

Detection of two cases of pulmonary tuberculosis caused by *Mycobacterium bovis* in a major livestock breeding hub in Iran

Noormohamad Mansoori^{1, 2*}, Bagher Pahlavanzadeh³, Shima Hadifar⁴

¹Department of Nursing, Gonbad Kavous School of Nursing, Golestan University of Medical Sciences, Gonbad Kavous, Iran. <https://orcid.org/0000-0002-0485-1166>

²Clinical Research Development Unit (CRDU), Shohada 12 Dey Hospital, Golestan University of Medical Sciences, Gonbad-e Kavous, Iran.

³Research Center for Environmental Contaminants (RCEC), Abadan University of Medical Sciences, Abadan, Iran. <https://orcid.org/0000-0002-4876-1058>

⁴Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran .<https://orcid.org/0000-0003-4702-634X>

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***Corresponding author:**

Noormohamad Mansoori, Ph.D.,

Department of Nursing, Gonbad Kavous School of Nursing, Golestan University of Medical Sciences, Gonbad Kavous, Iran. Clinical Research Development Unit (CRDU), Shohada 12 Dey Hospital, Golestan University of Medical Sciences, Gonbad-e Kavous, Iran. PO Box: 49711-16689, Fax: +98 17 32261175, Tel: +98 17 33551113, Email: noormohamad.mansoori@gmail.com.

ABSTRACT

Introduction: Bovine tuberculosis (bTB), a zoonotic disease mainly caused by *Mycobacterium bovis* (*M. bovis*), presents a considerable global economic and public health challenge. Golestan Province is a major livestock-breeding hub in Iran and a potential high-risk area for *M. bovis* transmission, while the incidence of human tuberculosis in the province remains moderate. This study sought to explore the presence of *M. bovis* in new pulmonary TB cases in Golestan province, Iran. **Materials & Methods:** Clinical specimens were cultured on Lowenstein-Jensen media, and suspected colonies were subjected to conventional biochemical testing. Identified *M. bovis* isolates were genotyped using the standard 24-locus MIRU-VNTR method, and demographic data were obtained from the Iranian TB registry.

Results: Of 165 culture-positive TB cases, two isolates were identified as *M. bovis* by conventional biochemical testing. Subsequent 24-locus MIRU-VNTR analysis generated distinct allelic profiles, both of which were assigned to the *M. bovis* (Bovis) genotype using the TBminer database. Epidemiological data revealed the bTB cases were a 19-year-old Afghan man and a 31-year-old Iranian man with no history of livestock contact.

Conclusions: The detection of two bTB cases in Golestan Province provides preliminary evidence of the circulation of *M. bovis* in this region; however, larger studies are required to determine its epidemiological significance and burden.

Keywords: Golestan province, Iran, MIRU-VNTR, *Mycobacterium bovis*, Tuberculosis, Zoonoses.

1. Introduction

Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* complex (MTBC), remains a formidable global health challenge associated with substantial morbidity and mortality [1]. According to the World Health Organization (WHO) report in 2024, an estimated 10.7 million people fell ill with TB, and 1.23 million died from the disease worldwide [2]. Among MTBC members, *Mycobacterium tuberculosis* (MTB), the main cause of human TB, and *Mycobacterium bovis*, a zoonotic pathogen, are of particular importance [1, 3].

M. bovis, as the causative agent of bovine TB (bTB), imposes high economic losses on livestock farmers and poses a public health threat due to the zoonotic nature of the disease [3]. Cattle are the primary host and reservoir for *M. bovis*, it can be transmitted to humans and other hosts through close contact with infected animals, ingestion of unpasteurized dairy products, and undercooked meat. [4]. In regions with high livestock farming and conventional dairy markets, these transmission routes may be intensified, which raises the risk of bTB. Although bTB is controlled through eradication strategies globally, it continues to be recognized as a public health threat in developing countries [5].

Iran, a country with a moderate TB burden in the WHO Eastern Mediterranean Region, has a national TB incidence rate of 11 per 100,000 population [2]. Golestan Province, located in northern Iran near the Caspian Sea, has a higher TB incidence (20.3 per 100,000 population) [6]. This province is also a major center for livestock farming and dairy products, suggesting a potential high-risk area for bTB transmission.

According to WHO estimates, zoonotic TB accounts for approximately 1.4% of global TB cases (about 140,000 people) and results in about 11,400 deaths [7]. The true burden of bTB among newly diagnosed TB patients is often underestimated, as the causative agent is often indistinguishable from *M. tuberculosis* based on clinical, radiographic, and histopathological

findings [8, 9]. Epidemiological studies are therefore crucial in many aspects, including disease management, identifying the source of infection, and tracking specific isolates of pathogens to understand the distribution of disease within populations [4]. Genotyping methods such as 24-locus Mycobacterial Interspersed Repetitive Units–Variable Number Tandem Repeats (MIRU-VNTR) provide a reliable tool for determining genotypes and the transmission dynamics of MTBC isolates [10]. However, data on the molecular epidemiology of bTB in new human pulmonary TB cases in Golestan Province with high livestock activity is still limited. Therefore, this study aimed to characterize *M. bovis* isolates among new pulmonary TB cases in Golestan Province, Iran, using 24-locus MIRU-VNTR typing and to investigate their epidemiological characteristics.

2. Materials and Methods:

2.1. Data and specimen collection

From July 2024 to July 2025, a cross-sectional study of new pulmonary TB cases in Golestan Province, Iran, were recovered 165 mycobacterial isolates. These isolates grown on Löwenstein–Jensen (LJ) medium then subjected to conventional biochemical tests; colony morphology, niacin production, catalase activity, and nitrate reduction, to preliminarily identify *M. bovis* isolates [11]. Isolates identified as *M. bovis* by conventional biochemical testing were subsequently genotyped using the standard 24-locus MIRU-VNTR method. Demographic characteristics were obtained from the Iranian TB registry program.

2.2. Genomic DNA preparation, and 24-locus MIRU-VNTR typing

Genomic DNA was extracted from *M. bovis* isolates [12]. Genotyping was performed using the standard 24-locus MIRU-VNTR typing method, followed by amplicon size determination via agarose gel electrophoresis [13]. After 24-locus MIRU-VNTR typing, the allelic profiles obtained for each isolate were entered into the TBminer database. The database was used to

compare the MIRU-VNTR profiles with reference genotypes and assign the corresponding genotype. Repeat unit sizes were determined using a 100-bp DNA ladder (CinnaGen, Iran). *M. tuberculosis* H37Rv and *M. bovis* BCG were used as positive controls, and deionized water as a negative control. MIRU-VNTR profiles was analyzed using the TBminer database (<http://info-demo.lirmm.fr/tbminer/>) [14].

3. Results

3.1. *M. bovis* isolate identification

Of 165 specimens with mycobacterial growth in LJ culture media, two isolates (1.2%) were identified as *M. bovis* based on colony morphology and biochemical reactions.

3.2. 24-locus MIRU-VNTR typing

The two *M. bovis* isolates exhibited distinct 24-locus MIRU-VNTR profiles. Comparison of these allelic profiles with the TBminer database assigned both isolates to the Bovis genotype (Table 1).

Table 1. Genotypes of *M. bovis* isolates that identified in Golestan Province, Iran using 24 MIRU-VNTR loci.

Loci*	MIRU-2	MIRU-4	MIRU-10	MIRU-16	MIRU-20	MIRU-23	MIRU-24	MIRU-26	MIRU-27	MIRU-31	MIRU-39	MIRU-40	ETR-A	ETR-B	ETR-C	OUB-11h	OUB-26	OUB-4156	Mtub04	Mtub21	Mtub29	Mtub30	Mtub34	Mtub39
Isolate (Code)																								
1 (MB-GOL-01)	2	2	2	3	2	4	2	5	3	3	2	2	6	3	3	4	5	1	2	3	3	4	3	2
2 (MB-GOL-02)	2	1	2	3	2	4	2	5	3	3	2	2	5	5	6	3	5	1	1	1	2	2	3	2
<i>M. tuberculosis</i> H37Rv	2	3	3	2	2	6	1	3	3	3	2	1	3	3	4	5	5	2	2	2	4	2	3	5
<i>M. bovis</i> BCG	2	2	2	3	2	4	2	5	3	3	2	2	5	5	6	3	5	0	0	1	2	2	3	2

* The copy numbers are mentioned for each isolate under the MIRU-VNTR loci.

3.3. Demographic information

Both *M. bovis*-infected patients were male residents of an urban area in Golestan Province with no professional livestock experience. The first case was a 19-year-old Afghan immigrant, and the second was a 31-year-old Iranian patient.

4. Discussion

In previous studies, we showed that Golestan province has a high TB incidence in Iran due to a large influx of immigrants from other provinces and neighbor countries such as Afghanistan [15]. Additionally, its status as a major livestock breeding center in Iran potentially elevates the risk of bTB. Herein, we found that the proportion of bTB among new pulmonary TB cases was 1.2%, consistent with the global estimate of approximately 1% of zoonotic TB [7]. Previous studies in central regions of Iran reported *M. bovis* prevalence among TB cases ranging from 1.3% to 1.9%, aligning with our findings [12, 16, 17], although prevalence varied more widely in northwestern and southeastern Iran (0.0%-7.4%) [18, 19]. However, these prior studies used small sample sizes. This consistency suggests that *M. bovis* contributes to a relatively stable prevalence of bTB cases across different settings, despite geographical variations. *M. bovis* prevalence among bTB cases in Turkey, Algeria, Egypt, the USA, and Canada was 4.3%, 4.1%, 4.5%, 1.5%, and 0.6%, respectively [1, 8, 20-22]. However, these prevalence estimates should be interpreted cautiously, as the studies varied in sample size, study design, populations examined, and methods used to identify *M. bovis*. These differences may partly explain the variation in reported prevalence. Nevertheless, the lower prevalence observed in many developed countries may also reflect the success of long-standing bovine tuberculosis control programs, including routine tuberculin testing and culling of infected animals [5].

In the current study, both *M. bovis*-infected cases were young, suggesting the likelihood of recent *M. bovis* transmission rather than reactivation of latent infection in the region. A 19-

year-old single student (Case-1) from Gonbad Kavous, Golestan Province, Iran, living with his parents and three siblings, was diagnosed with TB. He had no known close contact and no livestock experience. Sputum smear microscopy revealed a 3+ acid-fast bacilli grading, and he was successfully treated for the disease using the standard treatment regimen. Case-2 was a 31-year-old undergraduate bank clerk living in the same city as Case-1. He was diagnosed as a smear-negative TB case with undetectable acid-fast bacilli in sputum microscopy but a positive culture in LJ media, and "completed treatment" using first-line anti-TB drugs. Contact tracing revealed a history of TB in his father, but data on the *Mycobacterium* species in father was unavailable. Given that humans can contract pulmonary TB from inhaling *M. bovis* droplets [1, 21], aerosol transmission of *M. bovis* from father to son is plausible in this case. However, zoonotic exposure cannot be excluded, especially in this endemic Province, where trading of unpasteurized milk and dairy products is a common practice and various transmission routes may coexist.

Regarding MIRU-VNTR patterns of *M. bovis* isolates, they have been investigated using 12, 15, and 24-locus methods in several studies in Iran [4, 16, 18, 23, 24]. However, no clear similarity was found between the MIRU-VNTR profiles of those and the present study, possibly due to localized transmission dynamics and the presence of region-specific genotype patterns. Further investigations with larger sample sizes are crucial to determine the full spectrum of circulating *M. bovis* isolates within the study area.

It should be noted that in this study, we used conventional biochemical methods for the identification and screening of *M. bovis* isolates, which may have limited specificity compared to molecular techniques. Hence, misclassification or underestimation cannot be entirely excluded.

5. Conclusion

Taken together, these findings suggest the possible ongoing circulation of *M. bovis* among bTB cases in this region. It also reveals the potential gaps in current *M. bovis* control measures in the high-risk agricultural and livestock region. Therefore, differentiating *M. bovis* from *M. tuberculosis* using biochemical and molecular methods is crucial in human populations. This study also provide further data on zoonotic transmission of *M. bovis* to humans in Golestan Province, resulting in bTB infection. Given *M. bovis*'s capacity to cause extrapulmonary TB, future research should focus on determining its prevalence in such cases.

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Authors' Contribution

Noormohamad Mansoori: Research design, Data collection, Writing original draft, Bagher Pahlavanzadeh: Methodology and data analysis, Shima Hadifar: Draft revision.

Ethical considerations

This project is vetted by the Ethics Committee of Golestan University of Medical Sciences (IR.GOUMS.REC.1404.526).

Conflict of interest

The authors report no conflict of interest.

Data Availability

All available data have been shared in the manuscript.

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Declaration of generative AI and AI-assisted technologies

During the preparation of this work we did not use AI and AI-assisted technologies.

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