Evaluation of the Sensitivity and Specificity of Gamma Interferon Assay, Single Intradermal Tuberculin and Comparative Intradermal Tuberculin Tests in Naturally Infected Cattle Herds

P. Dandapat

Eastern Regional Station
ICAR-Indian Veterinary Research Institute
37 Belgachia Road, Kolkata-700037

INTRODUCTION

- bTB remains a global problem with >50 million infected cattle throughout the world (Lyashchenko *et al*., 2004).
- In India, prevalence of bTB is up to 16% in cattle and 25% in buffaloes (Lall, 1969).
- It is estimated that 5–10% of the global TB burden may be due to *M. bovis* (Warren *et al*., 2006)
- In some developed countries, *M. bovis* accounts for approximately 0.5 to 1.5 % of the entire human TB cases (Chen *et al*., 2009).
- Human TB due to *M. bovis,* infection remains heavily skewed towards developing countries where control strategies are neglected or do not exist at all (Cosivi *et al*., 1998).

INTRODUCTION (contd.)

- Diagnosis of bTB is still a challenge since available diagnostic tools have limitations on sensitivity and specificity and no single tests can diagnose bTB at all stages of infection (Bezos *et al.,* 2014).
- In India, single intradermal test (SIT) is recommended to diagnose bTB in large ruminants.
- In India, massive load of NTMs in animal population leading to false positive diagnosis by SIT.

OBJECTIVES

- To conduct comparative study on tuberculin tests, gamma interferon assay, bacteriological and molecular techniques for diagnosis of tuberculosis in cattle.
- To investigate the prevalence of bovine tuberculosis.

METHODOLOGY

Animals were selected from 5 neighbouring districts of Kolkata, *viz.,* Burdwan, Hooghly, Nadia, Purulia and South 24 Parganas.



Selection of Animals

- 175 cattle randomly selected from organized and backyard farming system.
- Each cattle was screened by single intradermal test (SIT), comparative cervical tuberculin test (CTT) and γ-interferon assay (γ-IFN).
- Ante mortem samples (nasal swab, buccal swab and aspirates from prescapular lymph node) collected from all animals in addition to milk samples from milching cows (96) for screening through bacteriological and molecular techniques.
- PM samples having tuberculous nodule like lesions collected from dead cattle.

Intradermal tuberculin testing



Testing of cattle by tuberculin test

- Both single intradermal test (SIT) and comparative cervical tuberculin test (CCT) was performed in each animal.
- The interpretation of the intradermal tuberculin test will be done as per OIE standards.

Interpretation of Tuberculin Tests

Interpretation of Single intradermal test (SIT)

X = (Bov3-Bov0)

X ≥ 4mm or if clinical signs like diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes, are observed = Positive.

2mm ≤ X < 4mm = Inconclusive

2mm > X without clinical signs, such as mentioned above = Negative

Interpretation of CCT

X = (Bov3-Bov0)-(Av3-Av0)

X ≥ 4mm = Positive

1 ≤ X < 4mm = Inconclusive

1 > X = Negative

γ-Interferon Immunoassay

The Bovigam® immunoassay was used to run γ-Interferon test.

Procedure:

- Jugular venepuncture performed to collect blood samples into heparinised vacutainers.
- Blood cultured in triplicate within 12 hours of collection. Blood of each animal stimulated by (a) PBS (negative control); (b) Bovine PPD antigen and (c) Avian PPD antigen.
- Samples incubated at 37℃ for 16-24 hours.
- Plasma harvested by centrifugation.
- Each sample assayed in duplicate and controls in triplicate.
- Microplates coated with monoclonal antibody to gamma interferon used for the assay.
- OD measured in ELISA reader with both 450nm filter and 620-650 nm filters for interpretation.

Processing of ante-mortem samples

(Milk, nasal swab, buccal swab and aspirates from prescapular lymph node)

Recovery of sediment by centrifugation (washing sediment with sterile NSS in case of milk)

Decontaminated By Petroff's ($NaOH$) & H_2SO_4 method

Cultural Isolation on L J-P & L J-G Media

Identification of isolates by biochemical & molecular tests

Processing of PM samples

Ziehl-Neelsen Staining

Decontamination

Petroff's (NaOH) & H_2SO_4 method

Cultural Isolation on L J-P & L J-G Media

Identification of isolates by molecular techniques

Speciation of *Mycobacterium* sp. by PCR

- Identification of *Mycobacterium* species and *M. tuberculosis* complex (MTBC) (Wilton and Cousins, 1992)
  - *Mycobacterium* species : 16S rRNA (1030 bp)
  - MTBC : MPB 70 gene (372 bp)
- Differentiation of *M. bovis* & *M. tuberculosis* detecting RD9 region and 500 bp fragment (Das *et al*., 2007)
  - *M. tuberculosis*: RD 9 present (333 bp)
  - *M. bovis* : RD9 absent (206 bp) & 500 bp

Statistical analysis

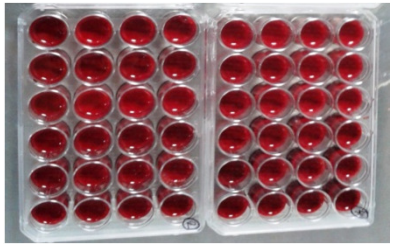
- Descriptive and inferential statistical analysis carried out in the present study.
- Results on continuous measurements presented on Mean ± SD (Min-Max) and categorical measurements are presented in number (%).
- Chi-square/ Fisher Exact test used to find the significance of study parameters on categorical scale between two or more groups.
- Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 used for the analysis of the data.

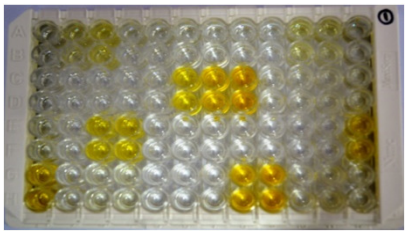
RESULTS

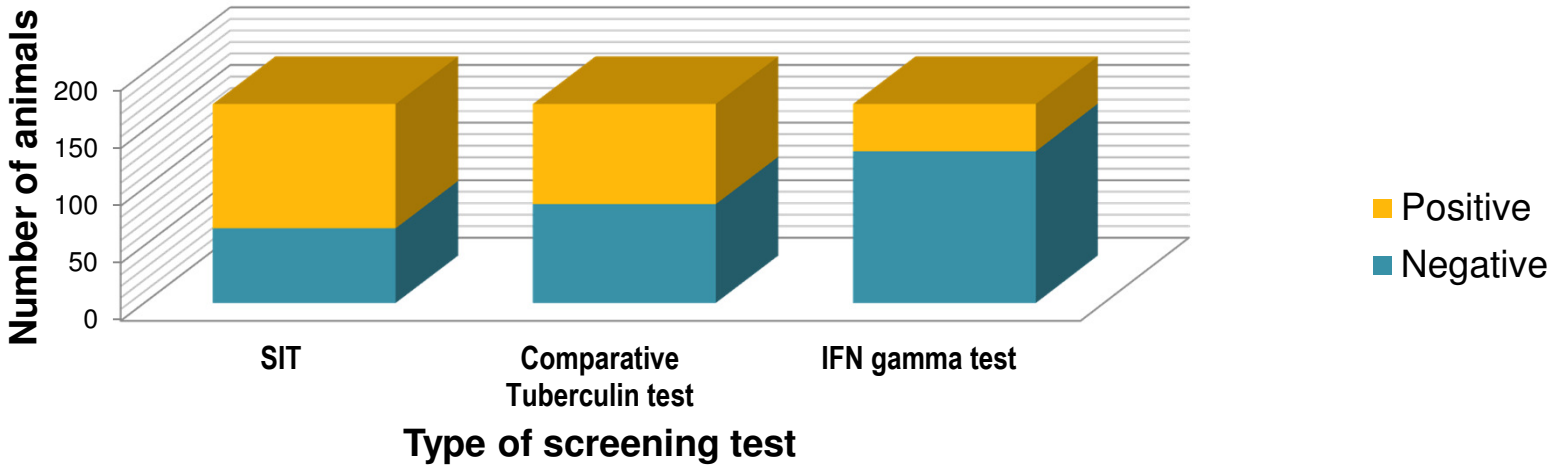
Screening of cattle by SIT, CTT and γ-IFN assay

Test of screening	No. of cattle screened	Negative	Positive	95%CI
SIT	173	65(37.6%)	108 (62.4%)	55.01-69.30
CTT	173	86 (49.7%)	87(50.3%)	42.91-57.65
γ-IFN Assay	173	132(76.3%)	41(23.7%)	17.98-30.56



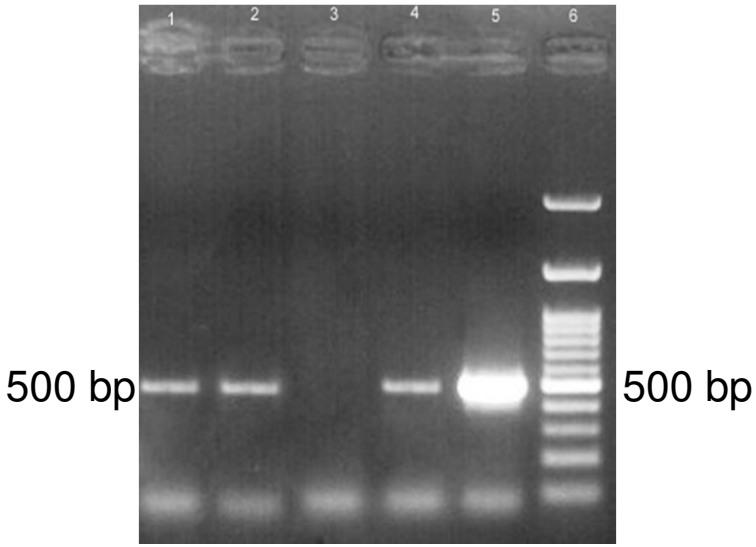


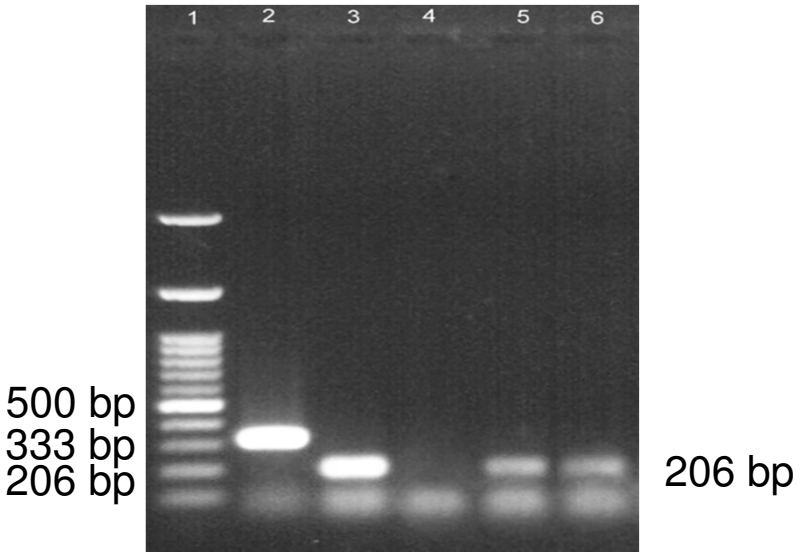




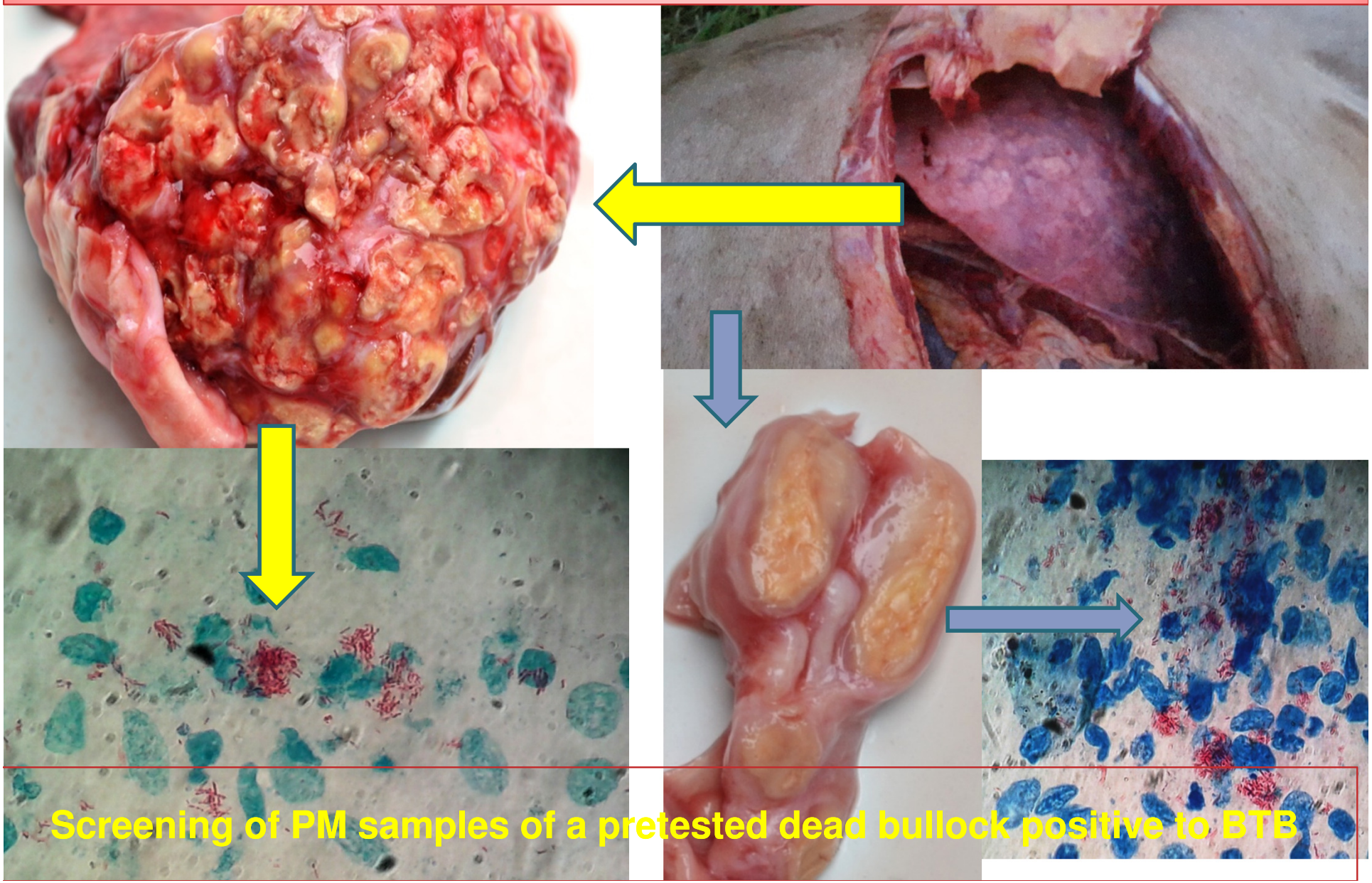
Identification of *Mycobacterium* sp in ante-mortem samples

- Milk from 4 cattle positive for 500bp fragment, suggestive of presence of *M. bovis*
- 2 milk isolates were positive for 206bp fragment
- None of other samples (nasal swab, buccal swab and aspirates from prescapular lymph node) turned positive.



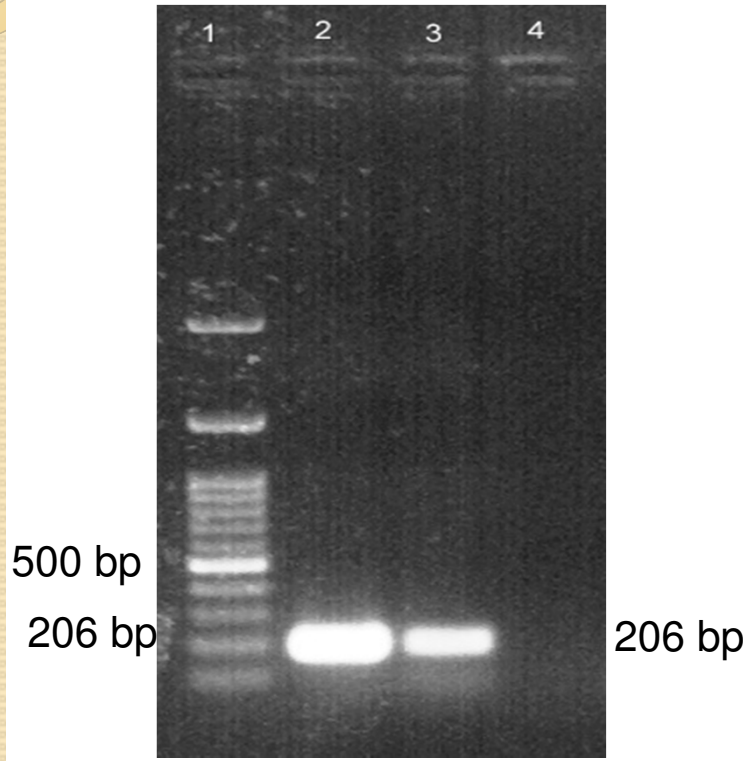


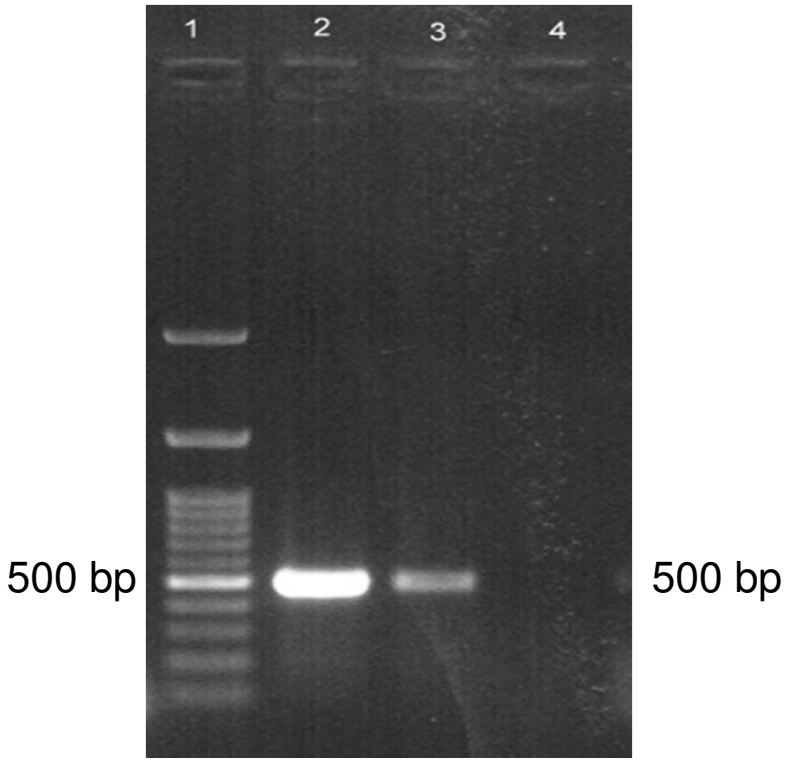
Identification of *Mycobacterium* sp in post-mortem samples



Screening of PM samples of a pretested dead bullock positive to BTB

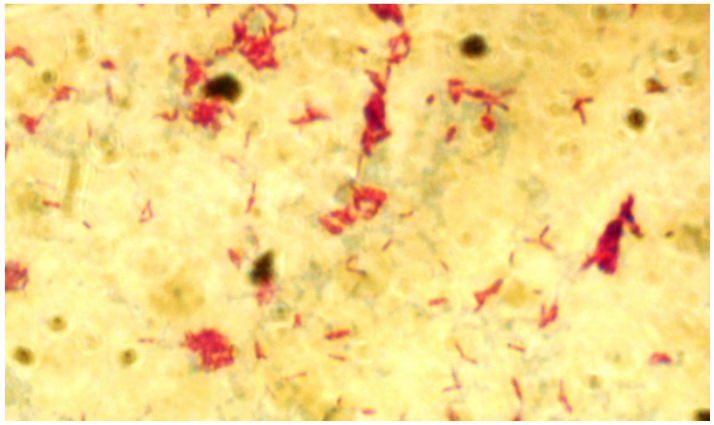
Speciation of *Mycobacteria* by molecular methods from lung tissue





Amplification of RD9 region (333 / 206bp) and 500bp fragment

Cultural isolation from ante and post-mortem samples & molecular detection





- 2 milk isolates were positive for 206bp fragment

Correlation of findings of SIT, CTT and γ-IFN assay

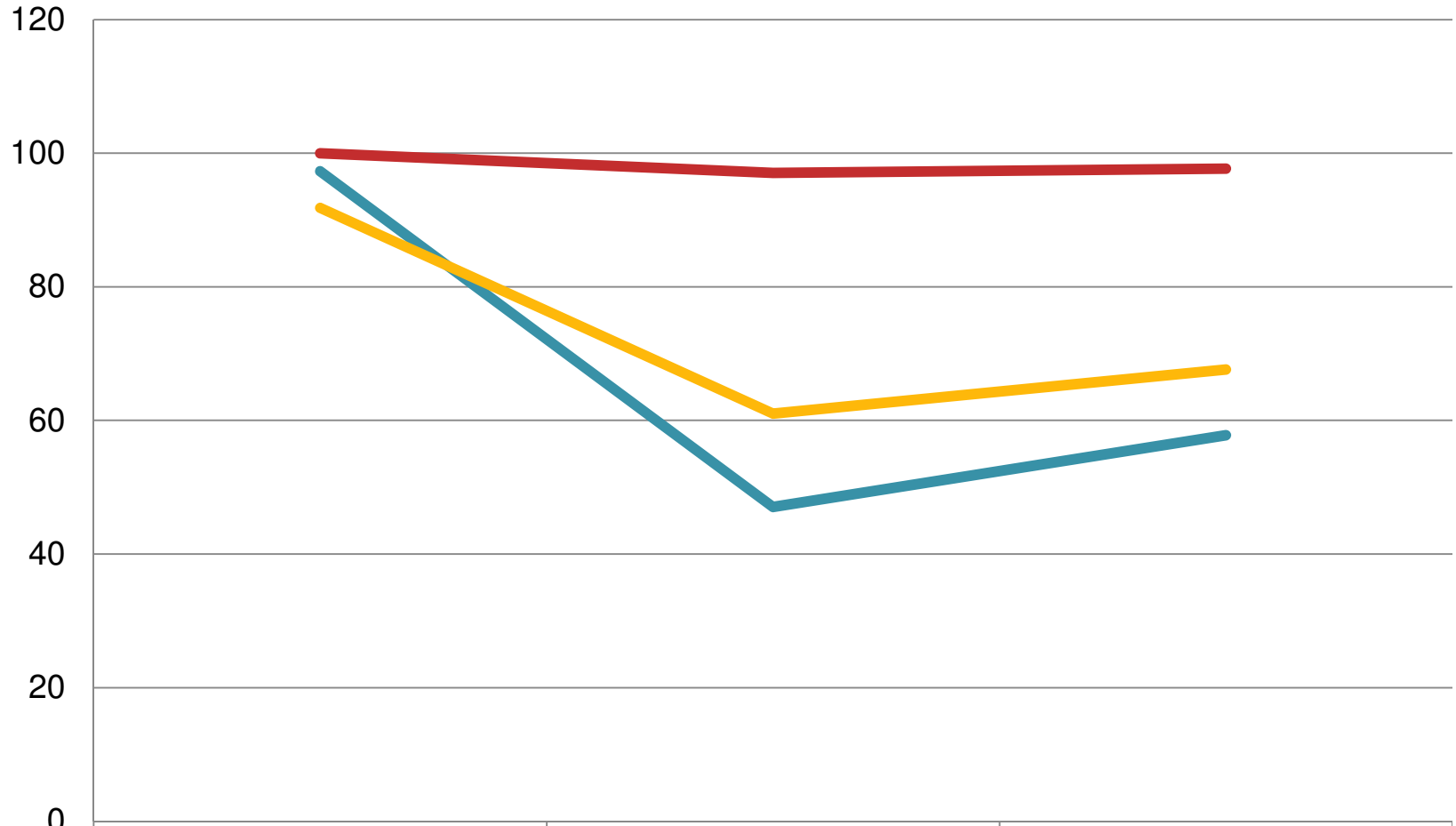
Through meta analysis of scientific reports and the observation of current study, case definition of positive BTB is defined as follows:

"The cattle is positive to BTB if it is positive to both SIT & γ-IFN assay or doubtful/positive to CTT & positive to γ-IFN assay".

	Negative	Positive
Final diagnosis	136 (78.6%)	37 (21.4%)

Tests	Final Diagnosis		Total (n=173)	P value
	Negative (n=136)	Positive (n=37)		
SIT				
• Positive	72(52.9%)	36(97.3%)	108(62.4%)	<0.001**
• Negative	64(47.1%)	1(2.7%)	65(37.6%)	
CTT				
• Positive	53(39%)	34(91.9%)	87(50.3%)	<0.001**
• Negative	83(61.1%)	3(8.1%)	86(49.7%)	
γ-IFN assay				
• Positive	4(2.9%)	37(100%)	41(23.7%)	<0.001**
• Negative	132(97.1%)	0	132(76.3%)	

Diagnostic evaluation of SIT, Comparative Tuberculin test and IFN gamma test in relation to final diagnosis



	Sensitivity	Specificity	Accuracy
SIT	97.3	47.06	57.8
CTT	91.83	61.03	67.63
γ-IFN assay	100	97.06	97.69

CONCLUSION

- bTB is mostly prevalent in organized farms.
- SIT is most sensitive test and hence, may be recommended for screening of herds
- Comparing sensitivity, specificity and accuracy of various diagnostic tests, γ-IFN may be recommended to screen individual cattle for BTB
- Battery of tests including CTT, γ-IFN assay and screening of milk by PCR may be recommended for confirmatory diagnosis of individual animal
- Screening of milk samples directly by PCR may be a better option in coming age for diagnosis in live milching cattle
- In West Bengal, maximum farming environment are contaminated with NTMs hampering BTB diagnosis by SIT
- High level of γ-IFN in normal blood hampers diagnosis of BTB by γ-IFN assay

Thank you





