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Full Length Research Paper

Zoonotic tuberculosis in Cameroon: a call for action

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ABSTRACT

Cameroon is among African countries where bovine tuberculosis (bTB) is still prevalent but in which human pulmonary tuberculosis due to *M. bovis* is not well documented while many risk factors as contact between human and animal or non-pasteurization of milk exist and can annihilate authorities' heath efforts against tuberculosis. We therefore make for the first time, a molecular epidemiology study by analyzing and comparing human and animal *M. bovis* strains by using a modified MIRU/VNTR loci system. Our result shows that there are some cases of bTB transmission between cattle and between human and cattle. This study was the first that shows a zoonosis case in tuberculosis in Cameroon. It also shows that the use of spoligotyping as the only typing technique could overestimate the cases of tuberculosis transmission but that its association with a good MIRU/VNTR system could find the true cases of transmission.

Keywords: Bovine tuberculosis, *M. bovis*, Zoonosis, MIRU/VNTR, Pulmonary tuberculosis, Cameroon

INTRODUCTION

Bovine tuberculosis (BTB) is still prevalent and under-evaluated in cattle destined for human consumption in Cameroon (Koro et al., 2015). This chronic disease principally caused by *M. bovis*, a member of *M. tuberculosis* complex, could affect wild and domestic animals as well as human (Popelka et al., 2016). In Africa impact of zoonotic tuberculosis due to *M. bovis* remains largely unknown while people live in conditions that favour direct contact with infected animals or animal products (Machado et al., 2018, Kelly et al., 2016). In Cameroon, *M. bovis* remains endemic and the principal cause of tuberculosis in Cattle destined for human consumption (Koro et al., 2013, Ndukum et al., 2005, Ndukum et al., 2010, Ndukum et al., 2012). The molecular typing of all *M.bovis* strains isolated from cattle in Cameroon identified three main lineages SB0944; SB1955 and SB0953 Which represent 95% of all these strains (Egbe et al., 2017, Koro et al., 2015, Njampop et al., 2001, Ndukum et al., 2013). Despite the high prevalence of *M. bovis* in cattle destined to human consumption, *M. bovis* remains very weakly isolated from the samples of human patients suffering from pulmonary or extrapulmonary tuberculosis in Cameroon. However, the few strains isolated from human patients belong to genotypes SB0944, SB1955, SB0953 that are endemic in cattle (Onana et al., 2018, Koro et al., 2013, Kamgue et al., 2013, Niobe et al., 2003).Knowing the phenomenon of homoplasy associated with spoligotyping technic we decided to analyze strains of M. bovis isolated from cattle destined to human consumption and those isolated from human using the technique of MIRU/ VNTR (Mycobacterial Interspaced Repetitive Units/ Variable Nucleotide Tandem Repeat). It is known that MIRU-VNTR typing has not yet been widely used in bTB research in sub-Saharan Africa, there is therefore little epidemiological data linked to genetic diversity at higher resolution that this method could provide (Muller et al., 2009).We used therefor a 16 MIRU/VNTR set differing in some loci from the standard one provide by Supply and collaborators in order to determine if the *M. bovis* isolated in cattle and those isolated in human

were not distinguishable isolates (meaning these isolates were implicated to same pockets of ongoing TB transmission). This system was chosen because it has a more reliable discrimination power between MTBC species, lineage, and clad when we analyze MTBC strains isolated from human and animal in our laboratory (personal data) compared to the conventional 12 and 15 loci that had been used before and gave the same haplotype for different species, lineages, or clad. Moreover, this system permits the recovery of another polymorphic locus compared to that given by 12 and 15 standard system when we analyse Cameroonian strains (Koro et al., 2016).

METHODS

Strains Collection and Culture

We collected all the existing conserves strains belonging to the SB0944, SB1955 and SB0953 isolated from cattle and human tuberculosis in our latest or others studies. This was recovered by culture on Lowstein Jensen Media supplemented or not by pyruvate as described earlier (Koro et al., 2016); Briefly *M. bovis* isolates conserved in milk were first activated at 37°C for six hours and then three Lowenstein-Jensen (L- J) slants, two containing 0.75% glycerol without pyruvate and one containing 0.4% pyruvate, were inoculated with approximately 0.1 to 0.3 ml of milk and incubated at 37°C. Cultures were considered negative when no colonies were seen after eight weeks of incubation. Strains from 1955 could not grow on culture media. Isolates were harvested and DNA extraction was performed as described in our previous report (Koro et al., 2013).

MIRU/VNTR Typing

A modified standard 16 MIRU/ VNTR loci system MIRU04, ETRC, QUB-26, QUB-11b, MIRU24, MIRU20, MIRU40, ETRA, MIRU27, MIRU26, MIRU31, MIRU39, Mtub30, Mtub34, and Mtub21 were individually amplified and analysed as previously described (Koro et al., 2016).

Data Analysis

Results from each of the 16 loci were combined to form a 16-digit allele profile. Each haplotype obtains from human *M. bovis* was compare to that of cattle *M.bovis* using Microsoft excel version 15.21.1. 2016.

RESULTS

Spoligotyping analysis of all molecular studies done on cattle tuberculosis in Cameroon have identified three main clusters of *M. bovis* strains minning SB0944(24 isolates), SB0953(12 isolates) and SB1955(16 isolates). In human tuberculosis (pulmonary and extra pulmonary tuberculosis) all identified *M. bovis* strains belonged to SB0944 and SB0953 and SB1955 (Table 1).

Table 1. Spoligotypes profile identified simultaneously in human and in Cattle in Cameroon.

Origin	Spoligotype profile	Total number	SB number
Cattle	■■□■■■■■□■■■■■■□■■■■■■■■■■■■■□■■■■■■■■□□□□□	24	SB0944
Human	■■□■■■■■□■■■■■■□■■■■■■■■■■■■■□■■■■■■■■□□□□□	1	SB0944
Cattle	■■□■■■■□□□□□□■□□□■■■■■■■■■■■■□■■■■■■■■□□□□□	16	SB1955
Human	■■□■■■■□□□□□□■□□□■■■■■■■■■■■■□■■■■■■■■□□□□□	1	SB1955
Cattle	■■□■■■■□□□□□□□□□□■■■■■■■■■■■■□■■■■■■■■□□□□□	12	SB0953
Human	■■□■■■■□□□□□□□□□□■■■■■■■■■■■■□■■■■■■■■□□□□□	1	SB0953

SB (vwxyz) Authoritative and unique number of spoligotype patterns assigned by Mbovis.org website (Smith & Upton 2011)

MIRU/VNTR Typing

SB0944 typing: knowing the homoplasy phenomenon due to spoligotyping, a MIRU/VNTR typing of human and cattle *M. bovis* spoligotype lineage SB0944 using a sixteen MIRU/VNTR loci set, gave a genetic diversity closed to 1(Table 2).

Table 2. MIRU/VNTR haplotypes of human and cattle *M. bovis* strain lineage SB0944 isolated in Cameroon

Origin	Spoligotype Lineage	MIRU 4	MIRU 31	VNTR 43	QUB-11b	QUB-26	MIRU 26	MIRU 27	MIRU 24	MIRU 10	MIRU 40	MIRU 16	MIRU 47	VNTR 48	ETRA	VNTR 49	VNTR 1955
Cattle	SB0944	1	3	5	3	3	1	3	2	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	3	2	3	4	3	2	3	2	5	4	5	5	2	3
Cattle	SB0944	3	3	3	3	2	5	3	2	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	5	3	3	5	3	2	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	5	3	3	5	3	1	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	6	2	3	5	3	2	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	6	3	2	5	3	2	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	6	3	3	5	3	2	3	2	5	4	5	4	3	3
Cattle	SB0944	3	3	6	3	3	3	3	2	3	2	5	4	5	3	3	3
Cattle	SB0944	4	3	6	2	3	5	3	2	3	2	5	4	6	4	3	3
Human	SB0944	3	3	5	3	4	2	3	2	3	2	5	4	5	3	3	3

MIRU: Mycobacterial Interspaced Repetitive Units/ Variable Nucleotide Tandem Repeat; QUB: Queen University of Bedfast; ETRA: exact Tandem Repeat

SB0953 typing: Typing of the human and cattle *M. bovis* spoligotype lineage SB0953 using the same MIRU/VNTR set, identified a haplotype with MIRU/VNTR profile 335345323254553, identical between human and cattle *M. bovis* strains (Table 3). Others haplotype of this spoligotype lineage differed by one loci or more loci repetitive units.

Table 3. MIRU/VNTR haplotypes of human and cattle *M. bovis* strain lineage SB0953 isolated in Cameroon

Origin	Spoligotype Lineage	MIRU 4	MIRU 31	VNTR 43	QUB-11b	QUB-26	MIRU 26	MIRU 27	MIRU 24	MIRU 10	MIRU 40	MIRU 16	MIRU 47	VNTR 48	ETRA	VNTR 49	VNTR 1955
Cattle	SB0953	3	3	4	3	4	5	2	2	3	2	5	4	3	4	3	3
Cattle	SB0953	3	3	5	2	4	5	3	2	3	2	5	4	5	6	2	3
Cattle	SB0953	3	3	5	2	4	6	3	2	3	2	5	4	6	5	3	3
Cattle	SB0953	3	3	5	3	2	5	3	2	3	2	5	4	5	5	3	3
Cattle	SB0953	3	3	5	3	4	5	2	2	3	2	5	4	5	3	3	3
Cattle	SB0953	3	3	5	3	4	5	3	2	3	2	5	4	1	5	3	3
Cattle	SB0953	3	3	5	3	4	5	3	2	3	2	5	4	5	5	3	3
Cattle	SB0953	3	3	5	3	4	5	3	2	3	2	5	4	5	5	3	3
Cattle	SB0953	3	3	5	3	4	3	3	2	3	2	5	4	5	5	3	3
Cattle	SB0953	4	4	5	3	4	5	3	2	3	2	5	4	6	5	3	3
Cattle	SB0953	3	3	5	3	4	5	3	2	3	2	5	4	5	5	3	3
Human	SB0953	3	3	5	3	4	5	3	2	3	2	5	4	5	5	3	3

MIRU: Mycobacterial Interspaced Repetitive Units/ Variable Nucleotide Tandem Repeat; QUB: Queen University of Bedfast; ETRA: exact Tandem Repeat

DISCUSSION

The survey and identification of *M. bovis* is critical for determining the impact of zoonotic transmission of bTB to humans even its transmission between cattle (Cousin et al., 1998) since bTB infectious disease could be responsible of economic losses from livestock deaths, trade restrictions. In Cameroon bovine tuberculosis is still endemic in many animals destined for human consumption and people live in conditions that favor direct contact with infected animal or animal products and sparse molecular studies had shown that

M. bovis is endemic in Cattle and base on Spoligotyping these studies identified three clusters among which SB0953 which was new in Cameroon (Koro et al., 2015). Moreover, all *M. bovis* strain lineage identified in human tuberculosis belonged to this three clusters (Niobe et al., 2003, Koro et al., 2013, Koro et al., 2016). Suggesting transmission even between human and cattle and between cattle. But knowing the homoplasy due to this technics a sixteen MIRU/VNTR typing loci system have been used to identify the possibility link between human and cattle *M. bovis* strains (Reyes et al., 2012). In fact it is knownthat MIRU/VNTR typing can be used to successfully distinguish between *M. bovis* isolates,and when appropriate loci are selected, this technic may have a great discriminatory power (Allix et al., 2006, Hilty et al., 2005, Roring et al., 2004). And can therefore be used to confirm conventional epidemiological links, trace transmission routes, and identify sources of infection (Koro et al., 2015, Koro et al., 2018).

In our study, the genetic population structure analyses of strains belonging to SB0944 Clad give the genetic diversity close to one. Moreover, haplotype obtained differs by more than three loci between human strains and animal strains, showing that the strains belonging to this clad were a distinguished strain and that it could not be implicated to the same chain of transmission. We also think therefor that existence of this *M. bovis* clad in human and in cattle is predominantly that of reactivated disease.

More interestingly genetic population structure analysis of the strains belonging to SB0953 reveals one haplotype (335345323254553) identical between animal strains and human strains meaning that based on the MIRU/VNTR system used, these strains are undistinguished strains and that they could be implicated to the same transmission shame between cattle and human or between cattle. But an epidemiological link could not be established since it remains difficult to trace back cattle movements in Cameroon. Nevertheless, it is well known in Cameroon that there is close contact between animals and human (Koro et al., 2015, Koro et al., 2018).

CONCLUSION

This study was the first that shows a possible zoonosis case in tuberculosis in Cameroon. It also shows that the use of spoligotype alone can amplify false transmission cases but it association to a good MIRU/VNTR genotype system, this can identify a transmission cases.

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CONFLICT OF INTEREST

I would like to undertake the responsibility for this submitted manuscript, I did not receive reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future. Moreover, I did not hold or was currently applying for any patents relating to the content of the manuscript even received reimbursements, fees, funding, or salary from an organization that holds or had applied for patents relating to the content of the manuscript. In the best of my knowledge I did not have any other financial and non-financial competing interests.

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