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Biomedicine

Zoonotic Potential of *Mycobacterium tuberculosis* Complex Isolated from Livestock in Georgia

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Abstract. Bovine tuberculosis (bTB) remains an important zoonotic disease with potential implications for public health in Georgia. This study investigated the geographic distribution and zoonotic potential of *Mycobacterium tuberculosis* complex (MTBC) isolated from livestock using integrated pathological, microbiological, molecular, and geospatial approaches. A total of 591 cattle carcasses were examined in 17 slaughterhouses across Georgia. Tuberculosis-like lesions were identified in 184 animals. Bacteriological culture confirmed MTBC infection in 11 samples, while real-time PCR detected three additional positive cases. The overall prevalence of bTB was 1.86%, with regional variability ranging from 0.00% to 3.23%. GIS-based spatial analysis revealed clustering of positive cases in western regions of the country. Statistical evaluation demonstrated a significant positive correlation between regional cattle TB cases and human pulmonary tuberculosis burden ($R = 0.72$, $p = 0.043$), suggesting shared epidemiological risk pathways. The findings confirm the continued circulation of wild-type *Mycobacterium bovis* strains in livestock populations and highlight the importance of coordinated veterinary and public health surveillance. Implementation of integrated One Health monitoring strategies, expanded molecular diagnostics, and regionally targeted control measures are essential to reduce zoonotic transmission risk and strengthen national tuberculosis control efforts in Georgia. © *2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: Bovine tuberculosis, *Mycobacterium bovis*, zoonotic tuberculosis, One Health, Georgia

Introduction

Worldwide, tuberculosis (TB) is a major animal and human health concern (World Health Organization, 2023; World Organization for Animal Health, 2021). There are nine known species in the *Mycobacterium tuberculosis* complex (MTBC) that cause TB in various animal species with *M. bovis* and *M. orygis* being the primary agents of animal and *M. tuberculosis* (M.tb) the primary cause of human TB (Rosenthal et al., 2017).

The human EPTB rates worldwide are about 16% of all TB cases, while in rural developing regions, the rates are well over 50% (Olea-Popelka et al., 2017). A systemic review of risk factors for culture-confirmed EPTB cases in rural areas of the world endemic for TB identified contact with patients with PTB, contact with live animals, consumption of raw milk, HIV-positive status, male sex, and low socio-economic status as independent factors (Diriba et al., 2022; Luciano et al., 2020). Thus, the absence of effective monitoring and surveillance programs for zTB – including improper sampling methods, coupled with limited laboratory capacity and improper culturing methods for *M. bovis* and *M. orygis* – leads to underdiagnoses and the subsequent mismanagement of this disease (Thoen et al., 2006; Müller et al., 2013; Dean et al., 2018). Thus, without a real understanding of the burden of zTB, control strategies at the human-livestock-widlife interface will be impossible (Rahim et. al., 2017; Häsler et. al. 2013).

Current status in Georgia. Despite improvements in national TB control indicators, tuberculosis remains a major public health challenge in Georgia. Recent epidemiological data indicate that TB incidence declined from approximately 65.8 per 100,000 population in 2019 to about 55 per 100,000 by 2023; however, these values remain substantially higher than in most industrialized countries (National Center for Disease Control and Public Health, 2020, 2024; World Health Organization, 2024; United Nations Development Programme, 2022).

Bovine tuberculosis. Bovine tuberculosis has been officially registered in Georgia since the mid-20th century, initially confined to the Kvemo Kartli and Javakheti regions. During 1953-1965, bTB was reported in 73 collective farm locations, followed by a substantial reduction by 1972. However, renewed expansion was documented in 1984 across 19 districts. After largescale livestock privatization in the 1990s, the epidemiological situation deteriorated due to the discontinuation of systematic diagnostic and preventive control programs (Tsitskishvili et al., 2019).

Recent findings from Project ISTC G-2312 (2017–2019) confirm persistent transmission. Examination of 1,700 slaughtered animals identified 429 suspected cases, with *Mycobacterium bovis* detected in all regions except Tbilisi. Regional prevalence ranged from 3.23% in Mtskheta-Mtianeti to 5.32% in Ajara-Guria, demonstrating that bTB remains a significant animal and public health challenge in Georgia (International Science and Technology Center, 2019; Makaradze et al., 2019).

Materials and Methods

Study population and design. This study was conducted in officially operating slaughterhouses located across multiple regions of Georgia. A cross-sectional, one-stage sampling design was applied, targeting potential zTB reservoir hosts, including livestock (cattle, sheep, and goats). Sampling intensity was determined based on district-level representativeness. All biological samples were collected exclusively from legally slaughtered animals.

On-site examination and sample collection. For each animal, standardized epidemiological data were recorded, including identification number, age category, sex, production class, geographic origin (district and GPS coordinates), and body condition score. Trained veterinarians performed ante-mortem inspection and systematic post-mortem examination for tuberculosis-like lesions (TBLL).

Post-mortem assessment included inspection and incision of major lymph nodes (head, thoracic, abdominal, and carcass groups) and examination of lungs and major organs. Suspected TBLL tissues were collected aseptically and transported at 4°C to the laboratory within 48-72 h or stored at –20°C prior to analysis.

Pathology and histopathology assessment. Gross pathological assessment was conducted using stan-

dardized lesion scoring systems. Lung lesions were graded from 0 (no lesions) to 5 (>200 lesions), while lymph node involvement was classified according to lesion number and size using species-specific criteria (Parlane et al., 2014; Corner et al., 2012; Ballesteros et al., 2009). Histopathological evaluation was performed on H&E-stained sections, with granulomas categorized into four stages (I-IV) based on necrosis, fibrosis, and mineralization. The highest lesion stage per lymph node was used to calculate cumulative histopathological scores (Wangoo et al., 2005).

Bacteriological and molecular diagnostics. All collected tissue samples underwent bacteriological culture regardless of the presence or absence of visible TB-like lesions. Samples from each animal were pooled, homogenized, decontaminated using hexadecylpyridinium chloride, centrifuged, and inoculated onto multiple solid culture media, including Lowenstein- Jensen with pyruvate, Stonebrink, Coletsos, and Middlebrook 7H11. Cultures were incubated at 37°C and monitored for mycobacterial growth for up to 12 weeks (Corner & Trajstman, 1988; Corner et al., 2012).

Molecular identification of *Mycobacterium tuberculosis* complex (MTBC) organisms was performed using PCR amplification targeting 16S rRNA and IS6110 sequences. Species differentiation was conducted using region-specific genetic markers. Spoligotyping was applied for strain classification and epidemiological clustering of MTBC isolates (Howard et al., 1998; Huard et al., 2003; Hashimoto et al., 1995).

Data analysis and spatial mapping. All collected data were entered into Microsoft Access and subsequently transferred to SAS version 9.2 and ArcGIS version 10.2 for statistical and spatial analysis. Livestock TB prevalence was calculated as proportions with corresponding 95% confidence intervals. Multivariate logistic regression models were applied to identify risk factors associated with TB infection status.

Associations between livestock and human TB incidence at regional and district levels were assessed using multivariate regression and spatial autocorrelation analyses. Geographic information system (GIS) tools were applied to generate spatial risk maps and identify potential transmission hotspots.

Results and Discussion

A total of 591 cattle carcasses were examined at slaughterhouses across the country during the study period. The selection of abattoirs was conducted using a random sampling approach to ensure representative coverage. Carcasses showing macroscopic lesions or suspicious findings were referred for additional diagnostic testing using microbiological and PCR methods. Among these, 11 cases tested positive by microbiology, and 3 were confirmed positive by PCR. The diagnostic agreement between microbiology and PCR was found to be moderate, with a kappa coefficient of 0.55, reflecting partial consistency between the two testing approaches.

Table 1. Number of examined animals and rate of positive cases by regions

Region	Investigated	Positives cases	Positive cases (%)
Ajara-Guria	93	3	3.23
Racha-Imereti	101	2	1.98
Javakheti	25	0	0.0
Kakheti	22	0	0.0
Kvemo Kartli	43	1	2.33
Mtskheta-Mtianeti	101	2	1.98
Samegrelo	103	2	1.94
Shida Kartli	102	1	0.98
Tbilisi	1	0	0.0
Total	**591**	**11**	**1.86**

Table 1 summarizes the number of examined animals and the rate of bTB-positive cases across regions. The overall positivity rate across all surveyed regions was 1.86% (11 positive cases out of 591 tested animals). Individual regional rates

ranged from 0.00% to 3.23%, with the highest observed in Ajara-Guria (3.23%). However, confidence intervals (95%) for each region's positivity rate were wide and substantially overlapping, due primarily to the low number of positive cases and limited sample sizes in several areas (Fig. 1). GIS-based spatial analysis revealed a clear clustering pattern of bTB-positive cases in the western regions of Georgia, with the highest intensity observed in the Ajara-Guria and Racha-Imereti regions. Central and eastern regions showed lower estimated densities, indicating spatial heterogeneity in disease occurrence and suggesting region-specific epidemiological risk factors (Fig. 2).

Notably, Javakheti, Kakheti, and Tbilisi reported zero positive cases, yet due to small sample sizes (n=25, 22, and 1, respectively), the statistical uncertainty is too high to conclude the true absence of disease. For instance, Kakheti had a confidence interval extending up to 14.87%, despite reporting no positives. Even regions with more robust sample sizes, such as Samegrelo and Mtskheta-Mtianeti, exhibited wide intervals (e.g., Racha-Imereti: 0.54%–6.93%). No statistically significant differences were found between regions when tested with a chi-square test (p = 0.968), confirming that the variations observed are likely due to random sampling variability rather than true geographic differences in prevalence.

Correlation of tuberculosis in livestock and humans. The analysis of the correlation between human tuberculosis (TB) burden and cattle TB cases across eight regions of Georgia, using absolute numbers for both human and cattle cases to ensure a consistent and fair comparison, focuses on pulmonary and non-pulmonary TB cases, as well as total human TB burden in relation to the absolute number of cattle TB cases detected regionally.

Table 2. Regional data: human and cattle TB cases (2024)

Region	Human pulmo cases	Human non-pulmo cases	Total human cases	Cattle TB cases
Ajara-Guria	586	21	607	3
Racha-Imereti	315	14	329	2
Javakheti	23	6	29	0
Kakheti	145	10	155	0
Kvemo Kartli	210	15	225	1
Mtskheta-Mtianeti	46	0	46	2
Samegrelo	227	7	234	2
Shida Kartli	98	3	101	1
Total	**1.650**	**76**	**1.726**	**11**

Note: The analysis was based on absolute numbers of human and cattle tuberculosis cases reported in 2024 to ensure consistent regional comparison. Tbilisi was excluded due to its predominantly urban setting and negligible cattle population, which could bias the livestock-human TB association.

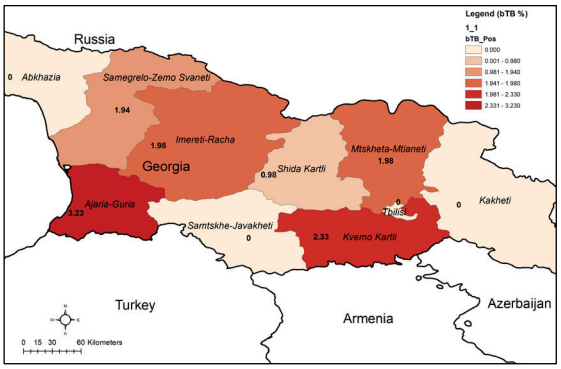

Fig. 1. Map showing the regional bTB-positive cases (%).

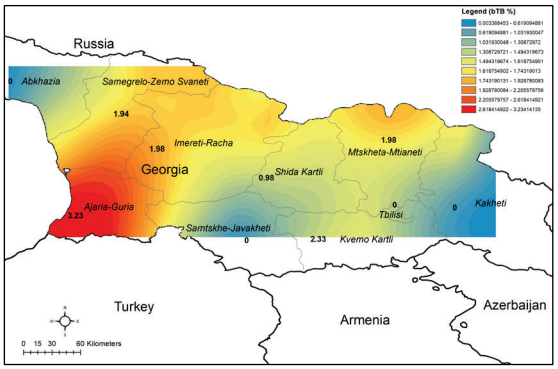

Fig. 2. Map showing clustering of bTB- distribution of positive cases in Georgia (Kernel density estimation).

The Table summarizes the distribution of pulmonary and non-pulmonary human TB cases alongside the absolute number of detected cattle TB cases. Regions like Ajara-Guria, Racha-Imereti, and Samegrelo demonstrate higher human TB burdens with corresponding cattle TB cases, suggesting a potential epidemiological link.

Table 3. Results of correlation between human TB cases and cattle TB cases

Analysis Type	Pearson R	p-value
Human Pulmo cases vs cattle TB cases	+0.72	0.043
Human Non-Pulmo cases vs cattle TB cases	+0.36	0.380
Total human cases vs cattle TB cases	+0.71	0.048

Statistical analysis revealed strong positive and statistically significant correlations between human pulmonary TB cases and cattle TB cases (Pearson $R = 0.72$, $p = 0.043$), as well as between total human TB cases and cattle TB cases (Pearson $R = 0.71$, $p = 0.048$). The correlation for non-pulmonary TB cases was weaker and not statistically significant (Pearson $R = 0.36$, $p = 0.380$).

Conclusion

This study provides new evidence on the circulation and zoonotic potential of *Mycobacterium tuberculosis* complex (MTBC) strains in livestock populations in Georgia. The detection of culture-confirmed and PCR-positive bovine tuberculosis (bTB) cases across multiple regions, combined with GIS-based spatial analysis, demonstrates ongoing transmission of wild-type *Mycobacterium bovis* in cattle. Although the overall prevalence observed in slaughterhouse surveillance was relatively low (1.86%), the wide confidence intervals and heterogeneous regional sampling highlight persistent uncertainty and the need for expanded systematic surveillance.

Importantly, the statistically significant positive correlations between cattle TB cases and human pulmonary TB burden at the regional level indicate shared epidemiological drivers and potential bidirectional transmission pathways at the human-livestock interface. These findings support the hypothesis that zoonotic tuberculosis remains an underrecognized public health concern in Georgia, particularly in western regions with higher human TB incidence and livestock exposure.

The results emphasize the critical importance of integrated One Health approaches that combine veterinary surveillance, public health monitoring, molecular epidemiology, and spatial risk mapping. Strengthening laboratory capacity for MTBC speciation, expanding routine slaughterhouse inspection programs, and implementing coordinated livestock-human TB control strategies are essential for improving early detection and reducing transmission risks. Future studies incorporating longitudinal sampling and whole-genome sequencing will further clarify transmission dynamics and inform evidence-based national control policies.

Overall, this study contributes to a more comprehensive understanding of zoonotic TB epidemiology in Georgia and provides a scientific foundation for strengthening cross-sectoral disease prevention and control efforts.

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ბიომედიცინა

საქართველოში შინაური ცხოველებიდან იზოლირებული *Mycobacterium tuberculosis* კომპლექსის შტამების ზოონოზური პოტენციალი

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მსხვილფეხა რქოსანი პირუტყვის ტუბერკულოზი (bTB) საქართველოში მნიშვნელოვან ზოონოზურ დაავადებად რჩება და წარმოადგენს რისკს საზოგადოებრივი ჯანმრთელობისთვის. კვლევაში შეფასებულია შინაური ცხოველებიდან გამოყოფილი *Mycobacterium tuberculosis* კომპლექსის (MTBC) შტამების გეოგრაფიული გავრცელება და ზოონოზური პოტენციალი. საქართველოს მასშტაბით მოქმედ 17 სასაკლაოზე გამოკვლეულ იქნა 591 მსხვილფეხა რქოსანი პირუტყვის ნაკლავი. ტუბერკულოზის მსგავსი დაზიანებები გამოვლინდა 184 ცხოველში. ბაქტერიოლოგიურმა კვლევამ MTBC ინფექცია დაადასტურა 11 ნიმუშში, ხოლო რეალურ დროში PCR მეთოდით გამოვლინდა 3 დადებითი შემთხვევა. bTB-ის საერთო გავრცელებამ შეადგინა 1,86%, რეგიონალური ცვალებადობით კი – 0,00%-დან 3,23%-მდე. GIS სივრცულმა ანალიზმა აჩვენა დადებითი შემთხვევების კლასტერიზაცია საქართველოს დასავლეთ რეგიონებში. სტატისტიკურმა ანალიზმა გამოავლინა მნიშვნელოვანი დადებითი კორელაცია მსხვილფეხა რქოსანი პირუტყვის ტუბერკულოზის რეგიონულ მაჩვენებლებსა და ადამიანის ფილტვის ტუბერკულოზს შორის ($R = 0,72$, $p = 0,043$), რაც მიუთითებს საერთო ეპიდემიოლოგიურ რისკ-ფაქტორებსა და გადაცემის პოტენციურ გზებზე. მიღებული შედეგები ადასტურებს *Mycobacterium bovis*-ის შტამების ცირკულაციას პირუტყვის პოპულაციებში და ხაზს უსვამს ვეტერინარული და საზოგადოებრივი ჯანმრთელობის სექტორებს შორის კოორდინირებული მეთვალყურეობის აუცილებლობას. მნიშვნელოვანია ინტეგრირებული „ერთიანი ჯანმრთელობის“ (One Health) მიდგომების დანერგვა, რეგიონულად მიზნობრივი მეთვალყურეობის სისტემების გაძლიერება და კოორდინირებული კონტროლის ღონისძიებების განხორციელება, რათა შემცირდეს ზოონოზური გადაცემის რისკი და გაძლიერდეს ტუბერკულოზის კონტროლის ეროვნული სისტემა.

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