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# Prevalence of Bovine Tuberculosis and Its Risk Factors in Georgia

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A B S T R A C T

The main goal of this study was twofold: 1) to assess regional distribution of bovine tuberculosis (bTB) in domestic cattle in Georgia; and 2) to describe the disease risk factors in the country. Study sample and methods: Randomly selected cattle carcasses were examined in all operational slaughterhouses. The information on cattle age, sex, breed, and origin were obtained from the national registry. Post-mortem sanitary inspection of carcasses was done for all randomly selected cases. In case of macroscopic changes, samples from internal organs and lymphatic nodes were collected for further histopathological and microbiological examination. Geographical coordinates for all participating slaughterhouses and farms were measured and integrated into GIS system. Descriptive statistical analysis was performed to describe bTB regional distribution in Georgia and stratified risk analysis was done to identify high risk strata for bTB in the country. In total, 2286 carcasses were examined in 36 slaughterhouses nationwide. Out of these, 552 cases were further investigated by histopathological and microbiological methods. Using this hybrid approach (histopathological and microbiological testing), an estimated bovine TB rate is 0.44% (0.36-0.54%) in slaughtered cattle in Georgia. Out of ten regions, only three had bTB cases. The estimated rates were 0.98% (0.27-2.49%), 1.36% (0.28-3.92%), and 1.84% (0.50-4.65%) for Kvemo Kartli, Shida Kartli, and Samstkhe-Javakheti regions respectively. The study revealed that the disease antecedents in the region, cattle female gender and older age (>2years old), are bTB risk-factors in Georgia. Stratified analysis shows that the stratum with the highest composite risk (>2 years old female cattle slaughtered in the region with bTB antecedents) has estimated bTB prevalence - 1.61%.

***Keywords:*** Cattle, Zoonosis, Bovine Tuberculosis, Risk factors, Pathology Assessment, Cross-sectional study.

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## Introduction

Natural milk production has been the most urGeorgia has one of the highest rates of human tuberculosis (TB) (156 per 100,000 WHO) in Eastern Europe. This rate is nearly three times higher than the regional average. Although TB is one of the major health priorities in Georgia, a rate of newly identified cases a year (88 per 100,000) remains unacceptably high. For comparison, the rate of all TB cases in industrialized countries is 23 per 100,000.

It is estimated that about 2 % of all TB cases and 10 % of all non-respiratory TB cases in humans have bTB origin in developing countries. The routes of bovine TB transmission from cattle to humans include direct exposure to infected cattle or consumption of contaminated animal products.

First cases of bTB have been recorded officially in two regions of Georgia (Samstkhe-Javakheti and Kvemo Kartli) around the mid-twentieth century. The following nationwide epidemiological assessment was conducted in 1953-1965 and revealed bTB cases in 73 farms of the country. As a result of bTB eradication measures by 1972, the number was reduced to 15 farms in five districts of the country. Then, the number of affected areas increased again, and bTB cases were recorded in 19 districts of the country in 1980-1985 (See Figure 1). The last known bTB study in Georgia showed multi-drug resistance of bovine mycobacteria strains isolated from carcasses of infected animals [1].

## Materials and Methods

**Study Design:** This was exploratory cross-sectional study in which randomly selected cattle carcasses were examined in all concurrently operational slaughterhouses. The information on cattle age, sex, breed, and origin were obtained from the national registry. Post-mortem sanitary inspection of carcasses was performed for all randomly selected cases. In case of macroscopic changes, samples of select internal organs and lymphatic nodes were collected for further histopathological and microbiological examination. Geographical coordinates of all participating slaughterhouses and farms were measured and integrated into GIS system.

**Bovine TB Diagnosis:** Pathology Assessment was done in accordance of Parlane, et al., 2014: I) lung lesion score was calculated by counting the total number of lesions as follows: 0 - no lesions, 1 - 1–9 lesions, 2- 10–29 lesions, 3 - 30–99 lesions, 4 - 100–199 lesions, 5 >200 lesions;II) A total lymph node lesion score per animal was calculated by pooling scores for four pulmonary lymph nodes (left and right bronchial and anterior and posterior mediastinal). Scores for individual lymph nodes was estimated as follows: 0 - no lesions, 1 - 1–19 small lesions (1–4 mm diameter), 2 ->20 small lesions (1–4 mm diameter) or medium size lesion(s) (5–9 mm diameter), 3 - large lesion(s) (>10 mm diameter) [2-4].

For histological examination sections were hematoxylin and eosin stained. Scoring of histopathological lesions in the lymph nodes was based on the scale described by Wangoo, et. al., 2005: Stage 0 - no granulomas are observed; Stage I - granulomas are composed of accumulations of epithelioid macrophages with low numbers of lymphocytes,neutrophils and Langerhans multinucleated giant cells and an absence of necrosis; Stage II - granulomas are similar to Stage I granulomas but also have central infiltrates of neutrophils and lymphocytes and necrosis could be present, Stage III granulomas exhibit complete fibrous encapsulation and significant necrosis and mineralization can be present; Stage IV - granulomas are characterized by multiple coalescing caseo-necrotic granulomas with multi-centric necrosis and mineralization. The histopathological score was based on the most severe lesion observed in each pulmonary lymph node section with scores ranging from 0 to 4, corresponding to Stages 0 to IV, respectively. A total histopathological score was compiled by pooling scores for each of the four pulmonary lymph nodes. Scoring of gross and histopathological lesions was assigned without knowledge of the slaughterhouse source [5].

Tissue and lymph node samples (mostly head and cervical lymph nodes) from every bTB suspected animal was subjected to bacterial isolation. Samples from all tissues were pooled, homogenized with sterile distilled water and decontaminated with 0.35% hexadecylpyridinium chloride for 30 minutes [2], centrifuged at 1,300 X g for 30 minutes and cultured onto Coletsos and 0.2% (w/v) pyruvate-enriched Lowenstein-Jensen media (Bio-Rad, Carlsbad, CA, USA) at $37^0$C. [3] Isolates wasassessed by determining traditional cultural and biochemical properties for Bovine Mycobacteria.

The primary outcome measure – positive bTB case - was defined as bovine TB characteristic changes by both histopathological and microbiological assessments.

**Statistical Analysis:** Proportions (%) of the randomly selected carcasses and of cases that were selected for further histopathological and bacteriological examination were computed for each region. Bovine TB mean rates and its 90% confidence intervals were estimated for each region by using Clopper-Pearson (exact) and Wilson tests. Bivariate relationship between bovine TB and select risk factors (age, gender, breed, and origin) as well as variation of the disease rates across regions for each risk factor were examined by chi-squire tests. All risk factors that were potentially associated ($p<0.20$) with primary outcome were used in stratified analysis. The later was performed to determine high risk strata of bTB in Georgia.

Statistical analyses were done in SAS 9.2 and all reported results are two-sided at 5% significance level.

## Results

During the study period, 322,074 cattle were slaughtered in ten regions of Georgia (more than 70% of these in four regions of the country: Imereti, Kakheti, Kvemo Kartli, and Shida Kartli). Although the rates were proportional to overall domestic cattle population in the regions, the numbers of the slaughtered animals varied from 1873 (Racha) to 70494 (Imereti) across the regions (see Table 1). 2286 animal carcasses (0.7% of all slaughtered cattle) were randomly selected from all slaughterhouses that were operational in all ten regions of the country during the study period. Animal ID number, age, gender, breed, and geographic origin information were obtained from the National Registry. The carcasses of all randomly selected animals were then inspected by a trained veterinarian and in the case of macromorphological changes (every 4th or 5th case), internal organ and lymph node samples were further examined by histopathologic and microbiologic methods. The number of the carcasses investigated varied from 41 (Racha) to 467 (Imereti) and exceeded 140 cases for seven regions (Table 1).

**Table 1.** *Frequencies and proportions of investigated animals by region during study period (02/2017-08/2018)*

| Region | Total number of slaughtered animals | Proportion of Cases (Percent of total number of animals slaughtered in the country) | Number of randomly selected carcasses | Proportion of inspected carcasses (Percent of animals slaughtered in the region) |
|---|---|---|---|---|
| Achara | 12574 | 3.9 | 141 | 1.1 |
| Guria | 7766 | 2.4 | 80 | 1.0 |
| Imereti | 70494 | 21.9 | 467 | 0.7 |
| Kakheti | 46643 | 14.5 | 381 | 0.8 |
| KvemoKartli | 52001 | 16.1 | 408 | 0.8 |
| Mtskheta-Mtianeti | 28489 | 8.8 | 102 | 0.4 |
| Racha-Lechkhumi | 1873 | 0.6 | 41 | 2.2 |
| Samtskhe-Javakheti | 6413 | 2.0 | 217 | 3.4 |
| Samegrelo-ZemoSvaneti | 29074 | 9.0 | 228 | 0.8 |
| ShidaKartli | 66747 | 20.7 | 221 | 0.3 |
| Total | 322074 | 100.0 | 2286 | 0.7 |

By applying hybrid histopathologic and microbiological diagnostic approach, out of the 552 examined cases 11 cases were identified to be bTB positive. Therefore, the estimated prevalence and its 95% confidence interval for bovine TB prevalence in cattle slaughtered in Georgia is 0.44% (0.36-0.54%). The rates vary across the regions: no bovine TB case was detected in seven regions of the country and bTB prevalences are 0.98% (0.27-2.49%), 1.36% (0.28-3.92%), and 1.84% (0.50-4.65%) for Kvemo Kartli, Shida Kartli, and Samtskhe-Javakheti, respectively (Table 2).

The distribution of bTB risk factors by region is shown in Table 3. Although most of the slaughtered cattle (79%) were female animals, the rate varied substantially across the regions. The lowest rate, 43.5%, was recorded in Imereti and highest rate, 91.7%, in Samegrelo. The gender difference among regions was statistically significant ($P<0.001$). One explanation of the previous fact might be the import of male cattle from some regions of Georgia to the neighboring countries.

**Table 2.** *Bovine TB prevalence in slaughtered animals by region*

| Region | Number of researched animals | Positive cases (%) | 95% CI* |
|---|---|---|---|
| Achara | 141 | 0.00 | 0.00 -2.58 |
| Guria | 80 | 0.00 | 0.00 -4.51 |
| Imereti | 467 | 0.00 | 0.00 -0.79 |
| Kakheti | 381 | 0.00 | 0.00 -0.96 |
| KvemoKartli | 408 | 0.98 | 0.27 -2.49 |
| Mtskheta-Tianeti | 102 | 0.00 | 0.00 -3.55 |
| Racha-Lechkhumi | 41 | 0.00 | 0.00 -8.60 |
| Samtskhe-Javakheti | 217 | 1.84 | 0.50 -4.65 |
| Samegrelo-ZemoSvaneti | 228 | 0.00 | 0.00 -1.60 |
| ShidaKartli | 221 | 1.36 | 0.28 -3.92 |
| Total | 2286 | 0.44 | 0.36-0.54** |

** Clopper-Pearson (Exact) test was used to compute 95% confidence intervals*
*** Wilson test with weight statement was used to compute 95% confidence intervals (weights were assigned based on ratio of the number of investigated cases in a region to the total number of slaughtered animals nationwide during the study period)*

**Table 3.** *Bovine TB prevalence in slaughtered animals by region*

| Risk factor | For all regions | Achara | Guria | Imereti | Samegrelo-ZemoSvaneti | Racha | Mtskheta-Mtianeti | ShidaKartli | Kakheti | Kvemo-Kartli | Samtskhe-Javakheti | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total | 2286 (100) | 141 (6.2 ) | 80 (3.5 ) | 467 (20.4) | 228 (10.0) | 41 (1.8 ) | 102 (4.5 ) | 221 (9.7 ) | 381 (16.7) | 408 (17.8) | 217 (9.5 ) | |
| Sex | | | | | | | | | | | | <0.001 |
| Male | 480 (21.0) | 43 (30.5) | 11 (13.8) | 203 (43.5) | 19 (8.3 ) | 9 (22.0) | 4 (3.9 ) | 77 (34.8) | 33 (8.7 ) | 62 (15.2) | 19 (8.8 ) | |
| Female | 1806 (79.0) | 98 (69.5) | 69 (86.3) | 264 (56.5) | 209 (91.7) | 32 (78.0) | 98 (96.1) | 144 (65.2) | 348 (91.3) | 346 (84.8) | 198 (91.2) | |
| Age | | | | | | | | | | | | <0.001* |
| <2 years | 332 (14.5) | 57 (40.4) | 16 (20.0) | 83 (17.8) | 4 (1.8 ) | 5 (12.2) | 11 (10.8) | 50 (22.6) | 35 (9.2 ) | 48 (11.8) | 23 (10.6) | |
| 2-4 years | 1145 (50.1) | 68 (48.2) | 17 (21.3) | 287 (61.5) | 98 (43.0) | 18 (43.9) | 55 (53.9) | 146 (66.1) | 68 (17.8) | 242 (59.3) | 146 (67.3) | |
| >4 years | 419 (18.3) | 13 (9.2 ) | 4 (5.0 ) | 97 (20.8) | 36 (15.8) | 18 (43.9) | 36 (35.3) | 25 (11.3) | 51 (13.4) | 91 (22.3) | 48 (22.1) | |
| Unknown | 390 (17.1) | 3 (2.1 ) | 43 (53.8) | 0 (0.0 ) | 90 (39.5) | 0 (0.0 ) | 0 (0.0 ) | 0 (0.0 ) | 227 (59.6) | 27 (6.6 ) | 0 (0.0 ) | |
| Breed | | | | | | | | | | | | |
| Local | 1378 (60.3) | 85 (60.3) | 54 (67.5) | 394 (84.4) | 82 (36.0) | 41 (100 ) | 91 (89.2) | 188 (85.1) | 131 (34.4) | 162 (39.7) | 150 (69.1) | <0.001* |
| Other | 86 (3.8 ) | 22 (15.6) | 0 (0.0 ) | 0 (0.0 ) | 0 (0.0 ) | 0 (0.0 ) | 11 (10.8) | 24 (10.9) | 11 (2.9 ) | 9 (2.2 ) | 9 (4.1 ) | |
| Unknown | 822 (36.0) | 34 (24.1) | 26 (32.5) | 73 (15.6) | 146 (64.0) | 0 (0.0 ) | 0 (0.0 ) | 9 (4.1 ) | 239 (62.7) | 237 (58.1) | 58 (26.7) | |

** Only the cases without Miss/Unknown information was used in testing statistical significance*

In seventeen percent of randomly selected animals, information of the animal age was missing. In the group with no missing age information vast majority (82%) were adult animals (>2 Years old). Across the regions rate of young adult animals (2-4 years old) varied from 44% (Racha and Kakheti) to 71% (Samegrelo), and the range rates of adult animals (>4years old) were 9% (Ajara) and 43% (Racha). Age variation across the regions was statistically significant (P<0.001).

More than one third of the investigated cases had missing/unknown animal breed information. As 94% of all cases without missing breed information were local breed, all other breeds were combined into one group – non-local breed. Also as the exclusion of cases with missing breed information might reduce the analytic sample significantly, the risk factor was used only in univariate and bivariate analysis, and was disregarded in stratified analysis. The overall proportion of local breed animals varied between 79% and 100% across the regions.

Table 4 reports the results of bivariate analysis. Bovine TB antecedents in a region is the most important risk factor for the disease (P=0.002). Additionally, cattle female gender (P<0.10) and adult age (P=0.17) are also associated with the disease. As the disease prevalence is very low and the rates for young adult and adult groups are similar, these two were combined in one adult group (>2 years old) for further analysis.

**Table 4.** *Bovine TB prevalence in slaughtered animals by region*

| Risk Factor | Number of researched animals | positive cases % | P-value |
|---|---|---|---|
| Total | 2286 | 11 (0.48 ) | |
| Sex | | | 0.09 |
| Male | 480 | 0 (0.0 ) | |
| Female | 1806 | 11 (0.61) | |
| Age | | | 0.17** |
| <2 years | 332 | 0 (0.0) | |
| 2-4 years | 1145 | 9 (0.79) | |
| >4 years | 419 | 2 (0.48) | |
| Breed | | | |
| Local | 1378 | 10 (0.73) | 0.65* |
| Other | 86 | 1 (1.16 ) | |
| bTB (Region) | | | 0.002 |
| Yes | 1059 | 11 (0.90) | |
| No | 1227 | 0 (0.0) | |

** Only the cases without missing/Unknown information was used for testing statistical significance*
***P-value derived from test that compares <2 and ≥2 years old animals*

As the low number of the primary outcome did not allow to do a multivariate examination of independent risk factors, stratified risk analysis was performed to identify high risk strata for bTB in the country. Animal gender, age, and bTBhistory of the region were used in the stratified analysis. The reason behind not using animal breed information is explained in a previous paragraph. The study showed that the stratum with the highest composite risk (>2 years old female cattle slaughtered in the region with bTB antecedents) has estimated bTB prevalence - 1.61%.

## Discussion

This study examined bTB regional distribution along with its animal (age, gender, breed) and region (bTB history) level risk-factors in Georgia.

In this study, we found that all bTB cases were diagnosed in adult animals. This agrees to the existing evidence that older animal age is one of the important risk factors of bTB. Studies that were conducted in both developing and developed countries showed that the disease rate is significantly higher in adult animals compared to young ones [6-7]. There are two possible explanations of this association: 1) older animals have high probability to get in contact with the infectious agent source; and 2) bovine TB has long latent period [8].

There is contradictory information of animal gender role in bovine TB infection. While a study found that male gender is a significant risk factor of bTB [9], another study showed that female gender was associated with the disease development [10]. Our study showed that all diseased cases were diagnosed in female cattle. This might be explained by the fact that, in Georgia, female cattle are kept for longer time because of milk production and reproductive purposes. Therefore, the relationship found in our study might be confounded by animal age.

There is evidence that non-local breed cattle have high bTB risk compared to local ones [11]. Due to a high rate of missing breed information and a very low number of animals categorized as non-local breed, we were not able to examine the relationship between cattle breed and bTB infection. However, this should not significantly affect the study findings as the vast majority of the cattle in Georgia belong to the local breed.

This study found that bTB antecedents in the region is the most important risk factor of the infection. Previous studies also showed that the regions with bTB history has a significantly higher risk of the disease outbreak [12,13].

Based on Stratified analysis, this study showed that bTB risk in the highest risk stratum (>2 years old female cattle slaughtered in the region with bTB antecedents) is about 4 times higher than nationwide rate of the disease.

## Future Studies

One of the main limitations of our study was that we were not able to confirm bacterial isolates by using molecular methods. Additionally, limited number of positive bTB cases did not allowed to perform multivariate analysis to examine independent relationship between the disease and its risk factors. This emphasizes the need of using more sensitive and accurate (e.g. PCR) molecular methods in future studies.

## Recommendation

Bovine TB remains a public health problem in Georgia and requires measures to minimize the risk of the disease transmission to humans.

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