www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 946-948

www.thepharmajournal.com
Received: 22-10-2021
Accepted: 24-11-2021

Dr. T Devi
Department of vet. Preventive medicine, Madras Veterinary College, Tamil Nadu, India

M Asokkumar
Department of Veterinary Public Health an Epidemiology, Madras Veterinary College, Chennai, Tamil Nadu, India

M Vijaya Bharathi
Department of Veterinary Preventive Medicine, Madras Veterinary College, Tamil Nadu, India

A Ramesh
Vaccine Research Centre -Viral Vaccines, Centre for Animal Health Studies, TANUVAS, Chennai, Tamil Nadu, India

KG Tirumurugaan
Zoonoses Research Laboratory, Madhavaram Milk Colony, TANUVAS, Chennai, Tamil Nadu, India

Corresponding Author
M Asokkumar
Department of Veterinary Public Health an Epidemiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Rapid diagnostic methods for detection of bovine tuberculosis in resource poor settings

Dr. T Devi, M Asokkumar, M Vijaya Bharathi, A Ramesh and KG Tirumurugaan

Abstract
A study was conducted in 30 numbers of Jersey cross cattle at Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University for rapid detection of *Mycobacteriosis*. Lymph node smear and sputum swabs were collected from the study population and were subjected to Auramine -O-Fluorescent staining and Loop mediated isothermal Amplification (LAMP). Out of 30 samples, 16 (53.33%) samples were found as positive by both test methods with 22 samples (73.33%) sparing positive only by Auramine O Fluorescent staining. There were only 6 (20%) samples which showed negative to Auramine O Fluorescent staining but positive to LAMP method. It was concluded that these two diagnostic methods may be considered as parallel tests for rapid diagnosis of *M*ycobacteriosis where early diagnostic methods like Interferon Gamma assay are not feasible for isolation and quarantine of positive animals before confirmation by gold standard culture method.

Keywords: *My* cobacteriosis, sputum, LN smear, LAMP, fluorescent staining

1. Introduction
Tuberculosis in bovine is caused by *Mycobacterium tuberculosis* complex (MTC) and more commonly associated with *M. tuberculosis* and *M. bovis*. This disease is a persistent problem in developing countries like India and it causes severe economic losses such as livestock deaths, chronic wasting disease and trade restrictions. In some situations, bovine tuberculosis may also be a serious threat to other species including human being as reverse zoonoses.
The tuberculosis diagnostics pipeline has grown rapidly in recent years with the development of several promising molecular diagnostic technologies. A simple, rapid, inexpensive POC test, however, is still not on the horizon (Wallis *et al*., 2010) [6]. The conventional gold standard diagnostic test like culture for confirmation of mycobacterium is time consuming because of their slow growth rate in selective media and it takes 2 to 8 weeks in normal culture to less than a week in Mycobacterial Growth Indicator Tube (MGIT) method developed by Becton Dickinson. To address the need for rapid and sensitive identification of *Mycobacterium tuberculosis* and other mycobacteria, numerous genotyping methods have been introduced for routine diagnosis during the past decade. Although PCR is highly sensitive, it needs sophisticated laboratory facility, hence there are pressing needs for point-of-care testing for MTB (Iwamota *et al*., 2003) [3].
Despite the long-established tradition of solid culture for Mycobacterium and more recent advances in tuberculosis diagnosis, direct sputum smear microscopy (>10^5 organisms/ml) and LAMP techniques play a significant role with good sensitivity in diagnosis of the same where poor resources are available. Loop-mediated isothermal amplification (LAMP) is a Novel Nucleic acid Amplification Test (NAAT) which relies on auto-cycling strand displacement DNA synthesis and it is performed by the Bst DNA polymerase large fragment without thermocycler, which allows DNA to be amplified rapidly at a constant temperature. Kaewphint *et al*. (2013) [4] found that LAMP has 100% specificity and 98.92% sensitivity against *M. tuberculosis*. LAMP test is robust and does not require sophisticated instrumentation and can be used at rural area, under biosafety requirements similar to microscopy (WHO, 2016) [7]. George *et al*. (2011) [2] stated that the overall efficacies of LAMP and fluorescence smear microscopy were high and broadly similar. LAMP and Auromine-O- fluorescent staining series can be used as a rule-in test combination for MTB (George *et al*., 2011) [2]. With this overview the present study was conducted to compare the sensitivity of LAMP and Fluorescent staining method for the detection of *M. tuberculosis*.

2. Materials and Methods

Lymph node smears and deep nasal swab were collected from 30 numbers of Jersey cross cattle which were suspected for Tuberculosis and isolated at Post Graduate Research Institute for Animal Science, Kattupakkam, TANUVAS. The smears were subjected to Auramine O Fluorescent staining as described by George *et al.* (2011) [2]. DNA was extracted from the Lymph node and sputum swab samples using TE boil extraction Method as described by Aldous *et al.* (2005) [1] and the isolated DNA was used as a template in the LAMP assay. In TE boil extraction, a 200µl aliquot of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) was added and the mixture was briefly mixed using vortex mixer. The suspension was placed in a boiling water bath for 15 min to destroy any viable mycobacteria and then centrifuged at 16,000 X g for 5 min. A 100 µl aliquot of the supernatant was transferred to a sterile tube and stored at -20 °C until PCR testing. Primers F3, B3, FIP(F1c/TTTT/F2) and BIP (B1c/TTTT/B2) procured from Biosource&surgical and Bst DNA Polymerase Large Fragment procured from New England BioLabs Inc were used.

Table 1: Primers and its sequences

Primer name	Sequence 5'-3'
F3	GCCAGATGCACCGTCGA
B3	GACACATAGGTGAGGTCTGC
FIP(F1c/TTTT/F2) and	AGCGATCGTGGTCCTG CGG-TTTTGATGACCAAACTCGGC
BIP (B1c/TTTT/B2)	TCCCGCCGATCTCGTCCA-TTTTTACCCACAGCCGGTT

Four sets of primers (B3, F3, BIP and FIP) recognizing a total of 6 distinct sequences (B1-B3 and F1-F3) on IS6110 gene of *M. tuberculosis* were used and reaction was carried out. The product was incubated at 63 °C for 60 min and was heated above 80 °C for 2 min to terminate the reaction as described by Kaewphinit *et al.* (2013)[4]. LAMP product was visualized by naked eye with BCG vaccine being used as positive control to have validated LAMP procedure. Results of Fluorescent staining and LAMP technique were compared.

3. Results and Discussion

Sixteen out of 30 samples (53.33%) showed positive with Auramine O Fluorescent staining and LAMP. But 22 samples (73.33%) were showing positive results only to Auramine O Fluorescent staining method. There were only 6 samples which showed negative to Auramine O Fluorescent staining but positive to LAMP method. One of the most attractive characteristics of LAMP is the visual judgment of nucleic acid amplification test (NAAT). This can be achieved due to the high specificity and high amplification efficiency of LAMP. Mori *et al.* (2001) [5] reported that the LAMP amplicons can be detected by confirming the presence of magnesium pyrophosphate, a white precipitate generated as a by-product during the reaction. Although this is a quite simple approach, detecting a small amount of the white precipitate by the naked eye is not always easy; therefore, the detection limit is apparently inferior to that of electrophoresis. This might be reason we could not get precipitate in almost 50% of samples tested with LAMP.

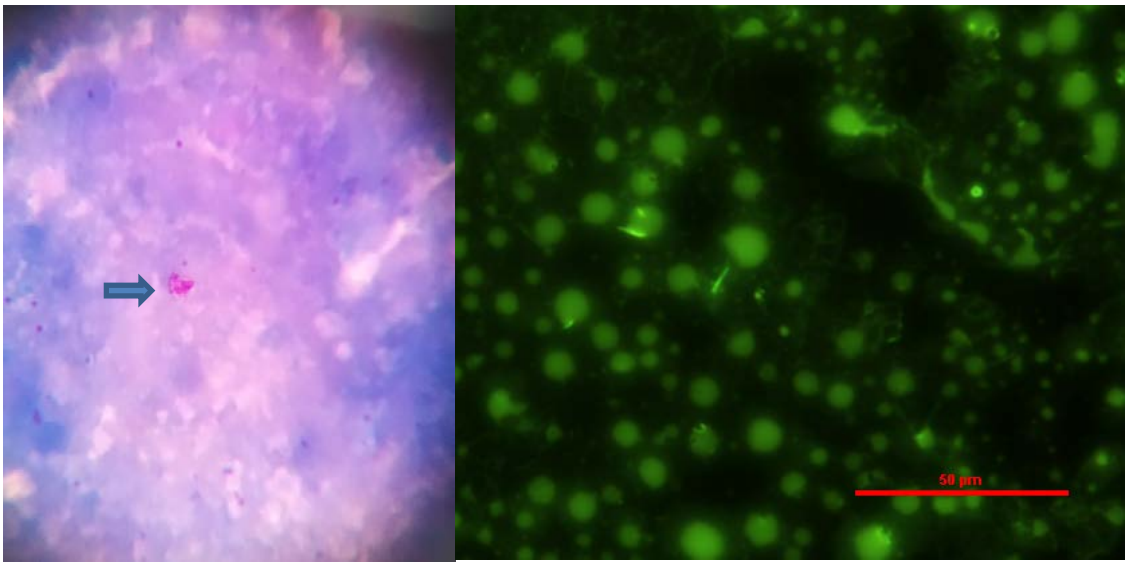

Fig 1: *M. tuberculosis* (Z-Nstaining) *M. tuberculosis* (Auramine O staining)

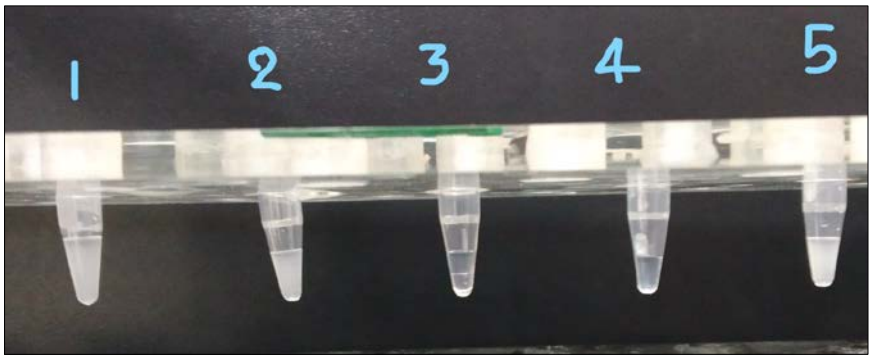

Fig 2: LAMP Method – 1: Positive control, 2 &5: Positive Control, 3&4: Negative Result

George *et al.* (2011) found that LAMP and Auramine O staining smear showed sensitivities of 79.5% and 82.1% respectively compared to culture and also found sensitivity of 82.1% when carried out LAMP and smear in parallel. This is in concurrence with our findings with the sensitivity of 53.33%.

Although long-established tradition of culture and direct sputum smear microscopy remains the cornerstone of global tuberculosis diagnostics options, the numerous more recent advances in tuberculosis diagnosis are found to be expeditious since the former tests have the limitations associated with low and variable sensitivity. The conventional acid-fast staining (ZN) may be useful when the sputum contains large number of bacilli (> 10^5 organisms/mL) and paves the difficulty of collecting sputum from animals compared to human beings as it requires special expertise on broncho alveolar lavage to have better sputum samples. The commercially available broth-based culture system (MGIT-BD, USA) is also found to be expensive and require sophisticated biosafety laboratories. Hence LAMP and smear can be used as a rule-in test combination for rapid diagnosis as a screening method before going to cultural confirmation.

4. Conclusion

The fluorescent Auramine-O and nucleic acid-based LAMP assay are having the potential to be used as point of care tests to replace the conventional diagnostics in mycobacterial diagnosis of animals as they offer less time consuming and enhanced sensitivity after validating these tests with large sample size through intra and inter technical diagnostic laboratories.

5. References

1. Aldous AK, Pounder JI, Cloud JL, Woods GL. Comparison of Six Methods of Extracting Mycobacterium tuberculosis DNA from Processed Sputum for Testing by Quantitative Real-Time PCR. Journal Clinical Microbiology 2005, 2471-2473.
2. George G, Mony P, Kenneth J. Comparison of the Efficacies of Loop-Mediated Isothermal Amplification, Fluorescence Smear Microscopy and Culture for the Diagnosis of Tuberculosis. PLoS One 2011;6(6):1-5.
3. Iwamoto T, Sonobe T, Hayashi K. Loop-Mediated Isothermal Amplification for Direct Detection of Mycobacterium tuberculosis Complex, *M. avium*, and *M. intracellulare* in Sputum Samples. Journal of Clinical Microboilogy 2003;41(6):2616-2622.
4. Kaewphinit T, Arunrut N, Kiatpathomchai W, Santiwatanakul S, Jaratsing P, Chansiri K. Detection of *Mycobacterium tuberculosis* by Using Loop-Mediated Isothermal Amplification Combined with a Lateral Flow Dipstick in Clinical Samples. Biomedical Research International 2013, 1-6.
5. Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochemical Biophysical Research Communications 2001;289:150-154.
6. Wallis RS, Menzies MD, Doherty TM, Walzl G, Perkins MD, Zumla A. Biomarkers and diagnostics for tuberculosis: progress, needs and translation into practice. Lancet 2010;29(375):1920-37.
7. Loop-mediated isothermal amplification for the detection of *M. tuberculosis* (TB-lamp). [Weblink: https;/www.who.int/tb/publications/factsheet_tb_lamp.pdf]. [Visited on October 2016].