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WP7 Workshop

VNTR/MIRUs and DVR spoligotyping for *M. bovis* typing

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The Wahlund effect - Why mixing populations is bad.

N. Smith, Veterinary Laboratories Agency (VLA, United Kingdom). Partner 30.

It is frequently an assumption of molecular epidemiology that a technique, working really well in one country, should work really well in another. Unfortunately population genetics says that this is not necessarily true. For example, spoligotyping \textit{M. tuberculosis} strains in Beijing, China is probably a waste of time - they will all be the same.

For multi-locus techniques - such as VNTR - the assumption is that it is possible to dictate a 'universal' set of loci that are best for all \textit{M. bovis} strains - everywhere in the world. Again, population genetics suggests that this may be a false assumption. I will describe the problems and outline the 'regional' approach that is proposed for Great Britain.
Epidemiological relevance of molecular typing of *Mycobacterium bovis* in a country with low bovine TB incidence.

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Sources of *Mycobacterium bovis* contamination remain unclear for many animal and human disease cases. A major limitation is the lack of sufficiently informative or epidemiologically well evaluated molecular methods for typing. We report an evaluation of a high-throughput method based on 29 Mycobacterial Interspersed Repetitive Unit – Variable Number of Tandem Repeat (MIRU-VNTR) loci to genotype 127 *M. bovis* isolates from cattle from 77 different Belgian farms, representative of a nation-wide collection obtained from 1995 to 2003. MIRU-VNTR stability was demonstrated by analyzing a series of 74 isolates in total, obtained from different animals from a single farm or from different farms with an identified epidemiological link. The genotyping results and the genotypic diversity (h) were compared with those obtained by IS6110 RFLP and spoligotyping. Among 68 isolates with no known epidemiological link, MIRU-VNTR typing discriminated better than either RFLP or spoligotyping, taken individually (32 vs 16 and 17 genotypes; h=0.91 vs 0.73 and 0.85, respectively) or in combination (32 vs 28 genotypes; h=0.91 vs h=0.92). Maximal resolution was already achieved with a subset of 9 loci, six of which were also ranked among the markers with the highest allelic diversities in Ireland. These loci found to be highly discriminatory in settings with radically distinct bacterial populations appear among the most interesting candidate markers to test to test the discrimination of *M. bovis* isolates on a more general scale. The observed congruence of the genetic relationships based on IS6110-RFLP, spoligotyping and MIRU-VNTR markers is consistent with a clonal population structure of *M. bovis*. These results support MIRU-VNTR typing as a convenient and discriminatory technique to analyse the population structure of *M. bovis* in much greater detail and to address some still unresolved issues in the epidemiology of the pathogen. Epidemiological interpretation of molecular results obtained using these typing techniques will be discussed.
Frequency and distribution of spoligotypes in United Kingdom affecting cattle from years 2000 to 2006.

M. Okker. Veterinary Laboratories Agency (VLA, United Kingdom). Partner 30.

Despite an intensive test and slaughter programme to prevent cattle-to-cattle transmission, Great Britain has one of the highest animal and herd incidences of bovine TB in the EU. VLA has seen an increase in isolates submitted for spoligotyping since 2002 following the occurrence of Foot and Mouth Disease in 2001. During most of that year (mid February to November) routine testing of cattle for bovine tuberculosis was suspended. Spoligotypes became more widespread and some of this may have been due to the restocking of herds with cattle from areas of high TB prevalence.

There are 35 main spoligotypes in the UK, with the dominant types being the UK types 9, 17 and 11. These three common spoligotype patterns all share the distinct loss of spacer 6 and spacers 8 to 12.

The spoligotyping database used at VLA Weybridge currently holds information for just over 23,000 bovine samples. It stores data which includes the county where the animal came from and its ear-tag number. The State Veterinary Service is gradually making more use of molecular typing data, in conjunction with cattle movement data, as an aid to tracing the origins of infection because spoligotypes are generally found in geographical clusters.

There has been an increase in submissions of samples from animal species other than bovine and badger. The Tuberculosis (England) Order 2006 (and equivalent instruments in Scotland and Wales) has extended the obligation to notify TB to those cases where lesions are found in the carcase of any farmed or pet mammal. The isolation of \textit{M. bovis} from any mammal other than man is also notifiable to VLA. Spoligotyping is able to distinguish between \textit{Mycobacterium tuberculosis}, \textit{M. bovis} and \textit{M. microti} (the vole TB bacillus). The latter is rarely found in cattle but is found in spill over hosts, particularly cats.
Spoligotyping diversity of Mycobacterium bovis in Spain.


Spacer oligonucleotide typing (spoligotyping) has been extensively used for characterization of Mycobacterium tuberculosis complex organisms; and it is a useful tool for molecular typing of Mycobacterium bovis.

In this study we have analyzed 4210 isolates of M. bovis isolated in Spain between 1992 and 2006 by DVR-spoligotyping. The isolates have been cultured from tissue samples from a wide range of domestic animals, wildlife, zoo animals and pets. A large part of the isolates was cultured in the “Laboratorio de Vigilancia Sanitaria” of the “Universidad Complutense” of Madrid and about 38% of the isolates was cultured and submitted by regional official laboratories as part of the bovine tuberculosis control program in Spain. DVR-spoligotyping was performed as described by Kamerbeek et al. (1997).

The results clustered the isolates into 210 spoligotypes. The frequency of each spoligotype was calculated, so that spoligotype patterns could be divided into the thirteen (6.2%) most frequent ones, 115 (54.8%) spoligotypes with an intermediate prevalence and 82 (39%) so-called orphans. The most frequent pattern, SB0121, has an outstanding prevalence of 25.6% of isolates, and was found all over Spain. By comparison of the origin of the isolates we found out that some spoligotypes only appear in determined regions. So do SB0339, the predominating strain in El Pardo (Madrid), and SB0135, which was exclusively found in Cantabria.

The large diversity of spoligotypes in Spain permits us to use spoligotyping at a large scale for epidemiological studies, yet the discriminatory power is calculated 0.86. Using spoligotyping as a epidemiological method we could establish a relationship between M. bovis infecting wildlife and domestic animals in the same geographic area, and, furthermore, detect relationships between animals of the same or neighbouring farm.
Improvement of spoligotyping with additional spacer sequences for characterization of *Mycobacterium bovis* and *M. caprae* isolates from Spain.

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Spoligotyping is a typing tool used worldwide for epidemiological studies on *Mycobacterium tuberculosis* complex organisms; however it has received little attention regarding improvement. In this study, we have evaluated a spoligotyping membrane prepared with 25 novel spacer sequences selected from a previous study (van den Zanden et al. 2002, J. Clin. Microbiol. 40:4628-4639) on 308 *M. bovis* and 88 *M. caprae* isolates in comparison with the traditional spoligotyping membrane.

The results obtained by combining the two membranes together revealed an improvement of 45% (45 patterns instead of 31). The spacers used in the second membrane were able to distinguish eight out of the 16 *M. bovis* types that had more than one isolate. Seven of these types were differentiated into two subtypes with the second-generation membrane, while spb-7, the most prevalent in Spain, was further differentiated into eight subtypes.

This second-generation membrane also differentiates *M. bovis* and *M. caprae*. A set of 39 spacers (1, 2, 4-8, 10-15, 17-21, 23, 26-32, 37, 44-49, 51-54, 56 and 57) contain all the discriminatory power for both *M. bovis* and *M. caprae* isolates; and a set of 35 spacers (1, 2, 4-8, 10-15, 17-21, 26-32, 37, 44-48, 52-54 and 57) had all the discriminatory power for the *M. bovis* isolates. Our results show that the research on new spacers and the design of a new membrane may be useful for epidemiological studies of *M. bovis* and *M. caprae* isolates.
Evaluation of the discriminatory power and epidemiological usefulness of VNTR/MIRUs typing and DVR spoligotyping on French M. bovis isolates.


Although 175 different spoligotypes exist among M. bovis strains in France, 3 of them, BCG-like (SB0120), GB54 (SB0121) and GB35 (SB0134) are the most representative ones in the last 6 years' BTB outbreaks (51%, 18%, 14%). This implies that quite regularly in the field, spoligotyping alone is an inadequate tool for molecular epidemiological follow up that needs to be complemented with additional molecular typing methods.

VNTR/MIRUs loci were analysed in a collection of mainly GB35 and BCG strains of different geographical, temporal and host species origins. In our hands, the most discriminative loci are ETR A, ETR B, ETR C, ETR D, QUB 3336, QUB 11a, QUB 11b, QUB 26.

We describe the practical uses of VNTR/MIRU typing in combination with spoligotyping for tracing cattle and wildlife outbreaks in France.
Experiences in establishing MIRU/VNTR typing of MTC isolates in the lab - results, difficulties, questions.


Germany has officially been recognised as being free from cattle tuberculosis. Nevertheless, sporadic outbreaks occur every year. Diagnosis is routinely made using cultivation which is estimated as “gold standard” followed by molecular specification of the isolates using polymerase chain reaction (PCR) and spoligotyping. Analysis of the restriction length polymorphism (RFLP) is done only when special questions of molecular epidemiology have to be answered, because this method is very complex and time-consuming. As alternative method mycobacterial interspersed repetitive unit (MIRU)/ variable number of tandem repeat (VNTR) typing was started to be established in the German tuberculosis reference laboratory. The type strain *M. bovis* BCG DSMZ 43990/ATCC27289 was used to establish and optimise the method. Nine MIRU/VNTR primer pairs selected from the literature were chosen as starting collection: MIRU4, MIRU26, ETR-B, QUB26, QUB11B, VNTR0577, VNTR2461, VNTR2165 and VNTR4156. Two additional primer pairs (QUB11A and VNTR3232) were used in some initial experiments and were rejected after repeated unsuccessful or unreproducible experiments. Primer concentrations and MgCl₂ concentrations and temperatures were optimised.

Eleven *M. bovis* field isolates and six *M. caprae* field isolates were then used as test strain collection. Experiments were repeated at least two times. The PCR products were visualised on agarose gels. With many of these primer pairs the reproducibility of the PCR was unsatisfactory in our hands with one or the other strain. A limited number of DNA sequence analyses of PCR products generated with the *M. bovis* BCG type strain was performed. One of the PCR products, VNTR 4156, did not show any repetitive unit. This primer pair did not generate any PCR product from the *M. caprae* strains and several negative results from the *M. bovis* strains.

In our hands MIRU/VNTR typing of *M. bovis/M. caprae* isolates seems to be more difficult than MIRU typing with *M. tuberculosis* or *M. pinnipedii* strains which we have established before in the lab.
Molecular typing by spoligotyping and ETR analysis of \textit{M. bovis/M. caprae} strains isolated in herd breakdowns has been routinely carried out since 2002 providing a data base of genetic profiles with which to support traditional epidemiological investigations.

In order to apply spoligotyping and ETR analysis, we have first verified the organization of DR and ETR loci by sequencing respectively 4 and 8 \textit{M. bovis} strains isolated in unrelated outbreaks in Italy. The results confirmed the stability and the genetic organization of these markers and allow us to design a reference table to calculate from amplicon sizes, the number of repetitive units present in each ETR locus.

We have characterized 1086 \textit{M. bovis} and 48 \textit{M. caprae} strains isolated from 2000 to 2005 in 637 cattle herds mainly located in Piemonte, Lombardia, Emilia Romagna and Veneto. We have identified 79 different spoligotypes. BCG-like is the predominant spoligotype in Italy (53.3%). ETRs analysis have shown 105 different profiles while the combination of spoligotyping/ETR typing has identified 209 genotypes; five hundred seven isolates have been grouped in 79 clusters while 130 are unique isolates.

A panel of BCG-like isolates have been later on characterized by MIRUs and QUBs analysis. Typing markers used in this work, have shown a different discriminatory capacity. The allelic diversity index of single loci has been evaluated to provide the most discriminative genotyping method with reference to locally prevalent strains.
Profiling *M. bovis* strains in North-Western Italy: an overview from 2000 to 2005.

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It will be focused on data and information obtained from molecular characterization of 664 (out of 1186) *M. bovis* strains collected during six years of activity in Tb investigation in North-Western Italy. All strains came from both ordinary and research diagnostic activity in veterinary and human field and they were identified and then characterized by mean of several molecular techniques. Spoligotyping and VNTR-MIRU-Qubs typing were differently applied in order to define molecular profiles of *M. bovis* strains isolated during operations of bovine tb eradication campaign, wild animals surveillance controls and routine activity on human health. Different evidences of *M. bovis* infection in cattle as well as in wild animals and human patients too were found.

Spoligotyping characterisation of all *Mycobacterium bovis* strains collected in Piedmont during bovine tb eradication campaign was applied since 2002 and it has confirmed a wide prevalence of BCG-like spoligotype (61%). Bovine strains were further differentiated by VNTR profile. The presence of a profile comparable to *M. bovis* subs *caprae* was detected in only two animals of a small cattle herd, where cows were usually imported from Germany. Spoligotyping gave the evidence of the source of infection, confirming the role of molecular biology procedures as epidemiological support.

In four *M. bovis* human Tb cases, anamnestic investigations, joint to molecular strain characterization, led to trace back the origin of the infection to previous cattle tb outbreaks: genetic identity of human strains to their respective bovine ones was shown in each case.

17 strains of *M. bovis* were isolated from wild boar: Spoligotype and VNTR strain profiles were compared to bovine ones detected in same areas, by using GIS method to localize isolates: trough a spatial analysis, in some cases we could observe homology between bovine and wild boar molecular profiles. Molecular strain characterization techniques confirm themselves as useful tools to verify transfer dynamics of infection not only in cattle and wild animal, but also in human *M. bovis* Tb cases.
Molecular typing of *M. bovis* and *M. caprae* Portuguese strains: main challenges.

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In a molecular epidemiology study of Bovine Tuberculosis (bTB) in Portugal, 134 *Mycobacterium bovis* and 9 *Mycobacterium caprae* strains, were typed by spacer oligotyping (spoligotyping) the Direct Repeat (DR) region. Twenty-two different spoligotypes were found. Thirteen *M. bovis* patterns have already been described in other countries, eight were never reported previously and, after several confirmations, only one *M. caprae* pattern, SB0157, was identified.

Spoligotyping presented a good discriminatory power (HGI=0.92) for our set of strains and clustering analysis showed the existence of three major groups. However, some puzzling results occurred, making definite interpretation of some patterns difficult. A standardisation of the method for animal strains is needed, to improve repeatability and reproducibility and reduce problems related with spoligo-membrane quality control and handling. Recommendations should be made concerning the most useful spacers for *M. bovis* animal strains differentiation.

Strains sharing the most frequent spoligotype, SB0886, were further analyzed by MIRU-VNTR typing using nine selected loci (VNTR 3232, ETR-A, ETR-B, MIRU-26, QUB11b, QUB11a, ETR-C, VNTR 4156, MIRU-4). The loci VNTR 3232, MIRU-4 and MIRU-26 presented low stability and robustness, with variable results for the same strain and conditions. The junction of both typing methods can improve the differentiation of *M. bovis* strains, but careful conclusions must depend on reliable results.
A comparison of VNTR typing and RFLP typing for the differentiation of Mycobacterium bovis strains.


The objective of this study was to identify VNTR loci which gave the best resolution of Mycobacterium bovis strains in the Republic of Ireland and compare this typing method with RFLP typing. A total of 16 VNTR loci were evaluated in 60 M. bovis isolates. This panel of isolates had previously been resolved by RFLP typing into 15 strain types. Seven of the VNTR loci did not show any allelic diversity, while two exhibited very limited diversity. However, seven VNTR’s achieved good discrimination of strains with discrimination indices of between 0.42 and 0.56. These were QUB-11a, QUB-11b, ETR-A, QUB-26, MIRU-26, QUB-1895 and QUB-3336. Evaluation of ETR-B and QUB-3232 is not yet completed.

VNTR typing with six of the highly discriminatory loci was compared with RFLP typing in a panel of 80 M. bovis isolates. Both methods achieved a similar level of resolution of the panel with 21 RFLP and 17 VNTR profiles, and a discrimination index of 0.86 for each method. The RFLP type which is most predominant and widespread in the Republic of Ireland, representing 20% of isolates, was subdivided into 7 different VNTR profiles. However, the isolates from the next most predominant RFLP type were not differentiated by VNTR typing and the majority of RFLP and VNTR profiles showed a high level of congruence.

This preliminary assessment has shown that VNTR typing is a suitable replacement for RFLP analysis, because it has achieved the same level of strain differentiation, and has the advantage of being a less labour intensive and faster procedure, with easier analysis of results.
High-resolution VNTR-based genotyping of Mycobacterium bovis strains in Northern Ireland.


In outbreaks of bovine tuberculosis, the significance of factors such as cattle movement, contiguous spread from neighbouring farms, persistence of infection on-farm and latency merits further investigation, as does the role of infected wildlife, most notably the badger, in the UK and Ireland. The ability to identify M. bovis strains and trace their transmission has the potential to clarify sources of infection and major routes of transmission.

We have recently developed a highly discriminating, highly reproducible and high-throughput, multi-locus genotyping assay based on a systematic evaluation of available variable number tandem repeat (VNTR) markers. This VNTR assay identified 40 VNTR types within a survey of 461 M. bovis isolates from Northern Ireland, compared to 14 spoligotypes. Different VNTR types were clustered in geographical areas. Further sampling from defined areas showed that, whilst certain VNTR types may predominate, there is still considerable VNTR type diversity within areas. Infected herds often contain multiple VNTR types, suggesting multiple sources. A disproportionate amount of bovine tuberculosis is caused by relatively few strains, suggesting that such strains have particular properties, which may influence diagnosis. We are examining the potential link between strain type and pathogenesis. This VNTR assay is now sufficiently discriminatory to address detailed epidemiological questions and has the desired performance characteristics to progress the molecular epidemiology of bovine tuberculosis.
Ten years of molecular epidemiology of bovine tuberculosis in Belgium.


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After decades of control and eradication programs, the herd incidence of bovine tuberculosis diminished progressively under the 0.1% threshold since 1997. Currently, bovine tuberculosis due to Mycobacterium bovis is still sporadic. Since Belgium was recognised officially free of bovine tuberculosis in 2003 (decision 2003/467/CE), 5 to 10 infected herds are identified yearly. In order to better understand the epidemiology of the infection in our country, the molecular typing of the strains of M. bovis, isolated since 1995, was done. Between August 1995 and November 2005, the isolation of M. bovis was done in 233 cattle herds. The isolates of M. bovis originating of 93% of these infected herds were typed by restriction techniques fragment length polymorphism (RFLP IS6110) and spoligotyping. Each of these techniques presents a different discriminatory power according to the genotypes, but the two techniques are complementary. Together, they distinguish more than 40 genotypes. The technique of mycobacterial interspersed repetitive unites - variable number tandem repeat analysis (MIKU-VNTR), evaluated on a sample of 128 isolates, differentiate the M. bovis strains, similarly to the two other techniques implemented together. Between 1995 and 2005, 12 lineages with distinct genotypes were observed in Belgium. A lineage is clearly dominating since it represents 48% of the infected herds. This lineage was associated with the new peak of incidence of bovine tuberculosis observed in the province of Liège between 1995 and 1996. Other lineages are more rarely observed (maximum 9% of the infected herds). A retrospective analysis shows that certain lineages can reappear several years after having been observed and that new type of strains appears punctually. Two type of strains that were never observed since 1995 appeared in 2004. This result suggests that in addition to the circulation of strains of M. bovis between Belgian flocks, other ways of introduction of bovine tuberculosis must be suspected in certain case. The continuous molecular typing of the isolated strains of M. bovis constitutes a precious tool for the re-orientation of the epidemiological investigations leading to take appropriate management measures.
MIRU-VNTR analysis of Spanish *M. bovis* and *M. caprae* isolates.


Partner 1.

The use of the MIRU-VNTR technique is increasing for the *M. bovis* typing because its discriminatory power seems to be higher than spoligotyping. However this measure depends on the combination of loci used and the geographic origin of the isolates.

Our preliminary study was developed to assess the discriminatory power of eight MIRU-VNTR markers (QUB 26, 3232, 11a, 11b, ETR-A, ETR-B, MIRU 4, MIRU 40) in a panel of 42 *M. bovis* and 5 *M. caprae* isolates from Spain. They were collected from goats (n=5), cattle (n=32), swine (n=3), red deer (n=2), fox (n=1), wild boar (n=2) and human being (n=2). We intentionally chose isolates with different spoligotyping patterns (35 spoligotypes) without an evident epidemiological link among them, except in the two following cases. Two multidrug-resistant (MDR) *M. bovis* isolates from human beings and three isolates from cattle, that share the spoligotyping profile (SB0426), were included in the study to determine if both groups were clonal.

The number of allelic variants of each locus ranged from 3 alleles (MIRU 40) to 8 and 10 (ETR-A and QUB 3232, respectively). The discriminatory power of the individual locus differed greatly, with allelic diversity (h) ranking from 0.07 (MIRU 40) to 0.81 (QUB 3232). The combination of solely four VNTR (QUB 3232, 11a, 11b and ETR-A) provided a maximal resolution of 36 profiles with a genetic diversity of 0.97.

The five human and cattle *M. bovis* isolates (SB0426), differed in a copy at ETR-A (6 and 5 copies) and at QUB-3232 (7 and 6 copies, respectively), showing the same profile at the other MIRU-VNTRs. Therefore, this technique rule out these cattle isolates as direct source of the MDR human outbreak. These results highlight that a combination of techniques is required for appropriate discrimination.
Molecular typing of *Mycobacterium tuberculosis* by Mycobacterial Interspersed Repetitive Unit-Variable-Number Tandem Repeat Analysis, a more accurate method for identifying epidemiological links between patients with tuberculosis.


IS6110 fingerprinting of *Mycobacterium tuberculosis* is the standard identification method in studies on transmission of tuberculosis. However, intensive epidemiological investigation may fail to confirm transmission links between patients clustered by IS6110-restriction fragment length polymorphism (RFLP) typing. We applied typing based on variable numbers of tandem repeats (VNTRs) of mycobacterial interspersed repetitive units (MIRUs) to isolates from 125 patients in 42 IS6110 clusters, for which thorough epidemiological data were available, to investigate the potential of this method in distinguishing epidemiologically linked from non-linked patients. Of seven IS6110 clusters without epidemiological links, five were split by MIRU-VNTR typing, while nearly all IS6110 clusters with proven or likely links displayed conserved MIRU-VNTR types. These results provide molecular evidence that not all clusters determined on the basis of multi-banded IS6110 RFLP patterns necessarily reflect transmission of tuberculosis. They support the use of MIRU-VNTR typing as a more reliable and faster method for transmission analysis.

The results described above were based on the conventional 12-loci MIRU/VNTR typing. However, in the coming months internationally standardized 15-loci typing will be published. Latter method combines a high level of discrimination with sufficient stability of the selected loci. In practice, the amplification of particular loci is technically demanding and subject of further investigation. In order to implement the 15-loci MIRU/VNTR typing as a routine tool in The Netherlands, a large part of the collection of *M. tuberculosis* isolates from the period 1993-2006 will be re-typed by MIRU/VNTR typing.
Molecular epidemiology of the multidrug-resistant tuberculosis in Spain.

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A *Mycobacterium bovis* strain in the 1990s was extremely dangerous with a high transmission and the highest mortality never sight (1-4). A variation of this *M. bovis* strain having a different genotype, RFLP pattern and spoligotype (one space difference) caused three cases. The explanation of why this varied *M. bovis* cut the chain of transmission remains unknown, but there are studies about the the *M. bovis* B strain, which let us explain that the second IS6110 inserted in the promotor region of the system phoP-phoR could be the cause of the major virulence of the strain (5).

After the detection of this epidemic including more than 100 cases, we organized a national surveillance net of MDR-TB in order to know how was the distribution of the MDR *M. tuberculosis* complex strains in our media.

We used spoligotyping and restriction fragment length polymorphism (RFLP) of the IS6110-insertion sequence, and MIRU-VNTR since 2003, to study the molecular epidemiology of multidrug-resistant (MDR) tuberculosis in Spain (6). Near 100% of the laboratories of the national sanitary system participate sending their MDR isolates. The immigrants play an important role in the dissemination of TB in some countries, and although until year 2000 only 18% of the presented/displayed cases were immigrants, in the last years this number has been tripled. The molecular typing and additional characterization of the resistance genes are necessary to identify their routes of transmission. (6).

The largest cluster including 25 patients corresponded to this *M. bovis*, but no new cases since 2003 were registered in the National MDRTB database, what make us believe that the control of this strain could be closed in our country.

Recently the discovery of a susceptible *M. bovis* strain has been published (7), with the same spoligotype than *M. bovis* B strain, although presenting other differences in other spacers not included in the current membrane and also in ETR-A. It could talk that, how it was foreseeable, the source of our *M. bovis* strain came from animals, but the origin of the so extremely resistance in this strain remains unknown.

Outbreak of acute tuberculosis in a goat herd; the first report of *Mycobacterium caprae* isolation in Greece.


Members of the *M. tuberculosis* complex can infect a wide range of domestic and wild animals. These mycobacterial infections cause zoonotic diseases that can represent a considerable Public Health threat. Tuberculosis in goats was formerly attributed exclusively to *M. bovis*. The bacterium originally named *M. tuberculosis* subsp. *caprae* and then *M. bovis* subsp. *caprae*, is now referred to as *M. caprae* and consists a distinct causative agent of caprine tuberculosis. Up to this day *M. caprae* has been identified in cases of tuberculosis in several animal species and humans in many countries.

Here we describe the first case of *M. caprae* isolation in Greece. Based on the evidence provided by spoligotyping, the isolate is similar to those commonly found in central Europe. In conclusion it can be stated that caprine tuberculosis caused by *M. caprae* can be manifested in an acute form, representing like in the case described here a serious economic and health problem.
Epidemiology of *Mycobacterium caprae* infection in livestock and wildlife in Spain.

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*Mycobacterium caprae* is a member of the *M. tuberculosis* complex that was first described as the main etiological agent of caprine tuberculosis in Spain; however, this pathogen can also infect other animal species and human beings.

In the present study we have characterised *M. caprae* isolates from 486 animals by DVR-spoligotyping. *M. caprae* infection was widespread in several geographic areas of Spain. Although the majority of the strains were identified in goats, we observed *M. caprae* affecting also other domestic and wild animal species [cows (n=29), sheep (n=2), pig (n=1), wild boars (n=9), red deer (n=1), and fox (n=1)]. In most cases, there was a geographical link with caprine flocks infected with the same spoligotype pattern. Unlike results found in reports from other EU countries, the epidemiology of *M. caprae* in Spain is driven by caprine infections.
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