ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 16, Issue, 05, pp.28418-28421, May, 2024
DOI: https://doi.org/10.24941/ijcr.47287.05.2024

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

INVESTIGATION OF TUBERCULOSIS INFECTION IN CATTLE IN TUNISIA USING INTERFERON-Γ TEST

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ARTICLE INFO

Article History:
Received 20th February, 2024
Received in revised form
25th March, 2024
Accepted 14th April, 2024
Published online 30th May, 2024

Key words:
Tuberculosis Infection, Cattle, Tuberculin Skin Test, Interferon Gamma Test, Tunisia.

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ABSTRACT

Bovine tuberculosis TB is a major concern in Tunisia and the Maghreb region, impacting both animal and human health. Despite a national program since 1984, knowledge gaps remain. This study aimed to assess the prevalence of bTB in cattle herds using the Tuberculin Skin Test (TST) and Interferon Gamma Release Assay (IGRA). TST testing revealed a 57% individual prevalence, significantly higher than the 11.72% positivity rate obtained with IGRA. This discrepancy highlights limitations in TST specificity. Our findings suggest the need for wider IGRA adoption alongside TST for a more accurate understanding of bovine TB distribution in Tunisia. Further research is crucial to validate these results, investigate transmission dynamics, and evaluate the economic impact of different diagnostic strategies.

Citation: Imen OUERTANI, Aymen MAMLOUK, Hanen NCIBI and Hichem BOUZGHAIA. 2024. "Investigation of tuberculosis infection in cattle in Tunisia using interferon-γ test*". International Journal of Current Research*, 16, (05), 28418-28421.

INTRODUCTION

Tuberculosis (TB) is a chronic and zoonotic bacterial infection that affects a wide range of animals, including livestock, wildlife, and even household pets(Pollock and Neill, 2002). The disease in cattle is caused by the *Mycobacterium tuberculosis* complex group, mainly *Mycobacterium bovis* but occasionally *M.caprae* and *M. tuberculosis*, which primarily attack the lungs but can also damage other organs. TB usually spreads through the inhalation of bacteria-laden droplets or the consumption of contaminated food water or fomites (Neill *et al.*, 2001). Clinical signs of the disease in animals can include weight loss, coughing, fever, lack of appetite, and lethargy. The control of TB is based on test and slaughter, and on the surveillance of carcasses in slaughterhouse(Collins, 2006).
Diagnosing tuberculosis in animals typically involves a combination of clinical examination and tuberculin skin testing.

The interferon-gamma release assay (IGRA) achieved approval and registration by the World Organization for Animal Health (WOAH) in 2015. To this day it remains the only blood test approved to supplement the skin test for TB in cattle (WOAH, 2022). The interferon-gamma is a cytokine that is released by different immune cells and plays a significant role in the elimination of pathogens after activating the production of reactive oxygen and nitrogen in macrophages (Yoneda and Ellner, 1998). Based on the activity of this cytokine, the IGRA is used as a supplementary test alongside the tuberculin skin test in specific TB breakdown herds. The aim of combining the skin and blood tests is two-fold: to shorten the duration of the TB breakdown and reduce the risk of leaving infected animals undetected in the herd by the time movement restrictions are lifted. Any skin test-negative but gamma test-positive animals must be slaughtered to increase the probability of correctly identifying all TB-infected cattle in the affected herd.

The gamma test is compulsory for every new TB breakdown herd with lesion and/or culture positive animals in several countries. The disease is endemic in Tunisian cattle and in humans. Indeed, there was an incidence of human tuberculosis of 13.91 reported cases per 100,000 population per year over 22 years (1995-2016) with projections to increase to 18.13 cases by 2030, according to a recent study (Ben Ayed *et al.*, 2019). Animal tuberculosis has received limited research attention in Tunisia, with a focus on molecular approaches (Kahla *et al.*, 2011; Lamine-Khemiri *et al.*, 2014; Djemal *et al.*, 2017). Notably, no published studies have tackled cattle tuberculosis prevalence directly, nor have researchers employed the gamma interferon test. Building upon existing protocols, we conducted a study employing the interferon-gamma test, a novel diagnostic tool for bovine tuberculosis in Tunisian cattle. By comparing it to the standardized tuberculin skin test (TST), we evaluated the effectiveness and congruence of both methods, aiming to advance diagnostic accuracy and disease management.

MATERIALS ET METHODS

Study region: Our research delves into the agricultural practices of 11 farms in Tunisia's Béja governorate (Figure 1), a region renowned for its agricultural bounty. Nestled in the northwest corner of the country, Béja boasts a rich agricultural heritage and consistently ranks among the top producers nationwide. This dedication to farming is evident in the 91% of land devoted to the sector, solidifying agriculture's position as the cornerstone of the local economy. To gain insight into the health status of the animals toward tuberculosis, we focused on ten farms. The veterinarian overseeing these farms ensured compliance with the regulation through regular tuberculin skin tests. All selected farms previously reported positive skin tests.

The interferon-gamma assay

Blood collection: Following a standardized protocol, blood samples were collected from 128 cattle over a one-year period (September 2019 - August 2020). After obtaining farmer consent and conducting thorough clinical examinations by a veterinarian, heparinized blood was drawn from the jugular vein. To maintain sample integrity, all samples were kept at an ambient temperature of 22°C ± 3°C and transported within six hours to the National School of Veterinary Medicine's laboratory for prompt culture, occurring within 30 hours of collection.

Interferon gamma test: To measure T-cell response to mycobacterial antigens, the IGRA test has two phases: stimulating T-cell production with mycobacterial antigens and quantifying interferon gamma release using an ELISA. First, we prepared a culture plate with mycobacterial antigens (PPD-B, PPD-A and ESAT-6 specific antigens 4 and CFP-105) and added duplicate blood samples from each animal. The plate was then incubated at 37°C for 20-24 hoursto allow T-cells to react to the antigens. Next, we separate the plasma from the blood cells by centrifugation. Plasma samples are then collected and stored for the final step, where the amount of interferon gamma produced will be measured by ELISA to assess the immune response to the antigens.

Statistical analysis: Data were analyzed with SPSS(Version 23.0. Armonk, NY: IBM Corp.). Univariate analysis through Pearson's chi-square test was first performed to assess the strength of the association between the farms and the tests' results at 5% significance threshold value. Then a Cohen's Kappa test was done to determine the concordance between the results of the two tests.

RESULTS

The study involved 128 cattle and resulted in 73 positive responses by TST, i.e. a positivity rate of 57%.The difference in prevalence between farms was statically significant ($p<0.001$).The IGRA test resulted in 15 positive responses, i.e. a positivity rate of 11.72%.The difference in prevalence between farms was not statically significant ($p=0.366$). The animal's infection rate was either 0% (farms B, C, G, H) or 100% (farms A, D, E, F, I and J) when tested by TST; and ranged from 0% (farms B, D, J) to 4/13 animals (farm A) when tested using IGRA test (Figure 1). Among the 10 farms in the study, 6 have all tested animals positive for TST; the other 4 farms showed no positive response. In TST positive farms, 4 of them showed at least one bovine with a positive result for IGRA test (Figure 1).In TST negative farms, 3 out of 4 farms showed at least one bovine with a positive result for IGRA test (Figure 2). The positivity rate calculated according to the health status of the animals shows that animals raised in farms with a history of bovine tuberculosis have the highest individual positivity rate 12.3% (9/73) compared to animals belonging to farms free from tuberculosis 10.9% (6/55). This difference was not statically significant ($p=0.805$). The evaluation agreement between the TST and the IGRA test using the Cohen's Kappa test showed none to slight agreement (Table I).

DISCUSSION

Tuberculosis in animals poses a major socio-economic and hygienic threat in Tunisia and the Maghreb region. It's endemic in both cattle and humans, and it's an area that deserves more attention. The issue of tuberculosis has been getting a lot of attention in the Tunisian media lately. This renewed public focus highlights the importance of addressing TB not just in humans, but also in animals. Tunisia has had a national program in place since 1984 to address bovine TB. The health status of cattle farms in Tunisia is defined following continuous monitoring by the TST under the supervision of the official veterinarian (ordinance of May 20th, 1975). This program targets state-run farms and private enterprises, thus relies on the cooperation of farmers, who voluntarily participate in a program of preventive measures.The core principles are: regular testing of cattle herds to identify infected animals, targeted slaughter of animals testing positive to prevent further spread, compensation for farmers to help offset the economic impact, and slaughterhouse surveillance of carcasses by veterinarians for signs of bovine TB lesions. One critical gap in our knowledge about TB in Tunisia is its actual prevalence in cattle herds. Our study is conducted within this framework as a preliminary essay to try to fill this gap. Our results showed that when infected herds are testes with TST, the result is either all sample or none are positive.

Table I. TST and IGRA test results

	Animals' prevalence in % (positive/tested [95% CI]	Farms' prevalence (positive/tested)
TST	57% (73/128)	6/10
IGRA test	11.71% (15/128)	7/10
Cohen's Kappa test coefficient(p value)	0.013 (0.805)	0.167 (0.596)

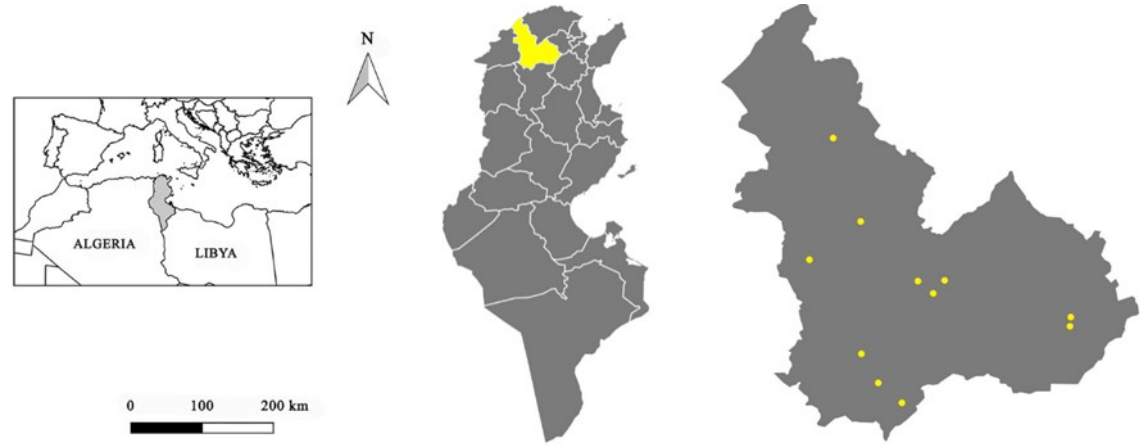

Figure 1. Map of Beja governorate, Tunisia, showing distribution of sampled farms

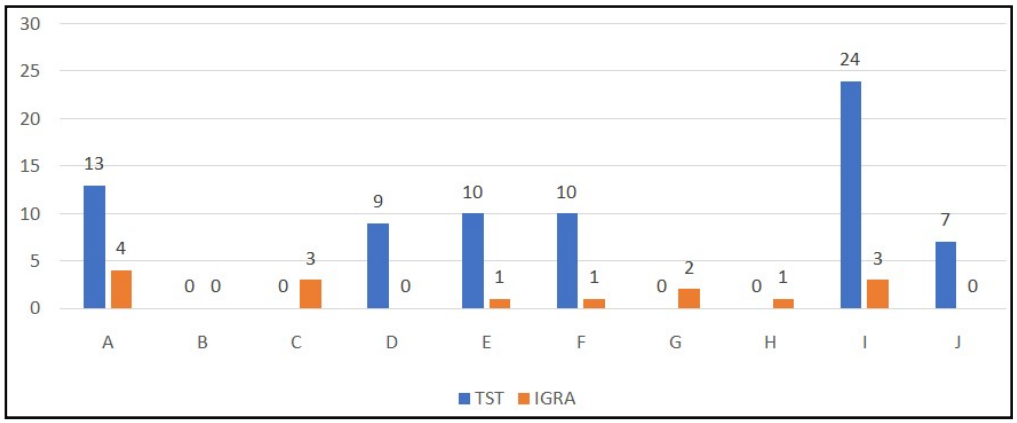

Figure 2. Positivity rate across farms

This highlights the problem of specificity of the TST test, which range from 91% to 98.2%(ANSES, 2019). Among other factors that can generate false positive responses are atypical mycobacteria like *M. avium* responsible for cross reactions(Klepp *et al.*, 2019). The individual prevalence rate in TST test animals was 57%, whereas it was 11.72% when they were IGRA tested. The last rate is closer to the national rate of 16% measured using TST according to (Abid *et al.*, 2019). These authors also reported a herds infection rate of 27% which is quite high. Our results showedthat only 12% (9/73)of TST positive animals were also IGRA test positive. The agreement between the test was poor. This was also reported by some research (Kelly *et al.*, 2022) sometimes this agreement was considered as fair (Kalis *et al.*, 2003).

CONCLUSION

Treatment options for tuberculosis in animals are absent, and slaughter is necessary to prevent further spread of the disease. Preventative measures such as vaccination and proper hygiene can help control the spread of tuberculosis in animal populations.

This study underscores the importance of bovine tuberculosis in Tunisia and emphasizes the need for improved diagnostic tools. While the national program has been in place since 1984, limitations in the specificity of the TST test might be inflating estimates of bovine TB prevalence. Our findings of a 11.72% positivity rate using the IGRA test suggest a need for wider adoption of this method alongside TST for a more accurate picture of bovine TB distribution.

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