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Assessing parallel testing to improve detection of bovine tuberculosis at a slaughterhouse in Lahore, Pakistan

Rubab Maqsood[1], Abdul Rehman[1], Hamad Bin Rashid[2], Farhat Nazir Awan[3], Shakera Sadiq Gill[1], Nimra Arshad[1], Rafia Akram[1], Chanda Jabeen[1], Gulshan Umbreen[1], Mobeen Sarwar[3], Mamoona Chaudhry[1#]

[1]Department of Epidemiology and Public Health, University of Veterinary and Animals Sciences, Lahore, Punjab, Pakistan
[2]Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Provincial Diagnostic Laboratory, Livestock and Dairy Development Department, Punjab, Pakistan

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Abstract

The current study was designed to evaluate the reliability of the interferon-gamma release assay (IGRA) as a screening test compared to intensive post-mortem inspection using agreement analyses. This study also aimed to determine the prevalence and risk factors of bovine tuberculosis (bTB) among bovines presented for slaughter in the largest abattoir in Lahore, Pakistan. After anti-mortem inspection, a total of 102 animals were randomly selected for sample and data collection. Selected animals were slaughtered and a thorough post-mortem examination was done for all carcasses to find TB-like lesions. Blood samples were processed by IGRA. Prevalence estimates were generated and Cohen's Kappa test was done for agreement analyses to compare the reliability of the two tests for bTB diagnosis. A substantial agreement (κ = 0.79) was estimated between the IGRA and intensive post-mortem inspection. The apparent prevalence was computed as 5.88% (95% CI; 2.59–11.97) and the true prevalence was estimated as 3.92% (95% CI; 1.35–9.47). A parallel testing strategy with IGRA and intensive post-mortem inspection is a useful approach for screening bTB.

Keywords: diagnostic test, post-mortem examination, prevalence, test agreement
[#]Corresponding author: mamoona.chaudhry@uvas.edu.pk

Introduction

Bovine tuberculosis is a global, communicable, progressive, and chronic bacterial disease (Ghebremariam *et al.*, 2016) with a resurgent impact on livestock (Didugu *et al.*, 2016). *Mycobacterium bovis* has been reported in many mammalian species but it is the most often isolated mycobacteria sub-species in cattle and is recognized as the primary causative agent for bovine tuberculosis (bTB) (Islam *et al.*, 2020; WOAH, 2022). It has been recognized as a zoonotic pathogen, particularly in the developing world (Diguimbaye-Djaibé *et al.*, 2006). Efforts have been made in developed countries to control bTB by using measures of regular screening, culling of infected herds, and compulsory milk pasteurization (Jajere *et al.*, 2018). Bovine tuberculosis remains a major problem for food animals in developing countries where the test and slaughter policy is not affordable (Anne *et al.*, 2017). According to recent data from various regions of Pakistan, the prevalence of bTB in abattoirs and the field ranges from as high as 12% to as low as 0.5% (Memon *et al.*, 2019).

Diagnostic tests for bTB have limitations in terms of sensitivity and specificity. Although a culture test is considered the gold standard, it can take months to produce results. The most widely-used primary diagnostic test for bTB at the herd level is the tuberculin skin test, but the interferon-gamma (IFNγ) release assay (IGRA) is recommended by the WOAH (2022) as an *in vitro* test that can

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detect positive animals from two weeks post-infection. However, IGRA involves the incubation of whole blood with purified protein derivative (PPD) antigens and then the IFNγ production is measured in the collected plasma, using a capture ELISA method. The Single Comparative Intradermal Skin test (SCIT) and IGRA are immunologic tests and both detect the early cell-mediated immune response in bTB infection (Singhla *et al.*, 2019; Kelly *et al.*, 2022). A post-mortem inspection is a visual inspection process based on examining organs and meat with the naked eye that can lead to missed lesions. The sensitivity and specificity of the detection of lesions during post-mortem examination in abattoirs is often questionable (Woldemariyam *et al*., 2021). For SCIT, 72 h is required to get an inflammatory response at the tuberculin-administered site, which is not practicable in abattoirs because animals cannot be withheld for such a long period. The IGRA test is advantageous over SCIT as logistics for revisits and recapturing of animals is required for measuring the immune response after 72 h. The *in vitro* test has shown promising results as a substitute for the SCIT in a shorter period and also minimizes the operator's errors, which are associated with intradermal inoculation of tuberculin and duration measurement. IGRA is reported to show a higher sensitivity with somewhat lower specificity as compared to the skin test. The drawback associated with IGRA is the time limitation for incubation and stimulation of blood samples (Kelly *et al*., 2022).

Improving bTB surveillance is essential for ongoing efforts to eradicate the disease globally. A parallel testing approach can be used to improve the overall sensitivity of any bTB surveillance method. The IGRA is gaining recognition internationally as a diagnostic test for bTB in combination with other screening tests. Slaughterhouse surveillance through post-mortem meat inspection is an important tool in detecting bTB in bovine herds and is a complementary approach to the live animal skin test program (McKinley *et al*., 2018). The objectives of this study were to calculate the prevalence of bTB at a selected abattoir using a parallel diagnostic testing strategy and to evaluate the reliability of IGRA as a diagnostic test compared to comparatively intensive post-mortem inspection. We also calculated the agreement between the intensive post-mortem inspections and the IGRA for the presence of disease.

Materials and Methods

Lahore (31°32′59″ N; 74°20′37″ E) is the capital and the second most populous city of Pakistan, with growing demands for meat, and there are eight abattoirs (three public and five private) in the Lahore district. These abattoirs were designated as "sentinel sites," and due to logistic and accessibility issues, only the largest public sector abattoir in Lahore was selected for a current study. A cross-sectional survey was carried out from March 2022 to July 2022 in the largest abattoir of Lahore district, Pakistan to detect bovine tuberculosis (bTB) among culled animals (cattle and buffalo) aged 2 years and above, destined for slaughter. A total of 102 bovines (cattle and buffalo) and respective carcass data were selected through a systematic random sampling procedure.

Individual animals in the lairage were allotted an identification number upon antemortem inspection by on-duty abattoir staff. During the antemortem inspection, physical examination and body condition scoring (presence of fat in the pelvic area, the scale was selected from 1 to 5, where 1 indicated lean and 5 obese body condition; Radostits *et al*., 2007) were performed and recorded. The information about the species, age, and gender of each selected animal was also recorded on a predesigned data sheet. All animals were declared fit for slaughter upon antemortem inspection. Whole blood (8 ml) was collected in sodium heparin-coated, individually-labelled vacutainers from the jugular vein of each selected animal and transported under refrigerating conditions to the BSL-2 laboratory at the Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, on the day of collection.

After slaughtering, with the help of an on-duty meat inspector veterinarian, carcasses of all preselected cattle and buffalo were laid open for the routine post-mortem inspection, which involves only visual examination and palpation of the suspected area. After the initial inspection, a more intensive post-mortem inspection was conducted on the organs of preselected cattle and buffalo. This examination involved inspecting and palpating each lobe of the lungs and liver, as well as the tracheobronchial, retropharyngeal, mediastinal, and mesenteric lymph nodes to detect visible and palpable TB-like lesions, such as granulomas or caseous masses (Fig. 1). Organs were also sliced into thin sections to further search for tuberculosis lesions (Pal *et al.*, 2017; Woldemariyam *et al.*, 2021). These observations were recorded by a trained veterinarian on a predesigned data collection sheet.

Upon arrival in the laboratory, each blood sample was divided into three aliquots of 1.5 ml each in 96-well plate. Commercial PPD-A (Avium) and PPD-B were added to aliquot A and B as stimulating antigens to produce IFN-γ in whole blood samples and phosphate buffered saline (PBS) was added to aliquot C as nil antigen control (Azami & Zinsstag 2018). All aliquots were mixed well and placed in an incubator at 37 °C for 16 h. The incubation was performed within 8 h post-collection of blood samples. On completion of incubation, samples were centrifuged at 902.98 × *g* for 5 min and plasma was

harvested in separate sterile Eppendorf tubes using the Eppendorf 5452 Minispin Centrifuge (Marshall Scientific USA).

The plasma from the upper layer was harvested and stored at -20 °C for further analysis. Plasma samples were tested in duplicate with a BOVIGAM™ TB Kit (Applied Biosystems, Foster City, CA) as per manufacturer guidelines. The optical density (OD) of each well was measured at 450 nm wavelength using a BIO-RAD microplate reader. PBS-stimulated plasma OD was subtracted from each corresponding PPD-stimulated sample OD. The OD of bovine PPD (OD_{PPD-B}) stimulated was subtracted from OD of avian PPD (OD_{PPD-A}) stimulated (OD_{PPD-B} - OD_{PPD-A}) and the calculated value ≥ 0.1 was kept as cut off for a positive, whereas for a negative result, the subtracted value would be < 0.1. Blood plasma collected from bovines with an OD_{450} value ≥0.1 were interpreted as positive for bTB.

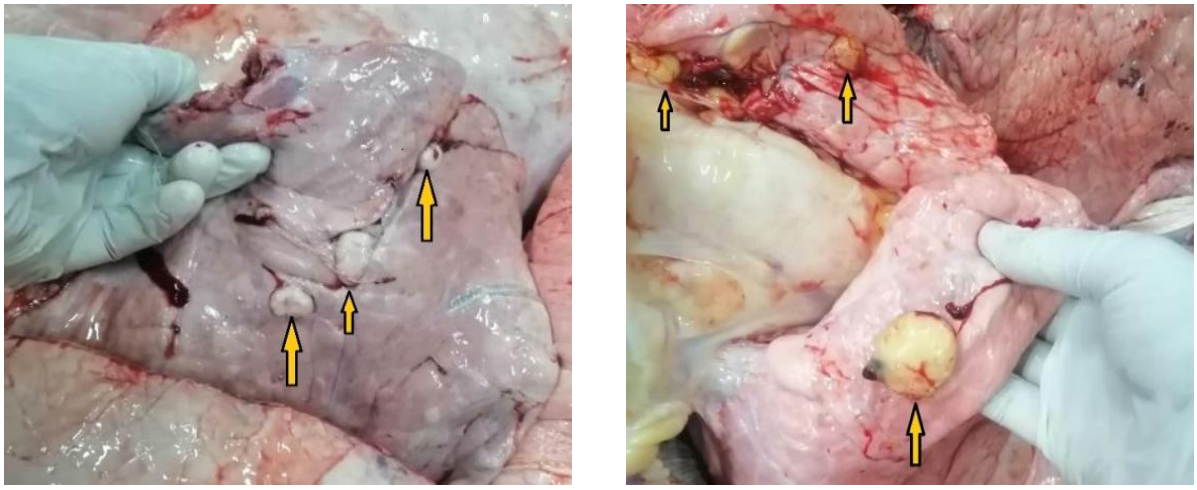

Figure 1 Generalized tuberculosis lesions present on all lobes of lungs of cattle and buffalo in the abattoir

Point prevalence estimates were calculated using the *epi.prev* function and the *prop.test* function in the epiR package in R (version 2.15.1.0) was used to compare the percentages of the reactor and non-reactor animals as determined by IGRA and intensive post-mortem inspection. Furthermore, frequencies of various characteristics of selected animals were calculated and compared to estimate significant differences using Fischer's Exact test. The Spearman rank correlation test was conducted to determine the correlation between various characteristics and IGRA OD values.

To evaluate the agreement between the intensive post-mortem inspection results and IGRA, Cohen's Kappa statistic was used to quantify the accuracy and reliability of the two methods. A positive IGRA result was defined as ≥0.1, whereas the presence of any visible TB-like lesions on post-mortem inspection was considered positive for bTB. Binary codes were assigned to the categorical data for analysis. Cohen's Kappa was interpreted as: 1 = perfect agreement, 0.81–1 = almost perfect agreement, 0.61–0.80 = substantial agreement, 0.41–0.6 = moderate agreement, 0.21–0.4 = fair agreement, 0.01–0.2 = poor agreement, and ≤0 = no agreement (Kelly *et al.*, 2022). The *ggplot2* package was used to create box and regression plots. The Shapiro–Wilk test was used to check the normality of age-related data of sampled animals (W close to 1 indicates normal distribution of data).

Results and Discussion

The study population consisted of cattle (18.6%, n = 19) and buffalos (81.37%, n = 83) aged >2 years. The mean age of sampled animals was 7.05 y with 2 and 12 years minimum and maximum age limits, respectively, and showed a normal distribution with the Shapiro–Wilk test (W = 0.97, *P*-value = 0.067). The intensive post-mortem inspection detected four carcasses with gross visible TB-like lesions out of 102 examined, whereas IGRA detected six samples as positive (OD_{450} >0.1). Out of the four samples found to be positive by IGRA also had TB-like lesions on post-mortem inspection. The prevalence estimate was 5.88% (95% CI; 2.58–11.97) using the IGRA method, whereas the prevalence was estimated as 3.96% (95% CI; 1.0–9.73) using intensive post-mortem inspection (Fig. 2). There was no significant difference in prevalence estimates using both tests (*P* >0.05).

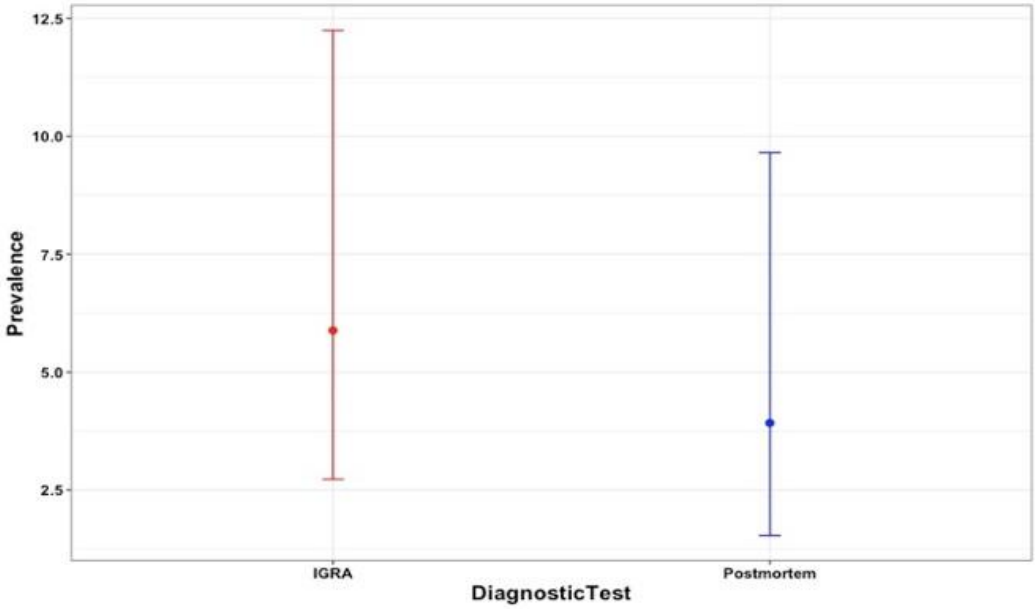

Figure 2 Comparison of prevalence based on interferon-gamma release assay and comprehensive post-mortem inspection of cattle and buffalo for bovine tuberculosis

The majority of the carcasses had multiple granulomas in retropharyngeal lymph nodes (n = 4). The other most affected organs were the lungs and tracheobronchial lymph nodes (n = 3). Mediastinal lymph nodes from two carcasses showed granulomas, and only one carcass had multiple caseous masses on slicing the liver. No TB-like lesions were found in mesenteric lymph nodes. Only two carcasses with multiple tuberculosis lesions on different lobes of the lungs were found throughout the routine postmortem inspection process. On performing a thorough inspection after slicing the organs further, two carcasses were observed with lesions (Table 1).

Table 1 Antemortem test results and distribution of lesions in different organs using two post-mortem inspection approaches

	Routine postmortem inspection						**Intensive postmortem inspection**					
IGRA	Retropharyngeal lymph nodes	Lungs	Tracheobronchial lymph nodes	Mediastinal lymph nodes	Liver	Mesenteric lymph nodes	Retropharyngeal lymph nodes	Lungs	Tracheobronchial lymph nodes	Mediastinal lymph nodes	Liver	Mesenteric lymph nodes
+		+					+	+	+	+	+	
+		+					+	+	+	+		
+							+	+	+			
+							+					
+												
+												

+: positive for interferon-gamma release assay and tuberculosis like lesions

The OD_{450} values of the IGRA test for all tested blood samples were between 0.0548 and 0.5808 with a mean OD_{450} of 0.0331 that was skewed towards the right. The OD_{450} of most of the IGRA-tested positive samples lay in the third quartile, above the median line. A possible outlier below the lower whisker was observed among the IGRA-tested negative samples (Fig. 3). The presence of TB-like post-mortem lesions on the carcass was moderately correlated with IGRA OD_{450} values (P = 0.3242).

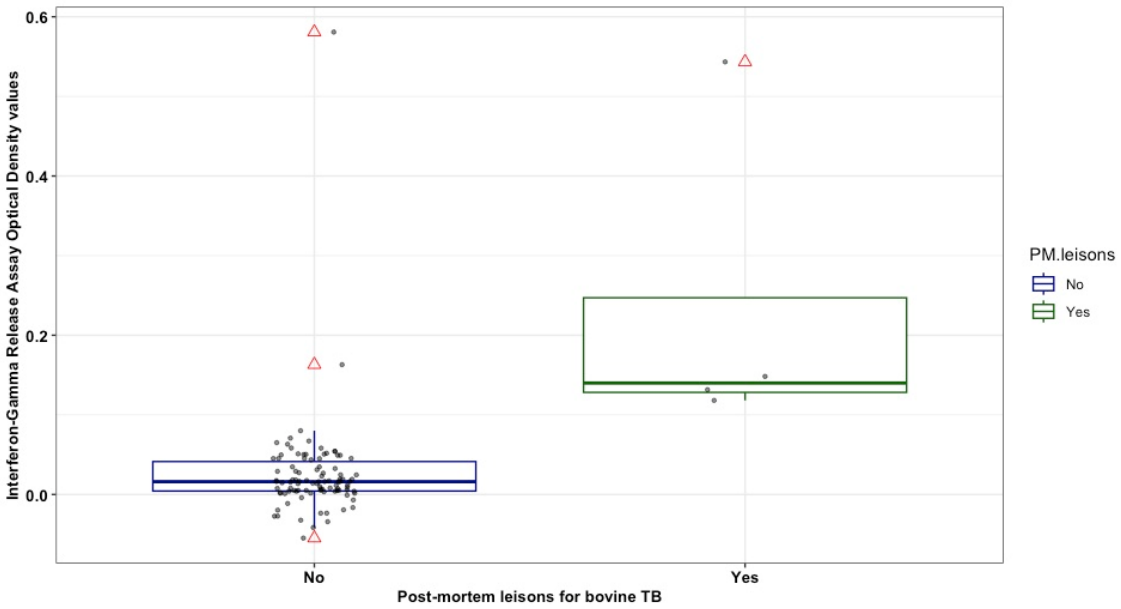

Figure 2 Distribution of optical density (OD_{450}) values among bovine tuberculosis (bTB) positive and negative bovines

In the absence of a gold standard test for the diagnosis of bTB, the carcasses with postmortem lesions were defined as diseased and used as a reference to calculate the sensitivity and specificity of the IGRA for this study. The calculated sensitivity (Se) was 100% and the specificity (Marcos *et al*.) was 98%. Positive predictive value (PPV) and negative predictive value (NPV) were calculated as 66.6% and 100% respectively. In this study, only 2% of tested samples were diagnosed as false positive by the IGRA.

The overall agreement between the IGRA and intensive post-mortem inspection of carcasses was considerably high (98.04%) with Cohen's Kappa value (κ) = 0.79 (95% CI; 0.60–0.979), which indicated substantial agreement between these two tests. A moderate agreement (κ = 0.48; 95%CI; 0.31–0.65) was obtained between the routine postmortem inspection and IGRA test (Table 2).

The parallel diagnostic testing strategy using both IGRA and intensive post-mortem inspection for bTB substantially improved the sensitivity (100%, 6/6) compared to single screening using intensive post-mortem inspection alone (66.7%, 4/6).

Table 2: Agreement between the comparatively intensive post-mortem inspection and IGRA

		Comparatively Intensive Post-mortem Inspection	
		Negative	**Positive**
IGRA	**Negative**	96	0
	Positive	2	4
Overall Agreement		98.04% (93.09–99.76)	
Kappa Value		0.79	

None of the variables (animal type, age, BCS, and milking status) had any association with bTB (P >0.05) upon univariable analysis and further multivariable analysis was not performed. The gender of animals as a risk factor was not analysed due to a zero cell value in the crosstabulation.

Despite the high prevalence of bTB, it is quite challenging to estimate the actual burden in developing countries due to the unavailability of surveillance and active control measures. Many epidemiological aspects of this disease may remain undocumented in such countries (Mekibeb *et al*., 2013). There is a scarcity of data about bTB and very few studies have reported the burden of bTB from abattoirs in Pakistan (Ramanujam *et al*., 2021). The current study aimed to fill this gap and calculated the burden of bTB at a selected abattoir using a parallel diagnostic testing strategy. Additionally, we

evaluated the reliability of IGRA as a screening test and the agreement between IGRA and intensive post-mortem inspection.

The prevalence of bTB in the current study was estimated as 5.88% using a parallel testing strategy compared to 3.96% using intensive post-mortem inspection. Previously, a 6.5% prevalence was reported from abattoirs in the Kohat District, Pakistan (Basit *et al*., 2015) and 5.88 % was reported in a herd of large ruminants from the central zone of Khyber Pakhtunkhwa province (KPK), Pakistan (Ullah *et al*., 2019). Compared to these estimates, different proportions of bTB have been reported from various countries of 5% to 28% (Mekibeb et al. 2013; Srinivasan et al. 2018; Abbate et al. 2020; Zhu et al. 2021). This variation in prevalence might be due to various factors including disease control policy, geographical and temporal differences, farming systems, sampling techniques, tests used for detection and data analysis methods (Borham *et al*., 2022).

In the current study, TB lesions were found most frequently (75%) in the lungs and associated lymph nodes, the presence of lesions in the lungs indicates the transmission of the disease through the respiratory route. These findings are close to 95% (Corner 1994) but substantially higher than 12.3% reported by Pal *et al.* (2017), who found TB lesions in the lungs and their associated lymph nodes on post-mortem examination of cattle.

IGRA and post-mortem inspection can both be used to diagnose bTB. However, they use different methodologies: one measures the cell-mediated immune response, whereas the latter is an observational method. It was difficult to explore the diagnostic reliability of both IGRA and post-mortem inspection in the current study in the absence of a “gold standard” test. We found a moderate correlation between IGRA and the presence of TB-like lesions in the carcasses examined (P = 0.3242).

There was substantial agreement (κ = 0.79) between the results of intensive post-mortem inspection and IGRA. A previous study assessed that only 43% of skin test-positive animals were found with gross lesions on post-mortem examination (O’Hagan *et al*., 2015). Another study observed that 58.3% of IFN-γ positive cattle had gross TB-like lesions on antemortem screening (Okafor *et al*., 2014). In the present study, the high sensitivity (100%) and specificity (98%) were contradictory to a previous study (Singhla *et al*., 2019). IGRA could be a test of choice for screening the animals destined to slaughter and also mass-level screening for bTB.

Results from the present study revealed a 50% probability of missing the carcasses with tuberculosis lesions during routine postmortem inspection as compared to intensive post-mortem inspection at the selected abattoir. A native study in Karachi abattoirs mentioned the lower sensitivity of routine postmortem inspection for the detection of TB-lesioned carcasses (Bhutto *et al*., 2019). Previous findings from Ethiopia suggested a higher probability (94.24%) of missing lesions during routine postmortem inspection (Pal *et al*., 2017). A poor agreement between routine and detailed postmortem inspection was documented earlier by Dechassa (2014). Smaller and embedded TB lesions go unnoticed on brief and posthaste routine inspections. The lower sensitivity of routine postmortem inspection has been attributed to the workload on veterinarian meat inspectors (Pal *et al.*, 2017). A parallel testing approach can be used to improve the overall sensitivity of both tests. Parallel testing can be defined as whether an animal is positive using two simultaneous testing systems.

Conclusions

To our knowledge, this was the first report from the abattoir of the study area that concluded that abattoirs could be used as a sentinel site for surveillance of bTB in Lahore, the most populous district of Punjab province. The data from abattoirs can be used as a proxy for the prevalence estimation of bTB in the general bovine population in resource-limited countries like Pakistan. Combined use of the IGRA test and post-mortem inspection can overcome the inaccuracies in prevalence estimates. Routine postmortem inspection is insufficient to find the TB-lesioned carcasses. The same logic can also be applied to large-scale, field-level screening for disease surveillance and control strategies. The generated baseline data indicated the underlying situation of bTB in the bovine population in the study area, which could be useful for policymakers in devising effective bTB surveillance programs and control measures.

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Author contributions

Conceived and designed the study; MC, RM. Data and sample collection and processing; RM, SSG, RA, NA, MA, MS, CJ, GU. Analysed the data; MC and RM. Supervised the study; MC. Drafted the manuscript; MC, RM, HBR, AR, FNA. All authors carefully reviewed and approved the final draft.

Conflict of interest declaration:

No competing interest was raised by all the co-authors.

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Ethical approval

Office of Research Innovation and Commercialization, University of Veterinary and Animal Sciences, Lahore found the study to comply with scientific and ethical requirements and approved with reference No. DR: 56.

References

Abbate, J.M., Arfuso, F., Iaria, C., Arestia, G. & Lanteri, G., 2020. Prevalence of bovine tuberculosis in slaughtered cattle in Sicily, southern Italy. Animals, 10(9), 1473.

Anne, N.S., Ronald, B.S.M., Kumar, T.S., Kannan, P. & Thangavelu, A., 2017. Molecular identification of *Mycobacterium tuberculosis* in cattle. Vet Microbiol, 198, 81–87.

Azami, H.Y. and Zinsstag, J., 2018. Economics of bovine tuberculosis: A One Health issue. In: Bovine Tuberculosis. CAB International. Wallingford, UK. pp. 31–42.

Basit, A., Hussain, M., Ayaz, S., Shahid, M., Rahim, K., Ahmad, I., Ullah, R., Hashem, A., Abd-Allah, E., Alqarawi, A.A. & Gul, N., 2015. Isolation and identification of *Mycobacterium bovis* and *M. tuberculosis* from animal tissues by conventional and molecular method. Indian J Anim Res, 49(5), 687–693.

Mujeeb-ur-Rahman Memon, A.L., Bhutto, M.G.S., Baloch, J., Leghari, R.A. & Soomro, S.A., 2019. 4. Prevalence and pathological lesions of bovine tuberculosis assessment through routine procedures of meat inspection in infected cattle in Karachi metropolitan corporation abattoirs. Pure Appl. Biol., 8(3), 1909–1918.

Borham, M., Oreiby, A., El-Gedawy, A., Hegazy, Y., Hemedan, A. & Al-Gaabary, M., 2022. Abattoir survey of bovine tuberculosis in Tanta, centre of the Nile delta, with *in silico* analysis of gene mutations and protein–protein interactions of the involved mycobacteria. Transbound. Emerg. Dis., 69(2), 434–450.

Corner, L.A., 1994. Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. Vet. Microbiol., 40(1-2),53–63.

Dechassa, T., 2014. Gross pathological lesions of bovine tuberculosis and efficiency of meat inspection procedure to detect-infected cattle in Adama municipal abattoir. J. Vet. Med. Anim. Health, 6(2),48–53.

Didugu, H., Ramanipushpa, R.N., Narasimha Reddy, C.E., Sagi, S.B.R., Venkateswara Reddy, M., Anitha Devi, M. & Nanda Kishore, K., 2016. Seroprevalence of bovine tuberculosis in Krishna district of Andhra Pradesh, India. Int J Sci Environ Techno, 5(2), 533–536.

Diguimbaye-Djaibé, C., Hilty, M., Ngandolo, R., Mahamat, H.H., Pfyffer, G.E., Baggi, F., Hewinson, G., Tanner, M., Zinsstag, J. & Schelling, E., 2006. *Mycobacterium bovis* isolates from tuberculous lesions in Chadian zebu carcasses. Emerg. Infect. Dis., 12(5), 769.

Ghebremariam, M.K., Rutten, V.P., Vernooij, J.C.M., Uqbazghi, K., Tesfaalem, T., Butsuamlak, T., Idris, A.M., Nielen, M. & Michel, A.L., 2016. Prevalence and risk factors of bovine tuberculosis in dairy cattle in Eritrea. BMC Vet Res., 12, 1–7.

Islam, S.S., Rumi, T.B., Kabir, S.L., van der Zanden, A.G., Kapur, V., Rahman, A.A., Ward, M.P., Bakker, D., Ross, A.G. & Rahim, Z., 2020. Bovine tuberculosis prevalence and risk factors in selected districts of Bangladesh. PLoS One, 15(11), e0241717.

Jajere, S.M., Atsanda, N.N., Bitrus, A.A., Hamisu, T.M. & Goni, M.D., 2018. A retrospective study of bovine tuberculosis at the municipal abattoir of Bauchi State, Northeastern Nigeria. Vet World, 11(5), 598.

Kelly, R.F., Gonzaléz Gordon, L., Egbe, N.F., Freeman, E.J., Mazeri, S., Ngwa, V.N., Tanya, V., Sander, M., Ndip, L., Muwonge, A. & Morgan, K.L., 2022. Bovine tuberculosis antemortem diagnostic test agreement and disagreement in a naturally-infected African cattle population. Front. Vet. Sci., 9, 877534.

Marcos, L.A., Spitzer, E.D., Mahapatra, R., Ma, Y., Halse, T.A., Shea, J., Isabelle, M., Lapierre, P. & Escuyer, V.E., 2017. *Mycobacterium orygis* lymphadenitis in New York, USA. Emerg. Infect. Dis., 23(10), 1749.

McKinley, T.J., Lipschutz-Powell, D., Mitchell, A.P., Wood, J.L. & Conlan, A.J., 2018. Risk factors and variations in detection of new bovine tuberculosis breakdowns via slaughterhouse surveillance in Great Britain. PloS One, 13(6), e0198760.

Mekibeb, A., Fulasa, T.T., Firdessa, R. & Hailu, E., 2013. Prevalence study on bovine tuberculosis and molecular characterization of its causative agents in cattle slaughtered at Addis Ababa municipal abattoir, Central Ethiopia. Trop. Anim. Health Prod., 45, 763–769.

Mujeeb-ur-Rahman Memon, A.L., Bhutto, M.G.S., Baloch, J., Leghari, R.A. & Soomro, S.A., 2019. 4. Prevalence and pathological lesions of bovine tuberculosis assessment through routine procedures of meat inspection in infected cattle in Karachi metropolitan corporation abattoirs. Pure Appl. Biol., 8(3), 1909–1918.

O'Hagan, M.J.H., Courcier, E.A., Drewe, J.A., Gordon, A.W., McNair, J. & Abernethy, D.A., 2015. Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Prev. Vet. Med., 120(3-4), 283–290.

Okafor, C.C., Grooms, D.L., Bolin, S.R., Averill, J.J. & Kaneene, J.B., 2014. Evaluation of the interferon-γ assay on blood collected at exsanguination of cattle under field conditions for surveillance of bovine tuberculosis. Transbound. Emerg. Dis., 61(6), e68–e75.

Pal, M., Zenebe, N., Amare, T. & Woldemariam, T., 2017. An abattoir based study on bovine tuberculosis in Debre Zeit, Ethiopia. World Vet, J., 7(3), 101–107.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. & Constable, P.D., 2007. A textbook of the diseases of cattle, horses, sheep, pigs and goats. Vet. Med., 10, 2045–2050.

Ramanujam, H., Thiruvengadam, K., Singaraj, R. & Palaniyandi, K., 2022. Role of abattoir monitoring in determining the prevalence of bovine tuberculosis: A systematic review and meta-analysis. Transbound. Emerg. Dis., 69(3), 958–973.

Singhla, T., Boonyayatra, S., Chulakasian, S., Lukkana, M., Alvarez, J., Sreevatsan, S. & Wells, S.J., 2019. Determination of the sensitivity and specificity of bovine tuberculosis screening tests in dairy herds in Thailand using a Bayesian approach. BMC Vet. Res., 15, 1–7.

Srinivasan, S., Easterling, L., Rimal, B., Niu, X.M., Conlan, A.J., Dudas, P. & Kapur, V., 2018. Prevalence of bovine tuberculosis in India: a systematic review and meta-analysis. Transbound. Emerg. Dis., 65(6), 1627–1640.

Ullah, A., Khattak, U.S., Ayaz, S., Qureshi, M.S., Khan, I., Jan, I.U., Khattak, I., Taj, R., Nigar, S., Khan, N.U. & Khan, M.A., 2019. Bovine tuberculosis (bTB): Prevalence and associated risk factors in large ruminants in the central zone of Khyber Pakhtunkhwa, Pakistan. Pak. J. Zool., 51(1).

WOAH. 2022. Mammalian tuberculosis (infection with *Mycobacterium tuberculosis* complex). In: OIE Terrestrial Manual. World Organization for Animal Health, France. pp. 22.

Woldemariyam, F.T., Markos, T., Shegu, D., Abdi, K.D. and Paeshuyse, J., 2021. Evaluation of postmortem inspection procedures to diagnose bovine tuberculosis at Debre Birhan municipal abattoir. Animals, 11(9), 2620.

Zhu, X., Yan, Y., Wang, Z., Zhang, K., Chen, Y., Peng, Y., Peng, Q., Guo, A., Robertson, I.D. & Aleri, J., 2021. An abattoir-based study on the prevalence of bovine tuberculosis from culled adult dairy cows in Wuhan, China. Prev. Vet. Med., 196, 105477.