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Prevalence of bovine tuberculosis and characterization of the members of the *Mycobacterium tuberculosis* complex from slaughtered cattle in Rwanda

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22 & These authors also contributed equally to this work

23 Abstract

24 Background

25 Bovine tuberculosis (bTB) is an endemic disease in Rwanda, but little is known about its
26 prevalence and causative mycobacterial species. The disease causes tremendous losses in
27 livestock and wildlife and remains a significant threat to public health.

28 Materials and methods

29 A cross-sectional study employing a systematic random sampling of cattle (n=300) with the
30 collection of retropharyngeal lymph nodes and tonsils (n=300) irrespective of granulomatous
31 lesions was carried out in six abattoirs to investigate the prevalence and identify mycobacterial
32 species using culture, acid-fast bacteria staining, polymerase chain reaction, and GeneXpert
33 assay. Individual risk factors and the origin of samples were analysed for association with the
34 prevalence.

35 Findings

36 Of the 300 samples, six were collected with visible TB-like lesions. Our findings demonstrated the
37 presence of *Mycobacterium tuberculosis* complex (MTBC) in 1.7% (5/300) of sampled slaughtered
38 cattle. *M. bovis* was isolated from 1.3% (4/300) animals while one case was caused by a rifampicin-
39 resistance (RR) *M. tuberculosis*. Non-tuberculous mycobacteria were identified in 12.0% (36/300)

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40 of the sampled cattle. There were no significant associations between the prevalence and abattoir
41 category, age, sex, and breeds of slaughtered cattle.

42 **Conclusions**

43 This study is the first in Rwanda to isolate both *M. bovis* and RR *M. tuberculosis* in slaughtered
44 cattle indicating that bTB is prevalent in Rwanda with a low prevalence. The isolation of RR *M.*
45 *tuberculosis* from cattle indicates possible zooanthroponotic transmission of *M. tuberculosis* and
46 close human-cattle contact. To protect humans against occupational zoonotic diseases, it is
47 essential to control bTB in cattle and raise the awareness among all occupational groups as well
48 as reinforce biosafety at the farm level and in the abattoirs.

49 # Author's summary

50 Tuberculosis in cattle (bTB) causes financial losses to livestock owners and is a disease
51 transmissible to humans especially those with an occupational risk through exposure to infected
52 animals and animal products. This study aimed to identify the prevalence of bTB and characterize
53 the mycobacterial species from cattle slaughtered in the six abattoirs in Rwanda. Four *M. bovis*,
54 as well as one rifampicin-resistant (RR) *M. tuberculosis*, were identified from slaughtered cattle
55 and, thus, the apparent bTB prevalence was 1.7% (5/300). Likely, the RR *M. tuberculosis* isolate
56 was mostly likely of human origin and transmitted to cattle during close human-cattle contact. It
57 is therefore essential to control bTB in cattle and reinforce the protection of farmworkers and
58 abattoir workers who are always exposed to infected animals.

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59 # Introduction

60 Apart from *Mycobacterium leprae*, the genus *Mycobacterium* comprises two groups,
61 *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM) also
62 known as atypical mycobacteria or mycobacteria other than tuberculosis (MOTT) (1). The bovine
63 tuberculosis (bTB) is a mycobacterial disease of cattle, other domestic and wild animals, as well
64 as humans (2-5). The disease is characterized by granulomatous lesions in affected tissues (2, 4,
65 6). The disease is primarily caused by *Mycobacterium bovis* (7), and occasionally by *M. caprae* (8-
66 10). *Mycobacterium tuberculosis* infection in cattle has been identified more recently from African
67 countries and is of concern (4, 11-13). These species belong to the MTBC whose members share
68 99.9% of their genome (14).

69 In Rwanda, only two studies are available on bTB prevalence including one that reported
70 0.5% prevalence in slaughtered cattle at société des abattoirs de Nyabugogo (SABAN
71 Nyabugogo) (15). This study also found that the disease was associated with financial losses
72 related to the condemnation of carcasses which negatively affected the livelihood of small
73 Rwandan farmers (15). Another study was a retrospective recording TB-like macroscopic lesions
74 at the same abattoir from 2006 to 2010 and reported the prevalence ranging from 1.4% in Kigali
75 city to 11.8% in Eastern Province (16). In Rwanda, the veterinary services lack the capacity and
76 facility to isolate airborne pathogens. Thus, the control program for bTB relies mostly on monthly
77 reports of gross TB-like lesions from the main private abattoir, SABAN Nyabugogo.

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78 The cattle population in Rwanda was estimated at 1,293,768 in 2018 (17). Although
79 informal slaughtering of goats, sheep, chicken, and rabbits for family or small bar consumption
80 does occur in Rwanda, it is estimated that 95.0% of slaughtered cattle are processed by abattoirs.
81 Determining the bTB prevalence and identification of MTBC members is essential to understand
82 the transmission dynamics at the animal-human interface and to design adequate control
83 programs. The objective of this study was therefore to determine the prevalence of bTB and
84 characterize MTBC members in slaughtered cattle in Rwanda. The findings of this study will
85 contribute to building the bTB database essential for policymakers to establish informed control
86 policies and strategies to mitigate bTB in Rwanda.

87 Materials and methods

88 Study area

89 The present study was carried out in six abattoirs in Rwanda. Rwanda is a member of the
90 East African Community (EAC) located in the southern hemisphere, near the equator. Six
91 abattoirs that consented to participate in this study included high throughput abattoirs (n=4)
92 slaughtering more than 50 cattle daily, and low throughput abattoirs (n=2), slaughtering 50 or
93 less every day. Three of these abattoirs slaughtered cattle and goats and three specialised in cattle.
94 The location of the six abattoirs is shown in Fig 1.

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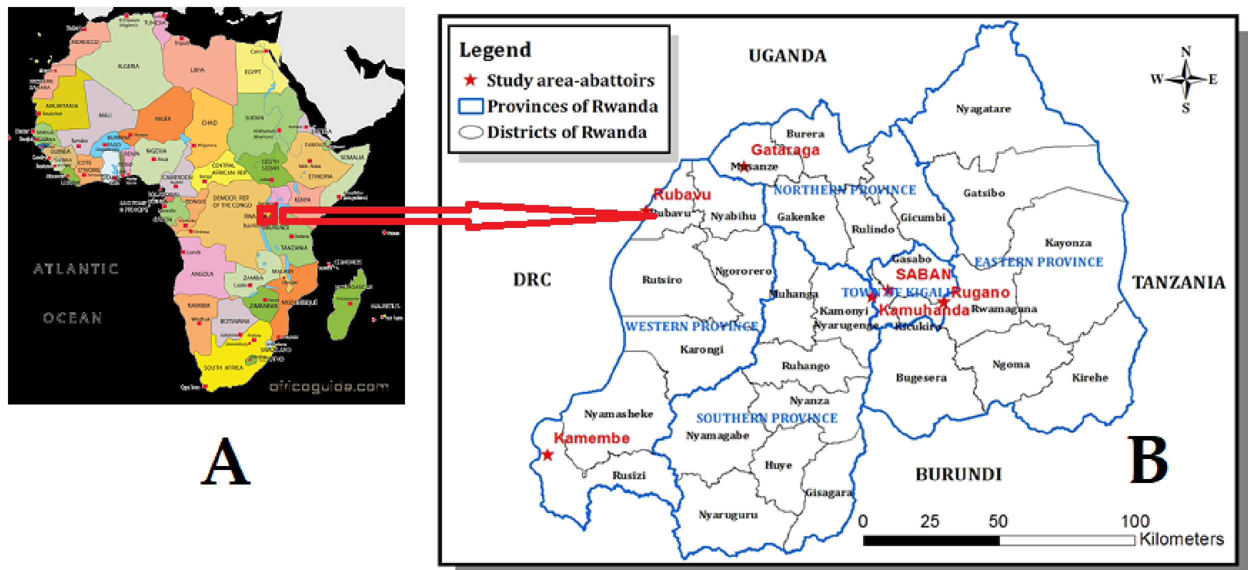

95

96 **Fig 1: Maps of (A) Africa with a red rectangle indicating the location of Rwanda (B) Rwanda**
97 **with provinces and districts and red stars show the locations of abattoirs visited in this study.**

98 Study design and sample size

99 A cross-sectional study was carried out from August 2018 through October 2019 to
100 determine the prevalence of bTB and characterize *Mycobacterium* spp. in cattle slaughtered at
101 abattoirs. The abattoirs that accepted to participate in the study were purposively selected based
102 on their strategic locations in the thirty districts of Rwanda (Fig 1) and their slaughtering capacity.
103 High throughput abattoirs received cattle from different districts. For instance, cattle that were
104 sampled at SABAN abattoir located in Kigali City were from 19 districts including Nyarugenge,
105 and Gasabo (Kigali City), Ngoma, Kirehe, Nyagatare, Gatsibo, Bugesera, and Kayonza (Eastern
106 Province), Gakenke, Burera, Rulindo, and Gicumbi (Northern Province), Rutsiro, Karongi,
107 Ngororero (Western Province), Ruhango, Nyanza, Kamonyi, and Muhanga (Southern Province).
108 Cattle that were sampled at Rugano abattoir located in Kigali City were from four districts

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109 including Gasabo, Kicukiro, Nyarugenge (Kigali City), and Rwamagana of Eastern Province.
110 Cattle that were sampled at Kamembe abattoir located in the Western Province, were from eight
111 districts including Gisagara, Huye, Nyaruguru, Ruhango, Nyanza, Nyamagabe (Southern
112 Province), Nyamasheke, and Rusizi (Western Province). Cattle that were sampled at Rubavu
113 abattoir located in the Western Province, were from two districts including Nyabihu, and Rubavu
114 (Western Province).

115 The sample size was calculated as previously described (18).

116
$$N = \frac{Z^2 \quad P(1-P)}{d^2}$$

117 Where N is the sample size, a 95% confidence level (z) equivalent to 1.96; P is an expected of 0.5%
118 based on a previous study in cattle in Rwanda (15), and the absolute precision (d = P/2) of 0.25%.
119 According to the formula, the total sample size was supposed to be 291 but it was rounded to 300
120 cattle to include and respect the systematic sampling of 10 cattle per each of the 30 districts of
121 Rwanda.

122 Sampling procedure and tissue collection

123 A systematic random sampling procedure among dairy cattle was adopted, and the
124 strategy was to sample five animals from the same district per day. The movement permit was
125 collected on arrival to record the origin of the animals. The age was determined using dentition
126 as previously described (19). Animals of two years and above were selected with a preference on
127 dairy except for some low throughput abattoirs that slaughtered mostly young male cattle.

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128 Animals were aligned in a crush pen and every fourth animal was selected, individual
129 demographic information (district of origin, age, breed, and sex) recorded, restrained, marked on
130 the head with original paint, and released for resting waiting for slaughter. Upon decapitation,
131 the marked head was inspected, and medial and lateral retropharyngeal lymph nodes and lingual
132 and palatine tonsils were aseptically collected into the same sterile 50 ml tube and stored at - 20°C
133 until processing.

134 Culture and detection of acid-fast bacilli (AFB)

135 Retropharyngeal lymph nodes and tonsils from each sampled animal (n=300) were
136 decontaminated as previously described (20). Briefly, tissues were thawed, sliced, grounded in a
137 sterile mortar with sea sand (Glentham Life Sciences, UK). An aliquot of each tissue sample was
138 kept at -20°C. Another aliquot was divided into two 50 ml falcon tubes. The content of one tube
139 was decontaminated by adding an equal volume of 2% hydrochloric acid and the other one with
140 an equal volume of 4% sodium hydroxide for 10 min at room temperature, then centrifuged at
141 3500 rpm for 10 min. The pellet was neutralized with 25 ml of sterile water centrifuged at 3500
142 rpm for 10 min (20). Löwenstein-Jensen (LJ) medium was prepared according to the
143 manufacturer's guidelines (Becton Dinkson, Franklin lakes, USA). Briefly, 37.3 g of LJ medium
144 were completely dissolved in 600 ml of distilled water containing 12 ml of glycerol by heating.
145 The dissolved LJ solution was then autoclaved at 121°C for 15 min. The homogenised sterile egg
146 content (1000 ml) was added to the cooled LJ solution, then, 7 ml of the mixture was distributed
147 into 14 ml – tubes (slopes) which were immediately inspissated at 85°C for 45 min. The slopes
148 were checked for sterility and stored in the fridge until use. Glycerol was replaced by 4g of

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149 sodium pyruvate (Labkem, Spain) for the preparation of LJ with pyruvate. The pellet that was
150 decontaminated by hydrochloric acid and sodium hydroxide were each inoculated onto duplicate
151 slopes of LJ with glycerol and duplicate slopes of LJ with sodium pyruvate, and then incubated
152 at 37°C for 10 weeks with weekly readings. Cultures were scored positive, negative, or
153 contaminated. When contamination occurred, the original sample was reprocessed and
154 reinoculated. Any suspected growth was tested for morphology using auramine O staining and
155 fluorescence microscope as previously described (21). All manipulations of samples including
156 processing, inoculation, and DNA extraction were performed in the biosafety level three at
157 National Reference Laboratory (NRL), Kigali, Rwanda.

158 Molecular Assays

159 DNA extraction

160 Lysate DNA was extracted from each AFB culture isolate as previously described (22).
161 Briefly, two loopful bacterial cells were suspended in 300 μl of distilled sterile water, then boiled
162 at 95°C for 25 min, quickly cooled, and stored at - 20°C until required. Genomic DNA was also
163 extracted from inactivated grown cultures using a DNA extraction kit according to the
164 manufacturer's instructions (Promega, USA).

165 Conventional PCR

166 Isolates that were confirmed AFB were screened for the presence of 16S rRNA sequence
167 specific for the genus *Mycobacterium* and a sequence encoding the MPB 70 antigen which is

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168 specific for members of MTBC using specific primers (Mycgen–F and Mycgen-R, TB1-F and TB2-
169 F, respectively) as previously described (23). PCR assay based on genomic deletions
170 differentiated members of MTBC (refer to as MTBC differential PCR assay) using primers
171 targeting the regions of difference, RD1, RD4, RD9, and RD12 as previously described (Table 1)
172 (Warren et al. 2006). *Mycobacterium tuberculosis* 25177 was used as a reference. For all multiplex
173 PCRs, the 15 µl PCR reaction mixture contained 1x MyTaq™ Red PCR Mix (Bioline, South Africa),
174 0.2 µM primers, and 2 µl of template DNA. The PCR cycling condition was as follows: initial
175 denaturation at 94°C for 5 min followed by 25 cycles of denaturation at 94°C for 30 sec, annealing
176 at 62°C for 1 min, and extension at 72°C for 1 min and a final extension step at 72°C for 8 min.
177 Primers amplified 1030 bp, 372 bp, 108 bp, and 268 bp fragments for the genus, MTBC, and *M.*
178 *bovis,* respectively, and were analysed by electrophoresis using a 2% agarose gel stained with red
179 gel nucleic acid stain and visualized under UV light. The PCR experiments were performed at
180 Rwanda Agriculture and Animal Resources Board, Department of Veterinary Services in the
181 Virology and Molecular Biology sections.

182 GeneXpert/MTB/RIF assay

183 MTBC isolates characterized by conventional PCR were also tested by
184 GeneXpert/MTB/RIF molecular diagnostic assay following the manufacturer's instructions
185 (Cepheid, Sunnyvale, USA). GeneXpert/MTB/RIF is a real-time PCR for the detection of MTBC
186 and rifampin resistance. Briefly, 0.5 ml of the cell suspension was transferred into a conical-
187 screwed tube and 1 ml of sample reagent was added. The mixture was vortexed for 10 sec and
188 incubated for 15 min with vortexing for 10 sec after 8 min of incubation. The liquefied sample

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189 was then dispensed into the sample chamber of the cartridge containing five probes (A-E),
190 integrated reagents tubes, a sample processing control, and a probe check control. Cartridges
191 were then installed into the GeneXpert^R Dx system version 4.8 (Cepheid, Sunnyvale, USA) and
192 the amplification was run for two hours by activating the software installed in the computer. This
193 assay was performed at NRL, Kigali, Rwanda.

194 Table 1. Oligonucleotides used to identify *Mycobacterium* species isolated from slaughtered cattle
195 in Rwanda

PCRs	**Primer name**	**Nucleotide sequence (5′-----------3′)**	**Target**	**Size (bp)**	**Tm (°C)**	**References**
Multi plex 1	MYCGEN-F	AGA GTT TGA TCC TGG CTC AG	16s rRNA	1030	62	(23)
	MYCGEN-R	TGC ACA CAG GCC ACA AGG GA				
	TB1-F	GAA CAA TCC GGA GTT GAC AA	MPB 70	372		
	TB2-R	AGC ACG CTG TCA ATC ATG TA				
Multi plex 2*	RD1-1	AAGCGGTTGCCGCCGACCGACC		146	62	(24)
	RD1-2	CTGGCTATATTCCTGGGCCCGG				
	RD1-3	GAGGCGATCTGGCGGTTTGGGG				
	RD4-1	ATG TGC GAG CTG AGC GAT G	Rv 1510	268 RD is absent	62	(25)
	RD4-2	TGT ACT ATG CTG ACC CAT GCG				
	RD4-3	AAA GGA GCA CCA TCG TCC AC				
	RD9-1	CAA GTT GCC GTT TCG AGC C	Rv 2073	108 RD is absent	62	
	RD9-2	CAA TGT TTG TTG CGC TGC				(24)
	RD9-3	GCT ACC CTC GAC CAA GTG TT				
	RD12-1	GGGAGCCCAGCATTTACCTC		306		(25)
	RD12-2	GTGTTGCGGGAATTACTCGG				
	RD12-3	AGCAGGAGCGGTTGGATATTC				

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196 MPB 70 stands for protein from *M. bovis* with 0.70 mobility by native polyacrylamide gel
197 electrophoresis at pH 9.4 gel but it is an antigen common to all MTBC, *MTBC (*Mycobacterium*
198 *tuberculosis* complex) differential PCR assay and Rv refers to rough morphology and virulent
199 MTBC strain.

200 Data analysis

201 Data were recorded in the Microsoft Excel spreadsheet and descriptive and inferential
202 statistics were performed using EpiInfo software version 7.2. The significance level of 95% and
203 p-value less or equal to 5% were considered for all analyses. The prevalence of isolation of
204 *Mycobacterium* and MTBC was tested for association with individual animal characteristics such
205 as age, sex, breed, and sampled abattoir in the univariate logistic analysis using Chi-square or
206 Fischer exact.

207 Ethical clearance

208 The authorization to conduct the study was obtained from the research screening and
209 ethical clearance committee of the College of Agriculture, Animal Sciences and Veterinary
210 Medicine, University of Rwanda (Ref:026/DRIPGS/2017), institutional review board of the
211 College of Medicine and Health Sciences, University of Rwanda (N° 006/CMHS IRB/2020), and
212 Animal Ethics Committee of the Faculty of Veterinary Science, University of Pretoria, South
213 Africa (V004/2020). Informed verbal consents were obtained from district officials, managers of
214 abattoirs, and owners of animals at the abattoirs.

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215 # Results

216 Out of the 300 sample sets collected from 300 cattle, 94.0% (282/300) were collected from
217 the high throughput abattoirs while 6.0% (18/300) were from the low throughput abattoirs. Of the
218 300 samples, 95.3% (286, 95% CI: 92.3 – 97.4) were collected from female cattle while 4.7% (14,
219 95%CI: 2.6 -7.7) were from male cattle. The majority of 90.3% (271/300, 95% CI: 86.4 – 93.4) were
220 from adult (3 years and above) cattle while 9.7% (29/300, 95% CI: 6.6 - 13.6) were collected from
221 young animals. Most samples, 67.9% (203/300, 95% CI: 62.3 – 73.2) were collected from
222 crossbreeds, 25.8% (77/300, 95%CI: 20.9 – 31.1) were collected from local breed "Ankole", while
223 6.7% (20/300, 95%CI: 3.8 – 9.5) were collected from a pure breed "Friesian".

224 Of the 300 samples, six were collected with visible TB-like lesions including three samples
225 with enlargement of all lymph nodes and one sample with granulomatous lesions in lungs. Other
226 two samples had a mucoid pus in the popliteal lymph nodes. Of the 300 sample sets that were
227 inoculated, 55.0% (165, 95% CI: 49.2 – 60.7) had bacterial growth of which 30.9% (51/165, 95% CI:
228 23.9 – 40.0) were AFB as indicated by the auramine fluorescence staining method. Of the 51 AFB,
229 the PCR identified 80.4% (41/51, 95% CI: 69.5 – 91.3) as *Mycobacterium* spp. (amplification of 1030
230 bp fragment, Table 1). Of the 41 *Mycobacterium* spp., 87.8% (36/41, 95%CI: 77.8-97.8) were NTM,
231 while the remaining 12.2% (5/41, 95% CI: 2.2 – 22.2) were MTBC (amplification of a 372 bp
232 fragment, Table 1). MTBC differential PCR assay identified 80.0% (4/5, 95% CI: 44.9 – 100.0) as *M.*
233 *bovis* (amplification of 108 bp, 146 bp, and 268 bp) and 20.0% (1/5, 95% CI: 0.0 – 55.1) as *M.*
234 *tuberculosis* (amplification of 146 bp, 172 bp, 235 bp, 369 bp) (Fig 2). Overall, NTM were identified
235 in 12.0% (36/300, 95%CI: 8.3-15.7) of the sampled cattle, MTBC were isolated in 1.7% (5/300) of the

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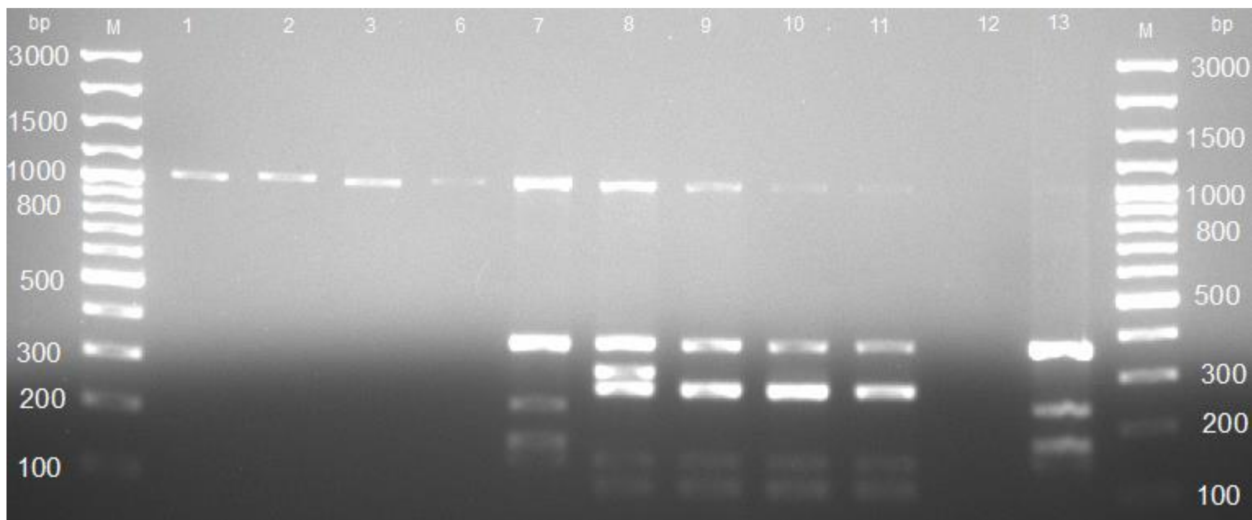
236 sampled cattle and among these, four were *M. bovis*, while one was *M. tuberculosis*. The GeneXpert
237 MTBC/RIF assay confirmed MTBC isolates. Resistance to rifampicin (RR) was detected in one
238 isolate, but such RR was not confirmed by GeneXpert testing of the original sample due to very
239 low bacterial load.

240 Among the six samples that were collected with visible TB-like lesions, only one was
241 confirmed as *M. bovis*, another one was NTM, while the remaining four were not identified as
242 *Mycobacterium* spp.



243

244 **Fig 2. Agarose gel electrophoresis of the *Mycobacterium tuberculosis* complex differential PCR**
245 **assay. Lane M: GeneRuler 100 bp (Invitrogen, ThermoFischer Scientific, South Africa), lanes**
246 **2-4: Non-tuberculosis mycobacteria (NTM) which amplified 1030 bp; lane 7: *M. tuberculosis***
247 **which amplified 1030 bp, 372 bp, 235 bp, 172 bp, and 146 bp; lanes 8 – 10: *M. bovis* which**
248 **amplified 1030 bp, 372 bp, 268 bp, 146 bp, and 108 bp; lane 11: negative control; lane 12: *M.***
249 ***tuberculosis* reference strain 2517.**

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250 *Mycobacterium* spp. isolates were found in 83.3% (5/6) of the abattoirs; 80.9% (38/47) in
251 high throughput abattoirs and 75.0 (3/4) in low throughput abattoirs, although the difference was
252 not significant (Table 2). All the MTBC isolates were found in the high throughput abattoirs.
253 MTBC were isolated in cattle from Nyarugenge district (n=1) of Kigali city, Karongi (n=1),
254 Nyabihu (n=1), and Rubavu (n=2) districts of the Western Province. All five MTBC isolates were
255 identified from adult and crossbred cattle (Table 2).

Table 2. Mycobacterial culture results for 300 slaughtered cattle and PCR results of AFB isolates stratified by the abattoir, age, breed, and sex of slaughtered cattle in Rwanda

Variables	Categories	Mycobacterial culture and AFB results		PCR results of AFB isolates for detection of *Mycobacterium* spp.				PCR results of AFB isolates for detection of *Mycobacterium tuberculosis* complex (MTBC)			
		Growth % (n)	**AFB positive**	**Positive % (n)**	**95% CI**	**Chi ²**	***p*-value**	**Positive n (%)**	**95% CI**	**Chi ²**	***p*-value**
Abattoirs	High throughput	53.9 (152/282)	16.7 (47/282)	80.9 (38/47)	0.3 - 4.8	0.80	1.0	10.6 (5/47)	0.0 - 16.3	0.00	1.00
	Low throughput	72.2 (13/18)	22.2 (4/18)	75.0 (3/4)				0.0 (0/4)			
	Total	**55.0 (165/300)**	**17.0 (51/300)**	**80.4 (41/51)**				**9.8 (5/51)**			
Provinces	East	55.7 (39/70)	17.1 (12/70)	83.3 (10/12)	-	1.60	0.9	0.0 (0/12)	-	10.20	0.04
	West	62.7 (44/70)	25.7 (18/70)	72.2 (13/18)				22.2 (4/18)			
	North	56.0 (28/50)	16.0 (8/50)	87.5 (7/8)				0.0 (0/8)			
	South	45.0 (36/80)	13.8 (11/80)	81.8 (9/11)				0.0 (0/11)			
	Kigali city	60.0 (18/30)	6.7 (2/30)	100.0 (2/2)				50.0 (1/2)			
	Total	**55.0 (165/300)**	**17.0 (51/300)**	**80.4 (41/51)**				**9.8 (5/51)**			
Age	Young	65.5 (19/29)	20.7 (6/29)	50.0 (3/6)	0.2-1.7	2.10	0.1	0.0 (0/6)	0.0–9.3	0.02	1.00
	Adult	53.8 (146/271)	16.6 (45/271)	84.4 (38/45)				11.1 (5/45)			
	Total	**55.0 (165/300)**	**17.0 (51/300)**	**80.4 (41/51)**				**9.8 (5/51)**			
Sex	Female	55.6 (159/286)	16.8 (48/286)	79.2 (38/48)	-	0.01	1.0	8.3 (4/48)	0.4 - 74.8	0.20	0.30
	Male	42.9 (6/14)	21.4 (3/14)	100.0 (3/3)				33.3 (1/3)			
	Total	**55.0 (165/300)**	**17.0 (51/300)**	**80.4 (41/51)**				**9.8 (5/51)**			
Breeds	Ankole	54.5 (42/47)	13.0 (10/77)	80.0 (8/10)	-	0.80	1.0	0.0 (0/10)	-	1.90	0.70
	Crossbred	56.2 (114/208)	18.7 (38/203)	79.0 (30/38)				13.2 (5/38)			
	Friesians	42.1 (8/19)	15.0 (3/20)	100.0 (3/3)				0.0 (0/3)			
	Total	**55.0 (165/300)**	**17.0 (51/300)**	**80.4 (41/51)**				**9.8 (5/51)**			

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242 # Discussion

243 Bovine tuberculosis (bTB) causes financial losses in livestock and remains a significant
244 threat to public health worldwide (26, 27). This study aimed to determine the prevalence of bTB
245 and identify *Mycobacterium* species in slaughtered cattle using bacterial culture and PCR assay.
246 This study identified for the first time in Rwanda *M. bovis* (n=4) and *M. tuberculosis* (n=1), as well
247 as non-tuberculous mycobacteria (n=36) in a total of 300 cattle from 30 districts slaughtered in six
248 abattoirs. Although the prevalence of bTB (1.7%, 5/300) was low, the identification of *M. bovis* and
249 *M. tuberculosis* with rifampicin resistance in cattle indicates the cross-transmission from humans
250 to cattle but also highlights the great risk of exposure of handlers of live infected cattle and animal
251 carcasses.

252 Considering the moderate sample size (n=300) and the random selection of animals found
253 without visible lesions (96.7%), the prevalence observed for MTBC in this study (1.7%) is higher
254 than 0.5% reported at SABAN Nyabugogo abattoir, Rwanda (15) given the small sample size
255 (n=36) selected on gross bTB-like lesions. However, considering that samples were collected at a
256 single point in time and the small sample size (n=10) per district, the prevalence in this study is
257 likely to be lower than the true prevalence in slaughtered cattle in Rwanda. The prevalence (1.7%)
258 obtained in this study is consistent with 2.1% obtained in Kenya using bovine TB-like lesions,
259 AFB staining, bacterial culture, and PCR (28) but lower compared to 7.6% obtained in Uganda
260 using bovine TB-like lesions, bacterial culture, AFB staining, and capillia TB-neo assay for
261 detection of MPT 64 antigens of the MTBC (29). The prevalence obtained in this study also falls
262 in the range of bTB herd prevalence (0.2 to 13.2%) reported in cattle in Tanzania (30). These

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263 findings confirm that bTB is prevalent in cattle of the East African region and the disease remains
264 a transboundary disease through animal trading and animal movement across porous borders
265 (31).

266 This study demonstrated that bTB is most probably endemic in Rwandan cattle but at low
267 prevalence since samples were collected from cattle slaughtered in the six abattoirs of which four
268 high throughput abattoirs received cattle from several districts including those from more than
269 90 km. As mentioned in materials and method section, only ten samples were collected from each
270 of the 30 districts but the animals at the sampled abattoir originated from different districts. Three
271 animals that were positive to MTBC were detected in cattle from Rubavu (n=2) and Nyabihu (n=1)
272 districts which supply cattle to the nearby Rubavu abattoir (less than 20 km) in the Western
273 Province. Of the other two cattle that were positive to MTBC, one was from the Ngororero district
274 of the Western Province and another one from Nyarugenge district of Kigali city, and both were
275 found at SABAN abattoir located in Kigali city. We, therefore, recommend a longitudinal study
276 considering a good sample size per district.

277 This study identified for the first time *M. bovis* (1.3%, 4/300) and *M. tuberculosis* (0.3%,
278 1/300) in slaughtered cattle in Rwanda. Similar studies in Africa isolated *M. bovis* (2.0%, 19/929)
279 and *M. tuberculosis* (0.2%, 2/929) in slaughtered cattle in Kenya (28), and *M. bovis* (4.0%, 12/300)
280 and *M. tuberculosis* (0.7%, 2/300) in slaughtered cattle in Cameroon (32). However, the prevalence
281 of *M. tuberculosis* in cattle is commonly below 1.0% (28, 32, 33), apart from some areas (27.0%) in
282 Ethiopia where cattle owners had the habit of chewing tobacco into the mouth of their cattle (34).
283 The prevalence of *M. tuberculosis* is also high in areas with a high prevalence of TB in humans

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284 owning cattle (35). For example, a study in the Eastern Cape province, South Africa, identified
285 more *M. tuberculosis* (41.8%, 157/376) than *M. bovis* (1.3%, 5/376) from slaughtered cattle (36). *M.*
286 *tuberculosis* (n=1) that was isolated in this study was resistant to Rifampicin which is a cornerstone
287 antibiotic of the first-line regimen (37). Resistance to rifampicin (RR) is considered multidrug
288 resistance tuberculosis (MDR-TB) (38) and has been reported in Rwandans with TB (39)
289 suggesting that RR *M. tuberculosis* was of human origin. The transmission of RR *M. tuberculosis*
290 from humans to cattle was not surprising since 92.0% of Rwandans owning cattle are small dairy
291 farmers practicing a zero-grazing system (40) which may promote close animal-human contact
292 with the risk of cross-infection from humans to cattle and vice-versa (41). Cattle can be considered
293 sentinels for *M. tuberculosis* in settings where TB is not effectively controlled in humans. It is
294 therefore also an alert for improved TB control in humans in rural settings. Despite the little
295 attention given to the zoonotic TB caused by *M. bovis* (42), several studies have isolated *M. bovis*
296 in extrapulmonary lymph nodes of humans in neighbouring Uganda (43), and Tanzania (44). It
297 is hence essential to raise awareness among veterinary and human health professionals about the
298 zooanthroponotic and anthropozoonotic transmission of TB in Rwanda. Further studies on the
299 identification of *M. bovis* in occupational groups are worth investigating in Rwanda to provide
300 epidemiological data that are indispensable for the eradication of tuberculosis by 2035 (45).

301 This study identified NTM in 12.0% of slaughtered cattle, and this prevalence is consistent
302 with 8.4% obtained in Uganda (29) but higher than 3.9% in Tanzania (46). This study considered
303 the presence of NTM in the environment, hence, tissues were aseptically (changing gloves and
304 sterilization of the knife into hot water) collected, stored, and processed, thus, it can be assumed

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305 that the identified NTM were recovered from the tissues of animals, but it does not prove any
306 pathological effect, it merely demonstrates colonization. Since these NTM were not speciated, it
307 would be important to determine their potential significance for the health of the cattle. NTM
308 have been isolated in cattle and sometimes cause localized lymphadenitis, skin infections, TB-like
309 pulmonary infections, and systemic diseases in immunodeficient cattle (47). The presence of NTM
310 in cattle may interfere with immune-diagnostic methods such as comparative tuberculin test and
311 may negatively impact vaccination (48).

312 In this study, among the samples with tuberculosis-like lesions (n=6), only one popliteal
313 lymph node was associated with NTM species, and one lung was associated with *M. bovis.*
314 However, a retrospective study reported the prevalence of 11.8% based on TB-like lesions
315 recorded during routine meat inspection from 2006 to 2010 at SABAN Nyabugogo abattoir,
316 Rwanda (16). TB-like lesions might therefore be a poor reflection of bTB in the absence of a
317 confirmatory laboratory test. TB-like lesions from routine meat inspection should, therefore, be
318 confirmed by laboratory tests to obtain accurate results essential for surveillance of bTB, but also
319 improve the knowledge of inspectors.

320 *Mycobacterium* spp. isolates were more frequently isolated in adult (76.7%) than in young
321 cattle (40.0%) and all MTBC were isolated from adult cattle consistent with a study in Ethiopia
322 (49). The isolation of *M. bovis* depends a lot on the dose and frequency of exposure, therefore,
323 higher infection rates in adult cattle result from a cumulative risk of infection. In other words, the
324 older an animal the more opportunities it had to contract *M. bovis*. Furthermore, the literature

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325 states that young cattle are less susceptible to mycobacteria owing to the high concentration of T
326 cells in the blood circulation and T cells play a role in the immunity against mycobacteria (50).

327 # Conclusions

328 This study demonstrated that bTB is prevalent in Rwanda at low prevalence. The present
329 study reports for the first time MTBC in cattle in Rwanda and the presence of RR *M. tuberculosis*
330 indicating possible cross-infection between humans and cattle. There is therefore a need for
331 raising awareness among veterinary and human health professionals about the zooanthroponotic
332 transmission and cross infection of TB in Rwanda. Further studies on the identification of *M. bovis*
333 in humans are worth investigating to provide epidemiological data that are indispensable for the
334 eradication of tuberculosis by 2035, a global movement led by the World Health Organization.

335 # Author's contributions

336 Conceptualization, JBN, AM, and HvH; methodology, JBN, AM, and HvH; Formal analysis JBN;
337 Investigation and data collection, JBN; Writing—original draft preparation, JBN; writing—review
338 and editing, JBN, IEM, JCSN, FBK, AM and HvH; Supervision, HvH, AM, and FBK; Project
339 administration, HvH; Resources, IEM, JCSN, HvH; Funding acquisition, HvH. All authors have
340 read and approved the manuscript.

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349 # Conflict of interest

350 The authors declare that there are no conflicts of interest. The funders had no role in the design
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486 Supporting information

487 Raw data. Excel file