

**Genomic Polymorphism Associated with the Emergence of Virulent Isolates of
Mycobacterium bovis in the Nile Delta.**

**Hazem F M Abdelaal¹, Daniel Spalink², Ali Amer³, Howard Steinberg¹, Emad A Hashish⁴, Essam A
Nasr⁵, Adel M Talaat^{1*}**

¹Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA.

²Department of Ecosystem Science and Management, Texas A&M University, College Station, TX, USA.

³Animal Health Research Institute, Dokki, Giza, Egypt.

⁴Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

⁵Veterinary Serum and Vaccine Research Institute, Bacterial Diagnostics Research Department
(Tuberculosis), Abbasia, Cairo, Egypt.

Running Head: Molecular Diversity of *M. bovis* Isolates from Egypt

#Address correspondence to:

Adel M. Talaat, M.V.Sc., Ph.D.

Lab of Bacterial Genomics

Department of Pathobiological Sciences

School of Veterinary Medicine

University of Wisconsin-Madison

Robert P Hanson Biomedical Science Laboratories

1656 Linden Drive, Madison, WI 53706-1581

Tel: 608-262-2861

Fax: 608-262-7420

adel.talaat@wisc.edu

1 **Supplemental Table 1. List of 137 virulence related genes¹ predicted in the *Mycobacterium***
 2 ***bovis* reference genome (*M. bovis* AF2122/97) used for the calculation of *dN/dS* ratios in the**
 3 ***M. bovis* isolates from the Nile Delta.**

#	Gene Name	Rv number	Mb number
1	treS	Rv0126	Mb0131
2	ptbB	Rv0153c	Mb0158c
3	mce1r	Rv0165	Mb0170c
4	fadD5	Rv0166c	Mb0172c
5	sigG	Rv0182c	Mb0188c
6	zmp1	Rv0198c	Mb0204c
7	pckA	Rv0211	Mb0217
8	hsp	Rv0251c	Mb0257c
9	mosR	Rv0348	Mb0356
10	hspR	Rv0353	Mb0361
11	pknG	Rv0410c	Mb0418c
12	sodC	Rv0432	Mb0440
13	icl1	Rv0467	Mb0467
14	pcaA	Rv0470c	Mb0479c
15	hbhA	Rv0475	Mb0485
16	-	Rv0485	Mb0495
17	senX3	Rv0490	Mb0500
18	regX3	Rv0491	Mb0501
19	mce2r	Rv0586	Mb0601
20	mmaA4	Rv0642c	Mb0661c
21	mmaA3	Rv0643c	Mb0662c
22	mmaA2	Rv0644c	Mb0663c
23	mmaA1	Rv0645c	Mb0664c
24	sigL	Rv0735	Mb0756
25	phoP	Rv0757	Mb0780
26	phoR	Rv0758	Mb0781
27	phoT	Rv0820	Mb0843
28	phoY2	Rv0821c	Mb0844c
29	ompA	Rv0899	Mb0923
30	pstA1	Rv0930	Mb0953
31	pknDb	Rv0931c	Mb0954c
32	pstS-1	Rv0934	Mb0959
33	ctpV	Rv0969	Mb0994
34	mprA	Rv0981	Mb1007
35	mprB	Rv0982	Mb1008
36	pepD	Rv0983	Mb1009

37	hsp22.5	Rv0990c	Mb1017c
38	sigE	Rv1221	Mb1253
39	lpqY	Rv1235	Mb1267
40	fadD33	Rv1345	Mb1380
41	irtA	Rv1348	Mb1383
42	irtB	Rv1349	Mb1384
43		Rv1410c	Mb1445c
44	lprG	Rv1411c	Mb1446c
45	pks5	Rv1527c	Mb1554c
46	lspA	Rv1539	Mb1566
47	pks10	Rv1660	Mb1688
48	pks7	Rv1661	Mb1689
49	pknE	Rv1743	Mb1772
50	plcD	Rv1755c	Mb1784c
51	eccD5	Rv1795	Mb1823
52	mgtC	Rv1811	Mb1841
53	secA2	Rv1821	Mb1852
54	modA	Rv1857	Mb1888
55	katG	Rv1908c	Mb1943c
56	-	Rv1931c	Mb1966c
57	tpX	Rv1932	Mb1967
58	mel2	Rv1936	Mb1971
59	yrbE3A	Rv1964	Mb1999
60	-	Rv1979c	Mb2001c
61	mpt64	Rv1980c	Mb2002c
62	nrdF1	Rv1981c	Mb2003c
63	vapC36	Rv1982	Mb2004c
64	dosT	Rv2027c	Mb2052c
65	acr1 (hspX)	Rv2031c	Mb2057c
66	acg	Rv2032	Mb2058
67	pks12	Rv2048c	Mb2074c
68	sigC	Rv2069	Mb2095
69	pafA	Rv2097c	Mb2124c
70	mpa	Rv2115c	Mb2139c
71	-	Rv2136c	Mb2160c
72	ctaC	Rv2200c	Mb2223c
73	caeA	Rv2224c	Mb2248c
74	ptpA	Rv2234	Mb2258
75	kasB	Rv2246	Mb2270
76	mbtB	Rv2383c	Mb2404c

77	aprA	Rv2395a	Mb2417a
78	aprB	Rv2395b	Mb2417b
79	ahpC	Rv2428	Mb2454
80	ndk	Rv2445c	Mb2472c
81	sigA	Rv2703	Mb2722
82	ideR	Rv2711	Mb2730
83	clgR	Rv2745c	Mb2766c
84	-	Rv2869c	Mb2894c
85	rip	Rv2869c	Mb2894c
86	fadD26	Rv2930	Mb2955
87	drrC	Rv2938	Mb2963
88	fadD28	Rv2941	Mb2966
89	mmpL7	Rv2942	Mb2967
90	lppX	Rv2945c	Mb2970c
91	pksI	Rv2947c	Mb2971c
92	virS	Rv3082c	Mb3109c
93	devR	Rv3133c	Mb3157c
94	nuoG	Rv3151	Mb3175
95	sigH	Rv3223c	Mb3250c
96	kefB	Rv3236c	Mb3264c
97	ctpC	Rv3270	Mb3298
98	sigF	Rv3286c	Mb3314c
99	choD	Rv3409c	Mb3443c
100	sigD	Rv3414c	Mb3448c
101	whiB3	Rv3416	Mb3450
102	yrb4A	Rv3501c	Mb3531c
103	ltp2	Rv3540c	Mb3570c
104	-	Rv3541c	Mb3571c
105	-	Rv3542c	Mb3572c
106	fadE29	Rv3543c	Mb3573c
107	fadE28	Rv3544c	Mb3574c
108	cyp125	Rv3545c	Mb3575c
109	hsaC	Rv3568c	Mb3599c
110	espD	Rv3614c	Mb3644c
111	espC	Rv3615c	Mb3645c
112	-	Rv3654c	Mb3678c
113	-	Rv3655c	Mb3679c
114	dppA	Rv3666c	Mb3690c
115	-	Rv3671c	Mb3695c
116	ponA2	Rv3682	Mb3707

117	lpqH	Rv3763	Mb3789
118	fbpA	Rv3804c	Mb3834c
119	pirG	Rv3810	Mb3840
120	mmpL8	Rv3823c	Mb3853c
121	espR	Rv3849	Mb3879
122	espF	Rv3865	Mb3895
123	espH	Rv3867	Mb3897
124	eccA1	Rv3868	Mb3898
125	eccB1	Rv3869	Mb3899
126	eccCA1	Rv3870	Mb3900
127	eccCB1	Rv3871	Mb3901
128	PE35	Rv3872	Mb3902
129	PPE68	Rv3873	Mb3903
130	esxB	Rv3874	Mb3904
131	esxB	Rv3874	Mb3904
132	esxA	Rv3875	Mb3905
133	esxA	Rv3875	Mb3905
134	eccD1	Rv3877	Mb3907
135	eccD1	Rv3877	Mb3907
136	mycP1	Rv3883c	Mb3913c
137	mycP1	Rv3883c	Mb3913c

5 **Supplemental Table 2. List of 67 *M. bovis* genomes retrieved from the NCBI public**
6 **database used in the comparative genomic analysis of *M. bovis* isolates from the Nile Delta.**

Used acronym	Accession number	Species	Strain	Contigs	%GC	Isolation date	Country	Host
M.Africanum-Gm041182	NC_015758.1	<i>Mycobacterium tuberculosis</i> var. africanum	GM041182	1	65.60	1968	West Africa	<i>Homo sapiens</i>
M.Canettii-Cipt-140010059	NC_015848.1	<i>Mycobacterium canettii</i>	CIPT-140010059	1	65.40	1997	Somalia	<i>Homo sapiens</i>
M.Caprae_Allgaeu	NZ_CP016401.1	<i>Mycobacterium tuberculosis</i> var. caprae	Allgaeu	1	65.60	1999	Spain	<i>Sheep, Ovis sp.</i>
M.Tb-Haarlem_V2	NC_022350.1	<i>Mycobacterium tuberculosis</i> var. tuberculosis	Haarlem	1	65.60	2005	USA	<i>Homo sapiens</i>
M.Tb-CDC1551	NC_002755.2	<i>Mycobacterium tuberculosis</i> var. tuberculosis	CDC1551	1	65.60	2002	USA	<i>Homo sapiens</i>
M.Tb-H37ra	NC_009525.1	<i>Mycobacterium tuberculosis</i> var. tuberculosis	H37Ra	1	65.60	1934	USA	<i>Homo sapiens</i>
M.Tb-H37rv	NC_000962.3	<i>Mycobacterium tuberculosis</i> var. tuberculosis	H37Rv	1	65.60	1934	USA	<i>Homo sapiens</i>
Mb-04-303-Argentina.La.Pampa-2004-Wild.Boar	NZ_AVSW0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	04-303	169	65.52	2004	Argentina	<i>Pig, Sus scrofa</i>
Mb-05-566-Argentina.Buenos.Aires-2000-Cattle	NZ_JPFR0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	05-566	89	65.50	2000	Argentina	<i>Bovine, Bovinae</i>
Mb-05-567-Argentina.Buenos.Aires-2000-Cattle	NZ_JPFQ0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	05-567	69	65.57	2000	Argentina	<i>Bovine, Bovinae</i>
Mb-08-08bf2-Brazil.Sao.Paulo.Boituva-2008-Cattle	NZ_JQET0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	08-08BF2	108	65.52	2008	Brazil	<i>Bovine, Bovinae</i>
Mb-0822-11-Brazil.Sao.Paulo.Porangaba-2012-Cattle	NZ_JQEW0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	0822-11	85	65.54	2012	Brazil	<i>Bovine, Bovinae</i>
Mb-09-1191-Argentina.Buenos.Aires.Balcarce-2009-Cattle	NZ_JPFP0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	09-1191	86	65.57	2009	Argentina	<i>Bovine, Bovinae</i>
Mb-09-1193-Argentina.Buenos.Aires.Trenque.Lauquen-2009-Cattle	NZ_JQEN0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	09-1193	89	65.50	2009	Argentina	<i>Bovine, Bovinae</i>
Mb-1595-S.Korea-2015-Cattle	NZ_CP012095.1	<i>Mycobacterium tuberculosis</i> var. bovis	1595	1	65.64	2012	Korea	<i>Cattle, Bos sp.</i>
Mb-18-08c-Brazil.Paran.Palmeiras-2008-Cattle	NZ_JQEQ0000000.1	<i>Mycobacterium tuberculosis</i> var. bovis	18-08C	63	65.58	2008	Brazil	<i>Bovine, Bovinae</i>

Mb-32-08- Brazil.Minas.Gerais.Uberlandia-2008- Cattle	NZ_JQER000 00000.1	Mycobacterium tuberculosis var. bovis	32-08	83	65.56	2008	Brazil	Bovine, Bovinae
Mb-35- Brazil.Mato.Grosso.Do.Sul.Dois.Irmaos.D o.Buriti-2003-Cattle	NZ_JQEV000 00000.1	Mycobacterium tuberculosis var. bovis	35	94	65.60	2003	Brazil	Bovine, Bovinae
Mb-45-08b-Brazil.Minas.Gerais.Arax- 2008-Cattle	NZ_JQEP000 00000.1	Mycobacterium tuberculosis var. bovis	45-08B	72	65.55	2008	Brazil	Bovine, Bovinae
Mb-49-09- Brazil.Sao.Paulo.Presidente.Venceslau- 2009-Cattle	NZ_JQES000 00000.1	Mycobacterium tuberculosis var. bovis	49-09	61	65.59	2009	Brazil	Bovine, Bovinae
Mb-50-Brazil.Acre.Tarauac-Sep2014- Cattle	NZ_JQEU000 00000.1	Mycobacterium tuberculosis var. bovis	50	84	65.55	2009	Brazil	Bovine, Bovinae
Mb-534-Argentina.Santa.Fe.Las.Colonias- 1994-Cattle	NZ_JQEM000 00000.1	Mycobacterium tuberculosis var. bovis	534	72	65.56	1994	Argentina	Bovine, Bovinae
Mb-61-09- Brazil.Sao.Paulo.Novo.Castilho-2009- Sheep	NZ_JQEX000 00000.1	Mycobacterium tuberculosis var. bovis	61-09	68	65.59	2009	Brazil	Sheep, <i>Ovis</i> sp.
Mb-Af2122.97	NC_002945.3	Mycobacterium tuberculosis var. bovis	AF2122/97	1	65.60	1997	UK	Bovine, Bovinae
Mb-An5-Brazil-2014-Cattle	NZ_AWPL00 000000.1	Mycobacterium tuberculosis var. bovis	AN5	70	65.60	1948	UK	Cattle, <i>Bos</i> sp.
Mb-Atcc-Baa-935-Usa-Sep2014	NZ_CP00944 9.1	Mycobacterium tuberculosis var. bovis	BAA-935	1	65.61	1997	UK	Cattle, <i>Bos primigenius</i>
Mb-B-3222-S.Korea.Hongseong-2013- Cattle	NZ_LNOF000 00000.1	Mycobacterium tuberculosis var. bovis	B-3222	56	65.60	2018	S.Korea	Cattle, <i>Bos taurus</i>
Mb-B2.7505-Uganda-Oct2006-Human	NZ_JKAL000 00000.1	Mycobacterium tuberculosis var. tuberculosis	B2.7505	243	65.50	2006	Uganda	Human, <i>Homo sapiens</i>
Mb-Bcg-Atcc-35743-Beijing-2011	NZ_CP00349 4.1	Mycobacterium tuberculosis var. bovis	BCG- 63839	1	65.70	2011	Beijing	Elk, <i>Cervus canadensis</i>
Mb-Bcg-Mexico1931-2010	NC_016804.1	Mycobacterium tuberculosis var. bovis - BCG	BCG- Mexico193 1	1	65.70	2010	Mexico	Cattle, <i>Bos</i> sp.
Mb-Bcg-Moreau-Rdj-2016	NZ_AM41205 9.1	Mycobacterium tuberculosis var. bovis - BCG	BCG- Moreau- RDJ	1	65.70	2016	Brazil	Cattle, <i>Bos taurus</i>
Mb-Bcg-Pasteur1173p2-2007	NC_008769.1	Mycobacterium tuberculosis var. bovis - BCG	BCG- Pasteur117 3P2	1	65.70	2007	France	Deer, <i>Odocoileus virginianus</i>
Mb-BCG-Russia-2015	NZ_CP01374 1.1	Mycobacterium tuberculosis var. bovis - BCG	BCG- Russia	1	65.70	2015	Russia	Deer, <i>Odocoileus virginianus</i>
Mb-Bcg-Tokyo172-2008	NZ_CP01456 6.1	Mycobacterium tuberculosis var. bovis - BCG	BCG- Tokyo172	1	65.70	2008	Tokyo	Deer, <i>Odocoileus virginianus</i>

Mb-Bcg3281-China-2015	NZ_CP00874 4.1	Mycobacterium tuberculosis var. bovis - BCG	BCG3281	1	65.70	2015	China	<i>Cattle, Bos taurus</i>
Mb-Bcg368-Russia-2014	NZ_CP00924 3.1	Mycobacterium tuberculosis var. bovis - BCG	BCG368	1	65.70	2014	Russia	<i>Cattle, Bos taurus</i>
Mb-Bz.31150-Uganda-Jun2007-Human	NZ_JKAM00 000000.1	Mycobacterium tuberculosis var. bovis	Bz.31150- Uganda	141	65.50	2007	Uganda	<i>Human, Homo sapiens</i>
Mb-MB1-Spain-2015-Cattle	NZ_CDHF00 000000.1	Mycobacterium tuberculosis var. bovis	MB1	47	65.50	2015	Spain	<i>Cattle, Bos taurus</i>
Mb-Mb3-Spain-2015-Wild.Boar	NZ_CDHH00 000000.1	Mycobacterium tuberculosis var. bovis	MB3	88	65.50	2015	Spain	<i>Pig, Sus scrofa</i>
Mb-Mb4-Spain-2015-Wild.Boar	NZ_CDHE00 000000.1	Mycobacterium tuberculosis var. bovis	MB4	109	65.50	2015	Spain	<i>Pig, Sus scrofa</i>
Mb-Mburu-001-Uruguay.San.Jose-2014- Cattle	NZ_LFGY000 00000.1	Mycobacterium tuberculosis var. bovis	MbURU- 001	100	65.50	2014	Uruguay	<i>Cattle, Bos taurus</i>
Mb-Sp38-Brazil.Sao.Paulo-2015	NZ_CP01577 3.1	Mycobacterium tuberculosis var. bovis	SP38	1	65.50	2015	Brazil	
Mb-W-1171-S.Korea-Jun2014-Wild.Boar	JXTK0000000 0	Mycobacterium tuberculosis var. bovis	W-1171	50	65.50	2014	S.Korea	<i>Pig, Sus scrofa</i>

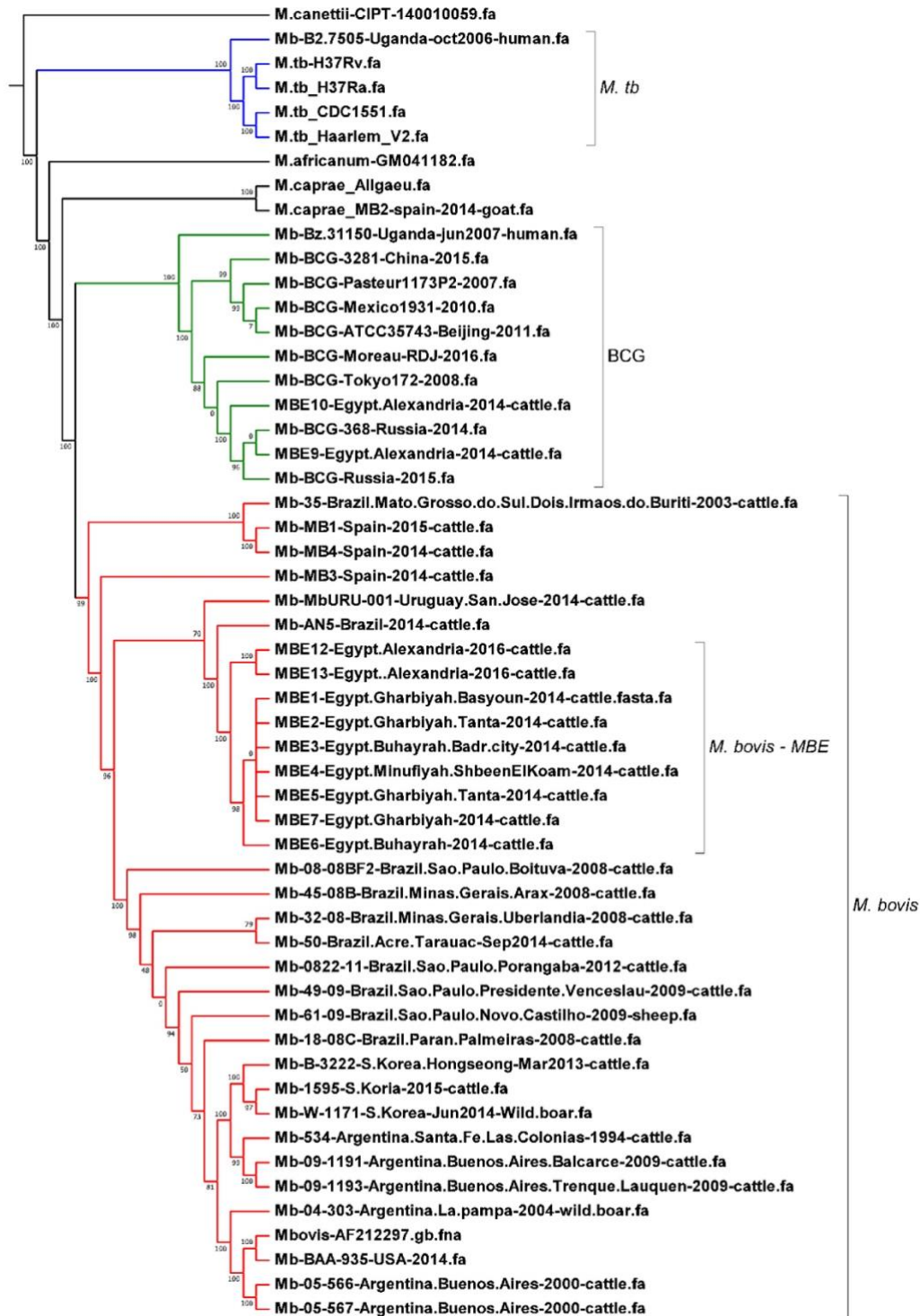
8 **Supplemental Table 3. List of 27 SNPs predicted to be uniquely associated with MBE4**
 9 **used for genotype comparison between MBE4, MBE5 and MBE7 relative to the reference**
 10 **virulence strain of *M. bovis*.**
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SNPs uniquely present in MBE4

#	Reference position	Reference allele	MBE4	MBE5	MBE7	Annotation	SNP type	Amino acid change
1	339620	C	G	C	C	BQ2027_MB0287c / PE_PGRS4	synonymous	
2	1165589	T	C	T	T	BQ2027_MB1071c	non-synonymous	Glu129Gly
3	1619153	A	G	A	A	BQ2027_MB1476c / PE_PGRS26	synonymous	
4	1619361	T	G	T	T		non-synonymous	Asn354Thr
5	2283587	G	T	G	G	BQ2027_MB2074c / pks12	non-synonymous	Asp3908Glu
6	2283590	G	A	G	G		synonymous	
7	2283596	G	C	G	G		non-synonymous	Phe3905Leu
8	2283598	A	G	A	A		non-synonymous	Phe3905Leu
9	2283607	A	G	A	A		non-synonymous	Phe3902Leu
10	2283616	T	G	T	T		non-synonymous	Thr3899Pro
11	3082946	T	A	T	T		intergenic	
12	3836507	T	C	T	T	BQ2027_MB3495	synonymous	
13	3878929	G	C	G	G	BQ2027_MB3537 / PE_PGRS53	non-synonymous	Lys775Asn
14	3878937	C	A	C	C		non-synonymous	Ala778Asp
15	3878946	T	A	T	T		non-synonymous	Ile781Asn
16	3878985	G	C	G	G		non-synonymous	Gly794Ala
17	3884294	A	G	A	A	BQ2027_MB3538 / PE_PGRS54	non-synonymous	Asp1106Gly
18	3884519	C	T	C	C		non-synonymous	Ala118Val
19	3890762	T	C	T	T	BQ2027_MB3541 / PE_PGRS55	synonymous	
20	3890767	G	C	G	G		non-synonymous	Gly834Ala
21	3893080	A	G	A	A		non-synonymous	Asp1605Gly
22	4259281	T	C	T	T	intergenic		

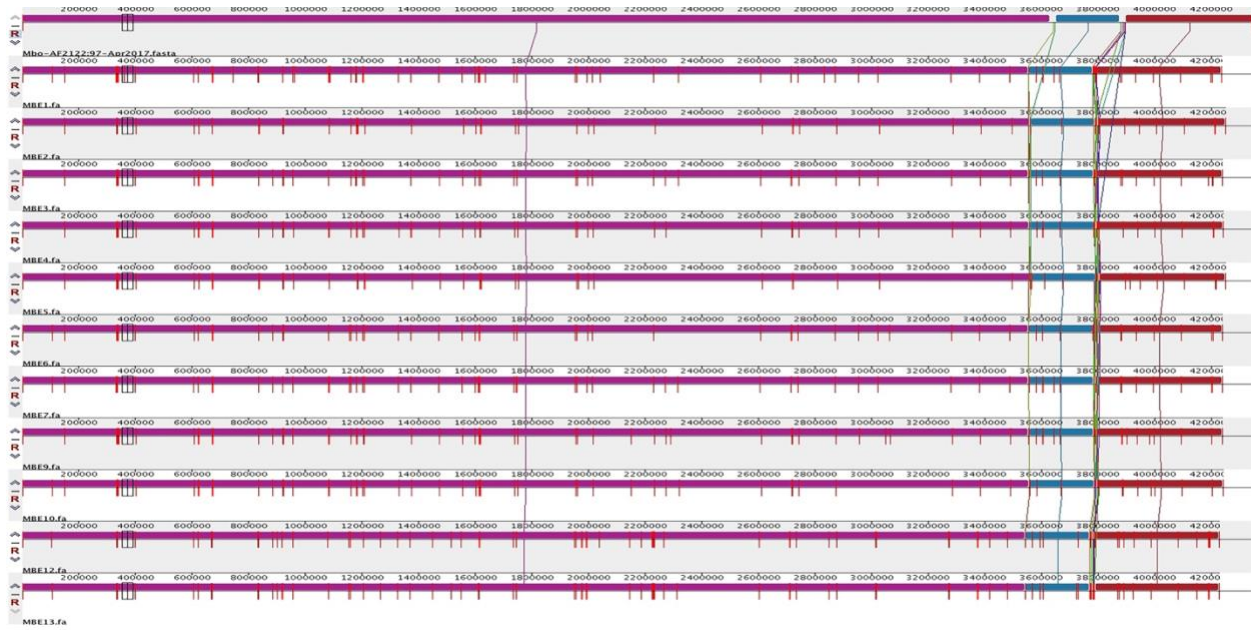
**SNPs
uniquely
absent in
MBE4**

1	928975	G	G	A	A	BQ2027_ MB0857C / PE_PGRS 14		
2	928981	G	G	A	A			
3	928985	A	A	T	T			
4	928987	G	G	A	A			
5	928991	A	A	G	G			

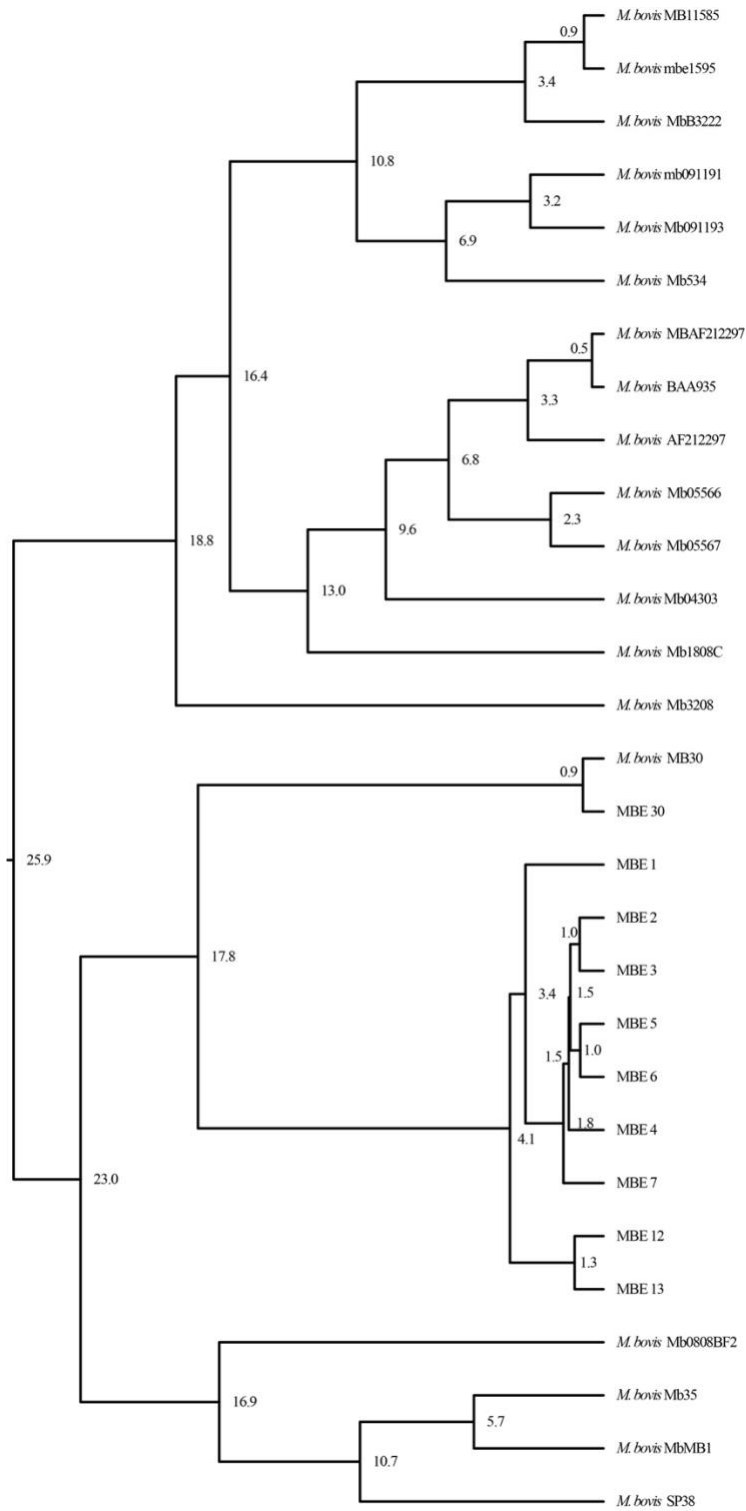


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Supplemental Fig. 1. Phylogenetic tree of MBE isolates and an archive of 67 sequenced *M. bovis* genomes. Whole genome sequences were analyzed with the Jukes-Cantor or generalized time-reversible (GTR) models of nucleotide evolution using Harvest software, and the tree was visualized with MEGA7, each strain identified by ID, origin of isolation, year of isolation, and species of isolation.



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 26 **Supplementary Fig. 2. Core-genome multiple alignment for all the *M. bovis* isolates from**
 27 **Nile delta, MAUVE alignment showing no major genomic rearrangements.**



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Supplemental Fig. 3. phylogeographic model of bovine tuberculosis in the Nile Delta.

30 **References.**

- 31 1. Forrellad, M. A. *et al.* Virulence factors of the Mycobacterium tuberculosis complex.
32 doi:10.4161/viru.22329

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