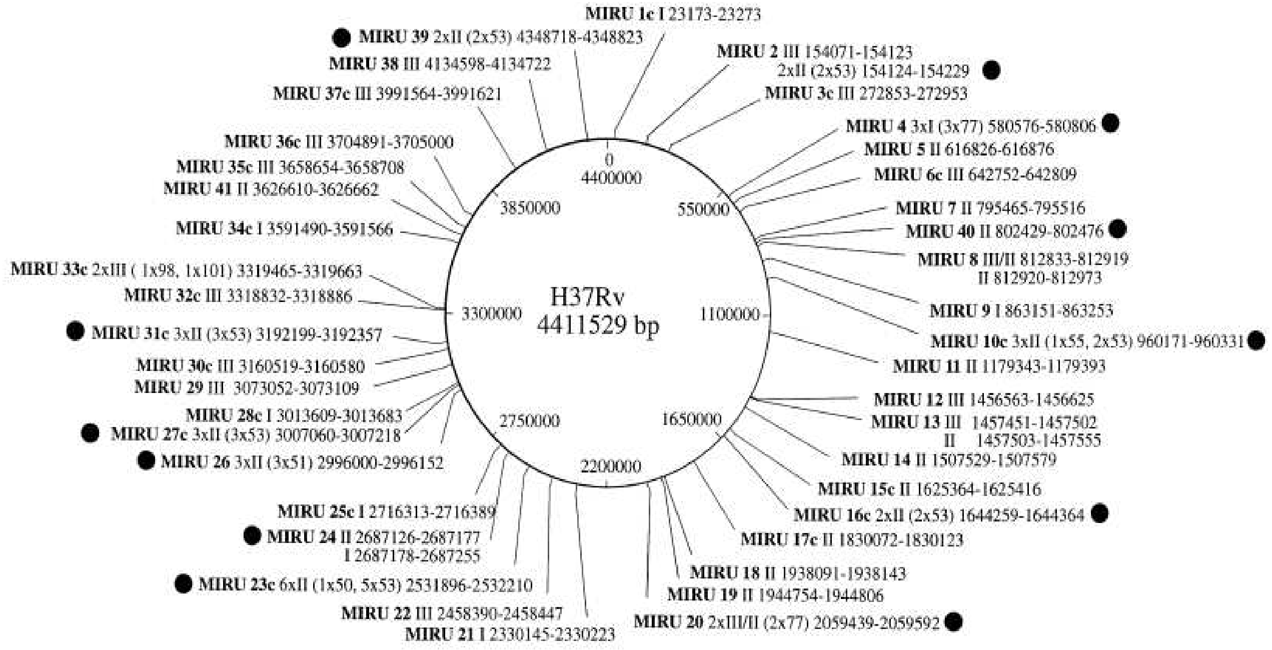
Spoligotype diversity of *Mycobacterium bovis* and *Mycobacterium caprae* animal isolates

E.L. Duarte[a,b], M. Domingos[a], A. Amado[a], A. Botelho[a,*]

MIRU-VNTR

(*Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeats*)

Polimorphisms in the number of tandem repeats



- PCR - eight *loci*:

  VNTR 3232
  ETR-A
  ETR-B
  ETR-C
  QUB 11a
  QUB 11b
  MIRU 4
  MIRU 26

- Nº tandem repeats

Supply *et al*., 2000

M. BOVIS DIVERSITY REVEALED BY SPOLIGOTYPING-MIRU ASSOCIATION

MIRU-VNTR typing of 12 spoligotypes (181 strains) provided 107 different profiles (discriminatory index *h*= 0.98)

MIRU-VNTR allelic profile

Spoligotype	MIRU profiles	VNTR3232	ETR-A	ETR-B	ETR-C	QUB11a	QUB11b	MIRU 26	MIRU 4	**Number of isolates**
SB0121 n=70	**44 MIRU profiles**	7	6	4	1	11	2	5	3	6
		7	6	4	2	*I*	1	2	3	5
		7	6	4	4	10	2	5	3	4
SB0119 n=20	**10 MIRU profiles**	7	6	4	2	*i*	1	2	3	6
		7	6	4	4	11	2	5	2	4
SB0886 n=27	**7 MIRU profiles**	8	7	2	4	7	4	6	3	21
SB0140 n=26	**8 MIRU profiles**	10	7	5	4	11	4	5	3	18
		10	7	5	2	11	4	5	3	2

E. L. Duarte [a, b], M. Domingos[a], A. Amado [a], M. V. Cunha[a] and A. Botelho [a*] (2009) . MIRU-VNTR typing adds discriminatory value to groups of *Mycobacterium bovis* and *Mycobacterium caprae* strains defined by spoligotyping. Vet. Microbiol doi:10.1016/j.vetmic.2009.11.027 (in press)

M. BOVIS DIVERSITY REVEALED BY SPOLIGOTYPING-MIRU ASSOCIATION

- High genotype diversity confirmed by MIRU-VNTR
- SB0121 represents a heterogenous group with genetically diverse strains
- *Locus* VNTR 3232 showed a very high diversity with 13 different alleles and several double alleles

INFORMATION FROM SPOLIGOTYPING-MIRU ASSOCIATION

- Detection of multi-genotype infected herds in the same outbreak
- Cases of possible reactivation in five herds
- Introduction of one infected animal from another herd
- Movement of animals between herds
- Existence of wild animal reservoirs

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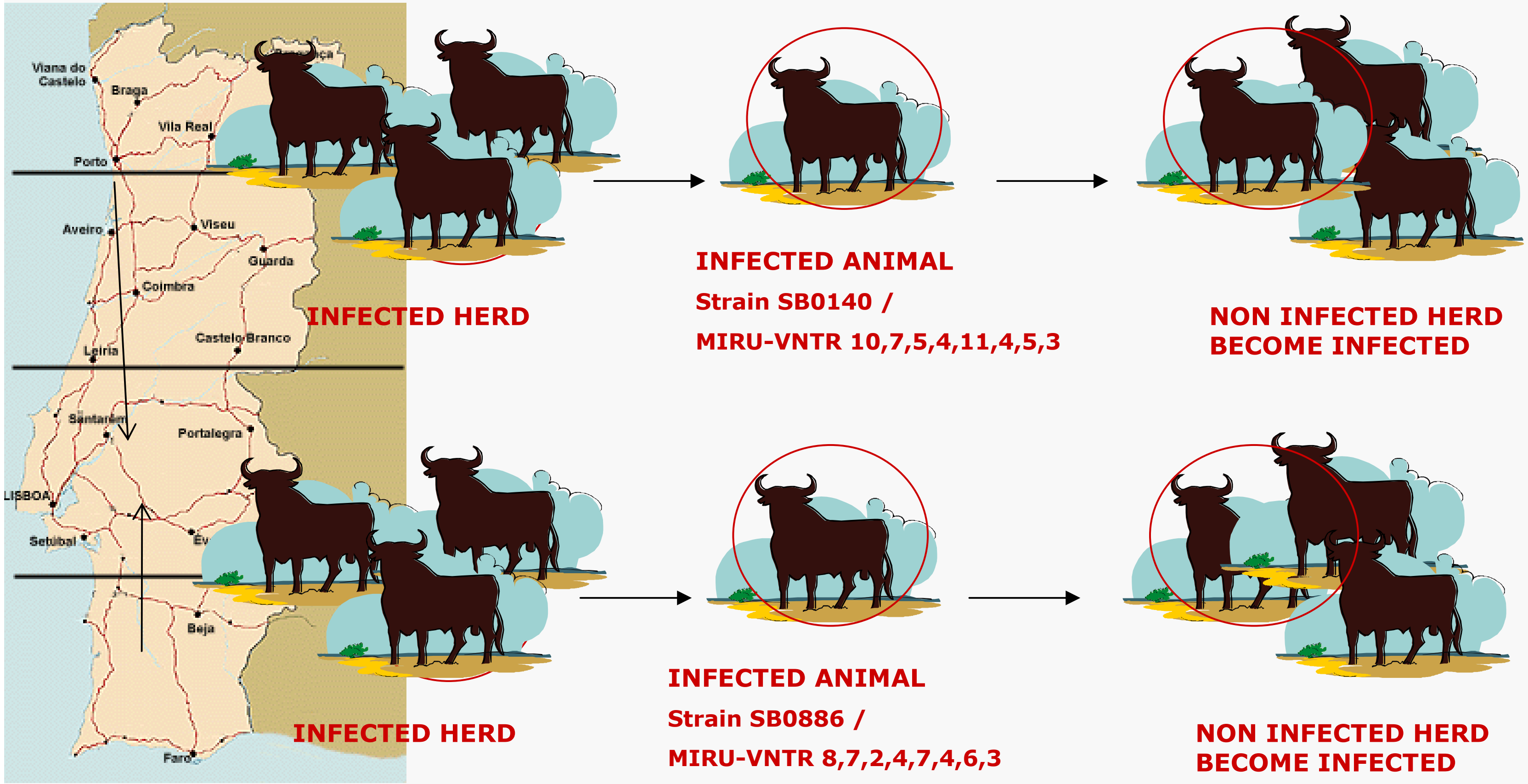
INFORMATION FROM SPOLIGOTYPING-MIRU ASSOCIATION

Introduction of an infected animal



INFECTED HERD

INFECTED ANIMAL

Strain SB0140 /

MIRU-VNTR 10,7,5,4,11,4,5,3

NON INFECTED HERD BECOME INFECTED

INFECTED HERD

INFECTED ANIMAL

Strain SB0886 /

MIRU-VNTR 8,7,2,4,7,4,6,3

NON INFECTED HERD BECOME INFECTED

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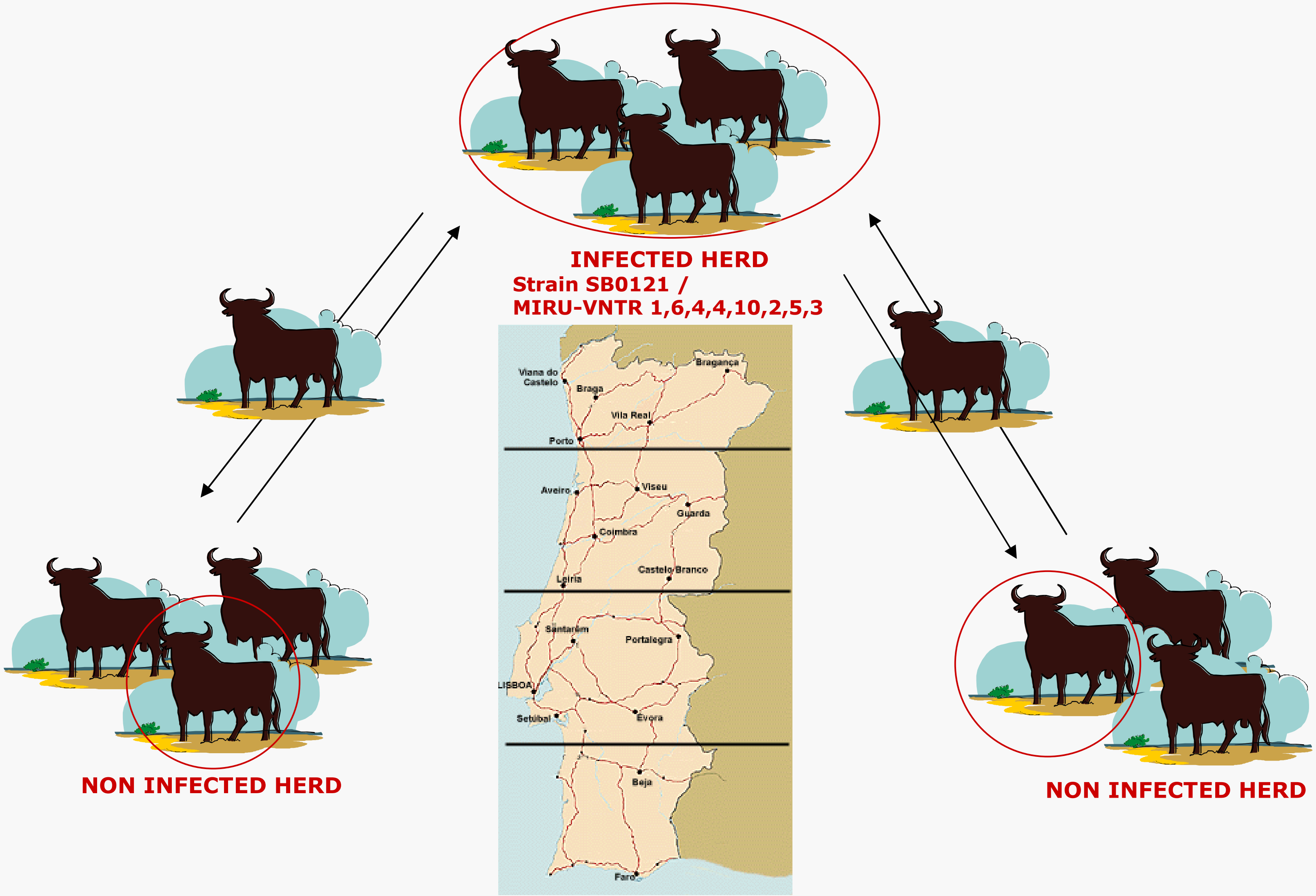
Movement of animals between herds



INFECTED HERD

Strain SB0121 /

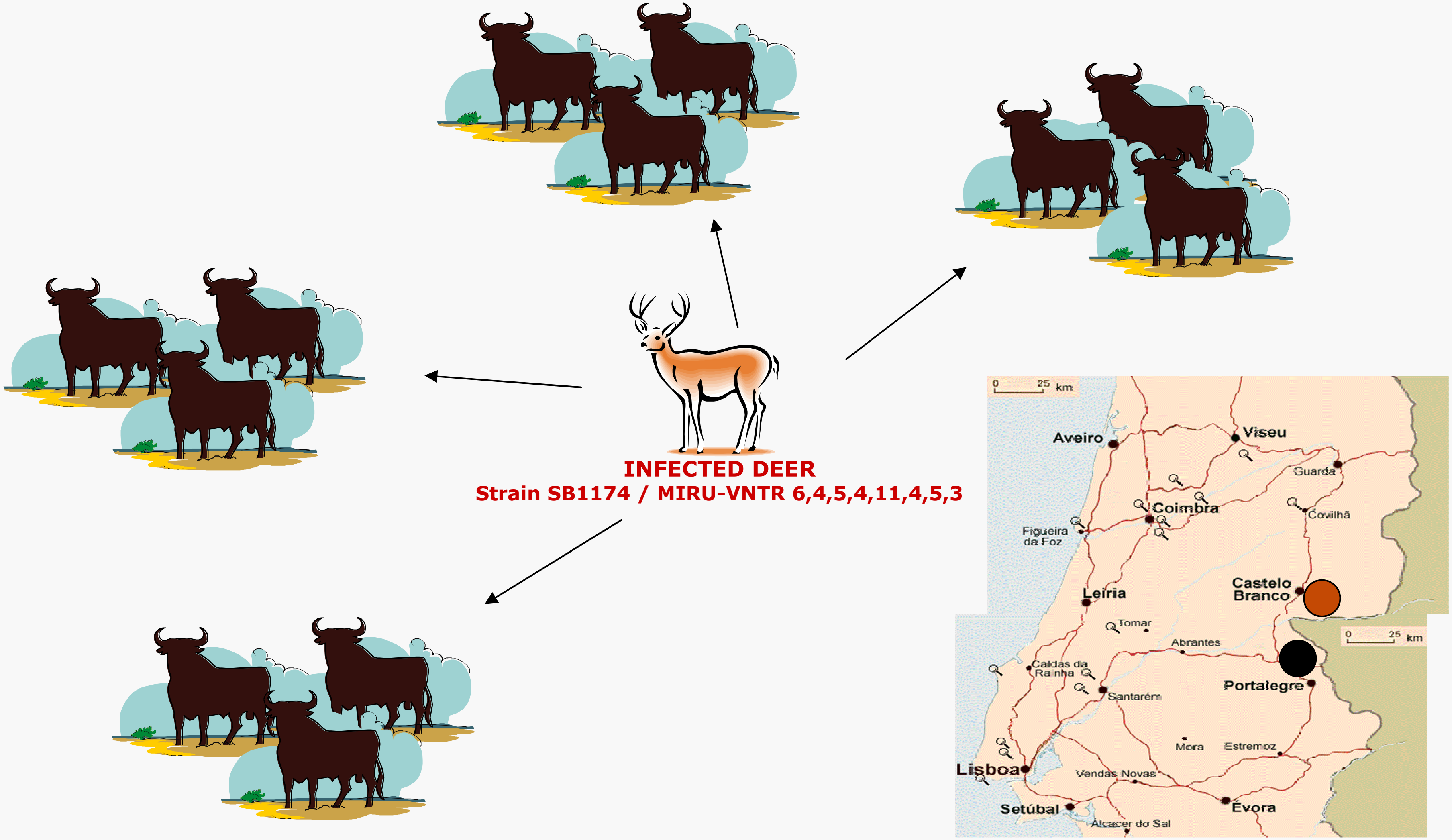
MIRU-VNTR 1,6,4,4,10,2,5,3

NON INFECTED HERD

NON INFECTED HERD

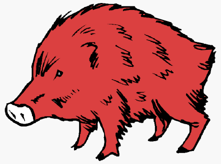
Cattle movements between herds occurred in 54.3% of the animals

Existence of wild animals reservoirs – *M. bovis* transmission between cattle and red deer

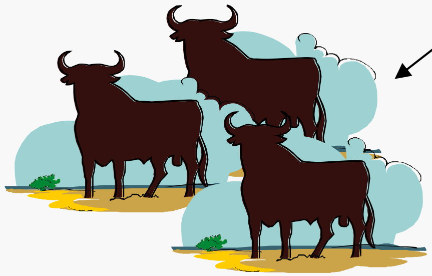


INFECTED DEER
Strain SB1174 / MIRU-VNTR 6,4,5,4,11,4,5,3

Existence of wild animals reservoirs – *M. bovis* transmission between cattle and wild boar



INFECTED WILD BOAR
Strain SB0265 / MIRU-VNTR 6,1,4,2,11,2,5,3



“Thought-provoking” aspects

- Interferon-gamma as a reliable screening method for bTB. Correspondence between this test and lesions, histopathology and bacteriological isolation.

- Analysis of the molecular epidemiology data in parallel with the traditional epidemiology (movement of animals, contact with different reservoirs)

- Importance of wild life reservoirs: control of movements, control of disease

- Animal reservoirs: wild boar and deer. Any others (badger, opossum…, pets in farms)?

- Coordination and integration of all the available information in a single data-base (Complementation of SNIRA/PISA)

Cooperation between different institutions

↓

Epidemiovigilance

BOVINE TB TEAM AT LNIV

Ana Botelho (MSc, PhD)
Teresa Albuquerque (Researcher)
Mónica V. Cunha (MSc, PhD)
Ana Canto (BSc)
Filipa Matos (DVM, MSc)
Madalena Monteiro (DVM)
Ana Jorge (Technician)
Alice Batalha (Technician)
Celeste Matos (Technician)
Ilda Lopes (Technician)