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**Proposed Title 1:** Molecular epidemiology and whole genome sequencing analysis of clinical *Mycobacterium bovis* from Ghana.

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30 **Abstract**

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

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

39 **Results:** *Mycobacterium bovis* was isolated from 3/560(0.5%) females and  
40 12/1195(1.0%) males with pulmonary TB. The average age of *M. bovis* infected cases  
41 was 46.8 years (7-72years). TB patients from the Northern region of Ghana (1.9%;4/212)  
42 had a higher rate of infection with *M. bovis* (OR=2.7,p=0.0968) compared to those from  
43 the Greater Accra region (0.7%;11/1543). Among TB patients with available HIV status,  
44 the odds of isolating *M. bovis* from HIV patients (2/119) was 3.3 higher relative to non-  
45 HIV patients (4/774). Direct contact with livestock or their unpasteurized products was  
46 significantly associated with bTB (p<0.0001,OR=124.4,95% CI=30.1-508.3). Two  
47 (13.3%) of the *M. bovis* isolates were INH resistant due to the S315T mutation in *katG*  
48 whereas one (6.7%) was RIF resistant with Q432P and I1491S mutations in *rpoB*. *M.*  
49 *bovis* from Ghana resolved as mono-phyletic branch among mostly *M. bovis* from Africa  
50 irrespective of the host and were closest to the root of the global *M. bovis* phylogeny. *M.*  
51 *bovis*-specific amino acid mutations were detected among MTBC core genes such as  
52 *mce1A*, *mmpL1*, *pks6*, *phoT*, *pstB*, *glgP* and *Rv2955c*. Additional mutations P6T in *chaA*,  
53 G187E in *mgtC*, T35A in *Rv1979c*, S387A in *narK1*, L400F in *fas* and A563T in *eccA1*  
54 were restricted to the 5 clinical *M. bovis* from Ghana.

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

57

58

## 59 **Introduction:**

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

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

88 About 85% of herds and 82% of humans live in close proximity in sub-Saharan Africa  
89 (SSA) in both rural and urban settings, driving the wide distribution of bTB compared to  
90 other global settings [15,16]. This is compounded by the inadequate sanitation practices  
91 such as the habit of sharing drinking water with beasts and consumption of non-  
92 pasteurized milk and dairy products [17–19] (. Despite the economic and public health

93 importance of bTB, little knowledge exists on the epidemiology and biology of *M. bovis*  
94 in relation to the human adapted MTBC (hMTBC) lineages spanning *M. tuberculosis*  
95 *sensu stricto* (*Mtbss*) and *M. africanum* (*Maf*) [20,21]. However, such information is  
96 critical for development of effective control tools for bTB.

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

## 102 **Materials and Methods**

### 103 **Ethical Statement and Participant Enrolment**

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

107

### 108 **Mycobacterial Isolation, Drug Resistance Profiling and Genotyping.**

109 Smear-positive sputum samples from the selected health centers in the Northern and  
110 Greater Accra regions of Ghana were decontaminated and inoculated on 2 pairs of  
111 Lowenstein Jensen (LJ) slants; one pair supplemented with 0.4% sodium pyruvate (to  
112 enhance growth of *M. bovis* and *M. africanum* (*Maf*)) the other with glycerol (for  
113 enhanced growth of *M. tuberculosis sensu stricto* (*Mtbs*)) and incubated as previously  
114 described [22]. MTBC cells growing in confluence were harvested and heat inactivated at  
115 95 °C for 60 min in nuclease-free water. After heat inactivation, chromosomal DNA was  
116 extracted using previously described protocol [23]. The isolates were confirmed as  
117 MTBC by PCR amplification of IS6110 and spoligotyping was carried out for lineage  
118 classification [24]. Isolates classified as *M. bovis* were confirmed with a large sequence  
119 polymorphism (LSP) assay using PCR detection of deleted regions of difference RD9,  
120 RD4 and RD12 [25]. Drug susceptibility testing against isoniazid (INH) and rifampicin  
121 (RIF) was carried out using the micro-plate alamar blue assay [23,26].

122

### 123 **Whole Genome Sequencing and Phylogenetic Analysis**

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

136

## 137 **Comparative Mutational Analysis of Selected MTBC Core-Genes.**

138 Coordinates of 147 MTBC core genes ([Supplementary Table S2](#)) previously reported to  
139 harbour amino acid mutations with phenotypic consequence on virulence and fitness of  
140 some laboratory strains of the MTBC [33–40] were compiled from the Tuberculist  
141 database [41]. Using the fasta file of H37Rv as reference, the paired end reads of the 5  
142 Ghanaian *M. bovis* genomes, 257 *M. africanum* [27] and global collection of 20 MTBC  
143 genomes [42] were screened for mutations within the compiled 147 core genes using  
144 ARIBA with default settings [43]. Amino acid mutations found to be present only among  
145 the 5 Ghanaian *M. bovis* genomes were suspected to be *M. bovis* specific. To confirm  
146 whether these mutations were widespread in *M. bovis*, the global collection of 767  
147 clinical and veterinary *M. bovis* genomes ([Supplementary data S1](#)) was screened for these  
148 specific mutations using ARIBA as described above. We further classified these amino  
149 acid mutations as *M. bovis*-specific if they were found in 100% of genomes in the global  
150 collection or core *M. bovis* mutations if found in at least 99% of genomes.

151

## 152 **Statistical Analysis**

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

156

157

158

159

160 **Results**

161 **Demography and Biological Associations of TB Patients infected with *M. bovis***

162 A total of 1755 MTBC isolates were obtained from 2074 smear positive TB patients  
 163 (84.6% isolation rate). Among the patients from whom a MTBC was isolated, 212  
 164 (12.1%) were from the Northern region and 1543 (87.9%) from the Greater Accra region  
 165 as previously described [27]. Fifteen (0.9%) of the isolates were genotyped as *M. bovis*  
 166 whereas the remaining 1740 (99.1%) were members of the hMTBC (*Mtbss* and *Maf*). The  
 167 average age of patients infected with *M. bovis* was 46.8 years (7 to 72 years) of which  
 168 12/1195 (1.0%) were from males compared to 3/560 (0.5%) from females ( $p = 0.412$ , OR  
 169 = 1.9). Four (1.9%) of the isolates from the Northern region ( $n = 212$ ) were *M. bovis*  
 170 compared to 11/1543 (0.7%) from the Greater Accra region ( $p = 0.0968$ , OR = 2.7).  
 171 Among the patients with known HIV status (893; 50.3%), 119 (13.3%) were HIV-  
 172 positive compared to 774 (86.7%) HIV-negative. The incidence of bTB among HIV and  
 173 non-HIV TB patients was 1.7% (2/119) and 0.5% (4/774) respectively with higher odds  
 174 of isolating *M. bovis* from HIV patients relative to non-HIV TB patients (OR = 3.3). Six  
 175 TB patients including 1 herdsman, 1 herds owner and 4 butchers representing 40% of 15  
 176 patients with history of direct contact with livestock were infected with *M. bovis*. This is  
 177 significantly higher compared to 0.5% (9/1740) of *M. bovis* infected TB patients without  
 178 such history ( $p < 0.0001$ , OR = 124.4, 95% CI = 30.1-508.3)

179

180 **Drug Resistance Profile of *M. bovis* Isolates**

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

185 **Table 1: Sensitivity of the MTBC Isolates to INH and RIF**

Drug	Total (1755)	hMTBC (1740)	<i>M. bovis</i> (15)	<i>P-value</i>	OR	95%CI
INH <sup>r</sup>	133; 7.6%	131;7.5%	2;13.3%	0.3163	1.9	0.2-8.5
RIF <sup>r</sup>	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

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



187

## 188 **Phylogenetic Distribution of Global Collection of *M. bovis***

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

200

201 **Fig 1: Phylogenetic tree of the Ghanaian clinical *M. bovis* amidst global collection of**  
202 **767 published *M. bovis* genomes.** *The whole genome phylogeny of 767 publicly*  
203 *available *M. bovis* genomes together with 5 clinical *M. bovis* from Ghana rooted on *M.**  
204 *africanum as an outgroup, shows the 5 Ghanaian clinical *M. bovis* genomes as a*  
205 *monophyletic group sitting in a clade consisting mostly of other African *M. bovis* isolates*  
206 *basal to the rest of the dataset.*

207

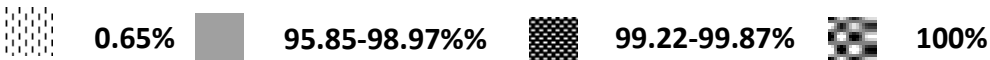
208

## 209 ***In silico* predicted *M. bovis*-Specific Amino Acid Mutations.**

210 We identified 41 *M. bovis* restricted amino acid mutations among 32 core-genes of the 5  
211 clinical *M. bovis* from Ghana when compared to 257 *Maf* [27] and 20 global MTBC  
212 genomes [42] (Supplementary data S3). However, when we screened our global  
213 collection of 772 *M. bovis* genomes (including the 5 from Ghana), only 8 of the  
214 mutations were found in all genomes, 20 mutations in 99.22% to 99.87% of the genomes  
215 and 7 mutations in 95.85% to 98.97% of genomes. A further 6 mutations (P6T in *chaA*,  
216 G187E in *mgtC*, T35A in *Rv1979c*, S387A in *narK1*, L400F in *fas* and A563T in *eccA1*)  
217 were restricted to the 5 clinical *M. bovis* from Ghana (Fig 2; Supplementary Table S4;  
218 Supplementary Figure S5).

219

## 220 **Figure 2: Distribution of selected core-gene amino acid mutations among *M. bovis*.**

221  0.65% 95.85-98.97% 99.22-99.87% 100%

222

223 Among the 41 mutations identified uniquely among 32 core-genes *M. bovis*, 17 were  
224 among 15 essential genes associated with important physiological processes such as lipid  
225 metabolism, cell wall and cell processes, intermediate metabolism, and cellular  
226 respiration, virulence, detoxification and virulence as well as regulatory proteins (**Table**  
227 **2**). These include *mce1A*, *phoT* and *eccA1* previously shown to be essential for the  
228 growth of *Mtbss* L4 strain H37Rv in primary murine macrophages [35]. In addition,  
229 mutations in other genes such as *pks6*, *pknD*, *pks4* and *glgP* have been shown to be  
230 associated with no production of phthiocerol dimycocerosates (PDIM) among mutant  
231 strains [36], attenuation in the central nervous system of BALB/c mice [40], no  
232 production of mycolic acid derivatives (mycolipanoic, mycolipenic and mycolipodienoic  
233 acids) among mutant strains [38] and *in vitro* slow growth [34].

234 **Table 2: Description of *M. bovis*-Restricted Amino Acid Mutations among Essential Genes**

Gene	Common Name	Mutation	Proportion of <i>M. bovis</i>	Function	Essentiality	Reference
<i>Rv0169</i>	<i>mce1A</i>	P359S	100%	virulence, detoxification, adaptation	required for survival in primary murine macrophages required for growth in C57BL/6J mouse spleen	[35] [34]
<i>Rv0405</i>	<i>pks6</i>	A456fs	100%	lipid metabolism	transposon mutant does not produce phthiocerol dimycocerosate (PDIM) essential gene for in <i>Mtbss</i> CDC1551 strain	[36] [37]
<i>Rv0820</i>	<i>phoT</i>	F35L	100%	cell wall and cell processes	required for survival in primary murine macrophages in H37Rv	[35]
<i>Rv0931c</i>	<i>pknD</i>	L376fs	99.9%	Regulatory	mutant <i>Mtbss</i> CDC1551 is attenuated in the central nervous system of BALB/c mice	[40]
<i>Rv1181</i>	<i>pks4</i>	D505A	99.5%	lipid metabolism	essential gene in <i>Mtbss</i> CDC1551 strain mutant aggregates in liquid culture and does not produce mycolipanoic, mycolipenic, or mycolipodienoic acids	[37] [38]
<i>Rv1328</i>	<i>glgP</i>	D532G	100%	intermediary metabolism and respiration	slow growth of <i>Mtbss</i> H37Rv mutant strain	[34]
<i>Rv1522c</i>	<i>mmpL12</i>	S947N	97.4%	cell wall and cell processes	essential gene for in vitro growth of <i>Mtbss</i> H37Rv	[44]
<i>Rv1661</i>	<i>pks7</i>	S1176P	95.9%	lipid metabolism	essential gene for in vitro growth of <i>Mtbss</i> H37Rv	[44] [34]
<i>Rv1662</i>	<i>pks8</i>	A808V	97.9%	lipid metabolism	essential gene for in vitro growth of <i>Mtbss</i> H37Rv	[44] [37]
<i>Rv1662</i>	<i>pks8</i>	D78Y	97.8%	lipid metabolism	essential gene for in vitro growth of <i>Mtbss</i> H37Rv	[44] [34]
<i>Rv1662</i>	<i>pks8</i>	Y1469C	99.6%	lipid metabolism	essential gene for in vitro growth of <i>Mtbss</i> H37Rv	[44] [34]
<i>Rv2339</i>	<i>mmpL9</i>	A44V	99.9%	cell wall and cell processes	essential gene for <i>in vitro</i> growth of <i>Mtbss</i> H37Rv	[44]
<i>Rv2524c</i>	<i>fas</i>	L400F	0.7%	lipid metabolism	essential gene in <i>Mtbss</i> H37Rv and CDC1551; essential gene for in vitro growth of <i>Mtbss</i> H37Rv	[34] [37] [44]
<i>Rv2956</i>	N.A	I237T	99.6%	information	essential gene for in vitro growth of <i>Mtbss</i>	[44]

				pathways	H37Rv	
<i>Rv3282</i>	N.A	A133S	99.7%	conserved hypothetical	<i>Mtbss</i> H37Rv mutants are slow growing	[34]
<i>Rv3666c</i>	<i>dppA</i>	E451G	97.8%	cell wall and cell processes	essential gene in <i>Mtbss</i> H37Rv	[34]
<i>Rv3868</i>	<i>eccA1</i>	A243V	99.5%	cell wall and cell processes	required for survival of <i>Mtbss</i> H37Rv in primary murine macrophages	[35]

235

## 236 Discussion

237 The global aim of reducing the impact of tuberculosis by the year 2030 cannot be  
238 achieved without considering the impact of zoonotic transmission and biology of *M.*  
239 *bovis*, the main causative agent of TB among cattle. The prevalence and incidence of bTB  
240 among humans is significantly lower across the globe compared to TB caused by the  
241 hMTBC [2]. Nevertheless, the association of bTB with compromised immunity and the  
242 innate resistance of *M. bovis* to pyrazinamide (PZA) (one of the four first line anti-TB  
243 drugs) underscore the need to adapt and implement TB control programs that encompass  
244 both bTB and TB caused by the hMTBC. Compared to other geographical regions, Africa  
245 has the highest burden of zoonotic transmission of bTB due to close contact of humans  
246 and animals (domestic and wild-life) as well as relatively poor hygienic practices  
247 [2,17,45–47]. We identified 15 *M. bovis* isolates among a total of 1755 MTBC isolated  
248 from pulmonary TB patients. Further molecular epidemiological analysis of these  
249 together with global collections of *M. bovis* and hMTBC showed (1) an association  
250 between close contact with livestock/animal carcasses and bTB infection in Ghana, (2)  
251 clustering of *M. bovis* of African origin close to the root of the global phylogeny and (3)  
252 the presence of *M. bovis*-specific amino acid mutations among both essential and non-  
253 essential core MTBC genes.

254 The finding of a significant association between bTB and close contact with animals ( $p <$   
255  $0.0001$ ) suggests zoonotic transmission and this calls for the implementation of  
256 preventive policies and strategies to reduce zoonotic transmission of TB among these  
257 high-risk groups [45]. This observation also calls for intensive education to create  
258 awareness of the disease about the risks of infection, the detection of infected  
259 animals/carcasses and prevention among farmers, butchers and the general population.  
260 Further emphasis should be placed on training butchers and animal handlers on the  
261 importance of adequate infection control measures, including the use of personal  
262 protective equipment (PPE) and the disposal of infected organs to avoid transmission of  
263 bTB among such personnel. An experienced butcher suffering from bTB in Australia  
264 gave an account of slaughtering many animals suspected of bTB and further cutting out  
265 the lungs for over 35 years without any proper precaution [48]. Also, some butchers in  
266 Nigeria, suffering from bTB, admitted eating visibly infected parts of the lung of cattle  
267 out of ignorance in order to convince customers to buy meat [49]. These instances  
268 highlight the importance of public education in the fight against bTB. This education  
269 should include veterinarians because there are instances of these professionals getting

270 infected with bTB due to a lack of precautionary measures during execution of their work  
271 as was the case of a veterinary surgeon who suffered cutaneous bTB after performing  
272 several examinations without proper PPE [50].

273 Our observation also confirms the importance of the test and slaughter (TS) control  
274 strategy for bTB. In addition to pasteurisation of dairy products, bTB has been controlled  
275 in developed countries due to the successful implementation of the TS policy of all  
276 infected cattle and compensation of affected farmers by governments [51]. However, this  
277 has not been implemented in SSA due to the costs involved. Nevertheless, our findings  
278 call for a reconsideration of the TS strategy for bTB control in SSA and Governments  
279 must respond to this call.

280 We found the proportion of *M. bovis* infected patients among participants from the  
281 Northern region (1.9%) of Ghana to be relatively higher (OR =2.7) compared to those  
282 from the Greater Accra region of Ghana (0.6%). The Northern region is home to over  
283 70% of the national cattle population [52], confirming the observation that there is a  
284 relationship between close animal contact and bTB. Even though we found no clear  
285 association between the *M. bovis* isolates and drug resistance and HIV infection, the  
286 proportions were relatively higher than among the hMTBC. However, the lack of  
287 association may be because of the relatively limited number of *M. bovis* isolates thus  
288 further investigation using a larger number of isolates is required.

289 The global phylogeny of *M. bovis* clusters most of the *M. bovis* of African origin at the  
290 root of the tree (Fig 1) which might be an indication that they are closest to the progenitor  
291 of this successful member of the MTBC with the widest host range. However, the limited  
292 number of genomes from Africa does not allow inference of ancestry. With the exception  
293 of the five clinical *M. bovis* from Ghana which clustered as a monophyletic branch at the  
294 base of the tree, the random distribution of *M. bovis* irrespective of the speciation of the  
295 host underscores the wide host range of *M. bovis* and indicates that there is no specific  
296 host adaptation. However, the geographical distribution may suggest transmission of  
297 specific clones within certain geographical locations which agrees with earlier reports  
298 [53–55].

299 The identification and implications of *M. bovis*-specific amino acid mutations among  
300 genes such as *mce1A*, *phoT* and *eccA1* [35], *pks6* [36,38] as well as *glgP* [34] highlights  
301 the potential attenuated virulence of *M. bovis* relative to the hMTBC [56]. It would be  
302 interesting to test the effects of these mutations on fitness of mutants using *ex vivo* human  
303 cell lines or *in vivo* bovine models. In addition to the potential phenotypic implications of  
304 the identified mutations among essential genes, the 8 *M. bovis*-specific mutations could

305 be utilized in developing either a rapid lateral flow diagnostic tool or a PCR-based tool  
306 specific for differential diagnosis of bTB among TB patients to advice an appropriate  
307 treatment regimen since *M. bovis* is innately resistant to pyrazinamide, a component of  
308 the DOTS regimen.

309

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

315

316

317 **References (to be completed after internal reviews)**

318

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538 **List of Figure Legends**

539

540

541 **Acknowledgements**

542

543 **Author contributions**

544

545 **Competing financial interests**

546 None declared

547

548 **Data availability.**

549 All the analyzed and/or generated data in this study are included in this article and its

550 supplementary information files.

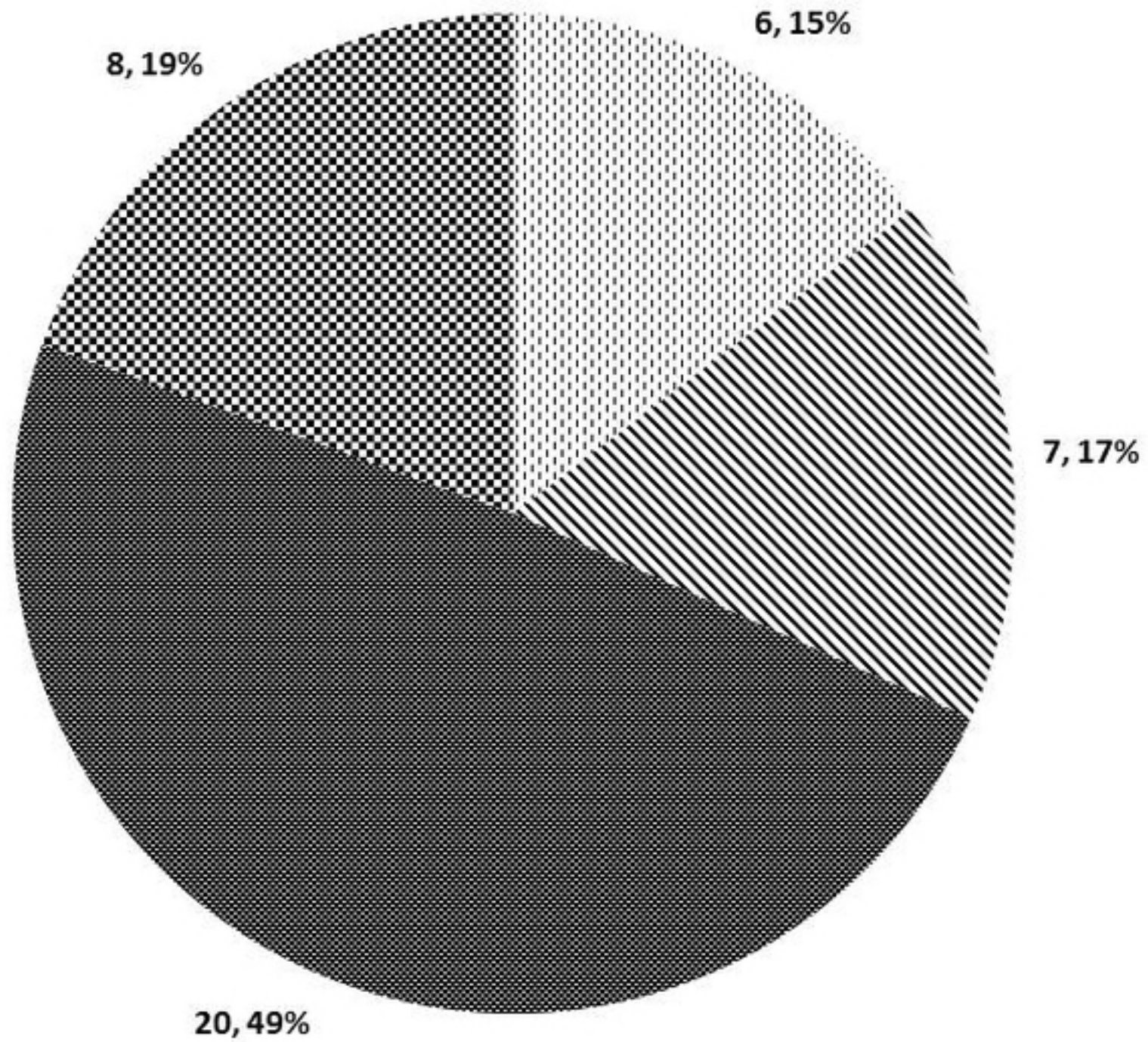


Figure 2



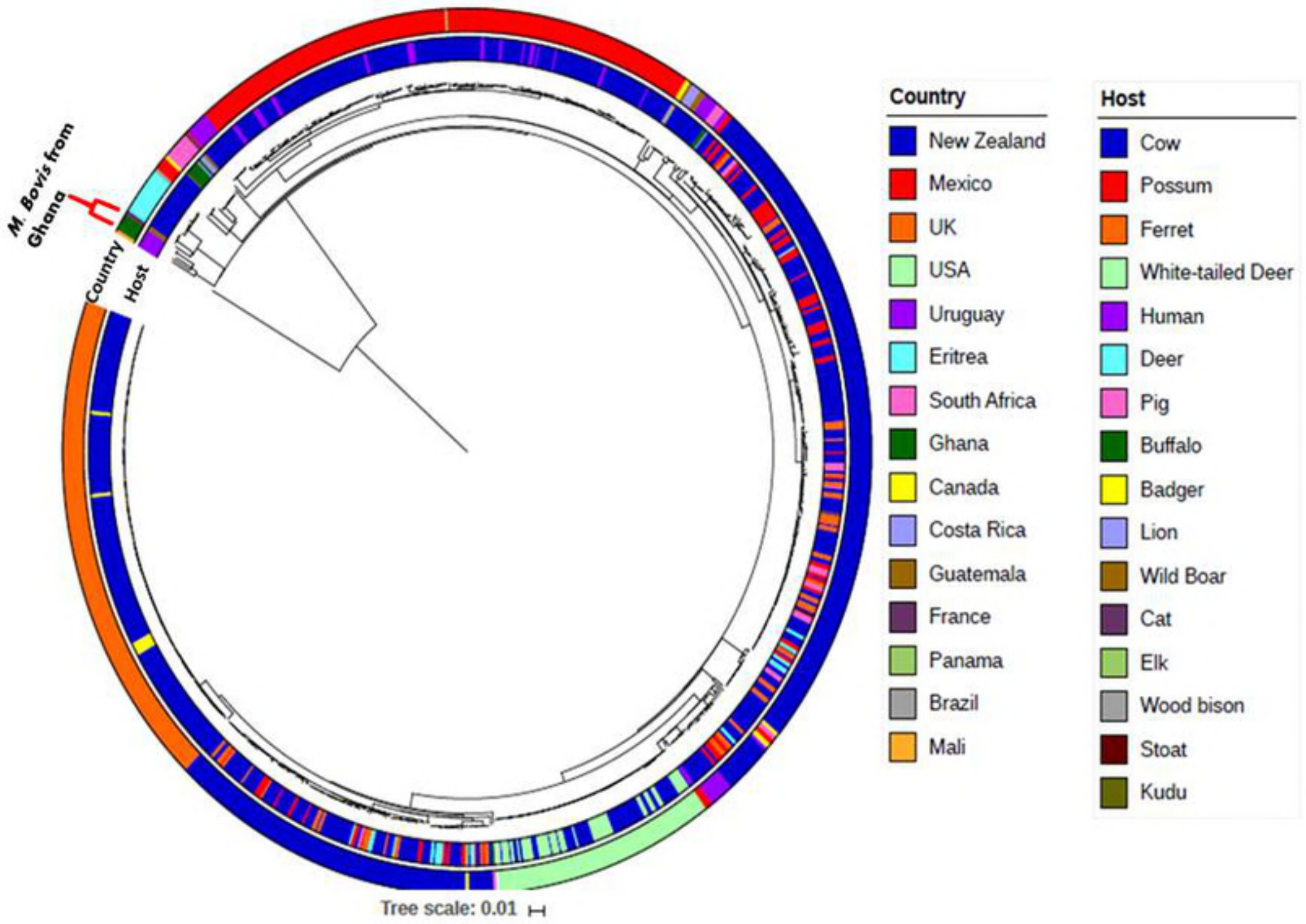


Figure 1