Preventive Veterinary Medicine 131 (2016) 75–78

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Genetic diversity based on MIRU-VNTR profile of isolates of *Mycobacterium bovis* from Mexican cattle

Alejandro Nava Vargas[a], Feliciano Milián Suazo[b], Germinal Jorge Cantó Alarcón[b], Yezenia Rubio Venegas[b], Roberto Guerrero Solorio[a], Elba Rodríguez Hernández[c], Oscar Pizano Martìnez[d,*]

[a] *Maestría en Salud y Producción Animal Sustentable, Universidad Autónoma de Querétaro, Querétaro, Mexico*
[b] *Facultad de Ciencias Naturales-Universidad Autónoma de Querétaro, Querétaro, Mexico*
[c] *CENID-FyMA-INIFAP, Querétaro, Mexico*
[d] *CUCS, Departamento de Clínicas Médicas, Universidad de Guadalajara, Jalisco, Mexico*

ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 9 July 2016
Accepted 11 July 2016

Keywords:
Genotyping
Mycobacterium bovis
Bovine tuberculosis
Variable number tandem repeats
MIRU-VNTR
Molecular epidemiology

ABSTRACT

Bovine tuberculosis (bTB) is a disease caused by *Mycobacterium bovis* (*M. bovis*), which affects cattle, animal species and humans. To determinate the genetic structure of strains of *M. bovis* in mexican cattle, 467 isolates obtained from 2009 to 2010 from different regions of Mexico with known spoligotype were included in the study. The isolates were genotyped by interspersed repeated mycobacterial units-variable number tandem repeats (MIRU-VNTR) obtaining 13 MIRU-VNTR groups. When combining MIRU-VNTR patterns with its spolygotypes, the Hunter genetic discrimination index (HGDI), we obtained 421 genetic patterns distributed in 17 groups. The HGDI for the total *loci* was 0.99. The locus that presented the higher HGDI was 2461 (0.857), while the locus with the lowest HGDI was 2686 (0.239). When we analyzed our results, using just 6 or 8 MIRU-VNTR we obtained an discriminatory power of 0.8499 and 0.8875 respectively indicating lower HGDI than 12 MIRU-VNTR locus.

1. Introduction

bTB is a disease caused by *M. bovis*, an intracellular pathogenic bacillus that forms granulomas in lungs and lymphonodes of animals and humans, even more, bTB is considered as a risk to public health by WHO (Lari et al., 2006). In the livestock industry, causes monetary losses and represents a non-tariff barrier to the free trade of animals and animals' products (Firdessa et al., 2012). Mexico has a national program for its control and eradication, based on the test and slaughter, but producers do not abide by it due to the lack of compensation for the slaughtered animals. Another big problem is the lack of a reliable way of tracing back animals found with lesions. In a commercial setting, animals are moved around the country, but its mobilization must be accompanied by official sanitary documents, this is not always the case, making difficult to establish quick actions to eliminate the source of infection (Bennett and Cooke, 2006). With the advent of molecular epidemiology, faster and more reliable methods for bTB diagnosis have been developed, which provide information about the genetic structure to establish relationship among strains. MIRU-VNTR is a molecular tool based on the amplification by PCR of 41 different *loci* of the *Mycobacterium* DNA sequence, which function as molecular markers obtaining fast, objective, and high resolution monitoring procedures in outbreaks or epidemiological emergencies (Acosta-Salinas et al., 2009). The aim of the study it was to identify the MIRU-VNTR in isolates of *M. bovis* from cattle of different regions of Mexico to stablish their spatial localization and its possible phylogenetical relation.

2. Material and methods

2.1. Samples included in the study

The study included 467 isolates of *M. bovis* obtained from 2009 to 2010 from cattle from different parts of Mexico, which were composed by Northern region, Central region and finally,

* Corresponding author at: Centro Universitario de Ciencias de la Salud, Departamento de Clínicas Médicas, Universidad de Guadalajara. Sierra mojada No. 950. Col. Independencia. CP. 44340, Guadalajara, Jalisco, México.
E-mail addresses: alexnavavargas@hotmail.com (A. Nava Vargas), miliansf@yahoo.es (F. Milián Suazo), gcanto99@prodigy.net.mx (G.J. Cantó Alarcón), yez.rubio@hotmail.com (Y. Rubio Venegas), caxixi_katzin@hotmail.com (R. Guerrero Solorio), rohe577@hotmail.com (E. Rodríguez Hernández), sorrento80@hotmail.com (O. Pizano Martìnez).

http://dx.doi.org/10.1016/j.prevetmed.2016.07.007
0167-5877/

Table 1
Isolates of *M. bovis* collected from Mexican cattle between 2009 and 2010 were included in the study.

Region	State	Abb	No. of isolates
Northern	Coahuila	Coah	41
	Baja California	BC	27
	Zacatecas	Zac	10
	Durango	Dgo	2
	Nuevo León	NL	1
	Hidalgo	Hgo	94
	Estado de México	Mex	75
	Querétaro	Qro	56
	Jalisco	Jal	51
Central	Guanajuato	Gto	43
	Aguascalientes	Ags	29
	Michoacán	Mich	14
	San Luis Potosí	SLP	14
	Nayarit	Nay	7
	Colima	Col	1
Southern	Chiapas	Chis	2
Total			**467**

Abb: abbreviation.

Southern region, (Table 1). The isolates were decontaminated using the Petroff modified method and then, were cultured in Stonebrink media for 6 weeks at 37 °C for its growth.

2.2. 12-locus MIRU-VNTR typing

The DNA extraction and PCR assays of each sample were performed as follows: 100 mg of each isolate were taken from culture, dissolved in 0.2 ml of TE buffer and underwent to a thermal shock during 45 min at 95 °C to obtain the DNA. Concentration and quality of the DNA were evaluated by a Nano Drop 2000c spectrophotometer (Thermo Scientific, EUA) and 1% agarose gel electrophoresis (Bio Rad, EUA). PCR reactions were carried out to final volume of 10 μl using 12 sets of primers, which amplified every locus individually (Table 2). Each reaction was composed of, 5 μl of Master Mix (Applied Biosystems, EUA) 1 μl of DMSO (Sigma Aldrich, EUA); 1 μl of sense primer 10 mM, 1 μl of primer antisense 10 mM (Invitrogen, Custom Primers, EUA) and 100 ng of DNA of each isolate were added. The reactions were transferred to a thermo-cycler C1000 Thermal Cycler (Bio- Rad, USA) under the following conditions: initial denaturation at 95 °C for 10 min; 95 °C for 1 min, alignment to 55 °C for 1 min and extension at 72 °C for 1.3 min; for 40 cycles, with a final extension of 72 °C for 10 min. The PCR products were electrophoresed in agarose gel at 2% and stained with ethidium bromide (Bio Rad, USA). Bovine BCG strain and human H37Rv were used as controls.

2.3. HGDI and correlation analysis

The HGDI was calculated according with the Hunter and Gaston method (Hunter and Gaston., 1988) and it was used to compare the discriminatory power of the MIRU-VNTR/spoligotypes of *M. bovis*. Likewise Sola et al., each *locus* were designated as highly discriminatory (HGDI > 0.6), moderately discriminatory (HGDI 0.3–0.6) and poorly discriminatory (HGDI < 0.3) (Sola et al., 2003).

2.4. Phylogenetic analysis

In order to establish whether there is a possible phylogenetic relationship between isolates by their genetic differences, each MIRU-VNTR profile was analyzed together with its corresponding spoligotype obtained in a previous study by Milián et al. (2012). To detect variations in its genome, we created a pattern that acts as an individual genetic fingerprint. We used the software MIRU-VNTRplus available in www.miru-vntrplus.org to analyze the data.

3. Results

3.1. MIRU-VNTR profiles and HGDI discriminatory power

All isolates were clustered into 7 different spoligoclusters with 434 singletons and 13 MIRU-VNTR clusters. When we combined both, the number of spoligoclusters and MIRU-VNTR clusters, we increased the HGDI because discrimination power increases if we use both data that if we use just one single technique, making genomic differences to obtain finally 17 clusters (Fig. 1). Each group included isolates from different regions but some clusters included isolates from a specific region, such as cluster 11, which includes isolates from Hidalgo, sharing similar MIRU-VNTR pattern. In most cases, the clusters share patterns from different region like clusters 4 and 6, which included isolates from Aguascalientes, Guanajuato and Querétaro.The number of copies for each locus of MIRU-VNTR showed variations ranging from 4 to 9 copies. The ETR-A locus was less expressed, because it was absent in 193 isolates (41%), while the most common locus was MIRU24 with 2 copies in 406 isolates (87%), (Table 3). To set the importance of this report we assign the power of discrimination, taking into account the classification of HGDI used by Sola et al.,. The HGDI for the 12 *loci* was 0.99, 9 of them have higher HGDI (>0.6), of which the ETR-B was the highest value with 0.857. The ETR-C and Mtub04 index showed a moderate discrimination with 0.514 and 0.596 respectively. Considering that it has been reported a good power of discrimination between isolates with just a few *loci* analysis in specific areas around the world, it is important to analyze the HGDI using 6 or 8 *loci* and discarding the rest. By using 6 MIRU-VNTR the discriminatory power was of 0.8499 and 0.8875, if we use 8 *loci* with highest HGDI, nevertheless, they do not have the HGDI from the 12 loci (Table 4).

Table 2
Primer sequences used to identify the MIRU-VNTR profile of *M. bovis* isolates obtained from cattle in different regions of Mexico.

VNTR locus	VNTR alias	Sentido (5′ – 3′)	Antisentido (5′ – 3′)
0424	Mtub04	CTTGGCCGGCATCAAGCGCATTATT	GGCAGCAGAGCCCGGGATTCTTC
0577	ETR-C	GTGAGTCGCTGCAGAACCTGCAG	GGCGTCTTGACCTCCACGAGTG
1644	MIRU 16	TCGGTGATCGGGTCCACTCCAAGTA	CCCGTCGTGCAGCCCTGGTAC
1955	Mtub 21	AGATCCCAGTTGTCGTCGTC	CAACATCGCCTGGTTCTGTA
2165	ETR-A	AAATCGGTCCCATCACCTTCTTAT	CGAAGCCTGGGGTGCCCGCGATTT
2401	Mtub 30	ACTTGAACCCCCACGCCCATTA	AGCCCCGGTCTCATCTGTCACA
2461	ETR-B	GCGAACACCAGGACAGCATCATG	GGCATGCCGGTGATCGAGTGG
2686	MIRU24	CGACCAAGATGTGCAGGAATACAT	GGGCGAGTTGAGCTCACAGAA
2995	MIRU 26	TAGGTCTACCGTCGAAATCTGTGAC	CATAGGCGACCAGGCGAATAG
3192	ETR-E	CTTCGGCGTCGAAGAGAGCCTC	CGGAACGCTGGTCACCACCTAAG
4052	QUB 26	AACGCTCAGCTGTCGGAT	GGCCAGGTCCTTCCCGAT
2163b	QUB 11b	CGTAAGGGGATGCGGGAAATAGG	CGAAGTGAATGGTGGCAT