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Current knowledge and pending challenges in zoonosis caused by *Mycobacterium bovis*: A review

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ABSTRACT

Mycobacterium bovis is both the causative agent of bovine tuberculosis (TB) and a zoonotic pathogen. In humans, considerably fewer cases of TB are caused by *M. bovis* than *M. tuberculosis*; nevertheless, diagnostic limitations mean that currently available data on prevalence grossly underestimate the true dimension of the problem. The routes of transmission from animals to humans are well known and include direct exposure to infected animals or consumption of contaminated animal products. Application of fingerprinting tools facilitates analysis of the molecular epidemiology of *M. bovis* in animal-to-human and human-to-human transmission. Apart from cattle and *M. bovis*, other animal species and members within the *M. tuberculosis* complex can contribute to the zoonosis. Improvements in diagnostic techniques, application of more advanced discriminatory genotyping tools, and collaboration between veterinary and human health care researchers are key to our understanding of this zoonosis.

1. General aspects and dimension of the problem

Mycobacterium bovis is the causative agent of bovine tuberculosis (TB). Therefore, it has received special consideration in livestock owing to the economic impact of infections in this context. Moreover, *M. bovis* can infect a wide variety of hosts, including wild animals, captive species, primates, and even humans. Consequently, the zoonotic potential of *M. bovis* has raised public health concerns.

In humans, TB caused by *M. bovis* is much less common than TB caused by *M. tuberculosis*, and the estimated prevalence of cases caused by *M. bovis* in Europe today has fallen considerably from the 30% recorded (O'Reilly and Daborn, 1995), before the introduction of milk pasteurization procedures and "test and slaughter" control programs in cattle. It is difficult to discern the true global load of human TB caused by *M. bovis*, because TB caused by *M. tuberculosis* and TB caused by *M. bovis* are indistinguishable clinically, radiologically, and histopathologically (de la Rua-Domenech, 2006). Therefore, the only way to determine the role of each pathogen is to identify isolates to species level. However, isolation and confirmatory culture of the pathogen is not routinely performed in the regions where human infections by *M. bovis* are more prevalent, thus making identification to species level problematic. Consequently the provided by the World Health Organization (*M. bovis* was responsible for 3.1% of all TB cases in humans) cannot reflect the real dimension of the problem.

Vulnerability to infection by *M. bovis* is greater in countries which generally lack bovine TB control programs and where exposure to infected animals or consumption of non-pasteurized products is expected to be more frequent. This hypothesis is supported by a comparison of the total number of annual cases in 10 EU countries (around 60 cases) (Eurosurveillance Editorial Team, 2005, http://www.eurosurveillance.org/) with estimations for Latin America (7000 cases) (de Kantor and Ritacco, 2006).

At a national level, the highest percentages are found for Mexico (13.8% (Perez-Guerrero et al., 2008)), Uganda (7%, (Oloya et al., 2008)), and Nigeria (5%; (Cadmus et al., 2006)). Similar studies on *M. bovis* in Europe showed that prevalence fell to 0.17–0.5% in the UK (Stone et al., 2012), 0.5–2% in France (Mignard et al., 2006), and 1.4% in the Netherlands (Majoor et al., 2011). Equally low values are found in the USA (1.4%, (Hlavsa et al., 2008)). The study by Hlavsa et al. provided a very robust dataset based on systematic genotyping of *M. bovis* in a nationwide population-based sample of 11,860 *M. tuberculosis* complex (MTBC) isolates over a 10-year period (1995–2005). Percentages in developed countries with a particularly well-developed meat-producing sector are above average: in New Zealand and Ireland, 2.7% and 3% of cases of human TB, respectively, were caused by *M. bovis* (Baker et al., 2006; Ojo et al., 2008).

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M. bovis can be transmitted from animals to humans directly through exposure to infected animals or indirectly through consumption of contaminated animal products. Direct exposure to animals leads to respiratory TB because it involves inhalation of infectious droplets from the infected animals or by handling their carcasses. Indirect exposure mainly affects individuals exposed at work (farmers, veterinarians, and meat industry and slaughterhouse workers) and during leisure activities (hunters handling infected carcasses).

Ingestion of raw dairy products from infected cattle (Tohen and Barletta, 2005) is more likely associated with the development of extrapulmonary TB. Infection through the digestive tract is less frequent in developed countries as a result of milk pasteurization procedures; however, travellers visiting countries without such control measures or immigrants travelling to their countries of origin can acquire TB by consuming unpasteurized dairy products (Mechai et al., 2011). Furthermore, the frequency of infection may be higher than the national average of the host country because of the cultural habits of immigrant populations. For example, in San Diego, which is close to the Mexican border, the number of persons infected by *M. bovis* (7%) was above the average values for the United States owing to the consumption of cheeses made from unpasteurized milk among the Hispanic population living in the city (LoBue et al., 2003; Dankner et al., 1993).

2. Diagnosis

Human TB caused by *M. bovis* is underdiagnosed: clinicians generally do not suspect the specific involvement of this entity, because the diseases caused by *M. tuberculosis* and *M. bovis* in humans are clinically, radiographically, and histopathologically indistinguishable (de la Rua-Domenech, 2006). Moreover, TB caused by *M. bovis* frequently has extrapulmonary involvement, which further hinders accurate detection and diagnosis (Allix-Beguec et al., 2010; Cicero et al., 2009). Finally, *M. bovis* is also expected to be underdetected when it coinfects a person already infected with *M. tuberculosis*. Although thought to be infrequent, coinfection with *M. bovis* was detected in 3 of 189 TB-infected patients (1.6%) in a prevalence study carried out in an urban area of Brazil between 2008 and 2010 (Silva et al., 2013). The authors warned about possible underdetection of coinfected cases.

Therefore, assignment of *M. bovis* as the causative agent of TB requires identification to species level, which is beyond the diagnostic capabilities of many laboratories in the most affected regions, since medium containing pyruvate (required for *M. bovis* culture) is not always available. Standard identification to species level of the different members of the *M. tuberculosis* complex is based on phenotypic and biochemical characteristics, such as dysgonic growth, slow growth rate, accumulation of niacin, nitrate reductase activity, and intrinsic resistance to thiophene-carboxylic acid hydrazide and pyrazinamide (Collins and Yates, 1997; Sreevatsan et al., 1996). These tests are laborious, accurate identification takes several weeks, and interpretation of the results is highly subjective.

The design of molecular techniques is complicated by the high similarity between some of the MTBC members at the nucleotide level (99.9%), which renders genetic regions commonly used for identification to species level (e.g., DNA coding for 16s rRNA) almost indistinguishable (Boddinghaus et al., 1990; Sreevatsan et al., 1997). This design problem has limited the development of commercial diagnostic kits. The only available kit is the GenoType MTBC line probe assay (Hain Lifescience GmbH, Nehren, Germany), which is based on DNA-STRIP technology and takes advantage of the presence of specific single-nucleotide polymorphisms (SNPs) in the *gyrB* gene, thus enabling genetic differentiation between the following members of the MTBC: *M. tuberculosis*, *M. bovis BCG*, *M. bovis*, *M. africanum*, *M. caprae*, and *M. microti*. However, the approach does not differentiate correctly between *M. canettii* and *M. tuberculosis* or between *M. pinnipedii* and *M. africanum* type I.

Efforts have been made to develop in-house molecular techniques to identify *M. bovis*. A diagnostic algorithm based on the analysis of the SNPs found in 5 genes (Rv0557, Rv1009, Rv0129, Rv1811, and Rv2629) has been developed (Homolka et al., 2012). The algorithm is inexpensive and enables identification with a high degree of confidence. In addition, it can classify the isolates into 17 phylogenetic lineages. The *Seek*TB method (Reddington et al., 2012) correctly identifies all members of *M. tuberculosis* complex. *Seek*TB is an internally controlled 2-stage multiplex real-time PCR method. Its results are 100% concordant with those of the GenoType MTBC assay. Its turnaround time is 1.5–3.5h after DNA extraction. Tanya et al. (Halse et al., 2011) developed a single-tube multiplex real-time PCR approach for rapid identification of members of the *M. tuberculosis* complex based on comparative genomics (specifically, regions of difference RD). The main advantage of this assay is that it enables identification of *M. bovis* directly from clinical specimens without culture, thus providing information within 2days of the reception of the sample. Other technologies, such as mass spectrometry, are also being evaluated as diagnostic tools. However, although several attempts have been made to apply matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the identification species within the MTBC, no robust results have been obtained to date (Shitikov et al., 2012; Saleeb et al., 2011).

Identification of the members of the MTBC to species level is relevant mainly for epidemiological and public health purposes; however, it can have a therapeutic impact in specific cases. *M. bovis* and *M. canettii* are intrinsically resistant to pyrazinamide (Barouni et al., 2004; Somoskovi et al., 2007), a drug included in standard first-line anti-TB treatment. Therefore, identification of the specific etiological agent of the infection is clinically important. Allix-Beguec et al. (Allix-Beguec et al., 2010) reported a case where non-identification of *M. bovis* compromised the efficiency of treatment, with fatal consequences.

3. Reactivation of *M. bovis* infection

As is the case with *M. tuberculosis*, only a small percentage of patients exposed to *M. bovis* will finally develop the disease, which is generally a reactivation resulting from immunosuppression. Infection can be controlled in most cases. Reactivations are more likely in persons with a history of possible exposure to *M. bovis*: elderly persons from rural areas who consumed raw milk before pasteurization became widespread, persons who were occupationally exposed to the microorganism, and persons who are immunosuppressed through anti-TNF alfa therapy or HIV infection and other diseases. Reactivation is also observed in immigrants arriving from countries with no effective bovine *M. bovis* control programs or pasteurization measures and leads to an increase in the frequency of TB caused by *M. bovis* in the host country. Application of molecular tools revealed importation to France of an *M. bovis* genotype (the African 1 clonal complex), which was unseen in France but prevalent in the country of origin (Chad) (Godreuil et al., 2010).

However, the frequency of reactivations in the host country of *M. bovis* infections likely acquired in the country of origin is still below the rate of *M. bovis* infection in the autochthonous population. For example, in London, 84% of all cases of TB involved immigrants, although the proportion of *M. bovis* infections was lower in immigrants (24% of all cases of *M. bovis* infection; (Mandal et al., 2011) and clearly below the rates for rural areas in the UK (Stone et al., 2012). In France, 55% of the TB cases involved immigrants, but 70% of all cases of *M. bovis* infection were autochthonous (Mignard et al., 2006). In New Zealand, most TB cases involved immigrants, who accounted for only 29% of all cases of *M. bovis* infection (Baker et al., 2006). In the Netherlands and Italy, 60% and 71.4% of