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Whole genome sequencing of *Mycobacterium bovis* to obtain molecular fingerprints in human and cattle isolates from Baja California, Mexico

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ABSTRACT

Objectives: To determine genetic diversity by comparing the whole genome sequences of cattle and human *Mycobacterium bovis* isolates from Baja California.
Methods: A whole genome sequencing strategy was used to obtain the molecular fingerprints of 172 isolates of *M. bovis* obtained from Baja California, Mexico; 155 isolates were from cattle and 17 isolates were from humans. Spoligotypes were characterized in silico and single nucleotide polymorphism (SNP) differences between the isolates were evaluated.
Results: A total of 12 *M. bovis* spoligotype patterns were identified in cattle and humans. Two predominant spoligotypes patterns were seen in both cattle and humans: SB0145 and SB1040. The SB0145 spoligotype represented 59% of cattle isolates ($n = 91$) and 65% of human isolates ($n = 11$), while the SB1040 spoligotype represented 30% of cattle isolates ($n = 47$) and 30% of human isolates ($n = 5$). When evaluating SNP differences, the human isolates were intimately intertwined with the cattle isolates.
Conclusions: All isolates from humans had spoligotype patterns that matched those observed in the cattle isolates, and all human isolates shared common ancestors with cattle in Baja California based on SNP analysis. This suggests that most human tuberculosis caused by *M. bovis* in Baja California is derived from *M. bovis* circulating in Baja California cattle. These results reinforce the importance of bovine tuberculosis surveillance and control in this region.

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Introduction

Mycobacterium bovis is one of several members of the *Mycobacterium tuberculosis* complex (MTC) and is known to be the species responsible for bovine tuberculosis (bTB). bTB is a global zoonotic disease that affects mammals, including humans (Pérez-Lago et al., 2014). In humans, the principal mode of infection is by ingestion of contaminated unpasteurized milk or dairy products and by direct contact with infected animals (Ayele et al., 2004; Cosivi et al., 1998; Cousins, 2001). Nonetheless, limited information is available regarding bTB and human tuberculosis caused by *M. bovis* (HTBMb) in Mexico (Tables 1 and 2).

In the Mexican cattle industry, it is estimated that bTB causes an annual loss of $450 million dollars, primarily due to the removal of infected cattle (SAGARPA, 2011). The prevalence of bTB among dairy cattle is estimated to be 16% in the country (Milián et al., 2000). However, higher prevalence rates (up to 40%) have been reported in regions of Baja California (Lopez-Valencia et al., 2010).

In Mexico, the median percentage of HTBMb is 7.6% (range 0–31.6%) (Müller et al., 2013). Suspected cases of tuberculosis (TB) in humans are determined mainly by microscopy (acid-fast bacilli, AFB) and mycobacterial culture, but this is only ordered by clinicians for subjects with a high risk of drug resistance or if the patient is experiencing treatment failure. Furthermore, most laboratories only use a culture medium containing glycerol (i.e., Lowenstein–Jensen), which typically fails to grow *M. bovis* during primary isolation, as this organism requires the presence of pyruvate for optimum growth. Furthermore, in Mexico, only a small number of laboratories have the capacity to perform the molecular procedures required to identify MTC at the species level (de Kantor et al., 2010).

Efforts made in the field of mycobacterial genomics in past years and the availability of whole genome sequences of MTC strains, including *M. bovis* (Cole et al., 1998; Garnier et al., 2003), have made it possible to address questions concerning genetic traits of the bovine tubercle bacillus, such as drug resistance and susceptibility (Walker et al., 2015).

Several methods for genotyping MTC, including *M. bovis*, have been described. Spoligotyping has been one of the most common methods for large-scale screening, since it can be used to analyze the distribution of *M. bovis* strains, and it can be used to compare isolates between different laboratories and countries. The characterization of strains in Mexico by spoligotyping is well documented in the literature (Zumárraga et al., 2013). This method is used to determine genetic diversity by PCR amplification of highly polymorphic direct repeat (DR) regions flanking the insertion

Table 1
Mycobacterium bovis in Mexican cattle.

Isolation site	Detection Years	Isolates (%)	Spoligotype (%)	VNTR
Qro (Milian-Suazo et al., 2000)	1992–1996	81 (79)		
Ags, Qro, Hgo, Coah (Milian-Suazo et al., 2000)	1996–1997	308 (77)		
Ags, Qro, EDM, Jal (Milián-Suazo et al., 2002)	1996–1997		Orphan (12), SB0663 (7), SB 1014 (7), SB0269, SB1109, SB1208, SB1493 (7), SB1697, SB1699, SB1700, SB1701, (7) SB1702, SB1704, SB1705, SB1706 (7) SB1707, SB1709, SB1710, SB1711 (7), SB1714, SB1715, SB1717, SB1718 (7), SB1719, SB1720, SB1721, SB1722 (7), SB1733, SB1114 (5), SB0669 (3), SB1110 (3), SB1111 (3), SB116 (3), SB1119 (3), SB1735 (3).	
Cd Juárez (Cobos-Marín et al., 2005)	2000	58 (58)	SB0121 (50), SB0140 (14), SB0673 (9), SB098 (7), SB0987, SB0272, SB0327, SB0807, SB0985 (20).	
Ver, Jal, EDM (Santillan-Flores et al., 2006)	1993–1998		SB0269 (12), SB0669 (12), SB0663 (10), SB1118 (7), SB1114 (7), SB1119, SB1120, SB1121 (7), SB1122, SB1123, SB1124 (7), SB1126, SB1127, SB1128 (7), SB1129, SB1130, SB1131 (7), SB1132, SB1133, SB1134 (7), SB0130, SB0140, SB0145 (7), SB1113 (5), SB1125 (4.5), SB0971 (5).	
13 out 32 states (Rodwell et al., 2010)	1997–2007		**SB0673** (23.8), SB0121 (9.7), **SB0140** (6.9), SB0669 (6.3), **SB0145** (6), **SB0971** (3.6), SB0296 (2.8), SB0327 (2.8), SB1165, SB0986, SB0663, **SB1040**, SB1116, SB 119 (9.2), SB1044, SB1496, SB0272, SB0130, SB111, SB114, SB0987, SB1118, SB1216 (6.2), SB1110, SB1696, SB1125, SB1113, SB1129, SB1503, SB0807, SB1757SB1755, SB0452 (3.8), **SB0152** (0.2) SS (18.5).	
B.C. (Martínez-Vidal et al., 2011)	2008–2010	21 (43)		8 loci
B.C. (Bermúdez et al., 2012)	2006–2007	166 (62)		
19 out 32 states (Gutiérrez Reyes et al., 2012)	2009–2010		Orphans (17), SB0673 (15), SB0669 (12), SS (11), SB0145 (7), SB0971 (7), SB0140 (6), SB0121 (6), SB0663 (4), SB0269 (4), SB116 (3), SB0119 (2), SB0327 (1), SB2055 (1), SB0484 (1), SB1165 (1)	
17 out 32 states (Zumárraga et al., 2013)	Nd		Orphan (24), Others (26), SB0121 (11), SB0673 (8), SB0140 (5), SB0269 (4), SB0663 (4), SB1112 (4), SB0971 (4), SB1165 (2), SB1116 (2), SB0669 (2), SB0145 (2), SB0327 (2)	
Chia, Tab, Ver (Vazquez-Chacon et al., 2015)	2011–2012		SB0673 (28), SB0971 (19), SB0140 (15), **SB0145** (8.5), SB1388 (6), Orphan (4.2), SB2374 (4.2), SB0121 (2), SB0120 (2), SB0268 (2), SB0130 (2), SB0662 (2), SB0327 (2), SB0669 (2)	24 loci
26 out 32 states (Milian-Suazo et al., 2016)	2003–2010		**SB0673** (11.8), **SB0669** (9.3), SB0121 (6), **SB0145** (5.8), **SB0971** (5.3), SB0140 (5), **SB0663** (4), SB0269 (3.3), SB1116 (3.2), **SB0120** (2.2), SB119 (2), SB1165 (1), Other (41.)	
16 out 32 states (Nava-Vargas et al., 2016)	2009–2010			12 loci
B.C. This study	2014-2015	155 (82)	**SB0145** (58), **SB1040** (31), SB0162 (2), SB1345 (2), SB0327 (1.3), SB1758 (1.3), SB2468[a] (0.6), SB2469[a] (0.6) **SB0971** (0.6), SB1760 (0.6), SB0140 (0.6), SB0986 (0.6).	

Isolation site: Ags, Aguascalientes; BC, Baja California; Chi, Chiapas; Coah, Coahulia; EDM, Estado de México; Hgo, Hidalgo; Jal, Jalisco; Qro, Querétaro; Tab, Tabasco; Ver, Veracruz. ND, no data; SS, single spoligotype; Bold letters, match with human spoligotype; Blank row, no data.

[a] New spoligotp patterns.