Review

Mycobacterium bovis at the animal–human interface: A problem, or not?

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ABSTRACT

Mycobacterium bovis is a pathogen of significant importance in livestock and a wide range of wild animal species worldwide. It is also known to cause tuberculosis disease in humans, a fact which has raised renewed concerns regarding the zoonotic risk for humans, especially those living at the animal-human interface. This review consolidates recent reports in the literature mainly on animal and zoonotic tuberculosis with an emphasis on evolution, epidemiology, treatment and diagnosis. The information presented reveals the fundamental differences in the complexity and level at which the disease affects the economy, ecosystem and human population of regions where animal tuberculosis control is achieved and regions where little or no control is implemented. In conclusion the review suggests that bovine tuberculosis has essentially been reduced to a disease of economic importance in the developed world, while low-income countries are facing a multifaceted impact which potentially affects the health of livestock, humans and ecosystems and which is likely to increase in the presence of debilitating diseases such as HIV/AIDS and other factors which negatively affect human livelihoods.

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1. Introduction

Tuberculosis in animals is primarily known from cases in cattle and other bovids for which the disease is generally referred to as bovine tuberculosis. The major causative agent of bovine tuberculosis is *Mycobacterium bovis*, a

member of the *Mycobacterium tuberculosis* complex (Smith et al., 2006). Animal tuberculosis is a disease of high economic relevance within the context of livestock farming as it directly affects animal productivity and also influences international trade of animal products. *M. bovis* infections have also been detected in wildlife and can have severe consequences for the ecosystem. Moreover, animal tuberculosis bears a zoonotic potential and is therefore of public health concern (Cosivi et al., 1998; Renwick et al., 2007).

However, although animal test-and-slaughter schemes have successfully reduced the prevalence of bovine tuberculosis in most industrialized countries, such expensive control programmes have been increasingly questioned considering their economic burden and increasing opposition by farmers (Torgerson and Torgerson, 2009; Bennett, 2009). Furthermore, despite occasional cases of *M. bovis* infections in humans, it is accepted that zoonotic transmission is negligible in most of the developed world (Anonymous, 2006).

The situation is profoundly different in developing countries. The WHO in conjunction with FAO and OIE recently classified bovine tuberculosis as a neglected zoonosis, with special reference to developing countries. In the world's most vulnerable communities, animal diseases, which are transmissible between livestock and humans, not only have the potential to impact on human health directly, but to threaten human livelihoods by compromising sustainable food supply, income and social status (http://www.who.int/zoonoses/Report_Sept06.pdf). Although recent studies have provided insights into the significance of zoonotic tuberculosis in developing countries in Africa (Cadmus et al., 2006), the extent to which zoonotic transmission contributes to the burden of human tuberculosis in these areas is still largely unknown. In Southern Africa, like other regions in Africa, communities facing a higher disease risk from *M. bovis* include those living at the livestock–human interface, consuming mostly unpasteurised milk and dairy products derived from cattle herds with an uncontrolled bovine tuberculosis disease status. At the same time they also include those population groups who are suffering from the world's highest HIV/AIDS infection rates and the associated increased susceptibility to co-infection with *M. tuberculosis*, the main cause of tuberculosis in humans (Ayele et al., 2004). To make matters worse, the risk groups mentioned are not mutually exclusive but may be identical in many cases.

In this short review, we will attempt to consolidate recent reports in the literature mainly on animal and zoonotic tuberculosis evolution, epidemiology, treatment and diagnosis with the aim to present the reader with a synopsis of the current knowledge in this field. Emphasis will be given to the question whether and to which extent animal and zoonotic tuberculosis is actually a problem in industrialized and developing countries.

2. Historical perspective

The *M. tuberculosis* complex is generally considered a family of "ecotypes" of very closely related Mycobacteria, with each ecotype being adapted to cause tuberculosis disease in a specific host species or group, even though inter-species transmission can occur (Smith et al., 2006). In contrast to the earlier hypothesis that tuberculosis has evolved from an originally animal disease to a human disease (Diamond, 2002), new findings indicate that in fact tuberculosis first emerged in humans and was subsequently transmitted to animals (Wirth et al., 2008). Recent studies suggest that the common ancestor of the *M. tuberculosis* complex emerged from its progenitor perhaps 40,000 years ago in East Africa. Some 10,000–20,000 years later, two independent clades evolved, one resulting in *M. tuberculosis* lineages in humans, while the other spread from humans to animals, resulting in the diversification of its host spectrum and formation of other *M. tuberculosis* complex member species, including *M. bovis* (Gutierrez et al., 2005; Wirth et al., 2008). This adaptation to animal hosts probably coincided with the domestication of livestock approximately 13,000 years ago.

Evidence in the form of skeletal lesions compatible with Pott's disease and especially the use of PCR-based DNA techniques date the occurrence of early documented cases of tuberculosis in both humans and animals to at least 3000 BC (Taylor et al., 2005, 2007). Pathognomonic bone lesions indicative of tuberculosis in bovids were found in skeletons of ice-age representatives of this genus but the link to hominids is currently unclear (Rothschild and Martin, 2006).

In modern history, cattle served as principle reservoir species for *M. bovis*, hence the name bovine tuberculosis. This term is also commonly used to describe *M. bovis* infection in other species including wildlife and humans to demonstrate the bovine source of the infection. Movement of cattle within and between countries and continents certainly facilitated the worldwide distribution of bovine tuberculosis, although the ultimate origin of *M. bovis* is unknown. However, progress has been made in our understanding of the population structure of *M. bovis* through the use of the PCR-based spoligotyping and VNTR typing methods, which allowed the identification of clonal complexes of *M. bovis* dominant in larger geographic locations. Recently, a clonal complex of strains of *M. bovis* named African1 (Af1) that is geographically localized to the Central-West African region has been described (Müller et al., 2009). Strains of Af1 were very frequent in this region and appeared to have nearly reached fixation in some areas of Central-West Africa. The most likely explanation for this observation is an introduction of *M. bovis* into cows that were originally naïve to tuberculosis (Müller et al., 2009). Similarly, a clonal complex provisionally named Eu1 appears to be dominant in the British Isles. We can expect that other groups are likely to be geographically localised to other regions of the world (Müller et al., 2009). Recent advances in our understanding of the population structure of *M. bovis* notwithstanding, the actual origin of these clonal complexes remains unknown.

Historical data could suggest that bovine tuberculosis actually emerged in Europe and was distributed throughout the world mainly during the colonial period. Myers and Steele (1969) suggested that *M. bovis* emerged in Europe and spread from northern Italy to Western Europe and the

UK (Myers and Steele, 1969). According to Webb (1936), *M. bovis* was thereafter distributed throughout the world through exportation of infected cattle from (mainly) the UK and the Netherlands to their former colonies (Renwick et al., 2007). *M. bovis* was reported in Africa at the beginning of the 20th century (Ostertag and Kulenkampff, 1941). During colonial times, the emigration of European settlers and their livestock facilitated the large-scale intercontinental movement of infected cattle. In the post-colonial era, cattle were exported from Europe into many African countries, mainly to improve the dairy production in these countries. As a result, strains representative of *M. bovis* clades which had evolved through clonal expansion in a restricted geographical location were subsequently shared between geographically distinct countries with political and economic ties (Müller et al., 2009) Examples have been documented in Algeria, Mali and South Africa where VNTR typing revealed a link between local *M. bovis* isolates and those described in France and the United Kingdom, respectively (Michel et al., 2008; Sahraoui et al., 2009). Intensification of the dairy industry in combination with movement of cattle (Gilbert et al., 2005) has contributed to the transmission of *M. bovis*, especially in the absence of suitable control measures. Cattle trade between neighbouring countries and trading partners probably lead to the regional dispersal of *M. bovis* and to the dominance of strains in large areas (Diguimbaye-Djaibe et al., 2006; Cadmus et al., 2006; Müller et al., 2008). However, these theories are still speculative and the evolutionary relationships between European and other strains of *M. bovis* may not be evident before sequencing of a number of strains from multiple sources allows in-depth genome analyses and the construction of comprehensive phylogenetic trees.

3. Disease in animals

3.1. Epidemiology and control in cattle

Bovine tuberculosis in cattle is an infectious, chronic but progressive disease characterised by the formation of typical granulomatous lesions with varying degrees of necrosis, calcification and encapsulation. Transmission between animals is thought to occur mainly by inhalation of contaminated aerosol and therefore affects the lungs primarily (Kaneene and Pfeiffer, 2006). However, infection can also occur via the gastro-intestinal tract or become systemic and affect other organs, such as the urinary tract or the mammary lymph nodes (Cousins et al., 2004). The number of severe cases of animals with clinical manifestation may be limited or absent in countries where active control measures are applied. Advanced disease and generalisation are usually more common in countries with insufficient or no control, adding to an increased risk for transmission to humans (Cosivi et al., 1998).

Risk factors contributing to difficulties in controlling bovine tuberculosis in cattle across continents can have their origin at farm-level, e.g. cattle breed (Ameni and Erkihun, 2007), age, behaviour, and nutrition of animals (Menzies and Neill, 2000). However, host independent factors are considered more important in most cases and include, amongst others, production types and management practices (Carrique-Mas et al., 2008; Elias et al., 2008), cattle movement (Green et al., 2008), existence of a wildlife reservoir (Porphyre et al., 2008), and possibly strain related differences (Andreevskaia et al., 2007) and survival of *M. bovis* (Tanner and Michel, 1999).

Bovine tuberculosis is widespread in cattle throughout the globe. According to information on the worldwide animal health information database of the OIE (WAHID Interface, http://www.oie.int/wahis/public.php?page=-home), 128 out of 155 countries reported the presence of *M. bovis* infection and/or clinical disease in their cattle population during the period between 2005 and 2008. In developed countries, the driving forces for the control and eradication of bovine tuberculosis from the national domestic herd are indisputably of economic and socio-political nature, based mainly on the negative economic impact of the disease (Reviriego Gordejo and Vermeersch, 2006). In large parts of the developed world, policies regulating the control of bovine tuberculosis are aimed at complete eradication of the disease from its livestock populations as part of an integrated approach to food safety. These policies follow an expensive test-and-slaughter strategy for the control of bovine tuberculosis and significant successes have been achieved in many countries (Reviriego Gordejo and Vermeersch, 2006; Radunz, 2006). On the other hand, the benefit and sustainability of such costly programmes have been increasingly questioned in the light of the rising economic burden and social impacts on and reduced acceptance by farmers (Torgerson and Torgerson, 2009; Bennett, 2009). However, in general, with the exception of a few countries with a wildlife reservoir of *M. bovis* (see further below), the prevalence of bovine tuberculosis has reached very low levels, in most developed countries (Eurosurveillance Editorial Team, 2005, http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2712).

The situation is profoundly different in developing countries, which are in general unable to apply expensive test-and-slaughter schemes for the control of animal tuberculosis. Although in parts of the Latin American and Caribbean countries there has been significant progress in bovine tuberculosis control and infection rates under 1% have been reported for 30% of the region's cattle, 70% of cattle are kept in areas where rates of infection are higher and where herd prevalence of up to 56% have been reported (de Kantor and Ritacco, 2006; de Kantor et al., 2008). On the African continent, more than 80% of the human population co-exists with cattle in the absence of any organized control of bovine tuberculosis (Cosivi et al., 1998). In recent years a growing awareness of neglected zoonoses including bovine tuberculosis has led to initiatives supported by the WHO/FAO/OIE to investigate, calculate and mitigate the unknown risk from these animal diseases on livestock productivity, human health and livelihoods (WHO, 2009). Overall, the presence and extent of bovine tuberculosis in the developing world has been poorly investigated in the past, but a number of recent studies have revealed new data confirming the presence of *M. bovis* in cattle (Diguimbaye et al., 2004; Oloya et al., 2007; Srivastava et al., 2008; de Kantor et al.,

2008) and moreover providing insights into the specific risk factors associated with tuberculosis in cattle in different countries and regions. In Africa, high prevalence rates of bovine tuberculosis (up to 50% at herd level) were reported in areas of Zambia where cattle and Kafue lechwe shared grazing and water as well as in areas where the traditional management of livestock in transhumant herds (herds which are moved to floodplains for grazing during the dry season) prevailed (Oloya et al., 2007; Munyeme et al., 2009). Under these often nomadic conditions, the risk of exposure to *M. bovis* was increased significantly by creating multiple herd contacts and increasing the total herd size. The latter has also been suggested as a driver of the disease prevalence in Ethiopia (Ameni et al., 2003) and Ecuador (Proano-Perez et al., 2006). On the other hand, in countries with a rapidly increasing livestock production and intensification of production systems such as Iran, the propagation and insufficient detection of circulating *M. bovis* strains may be the most important contributor to increasing economic losses from bovine tuberculosis, rather than the importation of infected cattle, as previously suggested (Tadayon et al., 2008).

Most importantly, in the mainly rural livestock producing areas of developing countries, bovine tuberculosis can have devastating impacts on the livelihood of millions of the world's most vulnerable communities as the disease compromises their sustainable food supply, income and social status (http://www.who.int/zoonoses/Report_-Sept06.pdf).

3.2. Disease in wildlife

At present, cases of *M. bovis* infection have been reported in more than 40 free-ranging wild animal species. Despite significant variations in size, appearance and distribution of the tuberculous lesions in different species, in the majority of affected wildlife species lesions closely resemble those in cattle (Zanella et al., 2008; Drewe et al., 2009). A consistently different pattern of pathological changes has, however, been described in lions where no histological evidence of necrosis was found (Keet et al., 1996). For detailed information regarding pathological changes, disease severity, epidemiology and implications we refer the reader to the specific literature. It is difficult to quantify the extent of disease in this large variety of animals, but measurement in predators as a surrogate to measure extent of disease in prey can possibly be considered (VerCauteren et al., 2008).

Tuberculosis in wildlife can pose serious difficulties for bovine tuberculosis control and eradication. Particularly noteworthy is the case of the British Isles, where the European badger represents an important and well-documented disease reservoir (Smith et al., 2006). In several industrialized countries that have adopted animal tuberculosis control programmes and in which wildlife has been involved, control programmes were designed to exclusively benefit the livestock sector with less importance given to wildlife conservation or protection (Radunz, 2006; Porphyre et al., 2008). This is mostly due to the fact that many of the wildlife maintenance host species, with the exception of badgers in the United Kingdom and

Ireland, score a low priority on their national wildlife conservation listings and enjoy, at best, the status of valued, sought-after hunting trophies (Rudolph et al., 2006) In some cases these reservoir species are even classified as alien or feral with well-documented examples being the brushtailed possums in New Zealand and feral water buffaloes in Australia (Radunz, 2006; Porphyre et al., 2008).

In sharp contrast to the spectrum of scenarios found in developed countries worldwide, bovine tuberculosis is an endemic disease in livestock in many African countries. In South Africa and other African countries, *M. bovis* has been transmitted from livestock to wildlife reservoirs in free-ranging ecosystems with potentially far reaching direct and indirect implications on wildlife, livestock and human populations (Michel et al., 2006). Perhaps most importantly, *M. bovis* has established itself in the African buffalo (*Syncerus caffer*), a wildlife species of outstanding economic and ecological value, also reflected in its ranking among the 'Big Five' wildlife species. Bovine tuberculosis in buffalo poses a threat not only to species conservation efforts and ecotourism but to commercial game farming which has, through the historically embedded prestige associated with keeping indigenous game, created a unique and sustainable niche in African agriculture. The wildlife industry in, e.g. South Africa nowadays enjoys the status of a specialised sector within agriculture and the land surface presently utilised for game farming is equal to or has, in some parts of the country, exceeded that of livestock farming. The potential for spillover of *M. bovis* from buffalo to other wildlife species extends the risk of infection to all types of wildlife and mixed livestock/wildlife operations.

4. Zoonotic tuberculosis

Tuberculosis in humans due to *M. bovis* is both clinically and pathologically indistinguishable from cases caused by *M. tuberculosis* (Wedlock et al., 2002). As for animals the primary location of lesions depends on the route of infection but also on subsequent dissemination of *M. bovis* to other organs. Transmission of tuberculosis from cattle to humans mostly occurs through the consumption of unpasteurized milk and close contact to infected animals.

The epidemiological link between tuberculosis in cattle and in humans, especially children, has long been recognised even before Robert Koch identified the tubercle bacillus in 1882. It appears that in previous centuries the easily noticeable so-called "TB grapes" in slaughter cattle, caused by nodular tuberculous lesions on the pleura or mesentery, were considered harmful to human health and were later associated with a living infectious agent "contagium vivum" transmitted to humans from cattle (Orland, 2003). Emil von Behring and leading paediatricians in the early 20th century thought of human tuberculosis caused by the bovine tubercle bacillus as an infectious disease, which was in many cases acquired in early childhood and could remain latent before causing pulmonary disease in adults (Zeiss and Bieling, 1940). This hypothesis received new support decades later when the rapid success in combating cattle tuberculosis was not

immediately paralleled by a decline of human *M. bovis* cases, especially in adults (Meissner, 1974).

During the first half of the 20th century bovine tuberculosis was considered one of the largest veterinary public health problems in Central Europe. Before the implementation of the eradication scheme 90% of the cattle herds in Germany were infected (Meissner, 1974). A significant percentage of tuberculosis cases in humans were thought to be caused by *M. bovis*, especially in children and cattle-tending persons in rural areas (Schmiedel, 1968). The breakthrough in the eradication of bovine and zoonotic tuberculosis in developed countries was achieved through mandated tuberculin testing of livestock and removal of positive reactors and compulsory pasteurisation of milk. As a result of these rigorous and expensive control efforts, the risk of contracting zoonotic tuberculosis has become extremely low in developed countries over the past few decades. The number of *M. bovis* cases in humans reported from 10 European Union member countries has exceeded 60 cases per year (sporadically only) between 1996 and 2003 (Eurosurveillance Editorial Team, 2005, http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2712). In contrast, it has been speculated that up to 7000 new human cases may occur in Latin America each year (de Kantor and Ritacco, 2006). Despite the fact that most of the human *M. bovis* cases in Europe occurred in the United Kingdom where bovine tuberculosis remains problematic to control, only a few of these are attributed to recent cattle-to-human transmission. Most cases are believed to be rather due to reactivation of latent infections contracted before 1960 or infections contracted outside the UK (de la Rua-Domenech, 2006; Jalava et al., 2007). The latter has also been confirmed in the USA, where the CDC reported 165 cases of BTB in humans out of 11,860 cases studied (Moonan et al., 2009), of which most if not all were attributed to consumption of unregulated and unpasteurised dairy products in foreign-born persons.

Human-to-human transmission of *M. bovis* as well as concurrent infection with *M. tuberculosis* is rare and quite probably occurs mostly in unusual cases (LoBue et al., 2001; Gibson et al., 2004; Sunder et al., 2009). Where the opportunity exists, transmission of *M. bovis* from humans back to cattle can occur and may, under these circumstances, complicate efforts to control bovine tuberculosis in cattle (see further below) (Szewzyk et al., 1995; Fritsche et al., 2004). Immuno-suppression due to HIV-infection is a known complication in humans affected by *M. tuberculosis* and has recently emerged as an aggravating factor in *M. bovis* infection in humans at the livestock–human interface, mainly in nosocomial outbreaks. Some of these were caused by multidrug-resistant *M. bovis* strains and caused complications in hospitalized HIV-infected patients (Cobo et al., 2001).

In developing countries, the conditions for *M. bovis* transmission to humans not only exist unchanged, but the human population has a greater vulnerability due to poverty, HIV and reduced access to health care (Ayele et al., 2004; WHO, 2009). The exact percentage of *M. bovis* in human tuberculosis cases is often difficult to determine, since generally the diagnosis of "TB" is made on the basis of

sputum smears only (Thoen et al., 2006). The WHO reported in 1998 that 3.1% of tuberculosis cases in humans worldwide are attributable to *M. bovis* and that 0.4–10% of sputum isolates from patients in African countries could be *M. bovis*. This is despite the fact that *M. bovis* is more often associated with extrapulmonary disease in humans (Cosivi et al., 1998).

More detailed data including strain characterization have recently been presented and confirm that the occurrence of *M. bovis* in the human population is a persistent, though insufficiently quantified feature in developing countries. The isolation rate of *M. bovis* from symptomatic human patients in specific studies was 13.8% in Mexico (Pérez-Guerrero et al., 2008), 6.9% in Uganda (Oloya et al., 2008), 5% in Nigeria (Cadmus et al., 2006), 0.5% in Taiwan (Jou et al., 2008) and between 0 and 2.5% in 10 Latin American countries (de Kantor et al., 2008). Through the use of epidemiological tools such as genetic typing of the *M. bovis* strains or case-control studies, epidemiological links between *M. bovis* infections affecting human and cattle populations have recently been demonstrated, for example, in Mexico, Uganda and Ethiopia, respectively. Genetic typing could not confirm the presence of common strains at the livestock–human interface in Chad (Diguimbaye et al., 2004) and further investigations, especially regarding the sample sizes, are necessary. Care should also be taken when using individual study results in the assessment of country situations as the findings may differ significantly between regions and ethnic groupings within countries as shown, e.g. in Uganda and Taiwan (Jou et al., 2008; Byarugaba et al., 2009). In some noteworthy studies in Tanzania and Uganda, *M. bovis* accounted for 18–30% of all *M. tuberculosis* complex strains isolated from human patients, in rural settings (Kazwala et al., 2001; Mfinanga et al., 2004; Cleaveland et al., 2007) whereas low prevalence rates of *M. bovis* infections were found in urban populations (Asiimwe et al., 2008). It is to be expected that the incidence of zoonotic tuberculosis in developing countries is heterogeneously distributed and that the livestock producing rural populations are mostly affected by *M. bovis* infections. However, there are only very few studies that have investigated the prevalence of zoonotic tuberculosis in rural communities of developing countries.

Although overall the proportion of *M. bovis* causing human tuberculosis is very low compared to *M. tuberculosis*, its potential impact on population groups at the highest risk should nevertheless not be underestimated. Exposure to aerosol-borne infection with *M. bovis* from cattle remains highest in farmers, veterinary staff and rural and slaughterhouse workers, while in developing countries, ethnicity, cultural and religious practices as well as socio-economic factors have been identified as additional contributors to an increased occurrence of *M. bovis* infections in humans. For Africa, general recommendations to combat bovine tuberculosis in humans, together with other neglected zoonotic diseases, have been formulated, which call on global, regional and national leadership to advocate and implement the "One Health" approach as an integrated strategy to improve human and animal health (WHO, 2009). Differential diagnosis should take priority in

control plans in order to enable the optimal use of veterinary intervention as a means to reduce the burden of human disease from an animal source. However, appropriate methods for differential diagnosis in developing countries do not exist (see further below).

The widespread co-infection of cattle and wildlife populations with *M. bovis* has raised the question of the risk of *M. bovis* transmission to humans through occupational or recreational exposure to wildlife. Few reports exist with limited information, but it is generally believed that the risk of direct disease transmission at the wildlife–human interface is probably negligible (Fanning et al., 1991; Szewzyk et al., 1995; Weyer et al., 1999).

4.1. Therapy

M. bovis reacts very similarly to *M. tuberculosis* in terms of sensitivity to antibiotics. In the case of *M. tuberculosis*, the standard therapy for antibiotic sensitive isolates consists of 2 months of isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z), followed by 4 months of H and R. *M. bovis* is sensitive to all these first-line antibiotics except Z (Daly et al., 2006). Furthermore, in the absence of acquired antibiotic resistance, wild-type *M. bovis* is sensitive to most if not all the other antibiotics used to treat TB, viz. streptomycin, ethambutol, ofloxacin, ethionamide (Parreiras et al., 2004; Romero et al., 2007). It is likely that despite natural resistance to Z, for most human cases, tuberculosis due to *M. bovis* will cure when a standard treatment is applied. Therefore, most clinics in high burden countries may well argue that *M. bovis* is not problematical from the human case management and therapy point of view. Whether more cases will relapse or develop drug-resistance because of innate resistance to pyrazinamide has not been properly investigated. However, large-scale comprehensive antibiotic trials show that the cure rate is high and relapse rate low when *M. tuberculosis* cases are treated with first-line drugs, even in the absence of Z (Fox et al., 1999). These trials suggest that the key antibiotics are isoniazid and rifampicin, and this evidence suggests to us that using the standard TB treatment regimen recommended for *M. tuberculosis* cases in humans could yield an expected relapse rate of between 2 and 6% for *M. bovis* cases, which is acceptable. Alternatively, addition of one other antibiotic in the intensive phase of treatment (such as ofloxacin or moxifloxacin; Rustomjee et al., 2008) or others such as streptomycin or ethionamide, could be a recommendation for the future. As with *M. tuberculosis*, it is clear that *M. bovis* can become drug-resistant and cause drug-resistant epidemics (Rivero et al., 2001).

The treatment of tuberculosis in animals depends on the specific reaction of individual species to the various antibiotics and the logistics and ease of administration of medication. It has been done for a few rare animal species in captivity, but it is not really viable for a herd or free-ranging animals.

5. *M. tuberculosis* infection in animals

Infection with *M. tuberculosis* occurs most frequently in animals living in close contact with humans and has

therefore been one of the most frequently recorded infectious diseases of captive wildlife (Kovalev, 1980). The risk of spillover of *M. tuberculosis* from humans to animals is considered high where tuberculosis in humans continues to be of great public health concern. This was demonstrated in an 11-year study on *M. tuberculosis* cases in the National Zoological Gardens of South Africa, which indicated that the disease was more frequently transmitted by visitors to animals than between animals (Michel et al., 2003). *M. tuberculosis* has been encountered as an emerging disease especially in Asian elephants in zoological collections in the USA (Mikota et al., 2001) but also in their native countries (Sarma et al., 2006).

Free-ranging wildlife is believed to be less prone to *M. tuberculosis* compared to those in captivity (Griffith, 1928), although this may change with the level of exposure of wild animals to human pathogens at the human–wildlife interface (Alexander et al., 2002).

In domestic animals, infection with the human tubercle bacillus has been known since the beginning of the previous century, when typical lesions were found in livestock exposed to garbage and effluents from tuberculosis hospitals (Kraus, 1942). Nowadays *M. tuberculosis* is still occasionally diagnosed in domestic pigs where they are exposed to humans shedding the disease (Mohamed et al., 2009). Hence screening of slaughter pigs for typical caseous lesions in the parotid lymph nodes can be of value as a sentinel detection system for human tuberculosis in low incidence countries. Likewise, infection of companion animals, especially dogs, but also other species including birds and monkeys, with *M. tuberculosis* is rather a reflection of the disease burden in the human population than a public health concern and has sometimes triggered diagnosis in the owner (Michel and Huchzermeyer, 1998; Parsons et al., 2008; Schmidt et al., 2008).

The number of documented cases of *M. tuberculosis* in cattle appears to have increased in recent years (Cadmus et al., 2006; Srivastava et al., 2008; Berg et al., 2009; Chen et al., 2009), which may be due to improved diagnosis by molecular tools and/or an actual increase in transmission from humans to cattle in these countries. According to information released by the WHO on the global tuberculosis burden, the incidence has increased in sub-Saharan Africa and in Southeast Asia, India, China, Bangladesh and Pakistan (http://www.who.int/vaccine_research/diseases/ari/en/index4.html). At the same time many developing countries have intensified their livestock production to meet the growing demand for food security, which has led to a higher risk of transmission for both *M. tuberculosis* as well as *M. bovis* at the human–livestock interface.

The isolation of *M. tuberculosis* from cattle raises a number of questions relating to the role of humans as a source of infection to cattle. It may be speculated that in some countries more cattle contract tuberculosis from humans than vice versa. The possible existence of cattle-adapted *M. tuberculosis* strains and subsequent cattle-to-cattle and cattle-to-human transmission still needs clarification. The pathological changes in cattle do not appear to support disease transmission, since *M. tuberculosis* infection usually does not progress beyond the development of small granulomas in several different

lymph nodes (Cousins et al., 2004). On the other hand, Srivastava et al. (2008) were able to isolate *M. tuberculosis* from milk samples in India, suggesting that in rare cases transmission to humans may occur in unpasteurised milk.

On a few occasions, *M. africanum*, another member of the *M. tuberculosis* complex supposed to primarily affect humans, has been implicated in the development of granulomatous lesions in lymph nodes and lungs of cattle and pigs (Alfredsen and Saxegaard, 1992; Rahim et al., 2007) which are indistinguishable from those caused by *M. bovis*. The true prevalence of *M. africanum* in livestock and its implications on animal and human health remain currently unknown, owing to national control measures being largely based on gross pathological examination of lesions.

6. Diagnostics

Despite our extensive knowledge on tuberculosis, disease diagnosis and the identification of the infecting mycobacterial species is not yet a simple matter. Species identification is indispensable for the study of the transmission of Mycobacteria between humans and animals. Moreover, diagnostics and species identification plays a major role in tuberculosis surveillance and control, in particular also at the animal–human interface.

Perhaps the first and best-known test for tuberculosis diagnosis is the tuberculin skin test. The same principle is used for testing in both, animals and humans and although imperfect, the tuberculin test has not yet been replaced by any other more accurate or satisfactory method. Some of the main deficiencies of the test are its inability to differentiate between distinct species of the *M. tuberculosis* complex, failure to distinguish between latent stages of infection and disease and failure to distinguish vaccinated and infected individuals (de la Rua-Domenech et al., 2006). In addition, anergy, exposure to environmental Mycobacteria and operator errors can lead to false results (de la Rua-Domenech et al., 2006). Two recent studies also suggested that the cut-off generally used for positive test interpretation (>4 mm) may not be applicable for at least some countries in sub-Saharan Africa and that a cut-off >2 mm could be more appropriate in some settings (Ameni et al., 2008; Ngandolo et al., 2009). The more recently developed Bovigam® test for cattle and its analog in humans, the QuantiFERON®-Tb-test, detect the production of interferon-gamma in (in vitro) stimulated blood samples. Applied in both standard commercial and customised test formats this assay has contributed significantly to the improved detection of early *M. bovis* infection in cattle as well as an increasing number of wildlife species (e.g. non-human primates, cervids) (Grobler et al., 2002; Morar et al., 2007; Denis et al., 2007; Lin et al., 2008; Waters et al., 2008). Recent improvements of the test include the use of two *M. tuberculosis* complex specific antigens, ESAT-6 and CFP-10, which has resulted in increased test specificity (Buddle et al., 2009). In contrast to the tuberculin skin test, the interferon-gamma test could also be used to differentiate between infected and vaccinated individuals. However, interferon-gamma release assays have not been found to assist significantly in diagnosing human tuberculosis in countries with a high endemic latent tuberculosis infection and high HIV prevalence (Barth et al., 2008). For diagnosis in animals in developing countries, the test also appears to be impractical as it requires sophisticated laboratory equipment and the need to quickly process the blood samples after collection (de la Rua-Domenech et al., 2006).

The tuberculin skin test and the interferon-gamma test are both based on the detection of the early cell-mediated immune response in tuberculosis infection. However, at late disease stages, the cell-mediated immune response can wane as opposed to a generally increasing humoral immune response and the tuberculin skin test or Bovigam® tests can therefore give false negative results (de la Rua-Domenech et al., 2006) This is of importance for the diagnosis of bovine tuberculosis in settings where no or little disease control measures are applied and where the percentage of late stage diseased animals is believed to be high. Therefore, in developing countries, serological tests, which are based on the detection of the humoral immune response, may be of particular use. Unfortunately, to date, no satisfactory serological test is available. Some of the problems related to the development of serological tests for tuberculosis diagnosis include the observed highly variable antibody responses between individuals to mycobacterial antigens and antigenic variation between mycobacterial strains (Pheiffer et al., 2005). However, a recently developed lateral flow test that is based on the detection of more than one antigen has shown promising results for tuberculosis diagnosis in certain animal species (e.g. in elephant), (Lyashchenko et al., 2008; Greenwald et al., 2009), although it may not be suitable for others, such as buffaloes (Michel and Simoes, 2009). Another recently developed serological test for animals is based on antibody detection using fluorescence polarisation but has shown variable effectiveness in different settings (Jolley et al., 2007; Ngandolo et al., 2009).

More direct methods for tuberculosis diagnosis are based on the isolation or detection of the bacterium in sputum samples or biopsies (mostly in humans) or at postmortem, from tuberculous organ lesions (generally in animals). The presence of Mycobacteria in a given sample can be assessed by Ziehl-Neelsen staining followed by light microscopy or auramine O staining and fluorescence microscopy. Recent work with *M. tuberculosis* suggests that the auramine O staining technique may be more sensitive and specific than Ziehl-Neelsen staining (Marais et al., 2008). However, microscopic detection of Mycobacteria shows a generally low sensitivity (from 50 to 70%) for human sputum samples. This is mainly due to the requirement of a high bacterial load for microscopy, which is particularly problematical in humans with HIV or in non-pulmonary tuberculosis. A much higher sensitivity can be achieved by prior culture of the bacteria. Culture is still regarded as the gold standard for tuberculosis diagnosis despite certain deficiencies. For example, the yield of culturable (quantities) of bacteria from blood, urine, lavage and cerebrospinal fluid is very low. Bacterial culture is also time consuming and does by itself not allow the differentiation between distinct mycobacterial species However, in many cases, culture is a prerequisite for further testing and characterization of Mycobacteria.

PCR-based techniques are indispensable for the accurate differentiation of mycobacterial species and molecular epidemiological investigations of tuberculosis transmission (for detailed technical information on this subject we refer to the more specific literature; Parsons et al., 2002; Warren et al., 2006). Although biochemical techniques also allow the differentiation between distinct mycobacterial species, these methods are very laborious, time consuming and appear to be erroneous. PCR can be used for any sample material in theory, but has some problems of its own, e.g. certain samples may contain PCR inhibitors, which could lead to false negative results. Conversely, the generation of a vast number of DNA amplicons can quickly give rise to false positive results. Moreover, the technique requires a relatively sophisticated laboratory and well-trained technicians. New methods have recently been developed, which allow a quick identification and differentiation of a range of common pathogenic mycobacterial species and some common antibiotic resistance mutations (Barnard et al., 2008; Hoek et al., 2008; Song et al., 2009). However, their usefulness as high throughput methods for tuberculosis diagnosis remains yet to be determined.

In this respect, it is particularly noteworthy to mention that many of the new developments in the field of tuberculosis diagnosis are not suitable for developing countries, either because they require expensive laboratory infrastructure or well-trained personnel. At the same time, developing countries are most severely affected by both, human and animal tuberculosis. Therefore, much of the investment made into the development of new diagnostic methods may not greatly contribute to disease surveillance and control in the most affected regions.

7. Conclusion

In this review, we have described the influence of animal tuberculosis on the economy, the environment (including populations of wildlife species) and on human health by considering the setting-dependent available resources for tuberculosis control. The information presented reveals the fundamental differences in the complexity and level at which the diseases affects the economy, ecosystem and human population of regions where animal tuberculosis control is achieved and regions where little or no control is implemented.

In industrialized countries, with a functioning animal tuberculosis control program, the disease is mostly of economic importance and losses in animal productivity, trade and food safety considerations have to be traded off against very costly interventions and the resistance of livestock producers to interference. Although reported evidence for *M. bovis* as a cause of human disease does exist, it is accepted that its role is negligible in most of the developed world (Anonymous, 2006). In developing countries, due to the absence of control, the prevalence rates of tuberculosis in livestock are much higher. This amplifies the economic losses and further increases disease incidence in animals. Moreover, as we have pointed out, the disease can directly affect the livelihood and health of millions of poor livestock holders.

In Africa, unpredictable consequences might result for entire ecosystems due to the presence of the disease in wildlife. The disease in wildlife may hamper future disease control efforts and unlike in most industrialized countries, wildlife and game farming also constitutes an important source of income for several African countries.

In low-income countries, the risk for contracting zoonotic tuberculosis is increased due to the higher infection rate in animals, absence of regular pasteurization of milk, cultural factors (e.g. often very close contact to animals) but also due to poverty, malnutrition and a higher HIV-infection rate. Nevertheless, currently available data suggests that the distribution of *M. bovis* infections in humans may be highly heterogenous and that mostly rural areas are affected. The higher occurrence of transmission between humans and animals in developing countries may be also mirrored by the fact, that apart from few anecdotal reports in industrialized countries, most studies describing *M. tuberculosis* infections in animals involve developing countries.

The profound differences between developing and industrialized countries are also mirrored by the availability of appropriate diagnostic means. Many of the newly developed methods seem to be inappropriate or out of reach for many developing countries, which lack laboratory infrastructure and well-trained technicians.

We conclude that developing countries are most dramatically affected by animal tuberculosis, however, most of the available information on animal tuberculosis comes from studies conducted in industrialized countries. Future research should be focused on further investigating tuberculosis in the most severely affected settings in order to efficiently target the major global burden of tuberculosis. Conventional cost intensive test-and-slaughter schemes do not appear to be appropriate control measures for animal tuberculosis in developing countries. Therefore, feasible strategies for the control of animal tuberculosis in developing countries need to be elaborated.

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