Comparative analysis of tuberculin and defined antigen skin tests for the detection of bovine tuberculosis in buffaloes (Bubalus bubalis)

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Research Article

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Abstract

Background: Bovine tuberculosis (bTB) is a chronic disease that results from infection with any member of the *Mycobacterium tuberculosis* complex and infected animals are typically diagnosed withtuberculin-based intradermal skin tests per World Organization of Animal Health or similar guidelines. Peptide-based defined skin test (DST) antigens, comprising of ESAT-6, CFP-10 and Rv3615c, are able to differentiate infected from BCG-vaccinated animals and sensitively and specifically identify tuberculin reactor cattle, but their performance in buffaloes remained unknown. To assess the comparative performance of the DST with the tuberculin-based single intradermal test (SIT) and the single intradermal comparative cervical test (SICCT), we screened 543 female buffaloes from 49 organized dairy farms in two districts of Haryana state in India.

Results: The results show that 37 (7%), 4 (1%) and 18 (3%) buffaloes were reactors with the SIT, SICCT and DST, respectively. Of the 37 SIT reactors, four were positive with SICCT and 12 were positive with the DST. The results further show that none of the animals tested positive with all three tests, and 6 DST positive animals were SIT negative. Together, a total of 43 animals were reactors with SIT, DST, or both, and the two assays showed moderate agreement (Cohen'sKappa 0.41; 95% CI: 0.23, 0.59). In contrast, only slight agreement (Cohen's Kappa 0.18; 95% CI: 0.02, 0.34) was observed between SIT and SICCT. Latent class analyses reveal test specificities of 95% for SIT and 99% each for DST and SICCT, but considerably lower sensitivities of 67%, 39%, and 19% for SIT, DST, and SICCT, respectively, albeit with broad and overlapping credible intervals.

Conclusion: Taken together, our investigation suggests that DST has a test specificity comparable with SICCT, and sensitivity intermediate between SIT and SICCT for the identification of buffaloes suspected of tuberculosis. Our studies also highlight an urgent need for future well-powered trials with detailed necropsy with immunological and microbiological profiling of reactor and non-reactor animals to better define the underlying drivers for the large observed discrepancies in assay performance, particularly between SIT and SICCT.

Introduction

Bovine tuberculosis (Bovine TB or bTB) is a chronic disease of cattle caused by members of the *Mycobacterium tuberculosis* complex (MTBC). It is a multi-host disease that can infect a diverse group of domesticated and wild animals. In cattle, this disease negatively affects milk production (reduces milk yield up to 10–20%) and fertility, thus leading to economic losses [1–3]. Importantly, bTB is also a neglected zoonotic disease that crosses the species barrier and can infect humans either by consumption of unpasteurized milk or undercooked meat [4].

The tuberculin-based intradermal skin test, recommended by the World Organization for Animal Health (WOAH), is currently used for screening of animals for bTB [5]. Tuberculin skin testing is based on delayed type hypersensitivity to purified protein derivatives (PPDs) of standard cultures of *M. avium*

(PPD-A) and *M. bovis* (PPD-B). The single intradermal test (SIT) involves PPD-B alone while in regions with high prevalence of environmental mycobacteria, the single intradermal comparative cervical test (SICCT) with both PPD-B and PPD-A is used to help improve test specificity. Importantly, the presence of cross-reactive antigens between field and vaccine strains causes inability to differentiate infected from Bacille Calmette-Guérin (BCG)–vaccinated animals (DIVA). This limits opportunities for the development and implementation of BCG vaccination-based control programs to help accelerate control of bTB.

Here, we tested female buffaloes in organized dairy farms in two districts of Haryana, India. The WOAH-recommended standard tuberculin-based test having PPD was used alongside peptide-based defined skin test (DST) antigens, comprising of ESAT-6, CFP-10 and Rv3615c, that have been recently shown to not only have utility in identifying infection in cattle and buffaloes but also possess DIVA capability [6–8].

Systematic evaluation of the performance of diagnostic tests for bovine tuberculosis is hampered by the lack of a proper gold standard for identification of animals that are truly infected versus those that are merely exposed and may have recovered. The Walter-Hui latent class model provides a theoretical framework to address this problem, allowing the sensitivity and specificity of a set of competing diagnostic tests to be estimated when samples are available from at least two populations with differing prevalence [9, 10]. In recent years this approach has been used to evaluate the relative performance of bTB diagnostics using field data from Ireland, Spain, France, Northern Ireland, Brazil, Pakistan and Egypt [11–20]. However, only one of these studies included buffalo (and did not evaluate the WOAH recommended tuberculin test) and no systematic performance of bTB diagnostics has previously been carried out in India [18]. We use the foundational Walter-Hui latent class model to provide first estimates of the relative sensitivity and specificity of the SIT, SICCT and DST tests in buffaloes in India.

Materials And Methods

Study population

Haryana, a state in Northern India, is located between 27° 37' to 30° 35' latitude and between 74° 28' to 77° 36' longitude. Based on agro-climatic zones in India, Haryana falls in Zone-VI (Trans-Gangetic Plains Region). The state is further subdivided into two zones i.e., Eastern and Western. District A is in western zone while district B is in eastern zone. A total of 49 organized dairy farms in two districts of Haryana viz., A and B were selected to compare performance of PPDs and DST in detecting bTB infection in buffaloes. A total of 543 female buffaloes (326 in A district and 217 in B district) from these organized dairy farms were included in this study, based on a likely prevalence assumption of 15% in female buffaloes at 20% precision, 95% confidence interval. The animals were grouped in three age groups viz. calves (6 months -one year of age), heifers (1–3 years of age) and adults (more than three years of age). At the time of testing, data such as age, breed, lactation stage, milk yield, pregnancy status etc. were collected. Female calves less than 6 months of age and adult buffaloes that were either in an advanced stage of pregnancy or recently calved were excluded from this study.

Skin testing

The intradermal skin test was performed on both sides of the neck. On the left side of the neck, bovine PPD (PPD-B) and avian PPD (PPD-A) (0.1ml each; Prionics, Switzerland) were administered intradermally using McLintock syringes (Bar Knight McLintock Limited, Scotland). On the right side of the neck, the peptide-based DST was injected. The DST contains overlapping chemically synthesized peptides of ESAT6, CFP10 and Rv3615c (40-mer length with a 20-residue overlap) at > 98% purity at 20 ug/peptide. Before administration, skin thickness was measured in millimeters (0-hour value) using a vernier caliper. Skin thickness was measured again at 72 ± 4 hours by the same operator. Difference in skin thickness (72 hour – 0 hour) was calculated as per WOAH protocol. An animal with increase in skin thickness of 4mm or more due to bovine PPD (Single intradermal test; SIT) or PPD-B minus PPD-A (Single intradermal comparative cervical tuberculin test; SICCT) was considered as a reactor. Bovine tuberculin PPD consisted of 3000 I.U. /dose while avian tuberculin PPD consisted of 2500 I.U./dose (WOAH, 2009). DST antigen was used at a concentration of 20ug/dose [21]. An animal with increase in skin thickness by 2mm or more due to DST antigen was considered as a reactor.

Statistical analysis

The agreement between SIT, SICCT and DST was estimated using Cohen's Kappa [22]. We carried out an exploratory analysis to test for associations between measured risk factors and positive status for the three test types. Risk factor model development was carried out in R [23]. For each test we built a multivariate logistic regression model using purposeful selection [24]. Firstly, we carried out a univariate screen with a generous cutoff for acceptance of 0.1, followed by a stepwise procedure (forwards and backwards) to select a parsimonious set of explanatory variables. Finally, to adjust for between herd variation in our study population we use a herd level random effect (intercept), estimating our final model using the lme4 R package [25]. The Hosmer-Lemeshow test as implemented in the Resource Selection R package was used to test for lack of model fit and classification ability of models was assessed through the area under the curve (AUC) of the Receiver-Operator-Characteristic (ROC) curve - calculated by the ROCR R package [26, 27]. Statistical significance was considered if $p < 0.05$. Effect sizes were calculated and reported as odds ratios (OR) with 95% confidence intervals.

The Walter-Hui latent class model was implemented in stan, estimated by Hamiltonian MCMC and analyzed in R using the rstan package [28, 29]. The key assumption of the Walter-Hui model is conditional independence between tests, i.e., the probability of a test k being positive for individual (i), $P\left(T_{i,k} = 1\right)$ only depends on the latent (true) disease status of the individual ($D \in \{0, 1\}$) and not the response of the other tests. Under this assumption the (conditional) probability of a positive test result given that an animal is infected ($D = 1$) or disease free ($D = 0$) can then be modelled by a single parameter for each test:

$$P\left(T_{i,k} = 1 | D = 1\right) = a_k$$

$$P\left(T_{i,k} = 1 | D = 0\right) = b_k$$

and the sensitivity of test k will then simply be a_k and the specificity will be $1 - b_k$.

Following [1, 30], and to allow for an extension to model any conditional dependence between tests, we parameterised the model using a probit (Φ) link function:

$$P\left(T_{t,k}=1|D=1\right)=\Phi\left(a_{t,1}\right)$$

$$P\left(T_{t,k}=1|D=0\right)=\Phi\left(a_{t,0}\right)$$

To ensure numerical stability we restrict the sensitivity parameters (on the probit scale) $a_{t,1}$ to the range $[-8,8]$. To force identifiability of the model (and avoid the label switching problem common with this class of models due to the symmetry of the likelihood) we make the assumption that no tests have a specificity of $<50\backslash\%$ or sensitivity $<20\backslash\%$ and thus restrict $a_{t,0}$ to the half-range $[-8,0]$ and $a_{t,1}$ to the range $[-1,0]$. Convergence was assessed through visual inspection of the chains and standard diagnostic statistics ($\widehat{R}=1$ for all parameters after $2,000$ iterations for 8 chains). Estimated parameters are presented as median posterior values with 95% Bayesian credible intervals (CI).

Model fit – and the central assumption of conditional dependence – was also assessed through calculating the pairwise probability of agreement between each pair of diagnostic tests (k, $k\prime$):

$$\alpha_{k,k\prime}=\frac{\sum_{i=1}^{N}T_{i,k}T_{i,k\prime}-\left(1-T_{i,k}\right)\left(1-T_{i,k\prime}\right)}{N}$$

Any systematic differences between the observed ($\alpha_{k,k\prime}$) and expected values from the estimated model ($\alpha_{k,k\prime}^{*}$) would imply a violation of the assumption of conditional independence. We can use draws from the posterior predictive distribution of $\alpha_{k,k\prime}^{*}$ for our fitted model to form a posterior predictive p-value [31]:

$$P\left(\alpha_{k,k\prime}^{*}>\alpha_{k,k\prime}\right)$$

If the model fits well, the value of $P\left(\alpha_{k,k\prime}^{*}>\alpha_{k,k\prime}\right)$ is expected to be close to 0.5, with extreme values close to 0 or 1 indicating a lack of fit (i.e., < 0.05 or > 0.95).

Results

Out of 543 female buffaloes screened for bTB in 49 organized dairy farms, 37 (7%) animals in both the districts were found to be reactors by SIT (Fig. 1). Only 4 (< 1%) animals were found reactors with the SICCT test; three of which did not show any response to PPD-A. By DST, 18 (3%) buffaloes were found to be reactors as per the cut-off of $\geq$2mm (Fig. 1). Considering SIT alone, 30 and 7 buffaloes were reactors in A and B district, respectively. Of the 30 reactor animals identified in district A, 21 were adult animals while eight were heifers and one was a calf. In district B, all seven reactors identified by SIT were adults. Of the 37 reactors identified by SIT, 23 (4%) were milch animals. Of the DST positive animals, 16 (12 adults, 02 heifers, and 02 calves) were in district A while two (both adults) were in district B. Seventeen animals showed higher PPD A response. Of the SICCT positive animals, three were adults and one was

heifer and all were from district A. Forty-five animals had a skin thickness difference of 2–3 mm by SIT; these animals were categorized as inconclusive reactors. PPD A response was high in 32 buffaloes (27 adults, 4 heifers, 1 calf) and it varied from 4–20 mm. Of the DST reactors, it was observed that six animals were negative by SIT. Out of the 49 dairy farms whose animals were tested, reactor animals by at least one of the tests used were identified in only 18 dairy farms. None of the tested animals in the remaining 31 dairy farms showed reactivity to tuberculins or DST.

The data was also analyzed with respect to the magnitude of skin thickness seen at 72 hours post-administration of antigens. With bovine PPD alone, 27 animals in both the districts had differences in skin thickness between 4-6mm while in the remaining 10 animals the difference was more than 7mm. Using SICCT, all four reactors had 4-6mm difference in skin thickness. With DST, 10 buffaloes were in the range of 2-3mm and 8 buffaloes showed 4-6mm increase in skin thickness (Fig. 1).

From the present study, we also observed discrepancies in reactions induced by the antigens injected (Fig. 2). Twenty-five animals that were reactors by SIT were negative by SICCT and DST (Fig. 2). None of the animals tested was found reactor both by SICCT and DST. Interestingly, there were a total of 6 animals that were DST positive but SIT negative. Combining two tests i.e., SIT and DST, 43 animals were found to be reactors. It was observed that SIT and DST showed moderate agreement with Cohen's Kappa of 0.41 (95% CI: 0.23, 0.59) for test positive cases (Table 1). Whereas, low Kappa agreement of 0.18 (95% CI: 0.02, 0.34) was found between SIT and SICCT.

After variable selection only two putative risk factors – the geographic region where the herd was located and lactation stage were associated with positivity to the SIT and DST tests. For the SICCT response, no variables passed the univariate screen likely due to the sparsity of positive results for this test. The final SIT and DST models including herd level random effect terms showed no evidence for a lack of fit with the Hosmer-Lemeshow test with p-values of 0.23 and 0.99, respectively. The SIT model demonstrates an excellent classification ability with an AUC = 0.88, while the DST model is outstanding with AUC = 0.96. However, this discrimination ability for the DST response appears to be driven purely be the random effects with neither of the selected risk factors being statistically significant. Results for the SICCT model are summarized in Table 2, which suggests that animals in district B have a reduced risk of being test positive to the SIT test (OR = 0.11, 0.02–0.55, 95% CI) while animals in the second lactation stage have a higher risk of testing positive (OR 4.39, 1.41–13.7, 95% CI).

The fitted latent class model demonstrates an excellent agreement with the apparent reactor status across all infected and uninfected herds (Supplementary Fig. 1). The entire observed values lie within the 95% posterior predictive intervals of the estimated model (Fig. 3). Posterior predictive p-values – based on the pairwise probability of agreement between each pair of diagnostic tests - are all within a 95% interval with no evidence of conditional dependence between the tests based on this data.

The latent class model estimates distinct differences in performance between the three diagnostic tests – albeit with relatively large overlaps in the posterior distributions (Fig. 3). Our analysis suggests that the DST test has lower diagnostic sensitivity (39%, 23–62 95% CI) compared to SIT (67% (43–96, 95% CI) but comparable specificity (99%, 96–100 95% CI) to the SICCT test (99.7, 98.4–100 95% CI) (Table 3). The DST has an apparent intermediate sensitivity to SIT and SICCT but with broad and overlapping predictive / credible intervals.

Discussion

The present study was undertaken to compare tuberculin skin test with defined skin antigen in buffaloes. Selection of test(s) used for screening animals is critical for control programs. Hence, it is crucial to validate new diagnostics in buffaloes which can accurately detect the case in order to develop effective control strategies for bTB in buffaloes. Lack of a gold standard test to define positive and negative animals in a herd is a concern in determining the accuracy of any screening test.

Here, we tested female buffaloes (*Bubalus bubalis*) in organized dairy farms in two districts of Haryana, India using the WOAH-recommended standard SIT and SICCT skin tests. It is important to note here the crucial differences in these tests in order to understand the limitations while interpreting results. While the SIT offers high sensitivity, PPD-B also elicits an inflammatory reaction in animals sensitized with non-tuberculous mycobacteria (NTM) due to the presence of cross-reactive antigens, resulting in decreased specificity of the test. In order to help improve diagnostic specificity, the SICCT is used wherein both bovine and avian tuberculins are injected simultaneously side-by-side into the skin of the neck. This allows better discrimination than the SIT between animals infected with members of the MTBC and those sensitized to tuberculin due to exposure to members of the *M. avium* complex or to environmental non-pathogenic mycobacteria. In regions with high prevalence of NTM, the SICCT is recommended; however, increased specificity of SICCT implies a drop in sensitivity. Moreover, these tuberculin antigens are unable to differentiate infection from BCG vaccination due to the presence of cross-reactive antigens. We compared the performance of a peptide-based defined antigen skin test (DST) with that of the tuberculins in a larger cohort of female buffaloes. This test has previously been assessed in both experimental and field trials in cross-bred cattle [32, 33]. A proof-of-concept study to evaluate DIVA capability of DST was performed in cross-bred cattle in India [34]. Recently, a pilot DST dose optimization trial was also conducted in domestic water buffaloes [21].

In the present study, a total of 543 female buffaloes from organized dairy farms in two districts in the state of Haryana were skin tested for diagnosis of bTB using both tuberculins and DST. A total of 6.81% and 0.73% buffaloes in two districts were found to be reactors by SIT and SICCT, respectively. In the present study, the peptide-based DST detected six additional animals as reactors which were negative by SIT and SICCT. Similarly, 25 animals detected as reactors by SIT were found non-reactors by DST. All SICCT positive animals were also DST non-reactors. These results raise important questions on performance of these tests and the underlying reasons behind these discrepancies. Firstly, the tuberculins themselves are crude reagents that are derived from culture supernatant of *M. bovis*AN5 strain (PPD-B)

and *M. avium* (PPD-A). A study comparing the potency of PPD-A and -B from various suppliers found that while PPD-A quality was relatively constant, PPD-B quality varied considerably, highlighting a lack of proper standardization [34]. We would like to mention that a single batch of PPDs was used in the current study. It has also long been recognized that exposure to environmental mycobacteria confounds the accurate interpretation of tuberculin-based skin test results [3, 35, 36]. Prevalence of environmental mycobacteria is particularly high in regions that have tropical weather [36]; the same is the case with the state where the study was carried out.

Our latent class analysis suggests that the DST has a sensitivity that is intermediate between the SICCT and SIT test and specificity comparable to the SICCT test. The uncertainty in these estimates, due to the relatively small sample and group sizes, is reflected in overlapping posterior distributions for diagnostic parameters and wide credible intervals for the bTB infection within each herd. The sample size may also contribute to the lack of evidence for conditional dependence between the diagnostic tests. The absence of any such evidence made exploration of alternative models to estimate such dependence between tests moot for this study, but cannot be ruled out. All three diagnostic tests measure different aspects of the animal's immune response to *M. bovis* rather than presence or absence of the organism itself. Indeed, the SIT and SICCT tests are designed to be dependent on each other in the sense that the avian response is used to increase the specificity of SICCT at the expense of sensitivity. The extent to which the sensitivity and specificity of the SIT and SICCT tests trade off against each other within this particular population is difficult to assess in the absence of microbiological or pathological confirmation of infection. The triangulation we carry out here against the DST test provides some insight into this trade-off, but validation of these estimates requires further studies including necropsies of reactor animals and culture of causative pathogens to both directly address this issue and begin to understand the other discrepancies in response between these alternative diagnostic tests.

It has been reported that specific antigens such as ESAT-6, CFP-10, and Rv3615c are present in field strains of *M. bovis* but are either absent or not immunogenic in BCG vaccine strain [37]. Srinivasan *et al*. (2019) assessed a peptide cocktail composed of 40-mer peptides covering the sequences of ESAT-6, CFP-10, and Rv3615c with a 20-residue overlap [peptide cocktail–long (PCL)] and a recombinant fusion protein of the same three antigens in animals experimentally infected with *M. bovis* and naive animals [2]. The cocktail was administered intradermally in the neck region. The results suggested that PCL performed better than the fusion protein and both were able to accurately detect infected animals and could differentiate them from uninfected animals with high sensitivity and specificity. Defined skin test, peptide-based cocktail of the above-mentioned antigens, has the potential to differentiate infected animals from BCG vaccinated animals i.e., DIVA capability [6]; the tuberculins lack the said potential.

Few animals in this study exhibited higher response to both bovine and avian PPDs and in some animals, PPD-A response was higher than PPD-B. It may be possible to get such a response from environmental mycobacteria. Proano-Perez *et al*. (2009) also reported that few animals exhibited higher PPD-A response and this response decreased significantly with age [38]. These authors opined that *Mycobacterium avium* complex (MAC) is more prevalent in the environment than *M. bovis*, and young animals are in contact

with these environmental mycobacteria early in life. It is to mention that recent studies report the presence of *M. orygis* rather than *M. bovis* in cattle and/or buffalo [39–42]. In south-east Asia *M. orygis* has been isolated from cattle and monkey in Bangladesh [43]. In India, *M. orygis* has also been reported from dairy in cattle [42]. The accuracy of DST for diagnosing infection other than *M. bovis* has yet not been established. Further studies ae needed to correlate the skin test reactions or outcome with the isolation of pathogen from the animals. Such studies in large cohorts can help to determine the performance of tuberculins or DST.

In conclusion, combined with the existing limitations of non-standardized and varying performance characteristics of current diagnostic tests, there is an urgent need for well-standardized skin tests to enable accurate monitoring of bovine tuberculosis over time. Defined antigen skin tests such as the peptide-based cocktail used in this study are specific and also provide the much-needed DIVA capability of implementation of vaccine-based intervention strategies in LMICs.

Abbreviations

bTB	Bovine Tuberculosis
DST	Defined Antigen Skin Test
PPD	Purified Protein Derivative
WOAH	World Organization of Animal Health
SIT	Single Intradermal Test
SICCT	Single Intradermal Comparative Cervical Test
MTBC	Mycobacterium Tuberculosis Complex
LMICs	Lower Middle-Income Countries
DIVA	Differentiating Infected from Vaccinated Animal
IAEC	Institutional Animal Ethics Committee
NTM	Non-Tuberculous Mycobacterium
BCG	Bacille Calmette and Guerin
MAC	Mycobacterium Avium Complex

Declarations

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Author Contribution

NJ, VK, SMB, SS, MV and DBa conceptualized the study. MK, TK, BLJ, DA, MS conducted the testing of animals in field. MK, NJ, YB and AC did the statistical analysis. MK and NJ prepared the first draft. All authors contributed to the article and approved the submitted version.

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Data Availability

The datasets generated during and/or analyzed during the current study are presented in the manuscript.

Ethical Approval

The study was approved by the Institutional Animal Ethics Committee (IAEC) vide proceeding no. VCC/IAEC/1630-58 dated 26.07.2018 of the Lala Lajpat Rai Veterinary and Animal Sciences University (LUVAS, Hisar, Haryana, India). All methods were performed in accordance with the relevant guidelines and regulations of IAEC.

Conflict of Interest

The authors declare that there is no conflict of interest.

Consent for publication

Not applicable.

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Tables

Table 1 Agreement of SIT with SICCT and DST

Test	SICCT		DST	
	Negative	Positive	Negative	Positive
SIT negative	506	0	500	06
SIT positive	33	04	25	12
Total	539	04	525	18
Cohen's Kappa (95% CI)	0.18 (0.02, 0.34), p = 0.001		0.41 (0.23, 0.59), p = 0.001	

SIT, Single intradermal test; SICCT, Single intradermal comparative cervical test; DST, Defined skin test

Table 2 Comparison of tuberculin's and defined skin test and associated risk factors for bovine tuberculosis in buffaloes.

Characteristic	OR	95% CI	p-value
Region			
A			
B	0.11	0.02, 0.55	0.007
Lactation stage			
0			
1	3.07	0.82, 11.4	0.095
2	4.39	1.41, 13.7	0.011
3	0.84	0.16, 4.49	0.8
4	3.04	0.91, 10.2	0.071
5+	1.62	0.44, 5.97	0.5

Table 3 Estimated sensitivity and specificity of bTB diagnostics from latent class analysis with 95% Bayesian credible intervals (CI)

Test	Sensitivity (95% CI)	Specificity (95% CI)
SIT	67% (43-96)	95.9 (92-99)
SICCT	19% (16 -29)	99.7 (98-100)
DST	39% (23-62)	99% (96-100)

SIT, Single intradermal test; SICCT, Single intradermal comparative cervical test; DST, Defined skin test

Figures

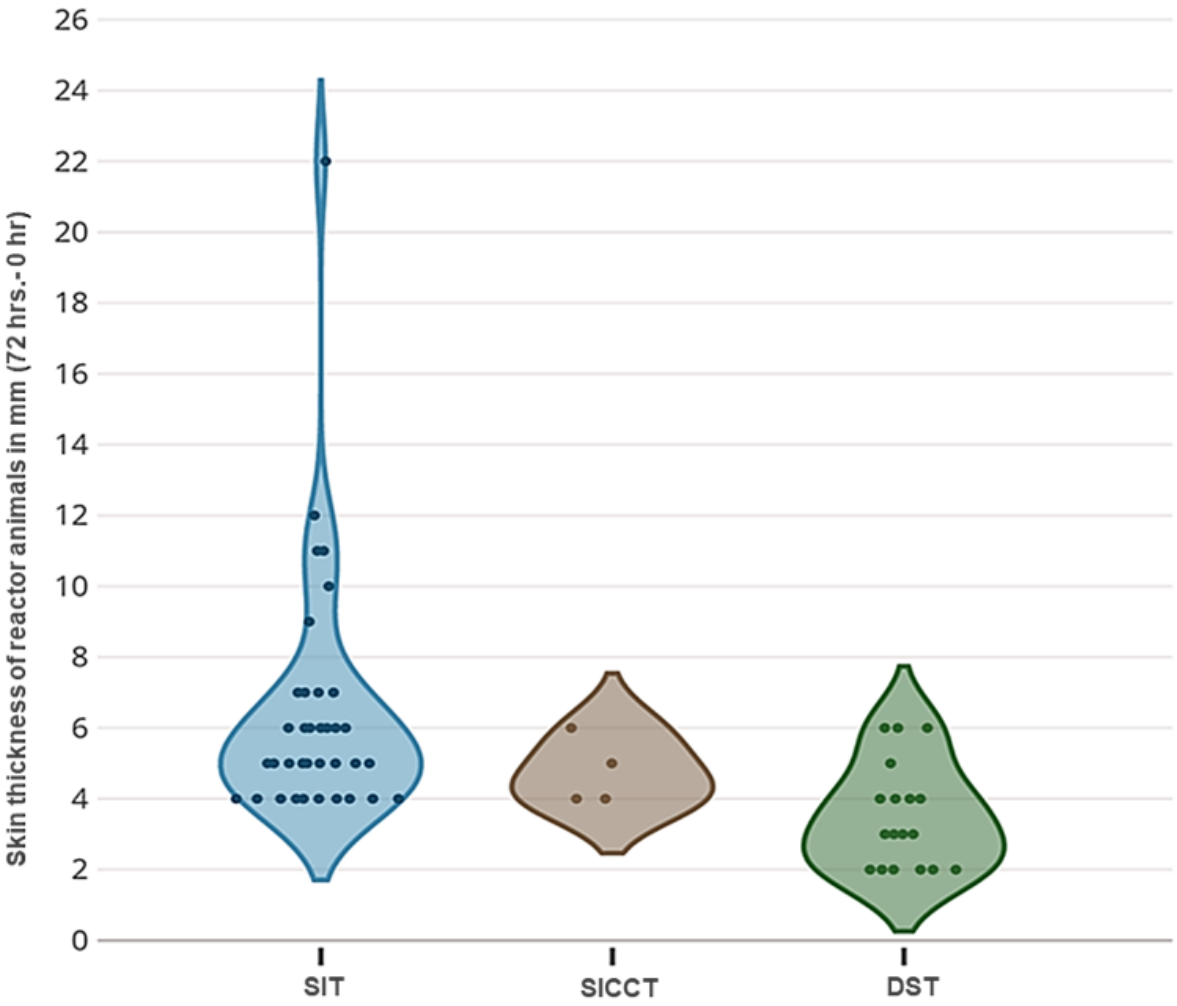


Figure 1

Distribution of skin thickness amongst the 43 buffaloes that were identified as reactors by different skin tests. SIT, Single intradermal test; SICCT, Single intradermal comparative cervical test; DST, Defined skin test

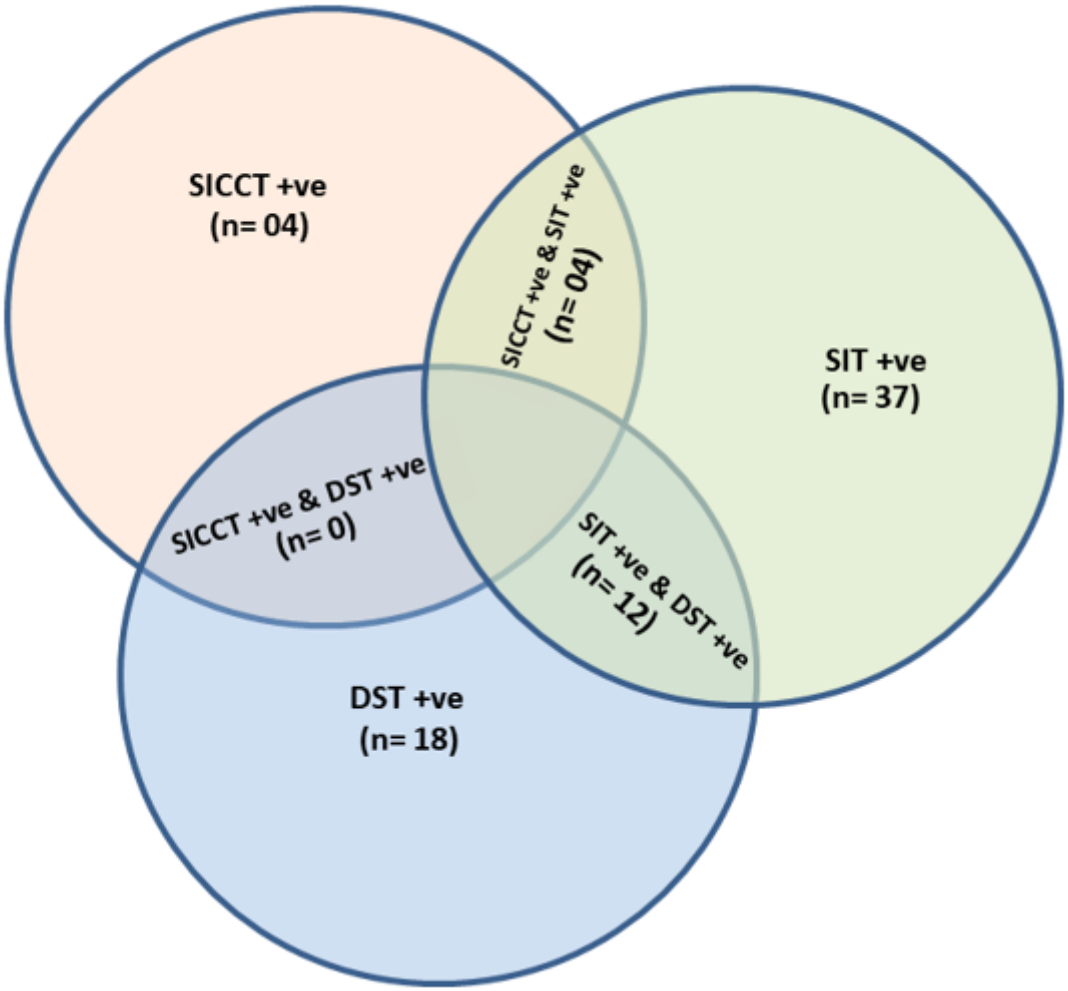
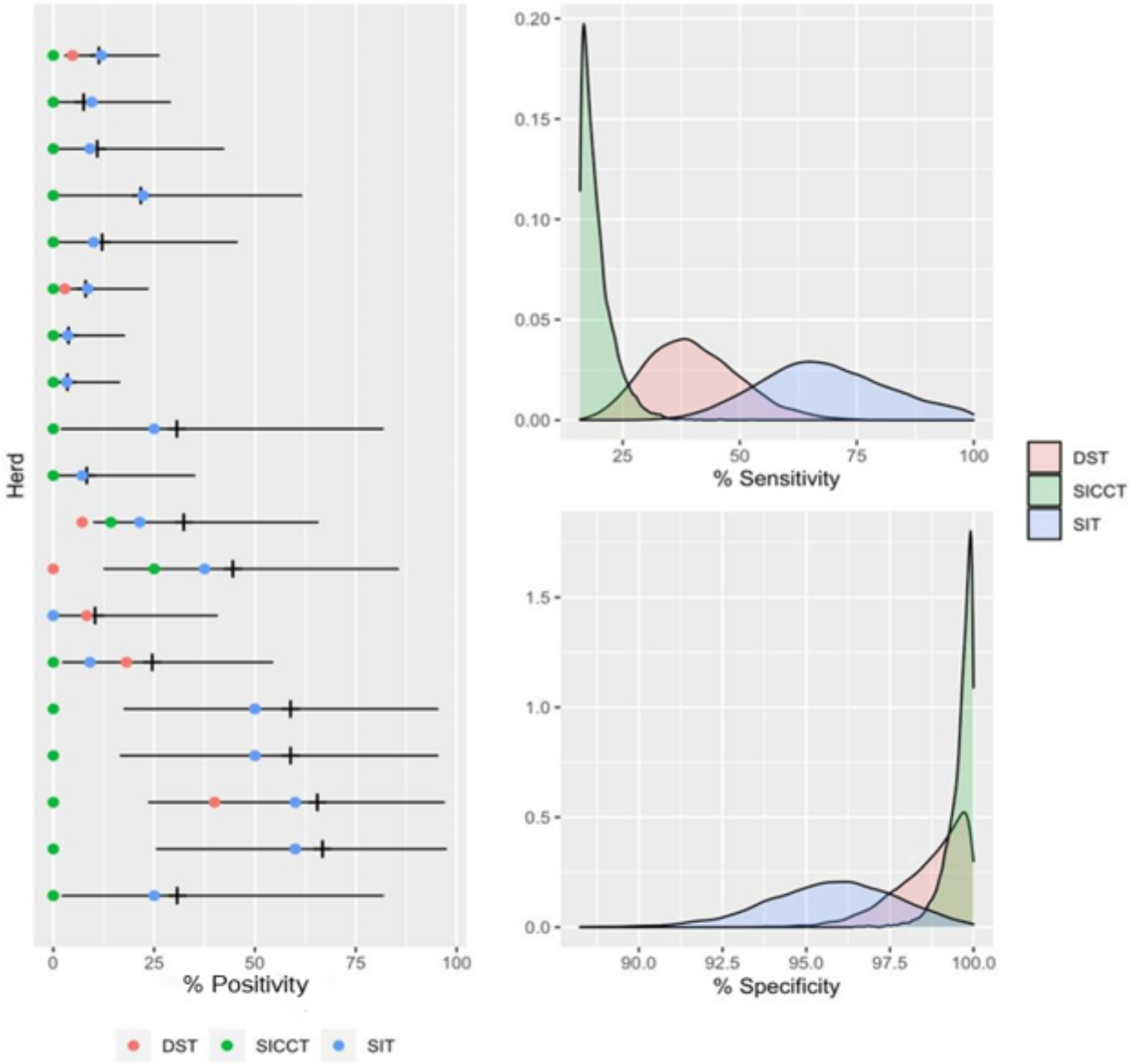


Figure 2

Number of adult buffaloes showing reaction to bovine and avian tuberculins and defined antigen skin test.

SIT, Single intradermal test; SICCT, Single intradermal comparative cervical test; DST, Defined skin test, +ve, Positive; -ve, Negative



Interaction	Posterior predictive p-value $P(\alpha^{*}_{k,k'} > \alpha_{k,k'})$
SIT-SICCT	0.89
SIT-DST	0.85
SICCT-DST	0.94

Figure 3

Left) Posterior estimates of the true within-herd reactor status, points indicate the observed reactor status within each herd for the DST (red), SICCT (green) and SIT (blue) tests. (Right) Posterior distributions for the sensitivity and specificity of the SIT, SICCT and DST diagnostic tests. Table shows Posterior predictive p-values (calculated from pairwise probability of agreement between each test).

Supplementary Files

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- SupplemantryFigure1.docx
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