International Journal of Comprehensive Veterinary Research

Article:

Mycobacterium Bovis in Sohag: P22 ELISA Serodiagnosis, Geographical Distribution, Genetic Diversities and Drug Resistance

Nahla Fouad [1]*, Mohamed Nayel [2], Akram Salama [2], Alyaa Elrashedy [2,3], Emad Sakr [4], Doaa Salman [1]

[1]Department of Animal Medicine, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt, [2]Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt, [3]Faculty of Health Science Technology, Borg Al Arab Technological University (BATU), Alexandria, Egypt, [4]Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt.

Received: 16 December 2024; Accepted: 26 January 2025; Published: 17 April 2025

Abstract

Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB), primarily in bovines; it has a wide host range and zoonotic features. In this report, we aimed to detect the antibodies specific against *M. bovis* in large ruminants using P22 ELISA, the geographical distribution of bTB-infected cattle and buffalo cases, found positive by GOVs to single intradermal tuberculin (SID) in Sohag governorate, and to carry out comprehensive and phylogenetic genome analysis to study the drug resistance genes of *M. bovis* strains in Egypt. Using P22 ELISA in large ruminants to detect *M. bovis* specific antibodies in serum samples of cattle and buffaloes; no positive cases were detected in either cattle or buffalo. Based on SID, no confirmed cases were detected from 2020 to 2023. In 2024, 24 positive cases (0.07%) and 37 positive cases (0.2%) were confirmed in Sohag Center and Akhmim respectively. After running the Pathosystems resource integration center (PATRIC) server on 11 *M. bovis* isolates, it was found that all isolates were susceptible to Streptomycin, Amikacin, Kanamycin, Capreomycin, Rifampin, Ethambutol, Isoniazid, and Ofloxacin, antibiotics, however, two resistant genes were identified in MBE12 and MBE7 strains. MBE12 strain showed monoresistance against Ethambutol antibiotic. However, more analysis is needed to understand *M. bovis* increased incidence, genomic diversities, and resistance in Sohag and other Governorates in Egypt.

Keywords: bTB, Drug Resistance, Geographical Distribution, P22 ELISA, Sohag.

Introduction

Bovine Tuberculosis (bTB) is a worldwide distributed disease; World Health Organization (WHO) considers it a notifiable disease due to zoonotic risk and its threats to livestock health. The disease has adverse effects on animal production, and it impedes the animal trade between the infected area and other areas to decrease the spread of the disease (Azami & Zinsstag, 2018; Dibaba et al., 2019). bTB is caused by *Mycobacterium bovis* (*M. bovis*) and is regarded as one of the most significant diseases confronting the agricultural sector, cattle proprietors, government authorities, abattoir personnel, and veterinary practitioners in Egypt (Hamed et al., 2021). In Egypt, the diagnosis of bTB is based on screening herds with a single intradermal tuberculin (SID) conducted by the General Organization of Veterinary Services (Govs). Antibody-based assays are regarded as a viable approach for diagnosing tuberculosis in infected animals through the analysis of serum, plasma, and milk samples for detecting specific antibodies against *M. bovis* (Ortega et al., 2021). The specificity and sensitivity of the P22 ELISA in detecting bTB have been evaluated in various studies, revealing promising results. The P22 ELISA, which utilizes a specific biomarker, demonstrates high sensitivity

IJCVR ***Corresponding author:** Nahla Fouad, ***Email**: nahlaelyan4@gmail.com ***Address**: Department of Animal Medicine, Faculty of Veterinary Medicine, Sohag University Sohag, Egypt*

and specificity, making it a valuable tool for bTB screening (Infantes-Lorenzo et al., 2019).

Abdellrazeq et al. (2016), highlighted the geographical distribution of bTB-dense areas with no infection foci detected in Sohag; however, the infection was present in Qena, El-Wadi El Gedid, and Asyut governorates of upper Egypt. Whole Genome Sequencing (WGS) recently provided new information about disease transmission dynamics (Biek et al., 2012; Bruning-Fann et al., 2017; Perea Razo et al., 2018). For instance, *M. bovis* clinical isolate genome sequencing provided a precise measure of the geographical distribution and expansion of such strain (Barbier & Wirth, 2017; Lasserre et al., 2018; Orloski et al., 2018; Patane et al., 2017; Price-Carter et al., 2018), which is not a common approach in Egypt, unlike European countries (Zimpel et al., 2017),

Bioinformatics plays a crucial role in managing large datasets and in the analysis, prediction, and interpretation of disease outcomes, as well as in evaluation and drug discovery (Khan et al., 2022). The resistance of *M. bovis* to anti-tuberculous drugs has been documented (Abdelsadek et al., 2020; El-Gedawy et al., 2024). *M. bovis* genome analysis through light on genome quality evaluation, evolutionary linkages, subsystem attributes, antimicrobial resistance (AMR) genes, and prospective candidate genes (Wang et al., 2020).

To the best of our knowledge, very limited research studied bTB in Sohag Governorate; (Mossad et al., 2009) conducted a study that revealed 3/100 positive animals using intradermal cervical tuberculin test from Sohag governorate, that study included cases from 2006 to 2008, with no other published data ever since. Therefore, this study aimed to detect *M. bovis* specific antibodies in large ruminants using P22 ELISA, the geographical distribution of bTB-infected cattle and buffalo cases using SID implemented by GOVS in Sohag governorate and conduct a thorough phylogenetic genome analysis of selected Egyptian strains to explore *M. bovis* genes associated with drug resistance.

Materials and Methods

1. Ethical approval

Ethical approval for this study was obtained from the Institutional Review Board Faculty of Veterinary Medical Research Ethics committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt, according to the OIE standards for use of animals in research with number Soh.un.vet/00017R1.

2. Sample collection

Five ml of blood was collected from 16 animals (6 cows and 10 buffalo) from jugular veins and placed into the sterilized vial with no anticoagulant addition. The tubes were labelled with the essential data (Species, Date of collection, Age, Sex, and Breed) and transferred immediately with ice to be centrifugated for 10 min at 1500 r.p.m. The serum was collected into Eppendorf tubes and stored at -20°c for further analysis.

3. The Indirect P22 ELISA

An in-house indirect P22 ELISA using P22 complex protein was performed according to (Infantes-Lorenzo et al., 2019; Thomas et al., 2019).

4. Data collection

a. GOVS Data

Bovine tuberculosis outbreaks data in Sohag governorate were collected from GOVS (2020-2024). The records included information such as governorate, location, species affected, index date, number of cases, outbreaks, and total at-risk population. Numbers of reactors for each month were recorded using database-generated sheets. These data were analyzed in the present study to discern the geographical areas where bTB foci have been detected based on the SID.

i. Data analysis

Microsoft Excel spreadsheets (Microsoft Corporation) and R 4.2.3 (R Core Team) were used to manage the data and draw charts. Descriptive methods were used to calculate outbreak incidence. Prevalence is the proportion of diseased cases in a particular population at a given time (MedicineNet, 2023).

The spatial distribution of bTB outbreaks in Sohag Governorate over the study period was constructed by administrative zones using ArcGIS 10.5 software (Environmental Systems Research Institute (ESRI), Inc., Redlands, CA, USA).

b. *M. bovis* whole genome sequence data retrieval

The complete genome sequence of *M. bovis* strain from Egypt was retrieved from the National Center for Biotechnology Information (NCBI) bio project database with the accession number PRJNA471317 (Abdelaal et al., 2019).

5. Comprehensive genome analysis

Comprehensive genome analysis was applied using the meta-service on the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) server (Davis et al., 2016).

6. Phylogenetic analysis

The "Codon Trees" phylogenetic tree from BV-BRC was utilized to ascertain the evolutionary relationships. It employs the established PATRIC protein global families (PGFams), selecting 10–1000 single-copy families from the constituents of a genomic group. Alignments for protein sequences of each family were generated using Muscle (Edgar, 2004), while BioPython's codon-align function was utilized for the corresponding nucleotide sequences (Cock et al., 2009).

Confidence values are generated by doing 100 rapid bootstrap iterations in RaxML (Stamatakis et al., 2008).

The accession numbers for sequences used for phylogenetic analysis were (MBE1: QFZD00000000, MBE2: QFZC00000000, MBE3: QFZB00000000, MBE4: QFZA00000000, MBE5: QFYZ00000000, MBE6: QFYY00000000, MBE7: QFYX00000000, MBE9: QFYW00000000, MBE10: QFYV00000000, MBE12: QFYT00000000, MBE13: QFYU00000000)

Results

1. Detection of bTB using P22 ELISA

Bovine tuberculosis detection using P22 ELISA was applied to large ruminants to detect *M. bovis*-specific antibodies in cattle and buffalo serum samples; no positive cases were detected in either cattle or buffalo using this test.

2. bTB prevalence in Sohag Governorate based on SID in the period between (2020 -2024)

No confirmed cases of bTB were recorded during 2020, 2021, 2022, and 2023, while 2024 recorded a total number of 61 positive cases of tuberculin skin test as illustrated in table and figure (1), with domination in the number of cases found in Akhmim 37 positive cases (0.18%) over 24 positive cases (0.07%) found in Sohag center.

Table 1. Bovine Tuberculosis prevalence in Sohag Governorate during 2024

Locality	Cases	Total population	Prevalence
Sohag	24	34802	0.07
Akhmim	37	20939	0.2

The prevalence of bTB among cattle and buffalos within Sohag Governorate was determined by analyzing the GOVs bTB database between 2020 and 2024.

3. Geographical bTB distribution in Sohag

According to the locations of reactors for bTB detected by the SID, bTB was located in Sohag and Akhmim centers (Figure 2). During 2024, bTB was distributed in two centers (Akhmim and Sohag) from a total of 12 centers in Sohag Governorate.

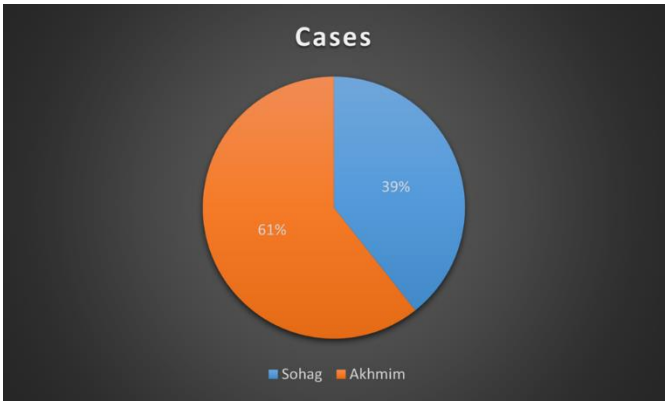

Figure 1. Bovine Tuberculosis distribution in different localities in Sohag Governorate during 2024. From 61 positive cases by SID, 24 were detected in Sohag and 37 were detected in Akhmim.

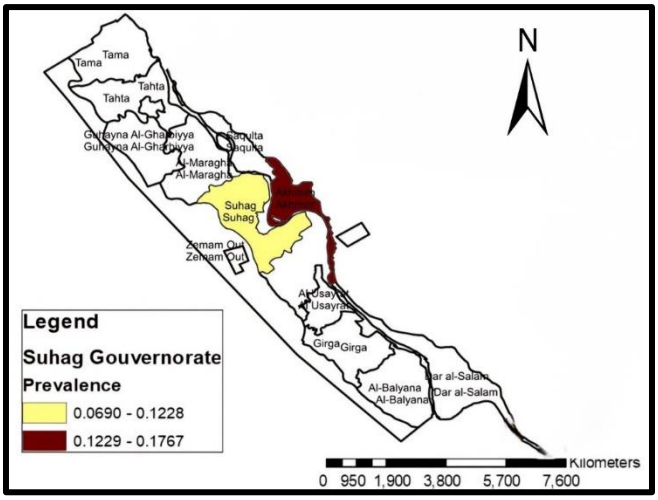

Figure 2. Geographical map of Sohag governorate displaying the distribution of Bovine Tuberculosis outbreaks during 2024

4. Comprehensive Genome Analysis

4.1. Genome Assembly and Annotation

A comprehensive genome analysis of eleven *M. bovis* isolates in Egypt was performed. The genome assembly of these strains showed almost similar characteristics: 65.63% GC content the genome length of 4,348,120 bp, except the MEB12 strain showed 69.19% and 5,460,188 bp in GC content and the genome length, respectively.

The genome annotation of field strains had an average of 4220 coding DNA sequences (CDS) and 46 repeat regions except for high numbers (159, 164, 163, and 183) in MBE1, MBE3, MBE4, and MBE10, respectively, and zero in MBE12. They also have 45 transfer RNA genes (tRNA), three ribosomal RNA genes (rRNA), an average of 846 hypothetical proteins, and 3370 functional proteins in almost all the strains except in MBE12 is 1685, and 4140, respectively **(Figure 3)**.

These functional proteins are estimated to have an average of 1071 Enzyme Commission (EC) numbers, 912 Gene Ontology (GO), 813 proteins linked to KEGG pathways, 3937 PLFams, and 4140 PGFams proteins mainly in all strains; however, MBE12 exhibited 1473, 1260, 1172, 5377, and 5592, respectively **(Table 2)**.

Table 2. Genome Assembly & Annotation of Mycobacterium bovis using BV-BRC server

	Genome Assembly				**Genome Features**					**Protein Features**						
Strains	Contigs	Contig L50	Contig N50	GC Content	Genome Length **(Dey & Parham)**	CDS	Repeat regions	tRNA	rRNA	Hypothetical proteins	functional Proteins	Proteins have an EC	Proteins have GO	Proteins with Pathway	PLfam	PGfam
MBE1	8	1	2,859,506	65.63	4,348,120	4,214	159	45	3	844	3,370	1,058	911	812	3,937	4,140
MBE2	24	2	852,716	65.62	4,353,842	4,224	45	45	3	841	3,383	1,061	913	813	3,942	4156
MBE3	8	1	2,858,537	65.63	4,346,664	4,215	164	45	3	844	3,371	1,058	911	812	3,937	4,138
MBE4	8	1	3,176,044	65.63	4,347,513	4,214	163	45	3	845	3,369	1057	910	812	3,934	4138
MBE5	15	1	2,858,883	65.61	4,347,510	4,219	49	45	3	848	3,371	1,060	913	814	3,934	4,139
MBE6	13	1	3,175,176	65.62	4,346,927	4,217	45	45	3	844	3,373	1,058	911	812	3,932	4135
MBE7	38	1	2,857,927	65.61	4,358,624	4,242	45	45	3	846	3,396	1072	925	826	3,940	4,165
MBE9	11	1	3,667,644	65.62	4,352,040	4,217	49	45	3	854	3,363	1053	906	807	3,930	4,133
MBE10	10	1	3,667,979	65.62	4,351,970	4,212	183	45	3	845	3,367	1,056	909	810	3,925	4,127
MBE12	**160**	**21**	**80,724**	**69.19**	**5,460,188**	**5,825**	**0**	**49**	**3**	**1,685**	**4,140**	**1,473**	**1,260**	**1,172**	**5,377**	**5,592**
MBE13	41	3	461,588	65.63	4,536,331	4,611	47	44	3	929	3,682	1,180	1,006	896	4,287	4,506

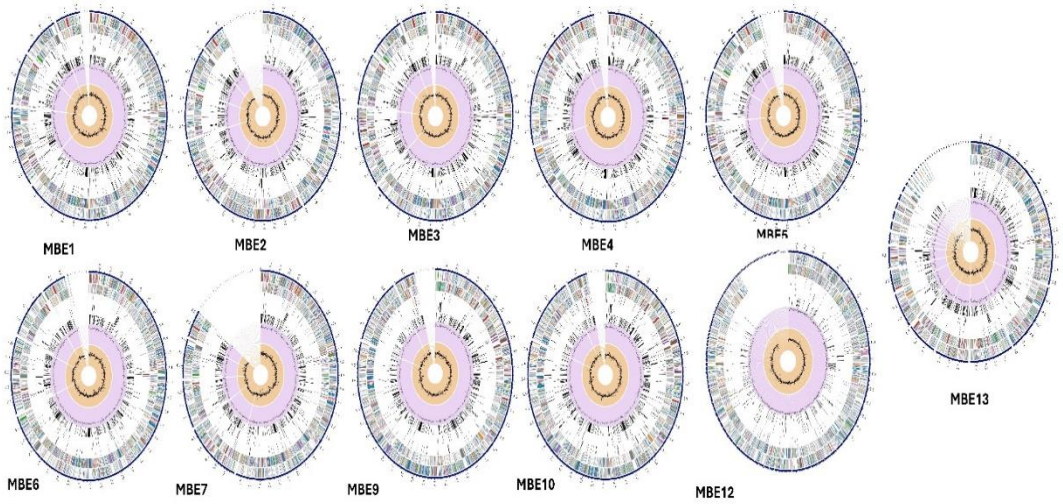

Figure (3) presents the circular genomes of eleven strains of M. bovis. The figure illustrates multiple genomic annotations organized from the outermost to the innermost layers, including contigs, coding sequences (CDS) on both forward and reverse strands, tRNA, CDS of antimicrobial resistance (AMR) genes, CDS of virulence factors, GC content, and GC skew.

4.2. Candidate Genes and Antimicrobial Resistance Genes

Numerous annotated genes in *Mycobacterium bovis* displayed similarities with well-known genes associated with antibiotic resistance, transporters, drug targets as well as virulence factors in all strains except the MBE12, as indicated in **(Table 3)**.

Table 3. Candidate genes of mycobacterium bovis strains

	Source	MBE1	MBE2	MBE3	MBE4	MBE5	MBE6	MBE7	MBE9	MBE10	MBE12	MBE13
		Genes	Genes	Genes	Genes	Genes	Genes	Genes	Genes	Genes	Genes	Genes
Transporter	TCDB	120	120	120	120	120	120	120	122	121	**51**	137
Drug Target	Drug Bank	70	70	70	70	70	70	71	70	70	**48**	74
	TTD	29	29	29	29	29	29	29	29	29	**19**	30
Antibiotic Resistance	CARD	29	29	29	29	29	29	30	29	29	**12**	30
	PATRIC	56	57	57	56	56	56	58	56	56	**54**	58
	NDARO	3	3	3	3	3	3	3	3	3	___	3
Virulence Factor	PATRIC_VF	460	459	459	460	460	461	459	461	461	**183**	495
	VFDB	70	71	70	70	70	70	70	70	70	**24**	70
	Victors	258	257	257	258	258	259	257	256	256	**107**	276

4.3. Antimicrobial Resistance Analysis

Antimicrobial resistance phenotypes refer to the resistance or susceptibility of *M. bovis* to one or more antibiotics. PATRIC provides AMR phenotype predictions using custom-built AdaBoost (adaptive boosting) machine learning classifiers **(Long et al., 2017)**. The predicted AMR phenotypes for these genomes are similar except MBE12. All of them showed susceptibility to Streptomycin, Amikacin, Kanamycin, Capreomycin, Rifampin, Ethambutol, Isoniazid, and Ofloxacin antibiotics, except the MBE12 exhibited resistance to Ethambutol and susceptible to the other antibiotics. Additionally, all strains' AMR patterns are identical except that of MBE12 **(Table 4)**. Interestingly, the MBE7 strain showed one more AMR gene (OxyR) in the regulator modulating the expression of antibiotic resistance genes.

Table 4. Antimicrobial Resistance Genes of all Mycobacterium bovis

	Source	MBE1 Genes	MBE2 Genes	MBE3 Genes	MBE4 Genes	MBE5 Genes	MBE6 Genes	MBE7 Genes	MBE9 Genes	MBE10 Genes	MBE12 Genes	MBE13 Genes
Transporter	TCDB	120	120	120	120	120	120	120	122	121	**51**	137
Drug Target	Drug Bank	70	70	70	70	70	70	71	70	70	**48**	74
	TTD	29	29	29	29	29	29	29	29	29	**19**	30
Antibiotic Resistance	CARD	29	29	29	29	29	29	30	29	29	**12**	30
	PATRIC	56	57	57	56	56	56	58	56	56	**54**	58
	NDARO	3	3	3	3	3	3	3	3	3	___	3
Virulence Factor	PATRIC_VF	460	459	459	460	460	461	459	461	461	**183**	495
	VFDB	70	71	70	70	70	70	70	70	70	**24**	70
	Victors	258	257	257	258	258	259	257	256	256	**107**	276

4.4. Subsystems in *Mycobacterium bovis* strains

A subsystem comprises a group of proteins collaborating to perform a particular biological function or form a structural complex. The organization followed a pattern of Subsystem Counts (Subsystems, Genes), indicating that the metabolism of the MBE1 strain involves a distinct biological process comprising 93 subsystems regulated by 779 genes (Figure 4).

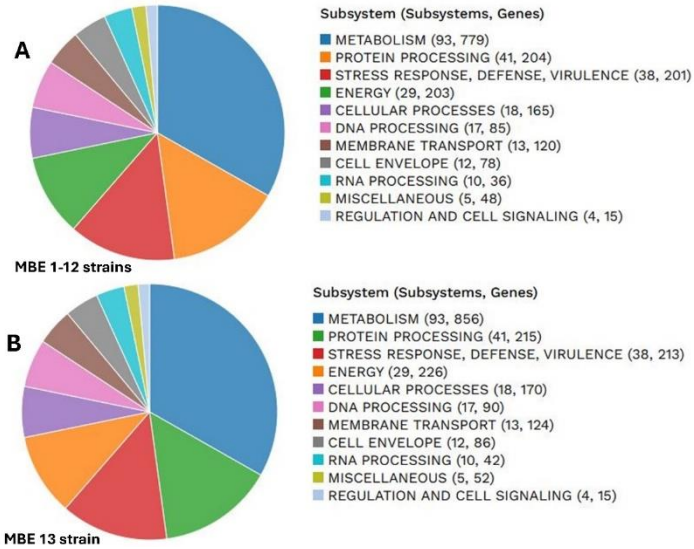

Figure 4. Distribution of subsystem categories for MBE from strains 1-12 (A) and MBE13 (B).

5. Phylogenetic analysis

A whole genome phylogeny was constructed (16 genomes) of *Mycobacterium bovis* from different hosts and other out-groups. These genomes were collected from different countries, years, and species. The tree was built on protein and gene sequences for those 100 genes. The whole genome phylogeny is clustered into three clades **(Figure 5)**. Clade one is involved with all the isolates except MBE12_Menoufia 2015, involved in clade three with *Mycobacterium avium subsp. Hominissuis*.

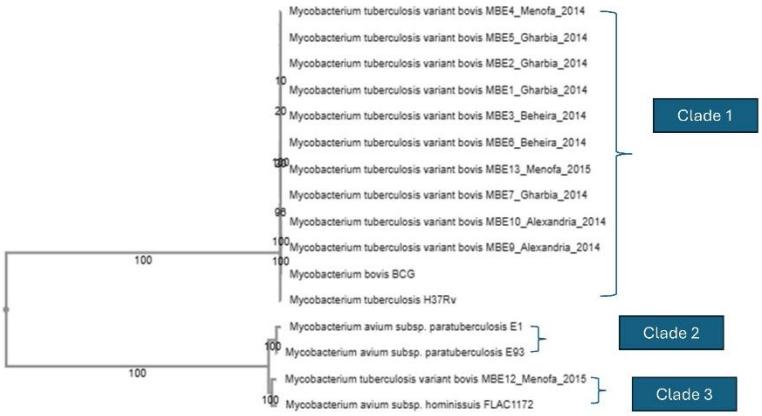

Figure 5. Whole-genome phylogeny of M. bovis strains with others. It is classified into three clades. Clade one is involved with all the isolates except MBE12_Menoufia 2015, involved in clade three with Mycobacterium avium subsp. Hominissuis.

Discussion

M. bovis is the primary causative agent of bTB, a severe infectious disease that is zoonotic and affects various animal species. In upper Egypt communities, it's common for animal owners to raise cattle and buffalo in a pen or room inside or near the owner's house with other domestic animals **(Farag et al., 2023)**. Animals can catch the infection and remain dormant for years before showing any symptoms **(Cousins, 2001).** Various diagnostic techniques, including post-mortem inspections, histopathology, molecular methods, and serological tests, are employed to detect *M. bovis*. The use of serological tests for bTB diagnosis in ruminants has been evaluated **(Infantes-Lorenzo et al., 2019)**. ELISA can detect infections that may be missed by conventional tests, thus improving overall diagnostic accuracy **(Griffa et al., 2020)**. The high sensitivity and specificity of the P22 ELISA suggest its potential as an ancillary test alongside traditional methods like the CFT, enhancing bTB control efforts **(Singhla et al., 2024)**. In the current study, bTB detection using P22 ELISA was applied; there were no positive cases detected in either cattle or buffalo using this test, which disagreed with **(El-Sify et al., 2013)** who revealed that 46/ 3474 were positive with 1.3% animals using an ELISA coated with ST-CF, while by using bovine PPD as a coating antigen in the same study 58 /3474 animals were positive with 1.67%. **Hekal et al. (2022)** showed a higher prevalence of the ELISA test. 94/2913 were positive, with 3.23% using PPD-B coated ELISA, and 97 /2913 were positive, with 3.33% using commercial polypeptide antigen. **Thomas et al. (2019)** revealed that the P22 ELISA induced a higher specificity and similar sensitivity compared to the bPPD ELISA at all the cut-off points considered. While the P22 ELISA shows high performance **(Singhla et al., 2024)**, other studies indicate variability in sensitivity and specificity among different ELISA tests, suggesting that no single test may suffice for comprehensive bTB detection **(Carneiro et al., 2021; Moens et al., 2023).** The gap between the current and other studies may be related to the numbers used. Dairy cattle experience significant hormonal changes and stresses throughout production, likely affecting their reaction to diagnostics **(Nasr et al., 2019)**.

Intradermal Tuberculin Test (ITT) is the standard diagnostics for antemortem diagnosis, with a sensitivity of 86% and specificity of 97% **(Pir & Yardımcı, 2024).** The present study was conducted in Sohag governorate from 2020 to 2024 to calculate the infection rate of bTB based on SID conducted by the GOVS. According to this study, no confirmed cases were detected from 2020 to 2023. In 2024, no positive cases were detected until June; on July, 24 positive cases (0.07%) were confirmed in Sohag center, and in September, 37 positive cases (0.2%) were confirmed in Akhmim. The total number of positive cases was 61 in 2024. A similar but broader data analysis was carried out by **Abdellrazeq et al. (2016);** their study included three governorates of upper Egypt with positive reactors to tuberculin test in Asyut, Quena, and El-Wadi El-Gedid; no positive cases were recorded in Sohag, which disagrees with the current study, which points out 61 positive cases in Akhmim, and Sohag center, which possess a public health risk to the livestock and humans in Sohag governorate. Rural populations in Upper Egypt lack awareness regarding the transmission routes of bTB. Their living regimes may increase the odds of transmitting infection to humans. Ruminant owners can profit from selling raw milk and unchecked meat after the animal is slaughtered by the community's butcher **(Farag et al., 2023)**. In developing countries, *M. bovis* causes human cases of tuberculosis **(Bobadilla-del Valle et al., 2015)**, which has been representing a public health threat for an extended period **(Vázquez-Chacón et al., 2021).** Humans catch infection by contacting infected hosts directly or indirectly or ingesting raw, unpasteurized milk **(Kasir et al., 2023)**.

There are reports that *M. bovis* is increasing in virulence and antibiotic resistance **(Otchere et al., 2019).** There is a routinely described regimen of four drugs for intensive TB treatment in humans, including pyrazinamide, rifampicin, Ethambutol, and isoniazid, that has been used in TB treatment since the mid-1960s. **(WHO, 2010)** these four groups of drugs are considered the first line of tuberculous drugs; they can turn tuberculous patients into noncontagious **(Munir et al., 2017)**, while the second line of anti-tuberculosis drugs includes fluoroquinolone, amikacin, kanamycin, and capreomycin. Ethambutol resistance is associated with *embB***;** in 1993, the WHO recognized tuberculosis as an emergency disease because the bacteria was resistant to first-line anti-tuberculosis drugs (Rifampicin and Isoniazid) **(Campbell et al., 2011).** Controlling and preventing the spread of *M. bovis* MDR is essential to protect humans **(Franco et al., 2017)** and livestock from such threats. In the current study, there were two antimicrobial-resistant genes identified using the PATRIC server in 11 *M. bovis* isolates; MBE1-Gharbia-2014, MBE2-Gharbia-2014, MBE3-Beheira-2014, MBE4-Menofia-2014, MBE5-Gharbia-2014, MBE6-Beheira -2014, MBE7-Gharbia-2014, MBE9-Alexandria-2014, MBE10-Alexandria-2014, MBE12-Menofia,2015, MBE13-Menofia,2015, all of isolates genes showed susceptibility to 8 antibiotics Streptomycin, Amikacin, Kanamycin, Capreomycin, Rifampin, Ethambutol, Isoniazid, and Ofloxacin antibiotics, except the MBE12 showed monoresistance to Ethambutol antibiotic while susceptible to the other antibiotics which align with the finding of **(Safi et al., 2013)**. Antibiotic resistance is principally due to mutations, with some exceptions, such as phenotypic drug tolerance **(Kester & Fortune, 2014; Sekiguchi et al., 2007)**. It was reported that the majority of mutations induct Ethambutol resistance resulted in amino acid replacements at position EmbB 306 or 406 **(Brossier et al., 2015; Ramaswamy et al., 2000; Starks et al., 2009)**. Hence, the resistance was only to a single antibiotic.

Drug resistance can be related to the low permeability through mycolic acid and the efflux pump action **(da Silva et al., 2016);** in this study, Based on sequence analysis, ten efflux pump genes have been identified in MBE12 strain of *M bovis* which include (MmpL5, MmpL7, MmpS5, Rv1258c, Rv1634, Rv1747, Rv1877, Rv2333c, Rv2994, Rv3239c), efflux pumps can pump out drugs to dodge antimicrobial effect, so it has a role in pathogenicity and virulence of bacteria **(Piddock et al., 2000),** overexpression of *Rv1258c* was found to show mild resistance to tetracycline and aminoglycosides **(Aínsa et al., 1998),** inhibiting efflux pump is an important approach to overcome the drug resistance problem.

The majority of mycobacteria have a functional oxyR, which is the central regulator of the bacterial oxidative stress response. The natural loss of oxyR during the evolution of *M. tuberculosis* participates in the high sensitivity of *M. tuberculosis* to isoniazid **(Pagán-Ramos et al., 2006)**; it belongs to the LysR family **(Domenech et al., 2001)** and is considered a pseudogene in *M. Tuberculosis* **(Li et al., 1981; Nei, 1987).** The current study's MBE7 strain showed one more AMR gene (OxyR) in the regulator modulating the expression of antibiotic resistance genes.

Catalase-peroxidase (katG) is a protein that contributes to mycobacterial metabolism by protecting the bacilli from reactive oxygen intermediates and toxic molecules for bacteria. KatG has an antimicrobial resistance role by detoxifying the reactive oxygen

resulting from respiratory bursts in phagocytic cells and impairing nitric oxide synthesis. **(O'Brien et al., 1996)**.

The resistance of MTBC to macrolides is related to Erm (37) **(Andini & Nash, 2006).** The GidB gene was reported to show resistance to streptomycin **(Okamoto et al., 2007)**; however, in this study, the MBE12 containing the GidB is susceptible.

Conclusion

In the current study, P22 ELISA detected no positive cases of tested large ruminants. To our knowledge, this is the first study in Sohag, Egypt, using the P22 IELISA, but this doesn't for sure exclude the possibility of infection. Based on the obtained data from GOVS, bTB in Sohag governorate during 2024 was geographically distributed in Sohag and Akhmim centers. The MBE12 showed monoresistance towards the Ethambutol antibiotic, and the OxyR, a resistant gene, was found in the MBE7 strain. MBE12 showed susceptibility to Streptomycin, Amikacin, Kanamycin, Capreomycin, Rifampin, Isoniazid, and Ofloxacin antibiotics. This study had some limitations including the SID test limitations in terms of sensitivity and specificity but this doesn't for sure exclude the possibility of infection, furthermore, low numbers of animals tested using p22 ELISA. The p22 ELISA used was constrained by the available resources Further investigations are required to elucidate the incidence, prevalence, and drug-resistant genes of *M. bovis* when financial support is available to increase and support the accuracy of this study's findings.

Conflict of interest

There is no conflict of interest.

Authors' contribution

The work was equally distributed among the authors. All authors have read and approved the final version of the manuscript.

References

1. **Abdelaal, H. F., Spalink, D., Amer, A., Steinberg, H., Hashish, E. A., Nasr, E. A., & Talaat, A. M. (2019).** Genomic polymorphism associated with the emergence of virulent isolates of Mycobacterium bovis in the Nile Delta. Scientific Reports, 9(1), 11657.

2. **Abdellrazeq, G., Elnaggar, M., Osman, H., Davis, W., & Singh, M. (2016).** Prevalence of bovine tuberculosis in Egyptian cattle and the standardization of the interferon-gamma assay as an ancillary test. Transboundary and emerging diseases, 63(5), 497-507.

3. **Abdelsadek, H. A., Sobhy, H. M., Mohamed, K. F., Hekal, S. H., Dapgh, A. N., & Hakim, A. S. (2020).** Multidrug-resistant strains of Mycobacterium complex species in Egyptian farm animals, veterinarians, and farm and abattoir workers. Veterinary World, 13(10), 2150.

4. **Aínsa, J. A., Blokpoel, M. C., Otal, I., Young, D. B., De Smet, K. A., & Martín, C. (1998).** Molecular cloning and characterization of Tap, a putative multidrug efflux pump present in Mycobacterium fortuitum and Mycobacterium tuberculosis. Journal of bacteriology, 180(22), 5836-5843.

5. **Andini, N., & Nash, K. A. (2006).** Intrinsic macrolide resistance of the Mycobacterium tuberculosis complex is inducible. Antimicrobial agents and chemotherapy, 50(7), 2560-2562.

6. **Azami, H. Y., & Zinsstag, J. (2018).** Economics of bovine tuberculosis: a one health issue. In Bovine tuberculosis (pp. 31-42). CAB International Wallingford UK.

7. **Barbier, M., & Wirth, T. (2017).** The evolutionary history, demography, and spread of the Mycobacterium tuberculosis complex. Tuberculosis and the Tubercle Bacillus, 453-473.

8. **Biek, R., O'Hare, A., Wright, D., Mallon, T., McCormick, C., Orton, R. J., McDowell, S., Trewby, H., Skuce, R. A., & Kao, R. R. (2012).** Whole genome sequencing reveals local transmission patterns of Mycobacterium bovis in sympatric cattle and badger populations. PLoS pathogens, 8(11), e1003008.

9. **Bobadilla-del Valle, M., Torres-González, P., Cervera-Hernández, M. E., Martínez-Gamboa, A., Crabtree-Ramirez, B., Chávez-Mazari, B., Ortiz-Conchi, N., Rodríguez-Cruz, L., Cervantes-Sánchez, A., & Gudiño-Enríquez, T. (2015).** Trends of Mycobacterium bovis isolation and first-line anti-tuberculosis drug susceptibility profile: a fifteen-year laboratory-based surveillance. PLoS Neglected Tropical Diseases, 9(9), e0004124. https://journals.plos.org/plosntds/article/file?id=10.1371/journal.pntd.0004124&type=printable

10. **Brossier, F., Sougakoff, W., Bernard, C., Petrou, M., Adeyema, K., Pham, A., Amy de la Breteque, D., Vallet, M., Jarlier, V., & Sola, C. (2015).** Molecular analysis of the embCAB locus and embR gene involved in ethambutol resistance in clinical isolates of Mycobacterium tuberculosis in France. Antimicrobial Agents and Chemotherapy, 59(8), 4800-4808. https://pmc.ncbi.nlm.nih.gov/articles/PMC4505224/pdf/zac4800.pdf

11. **Bruning-Fann, C. S., Robbe-Austerman, S., Kaneene, J. B., Thomsen, B. V., Tilden, J. D., Ray, J. S., Smith, R. W., Fitzgerald, S. D., Bolin, S. R., & O'Brien, D. J. (2017).** Use of whole-genome sequencing and evaluation of the apparent sensitivity and specificity of antemortem tuberculosis tests in the investigation of an unusual outbreak of Mycobacterium bovis infection in a Michigan dairy herd. Journal of the American Veterinary Medical Association, 251(2), 206-216.

12. **Campbell, P. J., Morlock, G. P., Sikes, R. D., Dalton, T. L., Metchock, B., Starks, A. M., Hooks, D. P., Cowan, L. S., Plikaytis, B. B., & Posey, J. E. (2011).** Molecular detection of mutations associated with first-and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy, 55(5), 2032-2041.

13. **Carneiro, P. A. M., de Moura Sousa, E., Viana, R. B., Monteiro, B. M., do Socorro Lima Kzam, A., de Souza, D. C., Coelho, A. S., Filho, J. D. R., Jordao, R. S., & Tavares, M. R. M. (2021).** Study on supplemental test to improve the detection of bovine tuberculosis in individual animals and herds. BMC Veterinary Research, 17, 1-8.

14. **Cousins, D. (2001).** Mycobacterium bovis infection and control in domestic livestock. Revue scientifique et technique (International Office of Epizootics), 20(1), 71-85.

15. **da Silva, P. E. A., Machado, D., Ramos, D., Couto, I., Von Groll, A., & Viveiros, M. (2016).** Efflux pumps in mycobacteria: antimicrobial resistance, physiological functions, and role in pathogenicity. Efflux-mediated antimicrobial resistance in bacteria: Mechanisms, regulation and clinical implications, 527-559.

16. **Davis, J. J., Gerdes, S., Olsen, G. J., Olson, R., Pusch, G. D., Shukla, M., Vonstein, V., Wattam, A. R., & Yoo, H. (2016).** PATtyFams: protein families for the microbial genomes in the PATRIC database. Frontiers in microbiology, 7, 118.

17. **Dey, B., & Parham, G. (1993).** Incidence and economics of tuberculosis in swine slaughtered from 1976 to 1988. Journal of the American Veterinary Medical Association, 203(4), 516-519.

18. **Dibaba, A. B., Kriek, N. P. J., & Thoen, C. O. (2019).** Tuberculosis in Animals: An African Perspective. Springer International Publishing. https://books.google.com.eg/books?id=SxegDwAAQBAJ

19. **Domenech, P., Honoré, N., Heym, B., & Cole, S. T. (2001).** Role of OxyS of Mycobacterium tuberculosis in oxidative stress: overexpression confers increased sensitivity to organic hydroperoxides. Microbes and infection, 3(9), 713-721.

20. **El-Gedawy, A. A., Elrefaie, A., El-Shazly, Y. A., Elkady, M. A., Abdelrahman, M. A., & Shalaby, M. (2024).** Highlighting on Mycobacterium Bovis in cattle, focusing on its antimicrobial resistance. Egyptian Journal of Animal Health, 4(1), 134-140. https://doi.org/10.21608/ejah.2024.327059

21. **El-Sify, A., Nayel, M., Hazem, S., Tarabess, R., Akram, S., Allaam, M., Hassan, H., & El Garhy, M. (2013).** Sero-diagnosis of bovine tuberculosis by ELISA using bovine PPD and ST. CF. Journal of Veterinary Medical Research, 22(1), 126-129.

22. **Farag, S. I., Cano-Terriza, D., Gonzálvez, M., Salman, D., Aref, N.-E. M., Mubaraki, M. A., Jiménez-Martín, D., García-Bocanegra, I., & Elmahallawy, E. K. (2023).** Serosurvey of selected reproductive pathogens in domestic ruminants from Upper Egypt. Frontiers in Veterinary Science, 10, 1267640.

23. **Franco, M. M. J., Ribeiro, M. G., Pavan, F. R., Miyata, M., Heinemann, M. B., de Souza Filho, A. F., Cardoso, R. F., de Almeida, A. L., Sakate, R. I., & Paes, A. C. (2017).** Genotyping and rifampicin and isoniazid resistance in Mycobacterium bovis strains isolated from the lymph nodes of slaughtered cattle. Tuberculosis, 104, 30-37. https://www.sciencedirect.com/science/article/abs/pii/S1472979216304371?via%3Dihub

24. **Govs. (1986).** the Ministerial Decision No. 517/1986 of the Minister of Agriculture and Food Security regarding the slaughter of animals, the meat trade, and the rules of meat inspection. Cairo Egypt. Ministry of Agriculture and Land Reclamation (MALR)

25. **Griffa, N., Moyano, R. D., Canal, A. M., Travería, G. E., Santangelo, M. d. l. P., Alonso, N., & Romano, M. I. (2020).** Development and diagnostic validation of an ELISA based on an antigenic mixture for the detection of bovine tuberculosis. The Veterinary Journal, 256, 105426. https://www.sciencedirect.com/science/article/abs/pii/S1090023320300034?via%3Dihub

26. **Hamed, Y. K., Nasr, E., Azooz, M. F., & Youssef, H. M. (2021).** Prevalence and risk factors of bovine tuberculosis in dairy cattle farms in Egypt. Iraqi Journal of Veterinary Sciences, 35(2), 351-359.

27. **Hekal, S. H. A., Dapgh, A. N., Abd-Elhafeez, M. B.-E., Sobhy, H. M., & Khalifa, F. A. (2022).** Comparative diagnosis of bovine tuberculosis using single intradermal cervical tuberculin technique, conventional methods, enzyme-linked immunosorbent assay, and the gamma-interferon assay. Veterinary World, 15(5), 1391. https://pmc.ncbi.nlm.nih.gov/articles/PMC9210844/pdf/Vetworld-15-1391.pdf

28. **Infantes-Lorenzo, J. A., Dave, D., Moreno, I., Anderson, P., Lesellier, S., Gormley, E., Dominguez, L., Balseiro, A., Gortázar, C., & Dominguez, M. (2019).** New serological platform for detecting antibodies against Mycobacterium tuberculosis complex in European badgers. Veterinary Medicine and Science, 5(1), 61-69. https://pmc.ncbi.nlm.nih.gov/articles/PMC6376137/pdf/VMS3-5-61.pdf

29. **Kasir, D., Osman, N., Awik, A., El Ratel, I., Rafei, R., Al Kassaa, I., El Safadi, D., Salma, R., El Omari, K., & Cummings, K. J. (2023).** Zoonotic tuberculosis: a

neglected disease in the Middle East and North Africa (MENA) region. Diseases, 11(1), 39.

30. **Kester, J. C., & Fortune, S. M. (2014).** Persisters and beyond: mechanisms of phenotypic drug resistance and drug tolerance in bacteria. Critical reviews in biochemistry and molecular biology, 49(2), 91-101.

31. **Khan, K., Alhar, M. S. O., Abbas, M. N., Abbas, S. Q., Kazi, M., Khan, S. A., Sadiq, A., Hassan, S. S. u., Bungau, S., & Jalal, K. (2022).** Integrated bioinformatics-based subtractive genomics approach to decipher the therapeutic drug target and its possible intervention against brucellosis. Bioengineering, 9(11), 633. https://pmc.ncbi.nlm.nih.gov/articles/PMC9687753/pdf/bioengineering-09-00633.pdf

32. **Lasserre, M., Fresia, P., Greif, G., Iraola, G., Castro-Ramos, M., Juambeltz, A., Nuñez, Á., Naya, H., Robello, C., & Berná, L. (2018).** Whole genome sequencing of the monomorphic pathogen Mycobacterium bovis reveals local differentiation of cattle clinical isolates. Bmc Genomics, 19, 1-14.

33. **Li, W.-H., Gojobori, T., & Nei, M. (1981).** Pseudogenes as a paradigm of neutral evolution. Nature, 292(5820), 237-239. https://www.nature.com/articles/292237a0.pdf

34. **Long, S. W., Olsen, R. J., Eagar, T. N., Beres, S. B., Zhao, P., Davis, J. J., Brettin, T., Xia, F., & Musser, J. M. (2017).** Population genomic analysis of 1,777 extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates, Houston, Texas: unexpected abundance of clonal group 307. MBio, 8(3), 10.1128/mbio. 00489-00417.

35. **Moens, C., Saegerman, C., Fretin, D., & Marché, S. (2023).** Field evaluation of two commercial serological assays for detecting bovine tuberculosis. Research in Veterinary Science, 159, 125-132. https://orbi.uliege.be/bitstream/2268/304366/1/1-s2.0-S0034528823001042-main.pdf

36. **Mossad, A., Akeila, M., Radwan, G., Samaha, H., Nasr, E., & El-Battawy, E. (2009).** Prevalence of bovine infection with Mycobacterium bovis in some Egyptian governorates.

37. **Munir, S., Mahmood, N., Shahid, S., & Khan, M. I. (2017).** Molecular detection of Isoniazid, Rifampin and Ethambutol resistance to M. tuberculosis and M. bovis in multidrug-resistant tuberculosis (MDR-TB) patients in Pakistan. Microbial Pathogenesis, 110, 262-274.

38. **Nasr, E. A., OR, S., & AM, S. (2019).** Prevalence Of Bovine Tuberculosis In Different Cattle Breeds In Dairy Farm In Egypt. Journal of Applied Veterinary Sciences, 4(2), 45-51.

39. **Nei, M. (1987).** Molecular evolutionary genetics. Columbia university press.

40. **O'Brien, L., Roberts, B., & Andrew, P. (1996).** In vitro interaction of Mycobacterium tuberculosis and macrophages: activation of anti-mycobacterial activity of macrophages and mechanisms of anti-mycobacterial activity. Tuberculosis, 97-130.

41. **Okamoto, S., Tamaru, A., Nakajima, C., Nishimura, K., Tanaka, Y., Tokuyama, S., Suzuki, Y., & Ochi, K. (2007).** Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. Molecular microbiology, 63(4), 1096-1106.

42. **Orloski, K., Robbe-Austerman, S., Stuber, T., Hench, B., & Schoenbaum, M. (2018).** Whole genome sequencing of Mycobacterium bovis isolated from livestock in the United States, 1989–2018. Frontiers in Veterinary Science, 5, 253. https://pmc.ncbi.nlm.nih.gov/articles/PMC6219248/pdf/fvets-05-00253.pdf

43. **Ortega, J., Roy, Á., Álvarez, J., Sánchez-Cesteros, J., Romero, B., Infantes-Lorenzo, J. A., Sáez, J. L., López, M., Domínguez, L., & de Juan, L. (2021).** Effect of the inoculation site of bovine and avian purified protein derivatives (PPDs) on the performance of the intradermal tuberculin test in goats from tuberculosis-free and infected herds. Frontiers in Veterinary Science, 8, 722825. https://pmc.ncbi.nlm.nih.gov/articles/PMC8429842/pdf/fvets-08-722825.pdf

44. **Otchere, I. D., van Tonder, A. J., Asante-Poku, A., Sánchez-Busó, L., Coscollá, M., Osei-Wusu, S., Asare, P., Aboagye, S. Y., Ekuban, S. A., & Yahayah, A. I. (2019).** Molecular epidemiology and whole genome sequencing analysis of clinical Mycobacterium bovis from Ghana. PLoS One, 14(3), e0209395.

45. **Pagán-Ramos, E., Master, S. S., Pritchett, C. L., Reimschuessel, R., Trucksis, M., Timmins, G. S., & Deretic, V. (2006).** Molecular and physiological effects of mycobacterial oxyR inactivation. Journal of bacteriology, 188(7), 2674-2680.

46. **Patane, J. S., Martins Jr, J., Castelao, A. B., Nishibe, C., Montera, L., Bigi, F., Zumárraga, M. J., Cataldi, A. A., Junior, A. F., & Roxo, E.** (2017). Patterns and processes of Mycobacterium bovis evolution revealed by phylogenomic analyses. Genome Biology and Evolution, 9(3), 521-535.

47. **Perea Razo, C. A., Rodriguez Hernandez, E., Ponce, S. I. R., Milian Suazo, F., Robbe-Austerman, S., Stuber, T., & Canto Alarcon, G. J. (2018).** Molecular epidemiology of cattle tuberculosis in Mexico through whole-genome sequencing and spoligotyping. PLoS One, 13(8), e0201981.

https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0201981&type=printable

48. **Piddock, L. J., Williams, K. J., & Ricci, V. (2000).** Accumulation of rifampicin by Mycobacterium aurum, Mycobacterium smegmatis and Mycobacterium tuberculosis. Journal of Antimicrobial Chemotherapy, 45(2), 159-165.

49. **Pir, H., & Yardımcı, H. (2024).** Differentiation of bovine Tuberculosis and Paratuberculosis infections with antemortem diagnostic methods. Etlik Veteriner Mikrobiyoloji Dergisi, 35(1), 8-18.

50. **Price-Carter, M., Brauning, R., De Lisle, G. W., Livingstone, P., Neill, M., Sinclair, J., Paterson, B., Atkinson, G., Knowles, G., & Crews, K. (2018).** Whole genome sequencing for determining the source of Mycobacterium bovis infections in livestock herds and wildlife in New Zealand. Frontiers in Veterinary Science, 5, 272.

51. **Ramaswamy, S. V., Amin, A. G., Göksel, S., Stager, C. E., Dou, S.-J., El Sahly, H., Moghazeh, S. L., Kreiswirth, B. N., & Musser, J. M. (2000).** Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy, 44(2), 326-336. https://pmc.ncbi.nlm.nih.gov/articles/PMC89679/pdf/ac000326.pdf

52. **Safi, H., Lingaraju, S., Amin, A., Kim, S., Jones, M., Holmes, M., McNeil, M., Peterson, S. N., Chatterjee, D., & Fleischmann, R. (2013).** Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations in decaprenylphosphoryl-β-D-arabinose biosynthetic and utilization pathway genes. Nature genetics, 45(10), 1190-1197.

53. **Sekiguchi, J.-i., Miyoshi-Akiyama, T., Augustynowicz-Kopec, E., Zwolska, Z., Kirikae, F., Toyota, E., Kobayashi, I., Morita, K., Kudo, K., & Kato, S. (2007).** Detection of multidrug resistance in Mycobacterium tuberculosis. Journal of Clinical Microbiology, 45(1), 179-192. https://pmc.ncbi.nlm.nih.gov/articles/PMC1828975/pdf/0750-06.pdf

54. **Singhla, T., Boonyayatra, S., Sthitmatee, N., Sirimalaisuwan, A., Maicharoen, N., Meemey, A., Muenthaisong, A., Rittipornlertrak, A., & Sreevatsan, S. (2024).** An ELISA test using a circulating Mycobacterium bovis peptide for detecting bovine tuberculosis in dairy cattle. Frontiers in Veterinary Science, 11, 1384537. https://pmc.ncbi.nlm.nih.gov/articles/PMC11150843/pdf/fvets-11-1384537.pdf

55. **Starks, A. M., Gumusboga, A., Plikaytis, B. B., Shinnick, T. M., & Posey, J. E. (2009).** Mutations at embB codon 306 are an important molecular indicator of ethambutol resistance in Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy, 53(3), 1061-1066. https://pmc.ncbi.nlm.nih.gov/articles/PMC2650568/pdf/1357-08.pdf

56. **Thomas, J., Infantes-Lorenzo, J. A., Moreno, I., Romero, B., Garrido, J. M., Juste, R., Domínguez, M., Domínguez, L., Gortazar, C., & Risalde, M. (2019).** A new test to detect antibodies against Mycobacterium tuberculosis complex in red deer serum. The Veterinary Journal, 244, 98-103. https://www.sciencedirect.com/science/article/abs/pii/S1090023318307822?via%3Dihub

57. **Vázquez-Chacón, C. A., Rodríguez-Gaxiola, F. d. J., López-Carrera, C. F., Cruz-Rivera, M., Martínez-Guarneros, A., Parra-Unda, R., Arámbula-Meraz, E., Fonseca-Coronado, S., Vaughan, G., & López-Durán, P. A. (2021).** Identification of drug resistance mutations among Mycobacterium bovis lineages in the Americas. PLoS Neglected Tropical Diseases, 15(2), e0009145. https://journals.plos.org/plosntds/article/file?id=10.1371/journal.pntd.0009145&type=printable

58. **Wang, S., Wang, W., Sun, K., Bateer, H., & Zhao, X. (2020).** Comparative genomic analysis between newly sequenced Brucella abortus vaccine strain A19 and another Brucella abortus vaccine S19. Genomics, 112(2), 1444-1453. https://www.sciencedirect.com/science/article/pii/S0888754319303301?via%3Dihub

59. **WHO. (2010).** Treatment of tuberculosis: guidelines. World Health Organization.

60. **Zimpel, C. K., Brandão, P. E., de Souza Filho, A. F., De Souza, R. F., Ikuta, C. Y., Ferreira Neto, J. S., Camargo, N. C. S., Heinemann, M. B., & Guimarães, A. M. (2017).** Complete genome sequencing of Mycobacterium bovis SP38 and comparative genomics of Mycobacterium bovis and M. tuberculosis strains. Frontiers in microbiology, 8, 2389.