Molecular epidemiology of bovine tuberculosis
II. Applications of genotyping

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Summary

The applications of genotyping of Mycobacterium bovis are reviewed. Published research to date has been conducted predominantly within the context of validating typing methods, and few studies have been specifically epidemiological. This is contrasted with the situation in human tuberculosis, where the application of restriction fragment length polymorphism typing using insertion sequence IS6110 has successfully led to insights into the epidemiology and molecular evolution of the pathogen. Based upon the medical experience, the adoption of an integrated approach which combines epidemiology and molecular biology is recommended for future studies. Accordingly, clear identification and explanation of type clustering should be possible, which should facilitate decisions related to disease control.

Keywords

Genotyping - Molecular epidemiology - Mycobacterium bovis - Restriction endonuclease analysis - Restriction fragment length polymorphism - Spoligotyping - Tuberculosis.

Introduction

Bovine tuberculosis has a complex epidemiology (39, 41). Problematic features include a variable incubation time, interaction with immuno-suppressive diseases and environmental stresses, and a wide potential host range. An additional difficulty was that until recently, no practical method existed of undertaking a subdivision of disease outbreaks by strains or types, an approach which is proving invaluable in understanding the intricacies of infectious disease dynamics (37). With advances in cell biology, this particular problem has now been overcome, and a number of techniques are currently available or in development. A review of those applicable to bovine tuberculosis has been presented by Durr et al. (22).

While the purpose of undertaking Mycobacterium bovis typing is epidemiological, few examples have been published of the use of typing for control of animal disease. This can be contrasted with typing in human medicine, where the method has been more widely and successfully used. In part, this reflects the relative importance of the diseases, as human tuberculosis is currently one of the most serious diseases, with an estimated one-third of the population of the world infected and therefore at risk of developing the disease (7). In contrast, bovine tuberculosis has been extensively controlled by test and slaughter policies in countries with a previously high prevalence of infection (39). However, a greater difficulty faced by veterinary epidemiologists has been the technical problem of the lack of a typing method that combines good discrimination with ease of use and interpretation. For human tuberculosis, restriction fragment length polymorphism (RFLP) typing, using the insertion sequence (IS) 6110 probe, was quickly established as the standard methodology. Only recently have comparable techniques become available for bovine tuberculosis.

While bovine tuberculosis is currently a relatively minor disease problem in the developed world, the disease remains problematic in several countries, particularly Great Britain, Ireland and New Zealand (11). Even in countries where the disease has been officially 'eradicated', such as the United States of America (USA), problems remain, due to relatively frequent, but sporadic outbreaks (23). Furthermore, the
Principles of using genotyping

The basic assumption underlying the practical use of genotyping is the clonal model resulting from asexual reproduction. This proposes that when an infectious bacterium is passed on to a second host, the bacterium retains the same genetic make-up. Consequently, if two individual hosts are found to have isolates with different genotypes, then infection has not spread from one host to the other, even if the two hosts have had close contact. Similarly, if two isolates are found in the same host, then two independent infection events have occurred.

The practical use of this model can be demonstrated by a hypothetical example (Fig. 1). Assume a farm previously free of bovine tuberculosis in which infection is detected. The epidemiological investigation shows that a cow had been previously introduced from a herd that also had a breakdown. However, tuberculosis had also been detected shortly before in a wildlife reservoir sharing part of the farm. The key epidemiological focus is to determine the source of infection. If the M. bovis isolated from the cattle on the farm all have the same genotype as that of the introduced animal, and this is different from that of the wildlife reservoir, then this provides strong evidence that the infection was introduced via the purchased animal (Fig. 1a). If the herd has a subsequent breakdown which is found to be the same M. bovis type as the wildlife reservoir, this supports the hypothesis of spread from the latter, in contrast to the alternative that the infection continued in the herd via a non-detected infected cow.

Although the assumption of clonality is central to the use of genotyping, this must be treated as a biological approximation. All bacteria undergo mutation of deoxyribonucleic acid (DNA) sequences, and if these mutations do not affect the functioning of essential enzymes or structural proteins, the daughter generation should survive. If the typing technique can detect this genetic change, then a 'new' clone is identified. Alternatively, if mutation occurs in part of the genome that the technique does not recognise, then the daughter generation will be classified as the same clone. An important consequence of this is that the typing technique used must be considered in relation to the rate of genetic change of the organism.

The problems posed by using a technique that is too sensitive to genetic change are illustrated by the hypothetical example in Figure 1b. In this case, mutations occur frequently in the genomic area targeted by the typing technique. As a consequence, although the typing can help support the hypothesis of infection originating in an introduced animal, the technique cannot add insight as to the 'path of transmission' of the infection within the herd. Similarly, the technique cannot provide any support to the competing hypotheses of the cause of the second breakdown (i.e., whether the breakdown was due to the wildlife reservoir or non-disclosed latent infection).

The problem posed by excessive sensitivity of the typing technique to genetic mutations can be contrasted to the alternative, where only major genetic shifts are detected. This results in insufficient discrimination, and similarly limits the utility of genotyping to answer the questions of source of infection and paths of transmission (Fig. 1c). The important concept is that, to be useful, the typing technique must fall between the two extremes. Alternatively, this compromise position between excessive sensitivity to mutation and insufficient discrimination may be resolved by using more than one technique, provided that the techniques used target different areas of the genome.

The above discussion serves to illustrate the point that the fundamental role of typing is to assist in choosing between competing hypotheses that arise as part of a thorough epidemiological disease investigation. In the hypothetical example, before the benefit of typing could be realised, the two critical steps were tracing the origin of the introduced animal and sampling the wildlife reservoir for evidence of infection. Without collecting this epidemiological data, typing isolates obtained from cattle at the farm where breakdown occurred would be of little use. In this way, molecular epidemiology can be viewed as an extension of, but not a substitute for, 'traditional' epidemiology.

An important piece of information, which assists in the reconstruction of a plausible sequence of infectious pathways, is the time-period during which a detected animal might have harboured the disease. This does not present a problem for many infectious diseases, in which the incubation period and disease course are both well established and subject to little variation. However, in mammalian tuberculosis, these disease characteristics are both highly variable, and at present, no simple methods are available to determine the length of time a particular animal may have had the disease and been infectious to others (27). Given this lack of information, a reconstruction of the sequence of infectious events must depend upon other time-based information obtainable by routine surveillance. In the hypothetical example, this is demonstrated by the need to know the disease status of the wildlife reservoir before the index case was detected. If this information is not available, then questions arise as to the direction of infection (Fig. 2). Accordingly, if the situation in wildlife was not investigated until after the infection had been detected in the cattle, an equally plausible scenario would be that infection spread from the cattle to the wildlife.
The example used above represents the most common epidemiological problem faced by disease managers during the latter stages of a bovine tuberculosis eradication campaign. In such a low incidence situation, the relative rarity of the disease can justify the considerable expense of a detailed 'case' investigation. In contrast, a broader, population-based approach may be more applicable in an endemic setting (Fig. 3). This approach differs in being more research orientated and directed at developing and exploring hypotheses. An important group of such population-based studies are those which are primarily exploratory, attempting to define patterns in space and time. These studies serve to generate hypotheses, the validity of which can be tested by specific, targeted studies.

Few population-based studies have been undertaken in bovine tuberculosis epidemiology. However, an important example can be found in M. tuberculosis molecular epidemiology, which serves to illustrate the general principles of such studies. When a resurgence of human tuberculosis was reported in North America and Western Europe in the late 1980s, an important factor was to determine the extent to which cases represented recent transmission, as opposed to re-activation of dormant infection acquired years previously (1, 48). The hypothesis used for these studies was that isolates from a group in which recent infection occurred should be 'clustered' (i.e. identical), while those from re-activated infections would occur as single examples, as the latter would have been acquired over a longer period of time and space. Such analyses demonstrated that, contrary to the prevailing theories, the majority of the new cases represented recent infection. This had important implications for public disease control. Although subsequent analyses have shown that the 'cluster equals active transmission' hypothesis is a simplification (10, 29), the conclusion remains valid nevertheless.

The difficulty of interpreting the path of infection from cross-sectional studies demonstrating identical types

While dissimilarity has only one interpretation (that transmission has not occurred), similarity may have three alternative interpretations.
Applications of *Mycobacterium bovis* typing

**Wildlife reservoirs versus cattle-to-cattle transmission in causing breakdowns**

By far the most common use of genotyping has been to find evidence for the role of wildlife reservoirs in outbreaks amongst cattle herds. This has been the case in several countries, particularly New Zealand and the British Isles (Great Britain and Ireland), where tuberculosis in cattle has remained a persistent problem, despite longstanding campaigns of eradication (39). In both cases, the primary problem is considered to be the persistence of infection in wildlife reservoirs, the Australian possum (*Trichosurus vulpecula*) and the Eurasian badger (*Meles meles*), respectively. The most controversial issue is the relative importance of these wildlife reservoirs as compared to cattle-to-cattle transmission.

In New Zealand, restriction endonuclease analysis (REA) has been used to study aspects of this problem. Comparison of *M. bovis* isolates from infected cattle herds and brushtail possums trapped in the locality usually showed identical patterns (14). Although this was taken to support the hypothesis of transfer of infection from possums to cattle, this was an inference which was based upon epidemiological rather than typing data (15). Certain outbreaks in cattle were characterised by the same REA pattern, which indicated that only a small number of infected possums were responsible for transmitting the disease to cattle, probably as a consequence of a single heavily-infected possum traversing pasture on which the cattle were grazing. A more detailed study of the role of possums, cattle and other wildlife was conducted in the MacKenzie Basin of the South Island (21). The area was free of tuberculosis prior to 1980, but in the subsequent years, the majority of cattle and deer herds in the area became infected with *M. bovis*. Typing of 125 isolates by REA and IS6110-RFLP revealed two principal groups, indicating that the infection was introduced from at least two distinct
sources. While several hypotheses were presented as to the source of infection, no definitive conclusion could be drawn regarding paths of transmission.

In parts of Ireland, while the badger is still considered to be the predominant source of infection, a significant amount of inter-bovine spread occurs, associated in part with frequent movements of animals between farms (40). Molecular typing has been used to try to determine the relative contribution of both factors. A study in Ireland using REA, compared isolates recovered from cattle and badgers in breakdowns in several counties (16). When an outbreak of tuberculosis occurred in cattle, the genotypes in infected badgers nearby were usually found to be the same. In a study in Northern Ireland, isolates from cattle, badgers and deer were typed using direct repeat (DR)-RFLP (46, 47). Most of the badger types were the same as those previously found in cattle, but no conclusion could be drawn regarding the direction of transmission between the two species.

Observations of the association between types and geographical areas have been explored in England and Wales, using geographical information system (GIS) technology (12). Since 1996, the majority of isolates from herd breakdowns have been spoligotyped, and using the map references of the farm locations held in the national veterinary disease database, detailed mapping has been undertaken (Fig. 4). Results at both a national and regional level reveal a distinct clustering of spoligotypes isolated from cattle, and often (but not always), these are the same as those isolated from badgers found nearby.

Perumaalla et al. investigated isolates recovered from cattle in Mexico and Texas using two RFLP techniques (IS6110 and DR probes) (42). Based upon the high frequency of isolates possessing multiple copies of IS6110, a hypothesis involving wildlife reservoirs was presented. However, a subsequent investigation of potential wildlife sources failed to find any evidence to support this hypothesis (43).

Transmission may potentially occur in the reverse direction, namely from farmed to wild animals. Whipple et al. investigated M. bovis tuberculosis in captive and free-ranging deer and coyotes in an outbreak in Montana, USA, and found similar types using RFLP (IS6110 and polymorphic guanine and cytosine-rich repeat sequences [PGRS] probes) (55). Although this result could not conclusively determine the direction of transmission, in the context of the whole case investigation, infection was considered more likely to have passed from the farmed animals to the free-ranging ones (44). The transmission of M. bovis between wild boars and cattle was suggested in a study in north-western Italy (45), where spoligotyping and RFLP were used to show commonality of types between the host species. In this case, the inference was that transmission occurred from the cattle to the boar and not vice-versa, although this was not proven.

**Associating types to geographical regions ('geotyping')**

In many of the studies investigating wildlife reservoirs, the alternative source of infection is purchased animals or spread from infected neighbouring farms. The ability to detect and assign certain types to distinct geographical regions provides the advantage that if a particular type is isolated outside the area in which it predominates, then the investigation can focus upon the hypothesis of an introduced infection, and hence detection of the animal responsible for the introduction.

To date, no specific studies of this kind have been reported. Nevertheless, geographical clustering has been reported in a number of studies, for example, in the Republic of Ireland (16), Northern Ireland (46, 47), New Zealand (14, 15), Argentina (25) and Great Britain (12).

The study by Clifton-Hadley et al. using a GIS (12), has been the most sophisticated demonstration of geotyping to date (Fig. 4). In particular, one spoligotype (type '17') was found overwhelmingly in a confined geographical location in south-central England, while the other types were scattered throughout the country. The potential of these results for systematic disease control is currently being investigated (P.A. Durr, unpublished findings).

However, geographical clustering of types has not been invariably demonstrated. Perumaalla et al. found little geographical clustering of types in a study of isolates recovered from cattle in Mexico and Texas (42). This was thought to reflect the extensive movement of cattle within regions and between the two countries. A similar lack of geographical clustering was shown in a study using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to examine isolates from six geographical regions in Mexico (38).

**Determining the source of Mycobacterium bovis in imported animals**

In countries free of bovine tuberculosis, most outbreaks are associated with imported animals. Detailed investigations are consequently undertaken to determine the source, so that lessons can be learnt to prevent repetition of the problem.

Bölske et al. used REA to undertake a thorough epidemiological investigation of an outbreak of bovine tuberculosis in several herds of farmed fallow deer (Dama dama) in Sweden (8). Detailed tracing was able to reveal that all the infected deer had either been imported into Sweden from the United Kingdom (UK) four years previously, or had been in contact with these imported animals. The REA patterns were identical for eight isolates from five affected farms, which indicated a common source of infection. Furthermore, the patterns were distinct and only resembled others that were similarly derived from isolates obtained from...
deer in the UK. A follow up study using IS6110-RFLP confirmed this conclusion (49).

In 1992, an outbreak of tuberculosis was observed among cattle housed at three farms in the Netherlands (54). Tracing studies showed that the probable source of infection was a young, diseased bull which was imported from Austria in 1991 and housed at all three of the farms. Using IS6110-RFLP, all the isolates from the infected bull and cattle were demonstrated to be clonal, confirming the bull as the source of infection.

Zumárraga et al. used spoligotyping to demonstrate that isolates from South America could be differentiated from those obtained from Spain (56). If similar associations could be made between types and countries, systematic assignment
of the source of infection in imported animals might be possible, thereby assisting epidemiological investigations when disease is detected in imported animals.

**Paths of transmission of Mycobacterium bovis outbreaks in zoos and wildlife parks**

*Mycobacterium bovis* infection is a common disease of captive wild mammals, possibly associated with the stress and relative overcrowding of zoological parks (50). Recently, typing has been utilised to establish paths of transmission between infected animals.

In Sweden, during an outbreak of *M. bovis* in a zoological garden involving tapirs and a gibbon, IS6110-RFLP was used to demonstrate that a single clone was involved (34). However, determination of the direction of transmission was not possible.

Thorel et al. described the use of typing in outbreaks of tuberculosis at two zoos in France (52). In the first zoo, an outbreak of tuberculosis occurred in a group of baboons, in the second, mortality occurred in three leopards and a sea-lion. Using IS6110-RFLP, the second outbreak was demonstrated to be caused by a single clone; however, the strain in the baboons contained only a single copy of IS6110, and thus the involvement of a clonal isolate could only be inferred.

Van Soolingen et al. reported the use of IS6110-RFLP to investigate an outbreak of tuberculosis in species of oryx and gazelle from two wild parks in Saudi Arabia (54). Most of the oryxes were thought to be infected during transport in a container from one wild park to another in Saudi Arabia by contact with a single diseased animal. Although not all the isolates had the same banding pattern, the hypothesis of a single point source during transport was considered a likely explanation of much of the outbreak.

Diagnosis of tuberculosis in a colony of captive seals (Australian sea-lions and New Zealand fur seals) at a marine park in Western Australia in 1986, led to a series of detailed studies using various typing methods (26). The REA showed that all the isolates from the six infected seals produced identical DNA patterns, indicating a common source of infection (17). The infection was considered most likely to have been introduced when one of the seals was captured from the wild, as tuberculosis was subsequently isolated from the same species of seal found dead in the area in which the seals of the marine park were originally captured (18). Furthermore, molecular typing by REA, PGRS-RFLP and IS6110-RFLP revealed identical or similar patterns (18).

**Transmission dynamics of zoonotic Mycobacterium bovis**

In the outbreak of *M. bovis* among the seals described above, typing was used successfully to show that a seal trainer contracted the same infection (51). Three years after having left the marine park, the trainer developed clinical pulmonary tuberculosis. Although the trainer had moved to the East Coast of Australia, the path of transmission was established by the use of REA, which revealed that the isolate was the same clone as that detected in the seals.

Zoonotic *M. bovis* remains a problem in Argentina, where infection is principally an occupational hazard among rural and abattoir workers (5). The hypothesis that cattle are the major reservoir for human infections is supported by a study which demonstrated that the majority of *M. bovis* isolates from humans had a single IS6110 copy, as did those examined from cattle (54). In contrast, in the Netherlands, only 40% of the *M. bovis* isolates from humans contained a single IS6110 copy, although the local cattle strains, like those in Argentina, contain only a single copy. Combined with the fact that the cattle population of the Netherlands has been free of tuberculosis for several decades, this indicates that an animal source other than cattle may be infecting humans.

However, in Spain, where *M. bovis* in cattle and goats remains a problem, evidence suggests that the source of most cases in humans is cattle. In a study of eighty *M. bovis* isolates from humans, spoligotyping was used to demonstrate that seventy-seven belonged to the bovine type (32). However, three isolates were typical of caprine strains. As subsequent tracing showed that all three patients had contact with goats, this suggests that goats (a previously unrecognised source of infection) have the potential to transmit the disease to humans.

Until as recently as the mid-1980s, the majority opinion held that human-to-human transmission of *M. bovis* occurred infrequently (30). However, several examples of this occurrence have now been documented, generally in HIV positive patients, and typing has proved essential in providing evidence for transmission. A serious case was documented in which a citizen of Canada acquired the infection after visiting a dying relation in Spain, and approximately one year after returning home was diagnosed with severe pulmonary tuberculosis (36). Spoligotyping was used to show the commonality of the isolates from Spain and Canada.

**Transmission dynamics in domestic pets**

Tuberculosis in domestic pets has particular importance, both due to the relative value of these animals and the risk of transmission to the owners. To date, few studies have used typing in such a clinical context. An interesting investigation was undertaken over a three-month period, on a cluster of twelve cases of tuberculosis in cats in a geographically localised area (20). On the basis of identical REA patterns, the study concluded that the cats were exposed to a single source of infection. This source could not be conclusively determined, although infection may have occurred while the cats were visiting the same veterinary clinics at which the cases were later diagnosed.
The potential of domestic cats to act as a source of infection for humans has been indicated. Distinct M. bovis types (as determined by IS6110-RFLP) found in urban domestic cats from Buenos Aires were also isolated from humans, indicating possible transmission (54).

**Clarifying the taxonomy of the Mycobacterium tuberculosis complex**

Discrimination between M. bovis and M. tuberculosis has traditionally been achieved by performing a series of cultural and biochemical tests. Nevertheless, some isolates are intermediate in these characteristics and fall between the two 'species'. For this reason, a degree of misclassification may have occurred in the past. This, in turn, has serious implications for subsequent epidemiological investigations and any consequent changes in public health policy. This is illustrated by a case study of a multidrug-resistant isolate from a patient with HIV-acquired immune deficiency syndrome (AIDS) (9). The isolate was initially reported to be M. bovis, as all the strains possessed all the indicated phenotypic characteristics. However, subsequent genetic analysis revealed that the isolates were in fact dysgonic, slow-growing M. tuberculosis strains which mimicked the M. bovis phenotype, probably as a consequence of cellular alterations associated with the multidrug resistance (33). Spoligotyping and IS6110-RFLP analysis confirmed that the outbreak was due to a single strain. As several typing techniques have been shown to consistently discriminate between M. bovis and M. tuberculosis, reference laboratories for human tuberculosis should introduce molecular typing as a routine confirmatory test on all suspected M. bovis isolates.

In Spain, both caprine and bovine tuberculosis remain serious problems (28). One particular epidemiological question of interest is the relation between the strains in goats and cattle, and through typing, the hypothesis that different subspecies were involved in the infection of the two host species was developed. Gutiérrez et al. demonstrated that a genetic difference was discernible in that, while the seventeen bovine strains had only a single IS6110, those from goats were found to carry six to eight copies of the element (31). A similar difference was shown between isolates from the two species when a more extensive data source (125 isolates) was investigated by Liébana et al. (35), even though almost half of the bovine isolates were shown to have more than one copy of IS6110. A third study looked at a wider range of species, using spoligotyping and identified two species clusters: the first comprising cattle, cats, wild boar and deer and the second confined to sheep and goats (2). As a result of these typing results, a thorough microbiological study, comparing the cattle and goat isolates, has recently been performed (4). The study concluded that the caprine isolates from Spain are sufficiently distinct to warrant the creation of a separate subspecies of the M. tuberculosis complex, namely, subspecies caprae.

Genotyping has also been useful in clarifying the taxonomic position of isolates recovered from seals with tuberculosis in the south-western Atlantic (57). Initially thought to be M. bovis, based on differences in the number of copies of IS6110, spoligotyping and gene polymorphism, the strain has been shown to represent a distinct subgroup of the M. tuberculosis complex.

**Association of types with pathogenicity**

Recent studies with M. tuberculosis have demonstrated an association of types with virulence. The most conclusive of these to date was the identification of a strain that was responsible for a large outbreak of tuberculosis in a rural community in the USA, which otherwise had a low risk of the disease (53). The RFLP genotyping showed the strain to be clonal, and laboratory studies with mice confirmed that it was of high virulence.

As yet, only preliminary studies have been undertaken regarding the association of the genetic variability of M. bovis and virulence. In a study conducted in Northern Ireland, the association between RFLP patterns and the nature of the disease in several herds was investigated (46). However, it was concluded that there was no evidence for a virulence-type linkage. Perumalla et al. discovered that isolates with multiple copies of IS6110 were mainly found in animals other than cattle (42). This suggested that these M. bovis isolates with multiple copies of IS6110 may have become attenuated for virulence in cattle. However, a study of isolates from Spain found no evidence to support this hypothesis (3).

**Discussion: research needs and future directions**

The above review of applications presents very few examples of the successful use of genotyping of M. bovis to resolve an epidemiological problem and to enable firm conclusions to be drawn. The exceptions are the study by Bolske et al., where the role of imported deer from the UK was clearly demonstrated (8); and the study by Thompson et al., which showed that the pulmonary tuberculosis in the seal trainer was the result of contact with infected animals (51). This contrasts with the plethora of successful molecular epidemiological studies conducted on M. tuberculosis.

One of the major factors responsible for the relative success of molecular epidemiology in M. tuberculosis, compared to M. bovis, was the early development of a successful typing technique (IS6110-RFLP), which possessed the twin desirable characteristics of isolate stability and population diversity. Consequently, successful molecular epidemiological studies of tuberculosis in humans were undertaken within a few years of the development of IS6110-RFLP. In contrast, veterinary molecular epidemiology has been greatly hampered by the lack of a comparable technique, despite the fact that the
earliest work in the genotyping of the *M. tuberculosis* complex was performed by veterinary researchers (13). Most research effort has therefore focused upon developing and validating new techniques, and a large proportion of scientific papers referring to the 'molecular epidemiology' of *M. bovis* are in fact genotyping validations (42, 46).

This problem of a lack of a 'gold standard' genotyping technique which combines ease of use, good discrimination and isolate stability, has still to be resolved (22). However, the progress in PCR techniques over recent years has been sufficient to inspire confidence that such a technique or combination will be developed soon. Traditionally, the validation of genotyping techniques has concentrated on a comparison with existing techniques, especially in terms of discrimination. While this was adequate in the earlier pioneering stages of *M. bovis* genotyping, progress has now been sufficient for a more rigorous approach. In particular, a greater emphasis on epidemiological usefulness as the key attribute is required. One method of assessing the usefulness of a technique to resolve competing hypotheses is through case studies for which a substantial amount of epidemiological information has been collected.

A critical requirement in the use of genotyping to resolve epidemiological problems is the existence of appropriate databases. The key databases needed are those providing base-line farm and disease data, and a reference typing library. At present, most developed countries now have established national animal health information systems. In contrast, no standard reference system exists for typing, even for spoligotyping, which is rapidly becoming one of the most popular typing techniques in veterinary and medical genotyping of mycobacteria, no standard nomenclature exists. Once an *M. bovis* typing library is established, one of the immediate benefits will be for countries and regions that have already eradicated bovine tuberculosis. When dealing with imported animals that are diagnosed with the disease, the ability to establish whether the type is 'common' in the exporting country will provide supporting evidence of whether infection occurred pre- or post-importation.

A database system that may have wide potential in the molecular epidemiology of *M. bovis* is GIS. The demonstration of geographical clustering of spoligotypes from both cattle and the principal wildlife reservoir, the Eurasian badger, has important implications in terms of understanding the dynamics of the disease (12). Comparable results may be found in other countries where wildlife reservoirs are important in the persistence of the disease, specifically Ireland and New Zealand. Nevertheless, GIS may not be relevant in all situations, especially those involving considerable movement of animals (24).

One result of the lack of traditional epidemiological input into *M. bovis* genotyping studies, has been a general lack of biostatistical analysis. This was not necessarily a problem in the earlier stages of the development of the technology, as the imposition of rigorous biostatistical criteria can restrict the range and extent of experimentation. For example, many of the pioneering studies were performed on very few isolates, due to the technical difficulties that were encountered, and if publication had been delayed until an adequate sample had been achieved, progress would have been much slower. However, bovine tuberculosis molecular epidemiology is entering a stage of greater maturity, and consequently, more rigorous biostatistical sampling and analysis is required.

A current trend in *M. tuberculosis* molecular epidemiology is the greater integration of research, in which macro-scale epidemiological studies inform research at the molecular level and vice-versa (6). Such a collaborative approach is particularly beneficial, as molecular biologists are now faced with a plethora of information regarding genomic sequences, but require some guidance as to which are of relevance in understanding pathogenicity, host resistance, etc. No comparable integrated, multidisciplinary *M. bovis* studies have yet been attempted, but such studies are undoubtedly essential if progress is to continue in molecular epidemiology.

The history of molecular epidemiological studies of bovine tuberculosis has been mixed. In the fifteen years since genotyping methods were first introduced, the technical achievement in developing and refining these methods has been impressive. However, much less progress has been achieved in the application of genotyping to provide insight into disease dynamics and improved methods of disease control. Nevertheless, sufficient positive examples can be found in the molecular epidemiology of human tuberculosis to lend confidence to the belief that practical utilisation of typing in routine control of bovine tuberculosis should be achievable in the near future.

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Épidémiologie moléculaire de la tuberculose bovine
II. Applications du typage génomique

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Résumé
Les auteurs examinent les applications du typage génomique de *Mycobacterium bovis*. Les travaux de recherche publiés à ce jour ont été effectués essentiellement dans le cadre de la validation de méthodes de typage et peu d'études ont été menées à des fins purement épidémiologiques. Il en va tout autrement de la tuberculose humaine pour laquelle l'application du typage par polymorphisme de taille des fragments de restriction utilisant la séquence d'insertion IS6110 a permis de mieux comprendre l'épidémiologie et l'évolution moléculaire de l'agent pathogène. L'expérience de la médecine humaine montre l'intérêt, pour les études à venir, d'une méthode intégrée alliant épidémiologie et biologie moléculaire. Il devrait donc être possible d'identifier avec précision les groupements par type et de les expliquer, ce qui devrait faciliter les décisions en matière de prophylaxie.

Mots-clés
Analyse par enzymes de restriction (endonucléases) — Épidémiologie moléculaire — Mycobacterium bovis — Polymorphisme de taille des fragments de restriction — Spoligotypage — Tuberculose — Typage génomique.

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Epidemiología molecular de la tuberculosis bovina
II. Aplicaciones de la tipificación genética

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Resumen
Los autores pasan revista a las aplicaciones de la tipificación genética de *Mycobacterium bovis*. Las investigaciones publicadas hasta ahora estaban destinadas principalmente a validar métodos de tipificación, por lo que de momento existen pocos estudios de carácter específicamente epidemiológico. Muy distinta es la situación en el campo de la tuberculosis humana, donde, gracias al uso de la secuencia de inserción IS6110 para analizar el polimorfismo de longitud de los fragmentos de restricción, se empieza a comprender la epidemiología y la evolución molecular del agente patógeno. Basándose en estos antecedentes, los autores recomiendan que de ahora en adelante se aborde la cuestión de forma integral, combinando estudios de epidemiología y de biología molecular. Ello permitiría identificar claramente los tipos bacterianos y determinar sus respectivas afinidades y relaciones, lo que sin duda facilitaría la adopción de decisiones relativas al control de la enfermedad.

Palabras clave
Análisis por endonucleasas de restricción — Epidemiología molecular — Mycobacterium bovis — Polimorfismo de longitud de fragmentos de restricción — Spoligotipificación — Tipificación genética — Tuberculosis.
References


