PLOS ONE

RESEARCH ARTICLE

Molecular epidemiology and whole genome sequencing analysis of clinical *Mycobacterium bovis* from Ghana

Isaac Darko Otchere[1], Andries J. van Tonder[2], Adwoa Asante-Poku[1], Leonor Sánchez-Busó[2], Mireia Coscollá[3], Stephen Osei-Wusu[1], Prince Asare[1], Samuel Yaw Aboagye[1], Samuel Acquah Ekuban[4], Abdallah Iddrisu Yahayah[4], Audrey Forson[5], Akosua Baddoo[5], Clement Laryea[6], Julian Parkhill[2], Simon R. Harris[2], Sebastien Gagneux[7,8], Dorothy Yeboah-Manu[1]*

1 Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana, **2** Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom, **3** Institute for Integrative Systems Biology (I2SysBio), University of Valencia-CSIC, Valencia, Spain, **4** Chest Department, Tamale Teaching Hospital, Tamale, Ghana, **5** Department of Chest Diseases, Korle-Bu Teaching Hospital, Accra, Ghana, **6** Public Health Department, 37 Military Hospital, Accra, Ghana, **7** Swiss Tropical and Public Health Institute, Basel, Switzerland, **8** University of Basel, Basel, Switzerland

* dyboah-Manu@noguchi.ug.edu.gh

Citation: Otchere ID, van Tonder AJ, Asante-Poku A, Sánchez-Busó L, Coscollá M, Osei-Wusu S, et al. (2019) Molecular epidemiology and whole genome sequencing analysis of clinical *Mycobacterium bovis* from Ghana. PLoS ONE 14(3): e0209395. https://doi.org/10.1371/journal.pone.0209395

Editor: Mark Spigelman, Hebrew University, ISRAEL

Received: November 25, 2018

Accepted: February 19, 2019

Published: March 4, 2019

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: This work was supported by the Wellcome Trust Intermediate Fellowship awarded to DYM (Grant Number 097134/Z/11/Z). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Background

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* is a re-emerging problem in both livestock and humans. The association of some *M. bovis* strains with hyper-virulence, MDR-TB and disseminated disease makes it imperative to understand the biology of the pathogen.

Methods

Mycobacterium bovis (15) among 1755 *M. tuberculosis* complex (MTBC) isolated between 2012 and 2014 were characterized and analyzed for associated patient demography and other risk factors. Five of the *M. bovis* isolates were whole-genome sequenced and comparatively analyzed against a global collection of published *M. bovis* genomes.

Results

Mycobacterium bovis was isolated from 3/560(0.5%) females and 12/1195(1.0%) males with pulmonary TB. The average age of *M. bovis* infected cases was 46.8 years (7-72years). TB patients from the Northern region of Ghana (1.9%;4/212) had a higher rate of infection with *M. bovis* (OR = 2.7,p = 0.0968) compared to those from the Greater Accra region (0.7%;11/1543). Among TB patients with available HIV status, the odds of isolating *M. bovis* from HIV patients (2/119) was 3.3 higher relative to non-HIV patients (4/774). Direct contact with livestock or their unpasteurized products was significantly associated with bTB (p<0.0001, OR = 124.4,95% CI = 30.1–508.3). Two (13.3%) of the *M. bovis* isolates were INH resistant due to the S315T mutation in *katG* whereas one (6.7%) was RIF resistant with

Q432P and I1491S mutations in *rpoB*. *M. bovis* from Ghana resolved as mono-phyletic branch among mostly *M. bovis* from Africa irrespective of the host and were closest to the root of the global *M. bovis* phylogeny. *M. bovis*-specific amino acid mutations were detected among MTBC core genes such as *mce1A*, *mmpL1*, *pks6*, *phoT*, *pstB*, *glgP and Rv2955c*. Additional mutations P6T in *chaA*, G187E in *mgtC*, T35A in *Rv1979c*, S387A in *narK1*, L400F in *fas* and A563T in *eccA1* were restricted to the 5 clinical *M. bovis* from Ghana.

Conclusion

Our data indicate potential zoonotic transmission of bTB in Ghana and hence calls for intensified public education on bTB, especially among risk groups.

Introduction

Among the *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium bovis* is the main causative agent of TB in cattle and sheep, albeit with the widest host range among other mammals including wildlife and humans [1]. *M. bovis* associated TB is a re-emerging global problem affecting both livestock and humans alike. The World Health Organization reported 147,000 new Bovine TB (bTB)) cases and 12,500 deaths among humans in 2016 [2]. Despite the low incidence of *M. bovis* associated TB (~2% globally), the mortality rate is high, especially among children and HIV co-infected patients [1,3,4]. Human-to-human transmission of *M. bovis* is mostly rare [5], thus human bTB is considered a zoonotic chronic disease characterized by lung infections and their draining lymph nodes as granulomatous necrotizing inflammatory disease [6,7]. Nevertheless, bTB among immunocompromised people and children are mostly extrapulmonary or disseminated affecting other organs other than the lungs and their draining lymph nodes. bTB in humans is mostly transmitted via the alimentary canal by the [4] consumption of unpasteurized dairy products from infected cattle [3,8,9] and or inhalation of aerosolised bacilli via direct contact with infected cattle and/or their carcasses [5]. However, a lack of knowledge or simply negligence of the dangers associated with being in close contact with livestock or wildlife and their unpasteurized products is apparent among some individuals who are constantly in direct contact with animals [10]. In addition, there is a growing association of *M. bovis* related TB cases with treatment failure due to intrinsic resistance to some commonly used anti-tuberculosis drugs [11].

Even though *M. bovis*, being a member of the MTBC, is genetically homogenous compared to other bacteria [12], molecular epidemiology of *M. bovis* infections in Great Britain has shown that they exhibit polymorphic metabolic profiles, such as differential rates of incorporation of propionate into membrane lipid components among different genotypes [13] as well as differential expression of some essential genes and accumulation of single nucleotide polymorphisms (SNPs) which could have functional implications [14].

About 85% of herds and 82% of humans in both rural and urban settings in sub Saharan Africa (SSA) live in close proximity to one another, thus driving the wide distribution of bTB compared to other global settings [15,16]. This is compounded by the inadequate sanitation practices such as the habit of sharing drinking water with beasts and consumption of non-pasteurized milk and dairy products [17–19]. Despite the economic and public health importance of bTB, little knowledge exists on the epidemiology and biology of *M. bovis* in relation to the human adapted MTBC (hMTBC) lineages spanning *M. tuberculosis sensu stricto* (*Mtbss*) and

M. africanum (*Maf*) [20,21]. However, such information is critical for development of effective control tools for bTB.

We determined the prevalence of bTB among pulmonary TB patients passively reporting to selected TB diagnostic/treatment facilities in Ghana, determined potential risk factors associated with bTB in Ghana and explored genomic similarities and differences among *M. bovis* strains from around the globe, irrespective of the host, using whole genome sequencing.

Materials and methods

Ethical statement and participant enrolment

The Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR) with Federal Wide Assurance number FWA00001824 reviewed this study and its protocols and accordingly gave ethical clearance in support of the work.

Mycobacterial isolation, drug resistance profiling and genotyping

Smear-positive sputum samples from the selected health centers in the Northern and Greater Accra regions of Ghana were decontaminated and inoculated on 2 pairs of Lowenstein Jensen (LJ) slants; one pair supplemented with 0.4% sodium pyruvate (to enhance growth of *M. bovis* and *M. africanum* (*Maf*)) the other with glycerol (for enhanced growth of *M. tuberculosis sensu stricto* (*Mtbss*) and incubated as previously described [22]. MTBC cells growing in confluence were harvested and heat inactivated at 95 ^{o}C for 60 min in nuclease-free water. After heat inactivation, chromosomal DNA was extracted using previously described protocol [23]. The isolates were confirmed as MTBC by PCR amplification of IS*6110* and spoligotyping was carried out for lineage classification [24]. Isolates classified as *M. bovis* were confirmed with a large sequence polymorphism (LSP) assay using PCR detection of deleted regions of difference RD9, RD4 and RD12 [25]. Drug susceptibility testing against isoniazid (INH) and rifampicin (RIF) was carried out using the micro-plate alamar blue assay [23,26].

Whole genome sequencing and phylogenetic analysis

Whole genome sequencing of 5 candidate *M. bovis* isolates was carried out as previously described [27]. The 5 genomes (ERR502499; ERR502526; ERR502529; ERR502538; ERR1203064) were added to a collection of 767 previously published clinical and veterinary *M. bovis* genomes (S1 Table) from around the world for analysis. Sequence reads were mapped to the *Mycobacterium bovis* AF2122/97 reference genome (NC0002945) using BWA (minimum and maximum insert sizes of 50 and 1000 respectively) [28]. Single nucleotide polymorphisms (SNPs) were called using SAMtools mpileup and BCFtools (minimum base call quality of 50 and minimum root squared mapping quality of 30) as previously described [28,29]. Variant sites in the alignment were extracted using snp-sites [30] and a maximum likelihood phylogenetic tree was constructed using FastTree2 [31] (nucleotide general time-reversible tree). The resulting tree was annotated and rooted using iTOL [32]

Comparative mutational analysis of selected MTBC core-genes

Coordinates of 147 MTBC core genes (S2 Table) previously reported to harbour amino acid mutations with phenotypic consequence on virulence and fitness of some laboratory strains of the MTBC [33–39] were compiled from the Tuberculist database [40]. Using the fasta file of H37Rv as reference, the paired end reads of the 5 Ghanaian *M. bovis* genomes, 257 *M. africanum* [27] and global collection of 20 MTBC genomes [41] were screened for mutations within the compiled 147 core genes using ARIBA with default settings [42]. Amino acid mutations

found to be present only among the 5 Ghanaian *M. bovis* genomes were suspected to be *M. bovis* specific. To confirm whether these mutations were widespread in *M. bovis*, the global collection of 767 clinical and veterinary *M. bovis* genomes (S1 Table) was screened for these specific mutations using ARIBA as described above. We further classified these amino acid mutations as *M. bovis*-specific if they were found in 100% of genomes in the global collection or core *M. bovis* mutations if found in at least 99% of genomes.

Statistical analysis

Where applicable, chi-square and Fisher's exact tests were used to establish statistical significance. *P-values* less than 0.05 were considered statistically significant with 95% confidence.

Results

Demography and biological associations of TB patients infected with *M. bovis*

A total of 1755 MTBC isolates were obtained from 2074 smear positive TB patients (84.6% isolation rate). Among the patients from whom a MTBC was isolated, 212 (12.1%) were from the Northern region and 1543 (87.9%) from the Greater Accra region as previously described [27]. Fifteen (0.9%) of the isolates were genotyped as *M. bovis* whereas the remaining 1740 (99.1%) were members of the hMTBC (*Mtbss* and *Maf*). The average age of patients infected with *M. bovis* was 46.8 years (7 to 72 years) of which 12/1195 (1.0%) were from males compared to 3/560 (0.5%) from females (p = 0.412, OR = 1.9). Four (1.9%) of the isolates from the Northern region (n = 212) were *M. bovis* compared to 11/1543 (0.7%) from the Greater Accra region (p = 0.0968, OR = 2.7). Among the patients with known HIV status (893; 50.3%), 119 (13.3%) were HIV-positive compared to 774 (86.7%) HIV-negative. The incidence of bTB among HIV and non-HIV TB patients was 1.7% (2/119) and 0.5% (4/774) respectively with higher odds of isolating *M. bovis* from HIV patients relative to non-HIV TB patients (OR = 3.3). Six TB patients including 1 herdsman, 1 herds owner and 4 butchers representing 40% of 15 patients with history of direct contact with livestock were infected with *M. bovis*. This is significantly higher compared to 0.5% (9/1740) of *M. bovis* infected TB patients without such history ($p < 0.0001$, OR = 124.4, 95% CI = 30.1–508.3)

Drug resistance profile of *M. bovis* isolates

Most of the *M. bovis* isolates (13) were susceptible to all the drugs tested except two isolates resistant to INH and one isolate resistant to RIF (Table 1). The two INH resistant isolates both had the S315T mutation in *katG* while the RIF resistant isolate had Q432P and I1491S mutations in *rpoB*.

Table 1. Sensitivity of the MTBC isolates to INH and RIF.

Drug	Total (1755)	hMTBC (1740)	*M. bovis* (15)	*P-value*	OR	95%CI
INH^r	133; 7.6%	131;7.5%	2;13.3%	0.3163	1.9	0.2–8.5
RIF^r	16; 0.9%	15;0.9%	1;6.7%	0.1288	8.2	0.2–61.0
MDR	40 (2.3%)	40;2.3%	0;0.0%	-	-	-
ANY	189 (10.8%)	186;10.9%	3;20.0%	0.2139	2.1	0.4–7.8

NB: ANY: Total number of isolates resistant to at least one drug.

https://doi.org/10.1371/journal.pone.0209395.t001

Phylogenetic distribution of global collection of *M. bovis*

The maximum likelihood phylogenetic tree of global collection of *M. bovis* spanning both clinical and veterinary isolates rooted on *Maf* L6 as an outgroup shows random distribution of both the clinical and veterinary *M. bovis* (Fig 1). The majority of the global collection of *M. bovis* analyzed were isolated from animals (predominately cattle). The *M. bovis* genomes of African origin (Ghana, Eritrea and South Africa) generally clustered together closest to the root of the phylogeny irrespective of the host. Nevertheless, there were few *M. bovis* from South Africa which were sporadically distributed far from the root of the tree. There were 2 major clusters of *M. bovis* from New Zealand and one major cluster each from the United Kingdom, Mexico and the United States of America. Interestingly, the 5 Ghanaian clinical *M. bovis* clustered together as a monophyletic branch among the African *M. bovis* group (Fig 1).

In silico predicted *M. bovis*-specific amino acid mutations

We identified 41 *M. bovis* restricted amino acid mutations among 32 core-genes of the 5 clinical *M. bovis* from Ghana when compared to 257 *Maf* [27] and 20 global MTBC genomes [41]

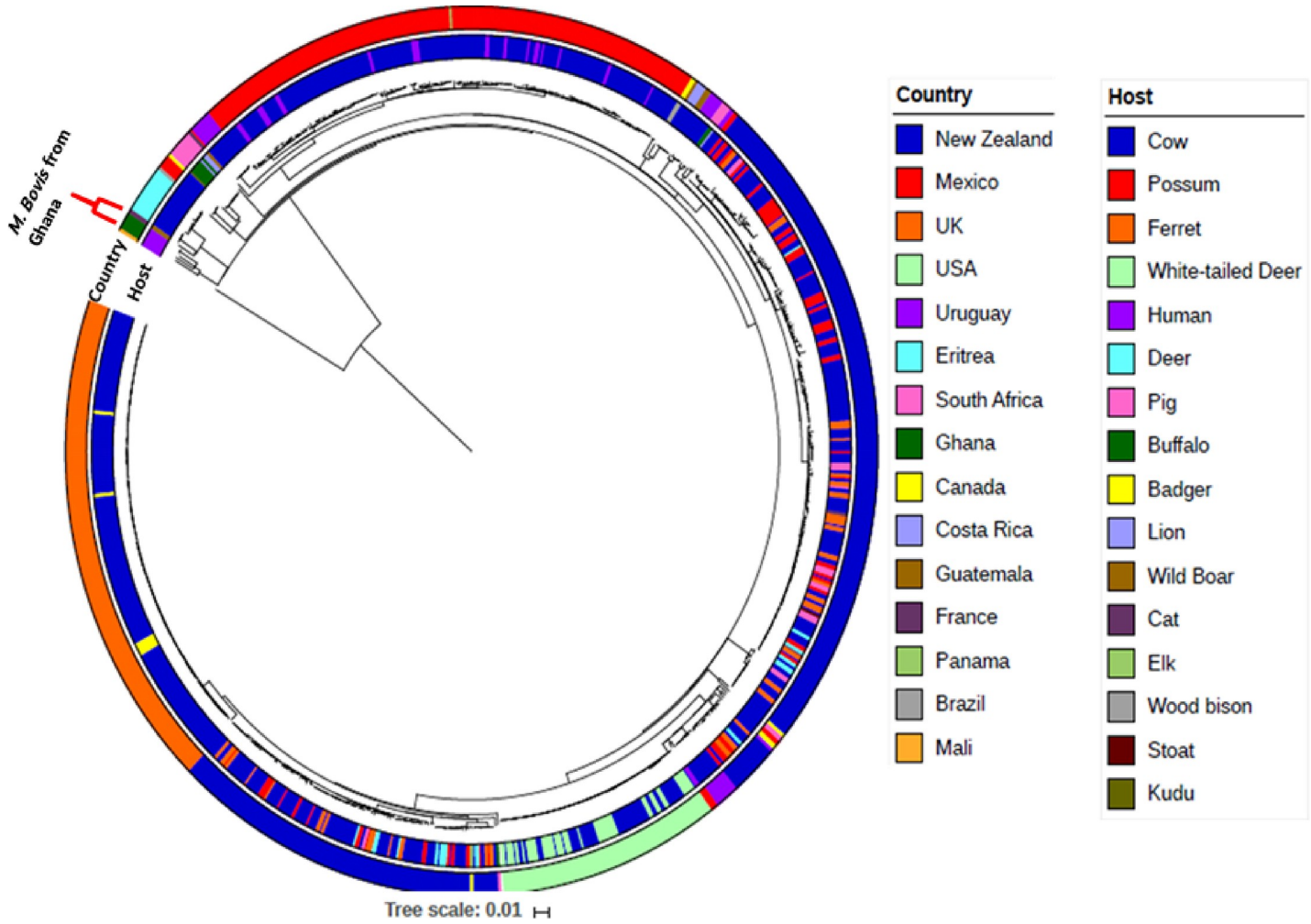

Fig 1. Phylogenetic tree of the Ghanaian clinical *M. bovis* amidst global collection of 767 published *M. bovis* genomes. The whole genome phylogeny of 767 publicly available *M. bovis* genomes together with 5 clinical *M. bovis* from Ghana rooted on *M. africanum* as an outgroup, shows the 5 Ghanaian clinical *M. bovis* genomes as a monophyletic group siting in a clade consisting mostly of other African M. bovis isolates basal to the rest of the dataset.

https://doi.org/10.1371/journal.pone.0209395.g001

(S3 Table). However, when we screened our global collection of 772 *M. bovis* genomes (including the 5 from Ghana), only 8 of the mutations were found in all genomes, 20 mutations in 99.22% to 99.87% of the genomes and 7 mutations in 95.85% to 98.97% of genomes. A further 6 mutations (P6T in *chaA*, G187E in *mgtC*, T35A in *Rv1979c*, S387A in *narK1*, L400F in *fas* and A563T in *eccA1*) were restricted to the 5 clinical *M. bovis* from Ghana (Fig 2; S4 Table; S1 Fig).

Among the 41 mutations identified uniquely among 32 core-genes *M. bovis*, 17 were among 15 essential genes associated with important physiological processes such as lipid metabolism, cell wall and cell processes, intermediate metabolism, and cellular respiration, virulence, detoxification and virulence as well as regulatory proteins (Table 2). These include *mce1A*, *phoT* and *eccA1* previously shown to be essential for the growth of *Mtbss* L4 strain H37Rv in primary murine macrophages [35]. In addition, mutations in other genes such as

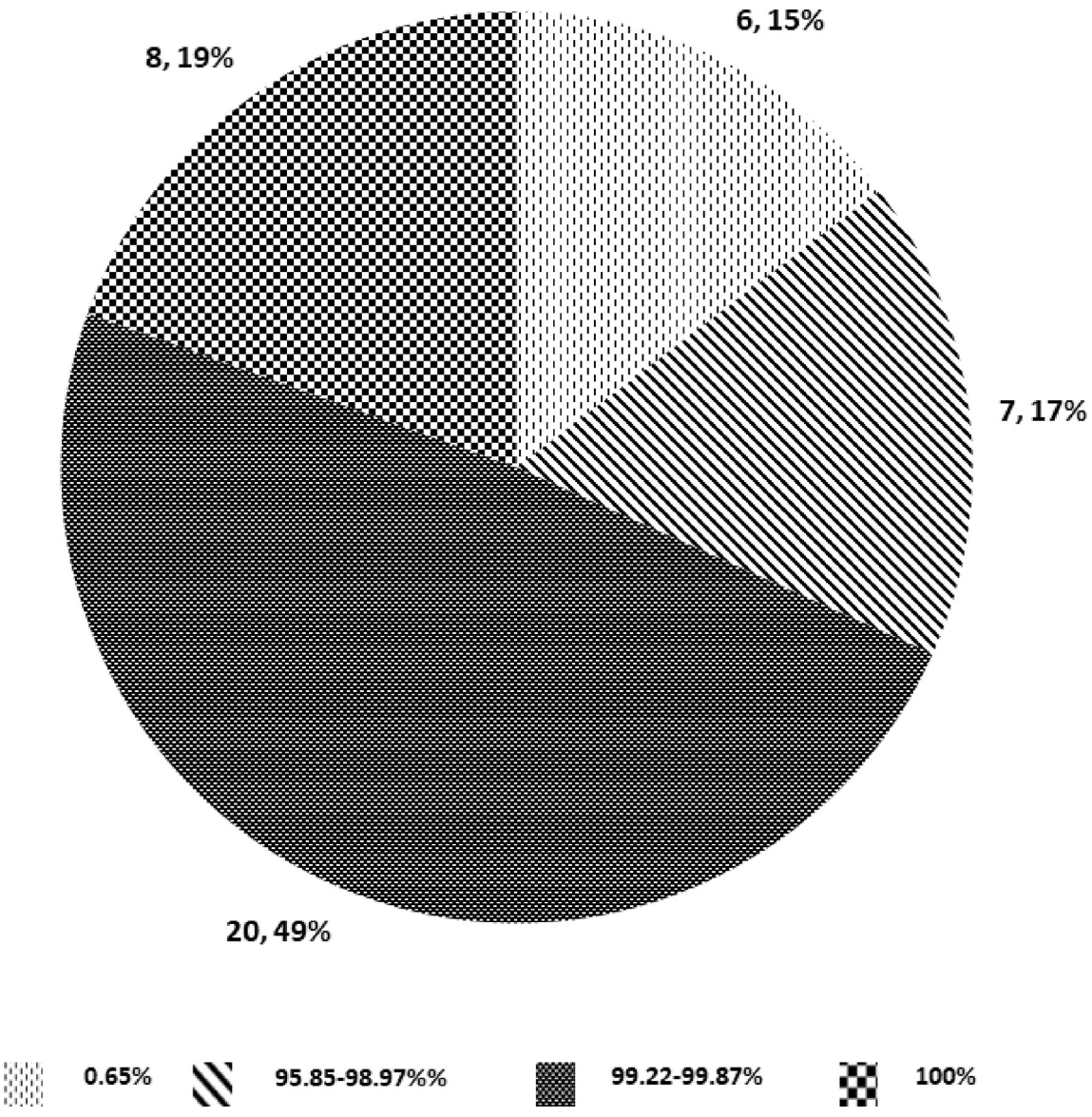

Fig 2. Distribution of selected core-gene amino acid mutations among *M. bovis*.

https://doi.org/10.1371/journal.pone.0209395.g002

Table 2. Description of *M. bovis*-restricted amino acid mutations among essential genes.

Gene	Common name	Mutation	*Proportion of M. bovis*	Function	Essentiality	Reference
Rv0169	*mce1A*	P359S	100%	virulence, detoxification, adaptation	required for survival in primary murine macrophages required for growth in C57BL/6J mouse spleen	[35] [34]
Rv0405	*pks6*	A456fs	100%	lipid metabolism	transposon mutant does not produce phthiocerol dimycocerosate (PDIM) essential gene for in *Mtbss* CDC1551 strain	[36] [37]
Rv0820	*phoT*	F35L	100%	cell wall and cell processes	required for survival in primary murine macrophages in H37Rv	[35]
Rv0931c	*pknD*	L376fs	99.9%	Regulatory	mutant *Mtbss* CDC1551 is attenuated in the central nervous system of BALB/c mice	[39]
Rv1181	*pks4*	D505A	99.5%	lipid metabolism	essential gene in *Mtbss* CDC1551 strain mutant aggregates in liquid culture and does not produce mycolipanoic, mycolipenic, or mycolipodienoic acids	[37] [38]
Rv1328	*glgP*	D532G	100%	intermediary metabolism and respiration	slow growth of *Mtbss* H37Rv mutant strain	[34]
Rv1522c	*mmpL12*	S947N	97.4%	cell wall and cell processes	essential gene for in vitro growth of *Mtbss* H37Rv	[43]
Rv1661	*pks7*	S1176P	95.9%	lipid metabolism	essential gene for in vitro growth of *Mtbss* H37Rv	[43] [34]
Rv1662	*pks8*	A808V	97.9%	lipid metabolism	essential gene for in vitro growth of *Mtbss* H37Rv	[43] [37]
Rv1662	*pks8*	D78Y	97.8%	lipid metabolism	essential gene for in vitro growth of *Mtbss* H37Rv	[43] [34]
Rv1662	*pks8*	Y1469C	99.6%	lipid metabolism	essential gene for in vitro growth of *Mtbss* H37Rv	[43] [34]
Rv2339	*mmpL9*	A44V	99.9%	cell wall and cell processes	essential gene for *in vitro* growth of *Mtbss* H37Rv	[43]
Rv2524c	*fas*	L400F	0.7%	lipid metabolism	essential gene in *Mtbss* H37Rv and CDC1551; essential gene for in vitro growth of *Mtbss* H37Rv	[34] [37] [43]
Rv2956	N.A	I237T	99.6%	information pathways	essential gene for in vitro growth of *Mtbss* H37Rv	[43]
Rv3282	N.A	A133S	99.7%	conserved hypothetical	*Mtbss* H37Rv mutants are slow growing	[34]
Rv3666c	*dppA*	E451G	97.8%	cell wall and cell processes	essential gene in *Mtbss* H37Rv	[34]
Rv3868	*eccA1*	A243V	99.5%	cell wall and cell processes	required for survival of *Mtbss* H37Rv in primary murine macrophages	[35]

https://doi.org/10.1371/journal.pone.0209395.t002

pks6, *pknD*, *pks4* and *glgP* have been shown to be associated with no production of phthiocerol dimycocerosates (PDIM) among mutant strains [36], attenuation in the central nervous system of BALB/c mice [39], no production of mycolic acid derivatives (mycolipanoic, mycolipenic and mycolipodienoic acids) among mutant strains [38] and *in vitro* slow growth [34].

Discussion

The global aim of reducing the impact of tuberculosis by the year 2030 cannot be achieved without considering the impact of zoonotic transmission and biology of *M. bovis*, the main causative agent of TB among cattle. The prevalence and incidence of bTB among humans is significantly lower across the globe compared to TB caused by the hMTBC [2]. Nevertheless, the association of bTB with compromised immunity and the innate resistance of *M. bovis* to pyrazinamide (PZA) (one of the four first line anti-TB drugs) underscore the need to adapt and implement TB control programs that encompass both bTB and TB caused by the hMTBC. Compared to other geographical regions, Africa has the highest burden of zoonotic transmission of bTB due to close contact of humans and animals (domestic and wild-life) as well as relatively poor hygienic practices [2,17,44–46]. We identified 15 *M. bovis* isolates among a total of 1755 MTBC isolated from pulmonary TB patients. Further molecular epidemiological

analysis of these together with global collections of *M. bovis* and hMTBC showed (1) an association between close contact with livestock/animal carcasses and bTB infection in Ghana, (2) clustering of *M. bovis* of African origin close to the root of the global phylogeny and (3) the presence of *M. bovis*-specific amino acid mutations among both essential and non-essential core MTBC genes.

The finding of a significant association between bTB and close contact with animals ($p < 0.0001$) suggests zoonotic transmission and this calls for the implementation of preventive policies and strategies to reduce zoonotic transmission of TB among these high-risk groups [44]. This observation also calls for intensive education to create awareness of the disease about the risks of infection, the detection of infected animals/carcasses and prevention among farmers, butchers and the general population. Further emphasis should be placed on training butchers and animal handlers on the importance of adequate infection control measures, including the use of personal protective equipment (PPE) and the disposal of infected organs to avoid transmission of bTB among such personnel. An experienced butcher suffering from bTB in Australia gave an account of slaughtering many animals suspected of bTB and further cutting out the lungs for over 35 years without any proper precaution [47]. Also, some butchers in Nigeria, suffering from bTB, admitted eating visibly infected parts of the lung of cattle out of ignorance in order to convince customers to buy meat [48]. These instances highlight the importance of public education in the fight against bTB. This education should include veterinarians because there are instances of these professionals getting infected with bTB due to a lack of precautionary measures during execution of their work as was the case of a veterinary surgeon who suffered cutaneous bTB after performing several examinations without proper PPE [49].

Our observation also confirms the importance of the test and slaughter (TS) control strategy for bTB. In addition to pasteurisation of dairy products, bTB has been controlled in developed countries due to the successful implementation of the TS policy of all infected cattle and compensation of affected farmers by governments [50]. However, this has not been implemented in SSA partly due to the costs involved. Nevertheless, our findings call for a reconsideration of the TS strategy and mass vaccination for bTB control in SSA and Governments must respond to this call.

We found the proportion of *M bovis* infected patients among participants from the Northern region (1.9%) of Ghana to be relatively higher (OR = 2.7) compared to those from the Greater Accra region of Ghana (0.6%). The Northern region is home to over 70% of the national cattle population [51], confirming the observation that there is a relationship between close animal contact and bTB. Even though we found no clear association between the *M. bovis* isolates and drug resistance and HIV infection, the proportions were relatively higher than among the hMTBC. However, the lack of association may be because of the relatively limited number of *M. bovis* isolates thus further investigation using a larger number of isolates is required.

The global phylogeny of *M. bovis* clusters most of the *M. bovis* of African origin at the root of the tree (Fig 1) which might be an indication that they are closest to the progenitor of this successful member of the MTBC with the widest host range. However, the limited number of genomes from Africa does not allow inference of ancestry. With the exception of the five clinical *M. bovis* from Ghana which clustered as a monophyletic branch at the base of the tree, the random distribution of *M. bovis* irrespective of the speciation of the host underscores the wide host range of *M. bovis* and indicates that there is no specific host adaptation. However, the geographical distribution may suggest transmission of specific clones within certain geographical locations which agrees with earlier reports [52–54].

The identification and implications of *M. bovis*-specific amino acid mutations among genes such as *mce1A*, *phoT* and *eccA1* [35], *pks6* [36,38] as well as *glgP* [34] highlights the potential attenuated virulence of *M. bovis* relative to the hMTBC [55]. It would be interesting to test the effects of these mutations on fitness of mutants using *ex vivo* human cell lines or *in vivo* bovine models. In addition to the potential phenotypic implications of the identified mutations among essential genes, the 8 *M. bovis*-specific mutations could be utilized in developing either a rapid lateral flow diagnostic tool or a PCR-based tool specific for differential diagnosis of bTB among TB patients to advice an appropriate treatment regimen since *M. bovis* is innately resistant to pyrazinamide, a component of the DOTS regimen.

The scarcity of *M. bovis* genomes from Africa limited our ability to infer ancestry of the Ghanaian clinical isolates. Nevertheless, our data indicates a potential zoonotic transmission of bTB hence highlights the need for public education among people at risk. Moreover, the identified *M. bovis*-specific mutations could be utilized in the development of rapid diagnostic assays for differential diagnosis of bTB.

Supporting information

S1 Table. Global compilation of published *M. bovis* genomes.
(DOCX)

S2 Table. List of MTBC core genes for comparative mutational analysis.
(DOCX)

S3 Table. Mutations restricted to the 5 clinical *M. bovis* from Ghana relative to hMTBC.
(DOCX)

S4 Table. Distribution of *M. bovis*-restricted amino acid mutations among global collection of *M. bovis*.
(DOCX)

S1 Fig. Distribution of amino acid mutations among core genes of *M. bovis*. Distribution of the *M. bovis* restricted amino acid mutations on the midpoint-rooted maximum-likelihood phylogeny of 772 global collection of *M. bovis* genomes. Mutation present and absent are represented by the red and white blocks respectively.
(TIF)

Acknowledgments

We would like to thank the Ghana Health Service, the national tuberculosis control program and especially the clinicians and lab technicians of the Korle-Bu teaching hospital (Accra), La general hospital (Accra), Tamale teaching hospital (Tamale) and Baptist medical center (Nalerigu) for their relentless support. Most importantly, we thank all the TB patients who consented to be part of this study.

Author Contributions

Conceptualization: Julian Parkhill, Sebastien Gagneux, Dorothy Yeboah-Manu.

Data curation: Isaac Darko Otchere, Andries J. van Tonder, Leonor Sánchez-Busó, Julian Parkhill, Sebastien Gagneux.

Formal analysis: Isaac Darko Otchere, Andries J. van Tonder, Adwoa Asante-Poku, Leonor Sánchez-Busó, Stephen Osei-Wusu, Prince Asare.

Funding acquisition: Dorothy Yeboah-Manu.

Investigation: Isaac Darko Otchere, Adwoa Asante-Poku, Leonor Sánchez-Busó, Stephen Osei-Wusu, Prince Asare, Samuel Yaw Aboagye, Dorothy Yeboah-Manu.

Methodology: Isaac Darko Otchere, Andries J. van Tonder, Adwoa Asante-Poku, Mireia Coscollá, Julian Parkhill, Simon R. Harris, Sebastien Gagneux, Dorothy Yeboah-Manu.

Project administration: Dorothy Yeboah-Manu.

Resources: Andries J. van Tonder, Mireia Coscollá, Samuel Acquah Ekuban, Abdallah Iddrisu Yahayah, Audrey Forson, Akosua Baddoo, Clement Laryea, Julian Parkhill, Sebastien Gagneux, Dorothy Yeboah-Manu.

Software: Isaac Darko Otchere, Leonor Sánchez-Busó, Mireia Coscollá, Julian Parkhill, Simon R. Harris, Sebastien Gagneux.

Supervision: Mireia Coscollá, Julian Parkhill, Sebastien Gagneux, Dorothy Yeboah-Manu.

Validation: Isaac Darko Otchere, Julian Parkhill, Dorothy Yeboah-Manu.

Writing – original draft: Isaac Darko Otchere.

Writing – review & editing: Isaac Darko Otchere, Andries J. van Tonder, Adwoa Asante-Poku, Leonor Sánchez-Busó, Mireia Coscollá, Stephen Osei-Wusu, Prince Asare, Samuel Yaw Aboagye, Samuel Acquah Ekuban, Abdallah Iddrisu Yahayah, Audrey Forson, Akosua Baddoo, Clement Laryea, Julian Parkhill, Simon R. Harris, Sebastien Gagneux, Dorothy Yeboah-Manu.

References

1. Müller B, Dürr S, Alonso S, Hattendorf J, Laisse CJM, Parsons SDC, et al. (2013) Zoonotic Mycobacterium bovis—induced tuberculosis in humans. Emerg Infect Dis 19: 899–908. Available: http://wwwnc.cdc.gov/eid/article/19/6/pdfs/12-0543.pdf. https://doi.org/10.3201/eid1906.120543 PMID: 23735540
2. World Health Organization (2017) Global Tuberculosis Report 2017. Geniva.
3. Thoen C, LoBue P, de Kantor I (2006) The importance of Mycobacterium bovis as a zoonosis. Vet Microbiol 112: 339–345. Available: https://www.sciencedirect.com/science/article/pii/S0378113505004086?via%3Dihub. Accessed 23 November 2018. https://doi.org/10.1016/j.vetmic.2005.11.047 PMID: 16387455
4. Grange JM (2001) Mycobacterium bovis infection in human beings. Tuberculosis 81: 71–77. Available: http://www.ncbi.nlm.nih.gov/pubmed/11463226. Accessed 23 November 2018. https://doi.org/10.1054/tube.2000.0263 PMID: 11463226
5. Evans JT, Smith EG, Banerjee A, Smith RM, Dale J, Innes JA, et al. (2007) Cluster of human tuberculosis caused by Mycobacterium bovis: evidence for person-to-person transmission in the UK. Lancet 369: 1270–1276. Available: http://linkinghub.elsevier.com/retrieve/pii/S0140673607605984. Accessed 8 November 2016. https://doi.org/10.1016/S0140-6736(07)60598-4 PMID: 17434402
6. Pesciaroli M, Alvarez J, Boniotti MB, Cagiola M, Di Marco V, Marianelli C, et al. (2014) Tuberculosis in domestic animal species. Res Vet Sci 97: S78–S85. Available: https://www.sciencedirect.com/science/article/pii/S0034528814001623?via%3Dihub. Accessed 23 November 2018. https://doi.org/10.1016/j.rvsc.2014.05.015 PMID: 25151859
7. Domingo M, Vidal E, Marco A (2014) Pathology of bovine tuberculosis. Res Vet Sci 97: S20–S29. Available: https://www.sciencedirect.com/science/article/pii/S0034528814000927?via%3Dihub. Accessed 23 November 2018. https://doi.org/10.1016/j.rvsc.2014.03.017 PMID: 24731532
8. Rodwell TC, Moore M, Moser KS, Brodine SK, Strathdee SA (2008) Tuberculosis from Mycobacterium bovis in binational communities, United States. Emerg Infect Dis 14: 909–916. Available: http://www.ncbi.nlm.nih.gov/pubmed/18507901. Accessed 23 November 2018. https://doi.org/10.3201/eid1406.071485 PMID: 18507901
9. Ritacco V, Sequeira MD, De Kantor IN (2008) Human Tuberculosis Caused by Mycobacterium Bovis in Latin America and the Caribbean. Mycobacterium Bovis Infect Anim Humans Second Ed 14: 13–17. https://doi.org/10.1002/9780470344538.ch3

10. Nuru A, Mamo G, Zewude A, Mulat Y, Yitayew G, Admasu A, et al. (2017) Preliminary investigation of the transmission of tuberculosis between farmers and their cattle in smallholder farms in northwestern Ethiopia: a cross-sectional study. BMC Res Notes 10: 31. Available: http://www.ncbi.nlm.nih.gov/pubmed/28061860. Accessed 23 November 2018. https://doi.org/10.1186/s13104-016-2349-z PMID: 28061860

11. Vazquez-Chacon CA, Martínez-Guarneros A, Couvin D, González-Y-Merchand JA, Rivera-Gutierrez S, Escobar-Gutierrez A, et al. (2015) Human multidrug-resistant Mycobacterium bovis infection in Mexico. Tuberculosis (Edinb) 95: 802–809. Available: http://www.sciencedirect.com/science/article/pii/S1472979215207482.

12. Smith NH, Gordon S V., de la Rua-Domenech R, Clifton-Hadley RS, Hewinson RG (2006) Bottlenecks and broomsticks: the molecular evolution of Mycobacterium bovis. Nat Rev Microbiol 4: 670–681. Available: http://www.nature.com/doifinder/10.1038/nrmicro1472. Accessed 26 July 2017. https://doi.org/10.1038/nrmicro1472 PMID: 16912712

13. Winder CL, Gordon S V., Dale J, Hewinson RG, Goodacre R (2006) Metabolic fingerprints of Mycobacterium bovis cluster with molecular type: implications for genotype-phenotype links. Microbiology 152: 2757–2765. Available: http://mic.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.28986-0. Accessed 23 November 2018. https://doi.org/10.1099/mic.0.28986-0 PMID: 16946270

14. Golby P, Nunez J, Witney A, Hinds J, Quail M A, Bentley S, et al. (2013) Genome-level analyses of Mycobacterium bovis lineages reveal the role of SNPs and antisense transcription in differential gene expression. BMC Genomics 14: 710. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3856593&tool=pmcentrez&rendertype=abstract. Accessed 31 October 2016. https://doi.org/10.1186/1471-2164-14-710 PMID: 24134787

15. Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D, et al. (1998) Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg Infect Dis 4: 59–70. Available: http://www.ncbi.nlm.nih.gov/pubmed/9452399. Accessed 27 March 2018. https://doi.org/10.3201/eid0401.980108 PMID: 9452399

16. Humblet M-F, Boschiroli ML, Saegerman C (2009) Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet Res 40: 50. Available: http://www.ncbi.nlm.nih.gov/pubmed/19497258. Accessed 23 November 2018. https://doi.org/10.1051/vetres/2009033 PMID: 19497258

17. Boukary AR, Thys E, Rigouts L, Matthys F, Berkvens D, Mahamadou I, et al. (2012) Risk Factors Associated with Bovine Tuberculosis and Molecular Characterization of Mycobacterium bovis Strains in Urban Settings in Niger. Transbound Emerg Dis 59: 490–502. https://doi.org/10.1111/j.1865-1682.2011.01302.x PMID: 22226088

18. Cleaveland S, Shaw DJ, Mfinanga SG, Shirima G, Kazwala RR, Eblate E, et al. (2007) Mycobacterium bovis in rural Tanzania: Risk factors for infection in human and cattle populations. Tuberculosis 87: 30–43. Available: https://www.sciencedirect.com/science/article/pii/S1472979206000497?via%3Dihub. Accessed 23 November 2018. https://doi.org/10.1016/j.tube.2006.03.001 PMID: 16618553

19. Kang'ethe EK, Ekuttan CE, Kimani VN (2007) Investigation of the prevalence of bovine tuberculosis and risk factors for human infection with bovine tuberculosis among dairy and non-dairy farming neighbour households in Dagoretti Division, Nairobi, Kenya. East Afr Med J 84: S92–5. Available: http://www.ncbi.nlm.nih.gov/pubmed/18338728. Accessed 23 November 2018. PMID: 18338728

20. Cadmus S, Palmer S, Okker M, Dale J, Gover K, Smith N, et al. (2006) Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. J Clin Microbiol 44: 29–34. Available: http://www.ncbi.nlm.nih.gov/pubmed/16390943. Accessed 6 October 2016. https://doi.org/10.1128/JCM.44.1.29-34.2006 PMID: 16390943

21. Müller B, Steiner B, Bonfoh B, Fané A, Smith NH, Zinsstag J (2008) Molecular characterisation of Mycobacterium bovis isolated from cattle slaughtered at the Bamako abattoir in Mali. BMC Vet Res 4: 26. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2483712&tool=pmcentrez&rendertype=abstract. Accessed 1 April 2014. https://doi.org/10.1186/1746-6148-4-26 PMID: 18637160

22. Asante-Poku A, Yeboah-Manu D, Otchere ID, Aboagye SY, Stucki D, Hattendorf J, et al. (2015) Mycobacterium africanum Is Associated with Patient Ethnicity in Ghana. PLoS Negl Trop Dis 9: e3370. Available: http://www.ncbi.nlm.nih.gov/pubmed/25569290. Accessed 9 January 2015. https://doi.org/10.1371/journal.pntd.0003370 PMID: 25569290

23. Otchere ID, Asante-poku A, Osei-Wusu S, Baddoo A, Sarpong E, Ganiyu AH, et al. (2016) Detection and characterization of drug-resistant conferring genes in Mycobacterium tuberculosis complex strains: A prospective study in two distant regions of. Tuberculosis (Edinb) 99: 147–154. Available: http://www.sciencedirect.com/science/article/pii/S1472979216301329.

24. Kamerbeek J, Schouls L, Kolk a, van Agterveld M, van Soolingen D, Kuijper S et al. (1997) Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and

epidemiology. J Clin Microbiol 35: 907–914. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=229700&tool=pmcentrez&rendertype=abstract. PMID: 9157152

25. Warren RM, Richardson M, Sampson SL, van der Spuy GD, Bourn W, Hauman JH, et al. (2001) Molecular evolution of Mycobacterium tuberculosis: phylogenetic reconstruction of clonal expansion. Tuberculosis 81: 291–302. Available: http://www.ncbi.nlm.nih.gov/pubmed/11584597. Accessed 12 October 2017. https://doi.org/10.1054/tube.2001.0300 PMID: 11584597

26. Franzblau SG, Witzig RS, Mclaughlin JC, Torres P, Madico G, Hernandez A, et al. (1998) Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. J Clin Microbiol 36: 362–366. Available: https://www.ncbi.nlm.nih.gov/pubmed/9466742 PMID: 9466742

27. Otchere ID, Coscollá M, Sánchez-Busó L, Asante-Poku A, Brites D, Loiseau C, et al. (2018) Comparative genomics of Mycobacterium africanum Lineage 5 and Lineage 6 from Ghana suggests distinct ecological niches. Sci Rep 8: 11269. Available: http://www.nature.com/articles/s41598-018-29620-2. Accessed 30 July 2018. https://doi.org/10.1038/s41598-018-29620-2 PMID: 30050166

28. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760. https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168

29. Harris SR, Feil EJ, Holden MTG, Quail MA, Nickerson EK, Chantratita N, et al. (2010) Evolution of MRSA during hospital transmission and intercontinental spread. Science 327: 469–474. Available: http://www.ncbi.nlm.nih.gov/pubmed/20093474. Accessed 1 November 2018. https://doi.org/10.1126/science.1182395 PMID: 20093474

30. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. (2016) SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb genomics 2: e000056. Available: http://www.ncbi.nlm.nih.gov/pubmed/28348851. Accessed 23 November 2018.

31. Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 5: e9490. Available: http://www.ncbi.nlm.nih.gov/pubmed/20224823. Accessed 23 November 2018. https://doi.org/10.1371/journal.pone.0009490 PMID: 20224823

32. Letunic I, Bork P (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 44: W242–W245. Available: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw290. Accessed 1 November 2018. https://doi.org/10.1093/nar/gkw290 PMID: 27095192

33. Sassetti CM, Rubin EJ (2003) Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci U S A 100: 12989–12994. Available: http://www.ncbi.nlm.nih.gov/pubmed/14569030. Accessed 23 November 2018. https://doi.org/10.1073/pnas.2134250100 PMID: 14569030

34. Sassetti CM, Boyd DH, Rubin EJ (2003) Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48: 77–84. Available: http://doi.wiley.com/10.1046/j.1365-2958.2003.03425.x. Accessed 23 November 2018. PMID: 12657046

35. Rengarajan J, Bloom BR, Rubin EJ (2005) Genome-wide requirements for Mycobacterium tuberculosis adaptation and survival in macrophages. Proc Natl Acad Sci U S A 102: 8327–8332. Available: http://www.pnas.org/content/102/23/8327.long. Accessed 26 November 2015. https://doi.org/10.1073/pnas.0503272102 PMID: 15928073

36. Waddell SJ, Chung GA, Gibson KJC, Everett MJ, Minnikin DE, Besra GS, et al. (2005) Inactivation of polyketide synthase and related genes results in the loss of complex lipids in Mycobacterium tuberculosis H37Rv. Lett Appl Microbiol 40: 201–206. Available: http://doi.wiley.com/10.1111/j.1472-765X.2005.01659.x. Accessed 23 November 2018. https://doi.org/10.1111/j.1472-765X.2005.01659.x PMID: 15715645

37. Lamichhane G, Zignol M, Blades NJ, Geiman DE, Dougherty A, Grosset J, et al. (2003) A postgenomic method for predicting essential genes at subsaturation levels of mutagenesis: application to Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 100: 7213–7218. Available: http://www.ncbi.nlm.nih.gov/pubmed/12775759. Accessed 23 November 2018. https://doi.org/10.1073/pnas.1231432100 PMID: 12775759

38. Dubey VS, Sirakova TD, Kolattukudy PE (2002) Disruption of msl3 abolishes the synthesis of mycolipanoic and mycolipenic acids required for polyacyltrehalose synthesis in Mycobacterium tuberculosis H37Rv and causes cell aggregation. Mol Microbiol 45: 1451–1459. Available: http://doi.wiley.com/10.1046/j.1365-2958.2002.03119.x. Accessed 23 November 2018. PMID: 12207710

39. Be NA, Lamichhane G, Grosset J, Tyagi S, Cheng Q, Kim KS, et al. (2018) Murine Model to Study the Invasion and Survival of Mycobacterium tuberculosis in the Central Nervous System. 198. https://doi.org/10.1086/592447 PMID: 18956986

40. Lew JM, Kapopoulou A, Jones LM, Cole ST (2011) TubercuList—10 years after. Tuberculosis (Edinb) 91: 1–7. https://doi.org/10.1016/j.tube.2010.09.008 PMID: 20980199

41. Comas I, Chakravartti J, Small PM, Galagan J, Niemann S, Kremer K, et al. (2010) Human T cell epitopes of Mycobacterium tuberculosis are evolutionarily hyperconserved. Nat Genet 42: 498–503. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2883744&tool=pmcentrez&rendertype=abstract. Accessed 11 July 2014. https://doi.org/10.1038/ng.590 PMID: 20495566

42. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. (2017) ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genomics. Available: http://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000131.v1.

43. Griffin JE, Gawronski JD, DeJesus MA., Ioerger TR, Akerley BJ, Sassetti CM (2011) High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. PLoS Pathog 7: 1–9. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3182942

44. Vayr F, Martin-Blondel G, Savall F, Soulat J-M, Deffontaines G, Herin F (2018) Occupational exposure to human Mycobacterium bovis infection: A systematic review. PLoS Negl Trop Dis 12: e0006208. Available: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0006208. Accessed 23 November 2018. PMID: 29337996

45. Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I (2004.) Bovine tuberculosis: an old disease but a new threat to Africa. Int J Tuberc Lung Dis. 8(8):924–937. Available: https://www.ingentaconnect.com/content/iuatld/ijtld/2004/00000008/00000008/art00002. Accessed 23 November 2018. PMID: 15305473

46. Boukari AR, Chaibou M, Marichatou H, Vias GF (2007) Caractérisation des systèmes de production laitière et analyse des stratégies de valorisation du lait en milieu rural et périurbain au Niger: cas de la communauté urbaine de Niamey et de la commune rurale de Filingué. Rev d'élevage médecine vétérinaire des pays Trop 60: 113. Available: http://revues.cirad.fr/index.php/REMVT/article/view/9963. Accessed 23 November 2018.

47. Ingram PR, Bremner P, Inglis TJ, Murray RJ, Cousins D V (2010) Zoonotic tuberculosis: on the decline. Commun Dis Intell Q Rep 34: 339–341. Available: http://www.ncbi.nlm.nih.gov/pubmed/21090190. Accessed 23 November 2018. PMID: 21090190

48. Hambolu D, Freeman J, Taddese HB (2013) Predictors of bovine TB risk behaviour amongst meat handlers in Nigeria: a cross-sectional study guided by the health belief model. PLoS One 8: e56091. Available: http://www.ncbi.nlm.nih.gov/pubmed/23409127. Accessed 23 November 2018. https://doi.org/10.1371/journal.pone.0056091 PMID: 23409127

49. Twomey DF, Collins R, Cranwell MP, Crawshaw TR, Higgins RJ, Dean GSet al. (2012) Controlling tuberculosis in a llama (Lama glama) herd using clinical signs, tuberculin skin testing and serology. Vet J 192: 246–248. Available: https://www.sciencedirect.com/science/article/pii/S1090023311001882?via%3Dihub. Accessed 23 November 2018. https://doi.org/10.1016/j.tvjl.2011.05.014 PMID: 21704542

50. Zamri-Saad M, Kamarudin MI (2016) Control of animal brucellosis: The Malaysian experience. Asian Pac J Trop Med 9: 1136–1140. Available: https://www.sciencedirect.com/science/article/pii/S1995764516304679. Accessed 21 March 2018. https://doi.org/10.1016/j.apjtm.2016.11.007 PMID: 27955740

51. Ministry of Food and Agriculture-Ghana (2004) Livestock development in Ghana policies and Strategies: 1–122.

52. Rodriguez-Campos S, Schürch AC, Dale J, Lohan AJ, Cunha MV, Botelho A, et al. (2012) European 2—A clonal complex of Mycobacterium bovis dominant in the Iberian Peninsula. Infect Genet Evol 12: 866–872. Available: http://dx.doi.org/10.1016/j.meegid.2011.09.004. Accessed 31 October 2016. https://doi.org/10.1016/j.meegid.2011.09.004 PMID: 21945286

53. Smith NH (2012) The global distribution and phylogeography of Mycobacterium bovis clonal complexes. Infect Genet Evol 12: 857–865. Available: https://www.sciencedirect.com/science/article/pii/S1567134811003170?via%3Dihub. Accessed 11 October 2018. https://doi.org/10.1016/j.meegid.2011.09.007 PMID: 21945588

54. Smith NH, Berg S, Dale J, Allen A, Rodriguez S, Romero B, et al. (2011) European 1: A globally important clonal complex of Mycobacterium bovis. Infect Genet Evol 11: 1340–1351. Available: https://www.sciencedirect.com/science/article/pii/S156713481100133X?via%3Dihub. Accessed 11 October 2018. https://doi.org/10.1016/j.meegid.2011.04.027 PMID: 21571099

55. Villarreal-Ramos B, Berg S, Whelan A, Holbert S, Carreras F, Salguero FJ, et al. (2018) Experimental infection of cattle with Mycobacterium tuberculosis isolates shows the attenuation of the human tubercle bacillus for cattle. Sci Rep 8: 894. Available: https://www.nature.com/articles/s41598-017-18575-5.pdf. Accessed 27 March 2018. https://doi.org/10.1038/s41598-017-18575-5 PMID: 29343690