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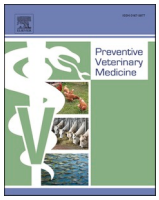

Accuracy of molecular diagnostic assays for detection of *Mycobacterium bovis*: A systematic review and meta-analysis

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ABSTRACT

Bovine tuberculosis (bovine TB) is a chronic wasting disease of cattle caused primarily by *Mycobacterium bovis*. Controlling bovine TB requires highly sensitive, specific, quick, and reliable diagnostic methods. This systematic review and meta-analysis evaluated molecular diagnostic tests for *M. bovis* detection to inform the selection of the most viable assay. On a per-test basis, loop-mediated isothermal amplification (LAMP) showed the highest overall sensitivity of 99.0% [95% CI: 86.2%-99.9%] and specificity of 99.8% [95% CI: 96.2%-100.00%]. Quantitative real-time polymerase chain reaction (qPCR) outperformed conventional PCR and nested PCR (nPCR) with a diagnostic specificity of 96.6% [95% CI: 88.9%-99.0%], while the diagnostic sensitivity of 70.8% [95% CI: 58.6–80.5%] was comparable to that of nPCR at 71.4% [95% CI: 60.7–80.2%]. Test sensitivity was higher with the input of milk samples (90.9% [95% CI: 56.0%-98.7%]), while specificity improved with tests based on major *M. bovis* antigens (97.8% [95% CI: 92.3%-99.4%]), the IS6110 insertion sequence (95.4% [95% CI: 87.6%-98.4%]), and the RD4 gene (90.7% [95% CI: 52.2%-98.9%]). The design of the currently available molecular diagnostic assays, while mostly based on nonspecific gene targets, prevents them from being accurate enough to diagnose *M. bovis* infections in cattle, despite their promise. Future assay development should focus on the RD4 region since it is the only target identified by genome sequence data as being distinctive for detecting *M. bovis*. The availability of a sufficiently accurate diagnostic test combined with the routine screening of milk samples can decrease the risk of zoonotic transmissions of *M. bovis*.

1. Introduction

Bovine tuberculosis (bovine TB) is a chronic granulomatous disease caused by bacteria of the *Mycobacterium tuberculosis* complex (MTC), in particular *Mycobacterium bovis* (Li et al., 2022). Although *M. bovis* is mainly a pathogen of cattle, a range of other domestic and wild animal hosts are also affected (Sichewo et al., 2020; 2019). Due to the global distribution of this pathogen, significant economic losses are reported from livestock production systems across the world, while conservation efforts are negatively impacted where wildlife is concerned (Clarke et al., 2021; Elsohaby et al., 2020). More importantly, human cases of tuberculosis stemming from infection with *M. bovis* are becoming increasingly common and are of great concern globally (Hlokwe et al., 2016). Veterinary officials, farmers, and abattoir workers, who encounter *M. bovis* as an occupational hazard, are often at risk of infection (Devi et al., 2021). Even though other routes of infection are possible, the main route of transmission in humans is the consumption of contaminated undercooked meat and unpasteurized milk and milk products (Silva et al., 2018).

Programs for the control and eradication of bovine tuberculosis in most countries, including South Africa, are based on the test and slaughter of positive reactors to the World Organization for Animal Health's (WOAH) recommended intradermal tuberculin skin test (TST) (Garbaccio et al., 2019). As an ancillary test to the TST, field-based detection is also achieved by the interferon gamma release (IFN-γ) assay, which detects the level of interferon gamma released by sensitized

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lymphocytes in infected animals (Sidhu et al., 2020). However, the sensitivity of both the TST and IFN-γ assays can be compromised during early infection and in animals with a poor immune response (Algammal et al., 2019).

Routine laboratory-based diagnosis of bovine tuberculosis is based on the conventional culture and biochemical characterization of the causative agent, which is considered the gold standard test for bovine tuberculosis (Araújo et al., 2014). Although highly specific, with wide application as the gold standard test, the culture of slow-growing bacteria such as *M. bovis* is time consuming, with a turnaround time of anywhere between six to eight weeks (Wards, 1995), while biochemical tests are not sufficiently sensitive, particularly in the case of intermediate strains that react differently to biochemical tests (Sales et al., 2014).

Considering that symptomatically, tuberculosis caused by *M. tuberculosis* is practically indistinguishable from disease caused by *M. bovis* (Kanipe and Palmer, 2020), timely differentiation of the two organisms is crucial for the administration of suitable treatment since *M. bovis* is resistant to pyrazinamide, the main treatment option for tuberculosis (Kapalamula et al., 2021). For this reason, the control and management of bovine tuberculosis in animals have a direct beneficial effect on their control in humans. Timely diagnosis and treatment of infectious diseases requires a robust approach to diagnostics using reliable tools that are rapid and accurate (Shah et al., 2002). Molecular assays offer considerable benefits as alternative forms of diagnosis, being relatively more rapid and sensitive (Zahran et al., 2014).

However, to the best of our knowledge, there is currently a scarcity of molecular-based assays for the specific detection of *M. bovis* infections, and those available have extensive application in research and epidemiological studies but are not well established as diagnostic tools (Tao et al., 2020; Taylor et al., 2007). Moreover, *M. bovis* shares >99.95% sequence identity with *M. tuberculosis*, which makes the development of diagnostic tests that are specific for *M. bovis* a challenge (Garnier et al., 2003; Guimaraes and Zimpel, 2020). Advances in the diagnosis of *M. bovis* in cattle and its differentiation from the closely related *M. tuberculosis* may have significant benefits for the development of suitable approaches for the control and management of bovine tuberculosis. Several reviews and meta-analyses report on available post-mortem and antemortem TB diagnostic tests (Bezos et al., 2014; de la Rua-Domenech et al., 2006; Downs et al., 2018a, 2018b; Farnham et al., 2012; Nuñez-Garcia et al., 2018; Roy et al., 2020; Schiller et al., 2010); however, none focus specifically on the accuracy of molecular diagnostic methods targeting large ruminants. Therefore, this systematic review seeks to determine the diagnostic accuracy of molecular assays designed for the diagnosis of *M. bovis* in cattle. These assays may also be useful for the diagnosis of *M. bovis* infections in humans and other animals.

2. Materials and Methods

2.1. Reporting procedure

The systematic review was performed by two independent reviewers following guidelines provided in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (Bossuyt et al., 2013). Reporting was based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines as outlined in Page et al., (2021).

2.1.1. Study period and location

The systematic review took place between March 2020 and October 2022. It was carried out at the Council for Scientific and Industrial Research, Veterinary Molecular Diagnostics and Vaccines group in Pretoria, South Africa.

2.1.2. Population, index test and target condition

This systematic review and meta-analysis forms part of the doctoral degree project of the first author (LM) with an objective of developing molecular diagnostic assays for the detection of bovine tuberculosis in cattle. Since the scope of the PhD only extends as far as cattle, the target population for this study was limited to cattle suspected of having bovine tuberculosis, regardless of age or sex. The index test was any molecular diagnostic assay that has been developed or used for the detection of *M. bovis*-related infections in cattle. A positive diagnosis for the target condition, bovine tuberculosis, was defined as positive bacterial isolation and identification of the causal agent as *M. bovis* by culture and/or a positive reaction to the TST test and/or a positive IFN-γ assays as gold standard tests for bovine tuberculosis.

2.1.3. Search strategy and selection

Online databases, namely, PubMed, ScienceDirect, and SpringerLink, were searched for eligible studies. The searches were restricted to research articles published between January 1990 and January 2022. Only articles published in English were eligible for further investigation. A combination of the search terms was used to search the databases: "*Mycobacterium bovis*", "Bovine Tuberculosis", "Molecular Diagnosis", "Molecular Detection", "PCR", "Polymerase Chain Reaction", "Real-time PCR", "quantitative PCR", "nested PCR", "nPCR", "Multiplex PCR", "RPA", "Recombinase Polymerase Amplification", "LAMP", and "Loop-mediated Isothermal Amplification". These key terms were used either individually or in combination with "AND" and/or "OR" operators. Articles were screened for relevance and downloaded after assessment of their titles and abstracts. A thorough search of the reference lists of each of the downloaded articles was conducted to identify additional studies of relevance.

2.1.4. Study inclusion/exclusion criteria

The titles of all the search results were screened for eligibility. Once all the articles that did not match the review question were eliminated based on titles, each of the remaining articles' abstracts was thoroughly investigated. Then, the contents of each research article that was considered eligible after abstract screening were thoroughly scrutinized to determine final eligibility for inclusion in the systematic review and meta-analysis. The following inclusion criteria were applied to each full-text article: (i) study employs the use of molecular diagnostic techniques for the detection of bovine tuberculosis, (ii) study detailing specific detection of *M. bovis* or the distinction of *M. bovis* from the BCG vaccine strain or other species of the MTC, (iii) study involving the comparison of molecular techniques with either of the gold standard tests as per the definition of the gold standard provided herein, (vi) full-text article available in English, (vi) assays validated on clinical samples collected from cattle (and buffaloes where cattle and buffalo samples could not be separated), and (vii) articles from which diagnostic test accuracy (DTA) data could be extracted (true positive (TP), true negative (TN), false positive (FP), false negative (FN)). Any other study that did not meet the inclusion criteria specified above was excluded from our study. Study inclusion was independently conducted by two of the authors (LM and MM); where decisive action could not be taken; a third independent reviewer was approached (ES) for a final decision.

2.1.5. Data extraction

Data extracted from the included studies were organized into an Excel spreadsheet. Data on the study characteristics were collected as follows: (i) author, (ii) publication year, (iii) index test, (iv) targeted gene, (v) clinical sample, (vi) gold standard test and (vii) diagnostic test accuracy data (TP, TN, FP, and FN). All studies from which sensitivity or specificity could not be calculated using the extracted DTA data were excluded from the meta-analysis. In some cases, the use or evaluation of two different index tests was reported in the same article, and data on both index tests were extracted such that a single article could contribute multiple studies to the meta-analysis.

2.1.6. Quality assessment of included studies

Quality assessment of the included studies was performed in accordance with a version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting, 2011) that has been adapted for veterinary use and is known as VETQUADAS (VQ) (Downs et al., 2018a, 2018b). Quality assessment of studies was performed independently by two of the authors (LM and MM) in accordance with the questions outlined in supplementary material 3 published by Roy et al., (2020). Agreement between authors to items 1–14 of the VQ tool was calculated using a 3×3 contingency table as described in Downs et al., (2018a), (2018b).

2.1.7. Data analysis and synthesis

Sensitivity and specificity estimates together with their 95% confidence intervals were computed using the random effects model on Open MetaAnalyst (Wallace et al., 2012) and plotted on forest plots. As a measure of between-study heterogeneity, the Higgins Statistic (I^2), which quantifies the percentage variation between studies, was employed (Higgins and Thompson, 2002). According to Deeks et al., (2019), the I^2 statistic can be classified as minor (0–40%), moderate (30–60%), significant (50–75%), and substantial (75–100%). Subgroup analyses were performed based on pooled accuracy estimates to determine diagnostic accuracy based on the following factors: (i) index test, (ii) clinical sample and (iii) target gene.

3. Results

In this study, searches of the different databases yielded a total of 6245 articles. After the removal of duplicates, 6173 articles remained. The titles of these articles were screened, leaving 134 articles eligible for further screening. The abstracts of these 134 articles were screened, and 85 articles were removed, leaving 49 eligible for full-text screening; thereafter, only 15 articles fulfilled the eligibility criteria for inclusion in the systematic review and meta-analysis (Fig. 1). Thirty-four articles were excluded for the following reasons: cannot compute a 2×2 contingency table (n=5), optimization of deoxyribonucleic acid (DNA) extraction methods (n=1), gold standard comparison not completed (n=4), use of nonbovine samples (n=16), assays targeting the *M. tuberculosis* complex (n=6), could not calculate a specificity value (n=1) and used for reasons other than diagnostic purposes (n=1).

3.1. Study characteristics

Study characteristics data from a total of 23 studies emanating from 15 eligible articles were recorded (Table 1). Different studies reported the use of index tests such as nested PCR (n = 4), conventional and multiplex PCR (n = 14), real-time PCR (n = 3), LAMP (n = 1), and a microsphere-based multiplex assay (n = 1). The target condition was detected in a variety of clinical samples (Table 1), including various tissues (n = 5), blood (n = 4), milk (n = 2), nasal mucus and nasal swabs (n = 4), oral, conjunctival and sputum swabs (n = 2), and lymph nodes and lymph node aspirates (n = 6). Regions of the *M. bovis* genome that are said to be specific for the identification and differentiation of *M. bovis* from other MTC members, including the *M. bovis* Bacillus Calmette-Guérin (BCG) vaccine strain, were targeted in these studies. They include the IS6110 insertion sequence (n = 5), the RD4 sequence that flanks a 12.7 kb deletion in *M. bovis* (n = 5), the major *M. bovis* antigens MPT83 (n = 2) and MPB70 (n = 3), the hupB gene (n = 4), the TbD1 gene (n = 1), and the RvD1-Rv2031c gene (n = 1) (Table 1). The reference standard was defined as positive bacterial isolation and identification of the causal agent as *M. bovis* by culture and/or a positive reaction to the TST and/or a positive IFN-γ assay.

3.2. Quality of methodological studies

All the studies considered eligible for inclusion in the meta-analysis were subjected to the VQ assessment tool, which assessed clarity in reporting, as well as internal and external validity of the eligible studies (Fig. 2). Clarity in reporting was fair, ranging between 50.0% and 81.3%. Internal validity was generally considered good except for items

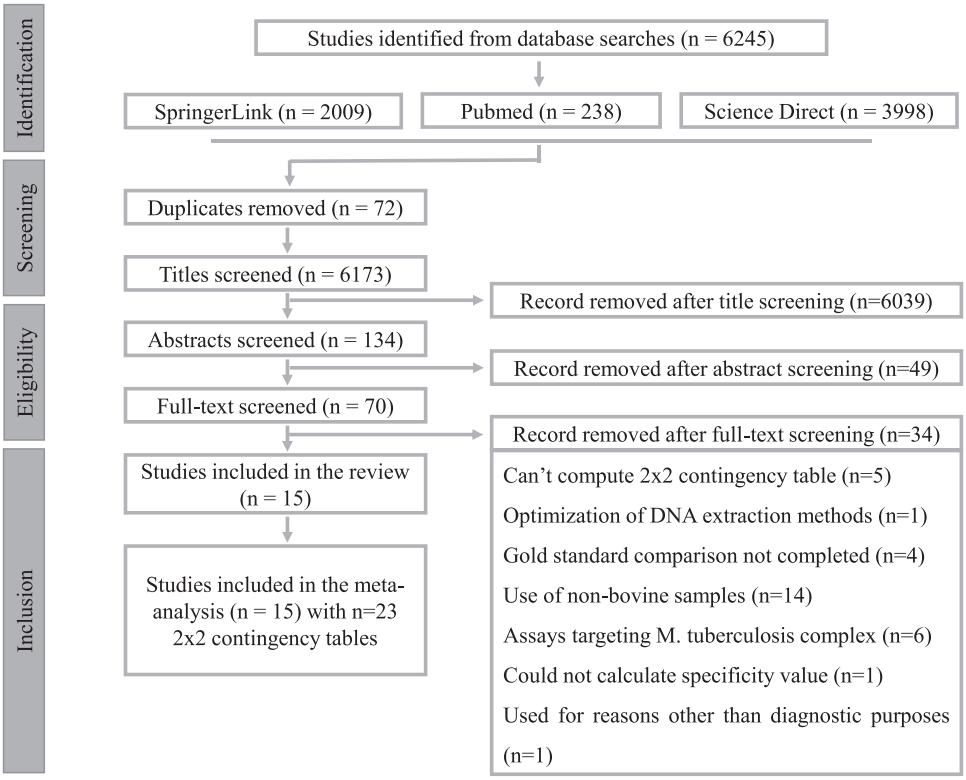

Fig. 1. PRISMA flow diagram.

Table 1
Summary of study characteristics.

Study	Year	Index test	Target gene	Clinical Sample	Reference Standard
Algammal et al.	2019	PCR	hupB	Tissue	Culture
Araujo et al.	2014	nPCR	TbD1	Tissue	Culture
Cedene et al.	2005	PCR	Unspecified	Nasal mucus	TST
Chen et al.	2010	MMA	RD4	Tissue	Culture
Costa et al.	2013	qPCR	IS6110 insertion	Tissue	Culture
de Souza Figueiredo et al.	2010	mPCR	RvD1-Rv2031c	Nasal swabs	TST
Elsohaby et al.	2020a	PCR	*M. bovis* antigen (MPB70 gene)	Blood	Culture
Elsohaby et al.	2020b	PCR	*M. bovis* antigen (MPB70 gene)	Blood	IFN-γ
Elsohaby et al.	2020c	PCR	*M. bovis* antigen (MPB70 gene)	Milk	Culture
Filia et al.	2016	PCR	Unspecified	Blood	IFN-γ
Mishra et al.	2005a	nPCR	hupB	Milk	Culture
Mishra et al.	2005b	nPCR	hupB	Blood	Culture
Mishra et al.	2005c	nPCR	hupB	Lymph node aspirate	Culture
Quan et al.	2016a	PCR	RD4	Nasal swabs	TST
Quan et al.	2016b	PCR	RD4	Nasal swabs	IFN-γ
Taylor et al.	2001a	PCR	IS6110 insertion	Lymph nodes	Culture
Taylor et al.	2001b	qPCR	IS6110 insertion	Lymph nodes	Culture
Taylor et al.	2007a	PCR	RD4	Lymph nodes	Culture
Taylor et al.	2007b	PCR	RD4	Lymph nodes	Culture
Thacker et al.	2011	qPCR	IS6110 insertion	Lymph nodes	Culture
Zahran et al.	2014	PCR	IS6110 insertion	Tissue	Culture
Zhang et al.	2011a	LAMP	*M. bovis* antigen (MPT83 gene)	Oral, conjunctival and sputum swabs	TST
Zhang et al.	2011b	PCR	*M. bovis* antigen (MPT83 gene)	Oral, conjunctival and sputum swabs	TST

PCR = Polymerase Chain Reaction, nPCR = nested PCR, mPCR = multiplex PCR, qPCR = quantitative real-time PCR, MMA = microsphere-based multiplex assay, LAMP = Loop-mediated Isothermal Amplification, TST = Intradermal Tuberculin Skin Test, IFN-γ = Interferon Gamma assay. The small letters a, b and c after the publication date indicate different sets of data from the same article. These data sets may differ in the clinical sample or gold standard test used.

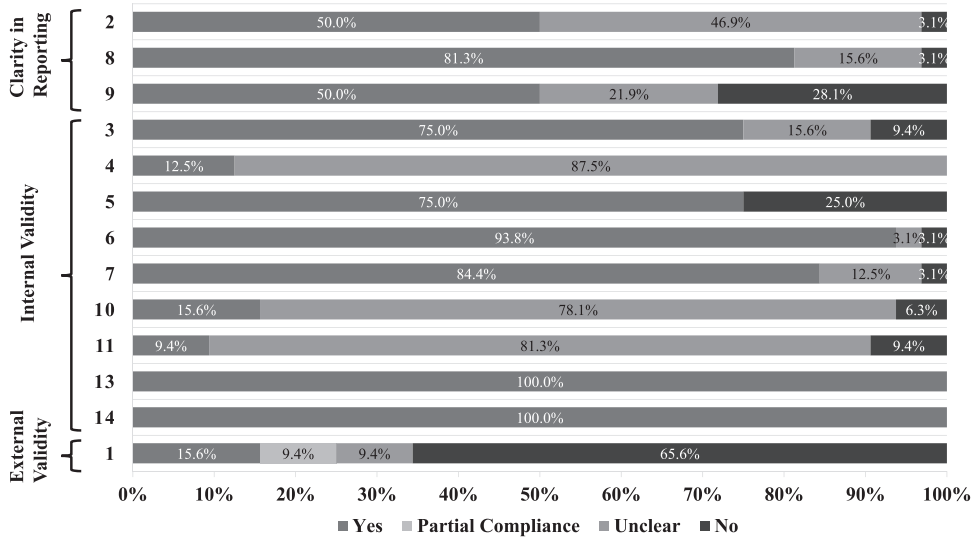

Fig. 2. Mean values of reviewers' responses to VETQUADAS items.

4 (time period between the reference standard and the index test short enough), 10 (index test interpreted without knowledge of reference standard results) and 11 (reference standard interpreted without knowledge of index test results), which scored 12.5%, 15.6% and 9.4%, respectively. Of the items measuring external validity (items 1 and 12), item 12 (same clinical data available when the test is used in practice) was excluded from the analysis because the index tests reported herein are semiautomated, with the results requiring little or no interpretation and no need for clinical data. Based on item 1 (representativeness of the spectrum of animals who will receive the test in practice) for measuring external validity, validity was generally poor at 15.6%. In relation to item 15 (source of funding for the study), six studies (40.0%) were reportedly funded by the public sector, one (6.7%) was funded by both the public and the private sector, seven (46.7%) omitted information, and in one study (6.7%), it was not clear whether the funding came from the public sector, the private sector, or both. Finally, all but one of the articles (93.3%) complied with item 16 (answers to items 1–14 are representative of all tests analyzed within the paper).

In Table 2, the percentage agreement between reviewers' responses to VQ items 1 through 14 (apart from 12) for all eligible studies is presented. The responses were dispersed unevenly, with "Yes" being the most typical response. As a result of the small number of responses, it was not possible to determine the percentage agreement between "No" and "Unclear" responses for some items. The total degree of agreement was highest for the "Yes" response (83.4%) when all items were considered and lower for the "No" and "Unclear" responses (56.0% and 67.2%, respectively).

3.3. Meta-analysis assessing the diagnostic accuracy of molecular assays for the detection of bovine tuberculosis

A summary of the accuracy estimates for each of the studies included

Table 2
Percentage agreement in possible responses to applicable VETQUADAS items studies with two reviewers.

VETQUADAS	Evaluation	Percent agreement between reviewers (%)			
Item	Category	Yes	No	Unclear	Overall
2	R	50.0	0.0	40.0	43.8
8	R	84.6	0.0	0.0	68.8
9	R	75.0	44.4	28.6	56.3
3	I	91.7	66.7	40.0	81.3
4	I	50.0	nd	85.7	81.3
5	I	83.3	50.0	nd	75.0
6	I	93.3	0.0	0.0	87.5
7	I	81.5	0.0	0.0	68.8
10	I	40.0	0.0	88.0	75.0
11	I	66.7	0.0	84.6	75.0
13	I	100.0	nd	nd	100.0
14	I	100.0	nd	nd	100.0
1	E	25.0	85.7	66.7	68.8
All		83.4	56.0	67.2	75.5

Partial compliance was coded to "Yes" for the analysis of item 1. R = clarity in reporting, I = internal validity, E = external validity. nd = not possible to determine due to the low number of responses.

in the meta-analysis is displayed in Table 3 below. Summary estimates of the diagnostic accuracy of molecular diagnostic methods for the detection of bovine tuberculosis are represented in forest plots in Fig. 3 and Fig. 4. The pooled sensitivity (Fig. 3) and specificity (Fig. 4) estimates of all the included studies were 64.4% [95% CI, 60.9%-67.8%] and 90.0% [95% CI, 88.6%-91.3%], respectively. LAMP had a higher sensitivity of 99.0% [95% CI: 86.2%-99.9%] and specificity of 99.8% [95% CI: 96.2%-100.0%] for the detection of *M. bovis* (Table 3).

3.4. Subgroup analyses

3.4.1. Accuracy among diagnostic tests based on the choice of index test

The diagnostic accuracy of the three categories of molecular diagnostic tests was analyzed, and the results are presented in Table 4. The pooled sensitivity and specificity for each of the index tests were in the range of 60.2%-71.4% [95% CI, 39.6%-80.5%] and 61.6%-96.6% [95% CI, 48.5%-99.0%], respectively. Of the 23 studies that were included in the meta-analysis, 14 reported the use of PCR and multiplex PCR with recorded sensitivity of 60.2% and specificity of 92.6%. Four studies reported on the diagnosis of bovine tuberculosis using nested PCR, and the pooled sensitivity and specificity were 71.4% and 61.6%, respectively. Furthermore, the performance of real-time PCR was also evaluated in 3 studies, and these yielded pooled sensitivity and specificity estimates of 70.8% and 96.6%, respectively. Due to the limited number of studies available, other assay types could not be included in the subgroup analysis and therefore were excluded from the meta-analysis. In this category, the pooled sensitivity and specificity were 98.5% and 94.0%, respectively.

3.4.2. Accuracy among diagnostic tests based on sample choice

The accuracy of molecular diagnostic assays was further evaluated based on the input clinical sample to determine whether sample choice influenced assay performance. The studies were divided into six groups based on the type and origin of the clinical samples used for diagnosis. The groups were blood, tissue, milk, nasal mucus/swabs, lymph nodes/aspirates and oral, conjunctival and sputum swabs. The sensitivity and specificity estimates in the six categories ranged between 19.4% and 90.9% and 71.9% and 96.5%, respectively (Table 4). Based on the data presented, higher diagnostic sensitivity was achieved with the use of milk samples at 90.9% [95% CI: 56.0%-98.7%], followed by tissues 88.7% [95% CI: 62.1%-97.4%].

3.4.3. Accuracy among diagnostic tests based on target gene selection

The choice of target gene was also taken into consideration to determine if it affected assay performance. The target genes chosen and employed for the precise identification of *M. bovis* were used to further separate the 23 studies that made up the meta-analysis into four groups. The pooled sensitivities for the different gene regions ranged between 48.8% and 92.1%, while pooled specificities were in the range of 51.6% and 97.8%. Among the four gene target groups, the assays designed based on the use of major *M. bovis* antigens (MPB70 and MPT83) had higher sensitivity at 84.3% [95% CI: 62.1%-95.4%] and specificity at 97.8% [95% CI: 92.3%-99.4%], followed closely by studies utilizing IS6110 at a sensitivity of 75.4% [95% CI: 59.1%-87.6%] and specificity of 95.4% [95% CI: 87.6%-98.4%]. The RD4 region had lower sensitivity at 49.0% [95% CI: 0.25.0%-73.2%], but the specificity was high at 90.7% [95% CI: 52.2%-98.9%], and the hupB gene displayed high sensitivity at 92.1% [95% CI: 73.7%-98.1%], while the specificity was low at 51.6% [95% CI: 42.8%-60.2%] (Table 4). Again, due to the unavailability of adequate reports, some studies could not be included in any of the subgroups and were therefore excluded from the meta-

Table 3
Summary of accuracy estimates for each study included in the meta-analysis.

Study	Year	TP	FN	FP	TN	Sensitivity [95% CI]	Specificity [95% CI]
Algammal et al.	2019	30	0	10	7	0.98 [0.79–1.00]	0.43 [0.22–0.65]
Araujo et al.	2014	51	22	36	120	0.70 [0.58–0.79]	0.77 [0.70–0.83]
Cedene et al.	2005	1	2	39	18	0.33 [0.04–0.85]	0.32 [0.21–0.45]
Chen et al.	2010	21	0	13	15	0.98 [0.72–1.00]	0.53 [0.36–0.70]
Costa et al.	2013	26	0	1	42	0.98 [0.76–1.00]	0.99 [0.84–1.00]
de Souza Figueiredo et al.	2010	2	32	0	16	0.07 [0.02–0.22]	0.97 [0.66–1.00]
Elsohaby et al.	2020a	13	0	31	201	0.96 [0.62–1.00]	0.87 [0.82–0.90]
Elsohaby et al.	2020b	41	24	3	177	0.63 [0.51–0.74]	0.98 [0.95–1.00]
Elsohaby et al.	2020c	5	0	7	233	0.92 [0.38–1.00]	0.97 [0.94–0.99]
Filia et al.	2016	1	0	3	117	0.75 [0.11–0.99]	0.97 [0.92–0.99]
Mishra et al.	2005a	4	0	22	22	0.90 [0.33–0.99]	0.50 [0.36–0.64]
Mishra et al.	2005b	2	0	11	17	0.83 [0.19–0.99]	0.60 [0.42–0.76]
Mishra et al.	2005c	2	0	16	17	0.83 [0.19–0.99]	0.52 [0.35–0.68]
Quan et al.	2016a	7	47	0	152	0.14 [0.07–0.25]	1.00 [0.95–1.00]
Quan et al.	2016b	7	10	0	189	0.42 [0.22–0.65]	1.00 [0.96–1.00]
Taylor et al.	2001a	26	2	0	10	0.91 [0.74–0.98]	0.96 [0.55–1.00]
Taylor et al.	2001b	20	8	0	10	0.71 [0.52–0.84]	0.96 [0.55–1.00]
Taylor et al.	2007a	49	49	3	8	0.50 [0.40–0.60]	0.73 [0.41–0.91]
Taylor et al.	2007b	51	35	4	5	0.59 [0.49–0.69]	0.56 [0.25–0.82]
Thacker et al.	2011	20	10	0	18	0.66 [0.48–0.80]	0.97 [0.69–1.00]
Zahran et al.	2014	4	4	0	1	0.50 [0.21–0.79]	0.75 [0.11–0.99]
Zhang et al.	2011a	50	0	0	200	0.99 [0.86–1.00]	1.00 [0.96–1.00]
Zhang et al.	2011b	36	14	0	200	0.72 [0.58–0.82]	1.00 [0.96–1.00]

TP = true positive, FN = false negative, FP = false negative, TN = true negative. The small letters a, b and c after the publication date indicate different sets of data from the same article. These data sets may differ in the clinical sample or gold standard test used.

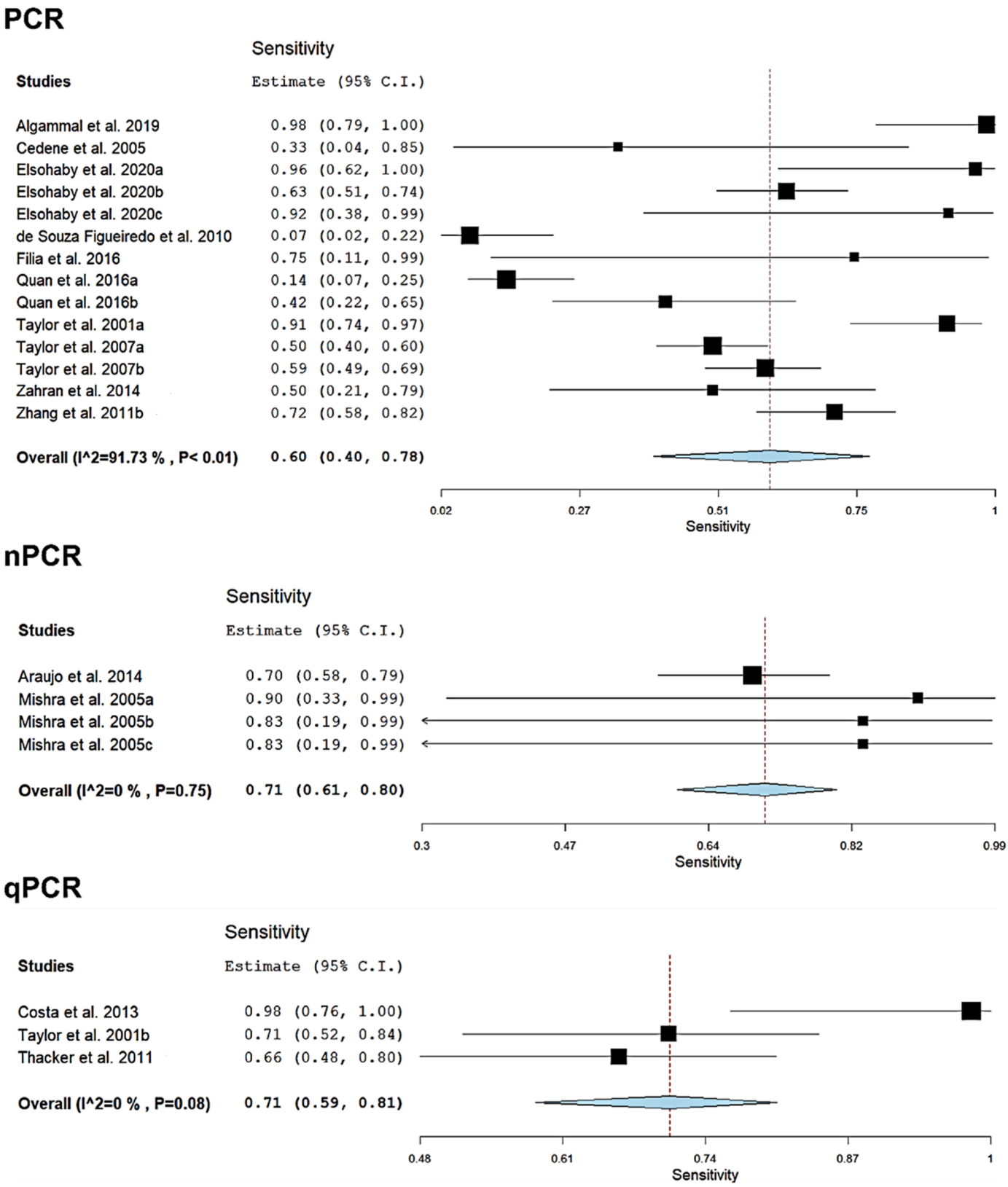

Fig. 3. Forest plots representing the sensitivity of molecular diagnostic methods (PRC, nPCR and qPCR) for the detection of *M. bovis* infections in cattle. The small letters a and b after the publication date indicate different sets of data from the same publication. These data sets may differ in the clinical sample or gold standard test used.

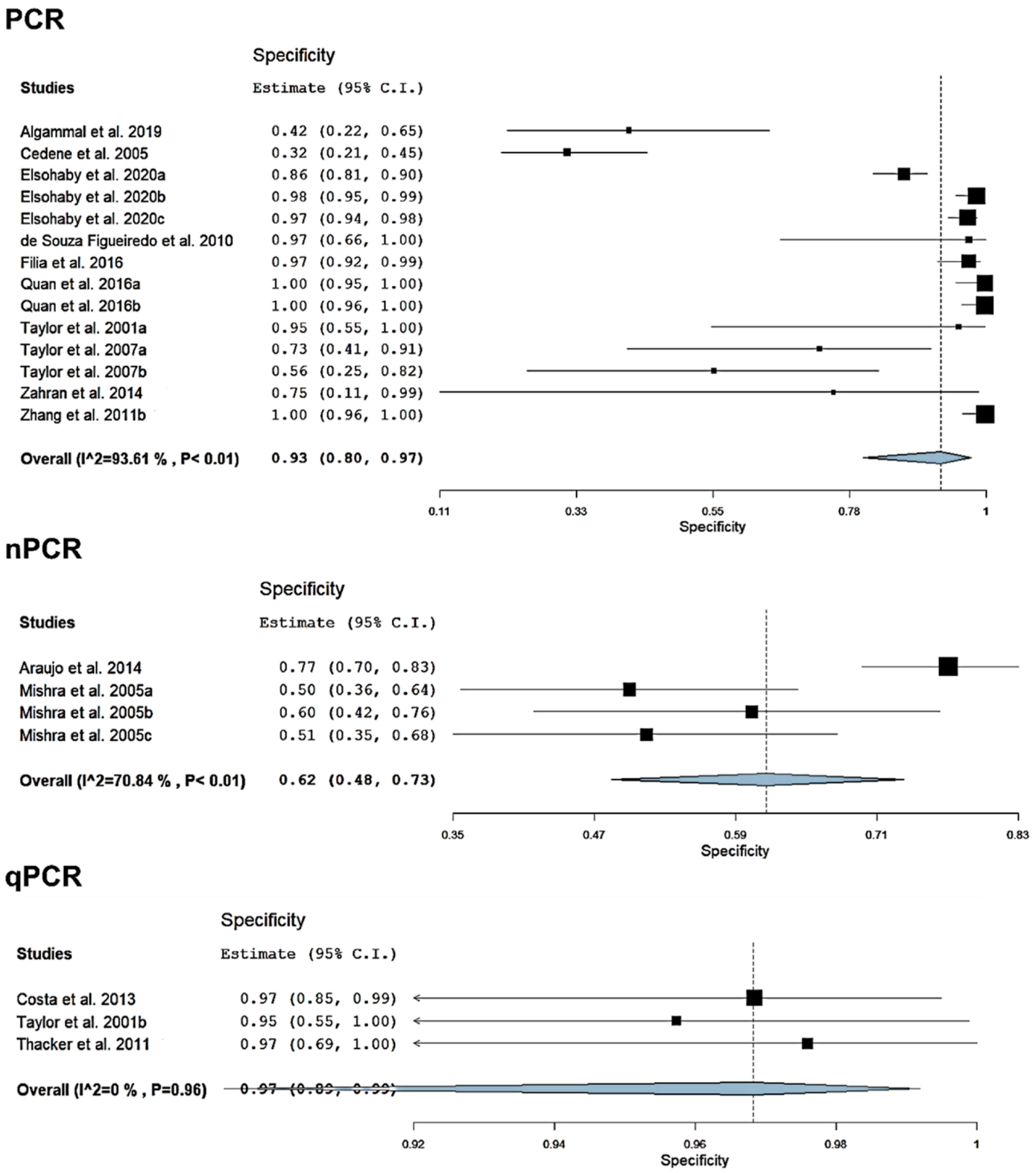

Fig. 4. Forest plots representing the specificity of molecular diagnostic methods (PRC, nPCR and qPCR) for the detection of *M. bovis* infections in cattle. The small letters a, b and c after the publication date indicate different sets of data from the same article. These data sets may differ in the clinical sample or gold standard test used.

analysis.

4. Discussion

The findings of the first systematic review and meta-analysis evaluating the accuracy of molecular diagnostic assays intended for the identification of bovine TB resulting from *M. bovis* infections in cattle are presented herein. A total of 23 different studies emanating from 15 eligible published articles (where more than one index test was evaluated in some articles) provided the data reported in this systematic

Table 4
Diagnostic accuracy estimates for subgroup analysis.

Group	Subgroup	Number of Studies	Sensitivity [95% CI]	Heterogeneity I^2 (*p*-value)	Specificity [95% CI]	Heterogeneity I^2 (*p*-value)
Index Test	nPCR	4	0.71 [0.61–0.80]	00.0% (0.747)	0.62 [0.49–0.73]	70.8% (< 0.001)
	PCR	14	0.60 [0.40–0.78]	91.7% (< 0.001)	0.93 [0.80–0.98]	93.6% (< 0.001)
	qPCR	3	0.71 [0.59–0.81]	00.0% (0.082)	0.97 [0.89–0.99]	00.0% (0.962)
Clinical Sample	Blood	4	0.66 [0.54–0.76]	00.0% (0.258)	0.92 [0.73–0.98]	92.6% (< 0.001)
	Tissue	5	0.89 [0.62–0.97]	83.4% (0.010)	0.72 [0.47–0.88]	95.0% (< 0.001)
	Milk	2	0.91 [0.56–0.99]	00.0% (0.924)	0.85 [0.34–0.98]	96.2% (< 0.001)
	Nasal swabs	4	0.19 [0.08–0.41]	58.3% (0.021)	0.97 [0.61–1.00]	87.0% (< 0.001)
	Lymph nodes	6	0.66 [0.54–0.76]	64.4% (0.010)	0.76 [0.54–0.90]	56.5% (0.022)
Target Gene	IS6110 insertion	7	0.75 [0.59–0.87]	53.1% (0.020)	0.95 [0.88–0.98]	00.0% (0.790)
	M. bovis antigens (MPT83 and MPB70)	5	0.84 [0.62–0.95]	79.1% (0.014)	0.98 [0.92–0.99]	87.1% (< 0.001)
	RD4	5	0.49 [0.25–0.73]	91.5% (< 0.001)	0.91 [0.52–0.99]	91.1% (< 0.001)
	hupB	4	0.92 [0.73–0.98]	00.0% (0.578)	0.52 [0.43–0.60]	00.0% (0.653)

PCR = Polymerase Chain Reaction, nPCR = nested PCR, qPCR = quantitative real-time PCR

review and meta-analysis. Utilizing the VQ assessment tool, the methodological qualities of these studies were evaluated. Clarity in reporting could be improved for items 2 (selection criteria of the animals clearly described) and 9 (execution of the index test described in sufficient detail) to allow replication of the test in the target population. If not appropriately addressed, these limitations have the potential to negatively impact the diagnostic process. In addition, the internal and external validity of the included studies were found to have certain limitations based on the VQ assessment. Measures of internal validity are key indicators of bias in the results of a study. Bias has been known to become a source of overestimation of diagnostic test accuracy (Downs et al., 2018a). In this study, it was frequently difficult to establish whether the results of the index test and reference standard were read without prejudice and thus interpreted objectively. Lack of blinding was identified as a frequently occurring limitation in a recent report by Roy et al., (2020). According to Westwood et al., (2005) insufficient blinding is highly and favorably correlated with estimated test performance, which in this case constitutes test sensitivity and/or specificity. Additionally, preferential testing of diseased animals or those that are highly likely to have a target condition will likely introduce selection bias, thus influencing the results of the index test (Downs et al., 2018a). Because many studies in this review selected their samples based on a positive reaction to the TST, they did not comply with item 1 of the assessment tool (animals in the study representative of animals who will receive the test), which potentially interfered with test accuracy estimates. To improve the diagnostic performance of molecular tests for the detection of bovine TB, it is crucial to design studies that eliminate these biases, which are verified in the target population and conducted and interpreted objectively.

The results of this research demonstrate substantial shortcomings in the molecular diagnostic methods currently in use for the detection of *M. bovis* in cattle. According to the literature studies, the genomes of *M. bovis* and *M. tuberculosis* share more than 99.95% sequence identity (Garnier et al., 2003; Guimaraes and Zimpel, 2020), which significantly reduces the number of reliable targets available for the development of specific *M. bovis* diagnostic assays. Comparative analyses of complete genome sequences of *M. tuberculosis* and *M. bovis* revealed that the genome sequence of *M. bovis* is interrupted at position 197 by a 12.7 kb deletion involving 11 genes (Rv1506c–1516c), which is unique to *M. bovis* (including *M. bovis* BCG) and thus allows for the differentiation of *M. bovis* from *M. tuberculosis* (Bakshi et al., 2005; Garnier et al., 2003; Guimaraes and Zimpel, 2020; Ru et al., 2017).Currently, this seems to be the only reliable target for the specific detection and differentiation of *M. bovis* from *M. tuberculosis*. In accordance with this logic, Table 1 demonstrates that only 20% (n=3) of the 15 research articles that were included in this study truly satisfy the criteria for *M. bovis*-specific assays, which has a substantial impact on how the subgroup analysis should be interpreted.

The analysis suggests that assays designed to target genes encoding the major *M. bovis* antigens, such as MBP70 and MPT83, as well as the IS6110 insertion sequence, display superior diagnostic performance compared to assays designed to target other gene regions (see Table 3 for the pooled sensitivities and specificities). Regrettably, these results cannot be accepted as true for *M. bovis* diagnosis, given the limitations mentioned above. IS6110 is a repetitive mobile element found exclusively in the genome sequence of members of the MTC (Comín et al., 2022; Thierry et al., 1990). While most members of the MTC have between 10 and 20 copies of the element, only a single copy is present in *M. bovis* (van Soolingen et al., 1993). Due to the display of a high degree of polymorphism with respect to copy number and insertion sites, the IS6110 element has been used extensively for diagnostic and typing purposes (Reyes et al., 2012). While it is highly diagnostic of MTC members, it is not specific for the identification of *M. bovis*. Similarly, the MBP70 gene present in *M. bovis* is identical to that found in *M. tuberculosis*, with only higher expression levels in *M. bovis* than in *M. tuberculosis*. From the analysis, it is evident that target gene choice influences diagnostic test performance, as suggested by Mugasa et al., (2012) and Mabe et al., (2022). However, utilizing assays designed on nonspecific target regions opens avenues for the overestimation of accuracy estimates, which might explain the high diagnostic sensitivity and specificity recorded for the IS6110 insertion sequence and major *M. bovis* antigens (MBP70 and MPT83) in this study. The assumption could be that detecting Mycobacterium in cattle correlates with diagnosing *M. bovis* infection, as *M. bovis* is the primary causative agent of tuberculosis in cattle. Given that there is evidence showing the presence of *M. tuberculosis* (Ibrahim et al., 2016; Lesslie, 1960; Lombard et al., 2021; Srivastava et al., 2008) and other nontuberculous mycobacteria in cattle tissue (Woolford et al., 2006), this assumption would be erroneous. However, this would explain why numerous assays in use today have been designed using targets that are only genus specific.

Since its discovery, several studies have investigated the potential of RD4, which encompasses Rv1506c-Rv1516c of *M. tuberculosis* H37Rv and is absent from *M. bovis* and *M. bovis* BCG (Ru et al., 2017), as a specific tool for identifying *M. bovis* infections in cattle samples (Bakshi et al., 2005; Chen et al., 2010; Kapalamula et al., 2021; Quan et al., 2016; Taylor et al., 2007; Thakur et al., 2012). Differentiation of *M. bovis* from *M. tuberculosis* is achieved by amplifying a sequence of the *M. bovis* genome that flanks the 12.7 kb deletion known as the RD4 region. This 229 bp sequence is present in both *M. bovis* and *M. tuberculosis*, but in *M. tuberculosis* it is interrupted at position 197 by a unique 12.7-kb fragment and thus generates a smaller amplification product in *M. bovis* and a larger product in *M. tuberculosis* (Ru et al., 2017). While

there is considerable potential for enhancing *M. bovis* diagnostics through focusing on the RD4 region, the meta-analysis highlighted a notable level of variability in the pooled sensitivity and specificity of these assays, as evidenced by the high heterogeneity (I^2) index. However, it's important to note that only three articles (comprising 5 studies) featuring this target gene met the inclusion criteria for this study. Consequently, the elevated I^2 index may partly stem from the limited number of datasets analyzed and the significant variation among them, with some datasets acting as outliers and leading to an overestimation of the I^2 index. Nevertheless, the RD4 region remains a promising candidate for future *M. bovis*-specific diagnostic assays, warranting thorough evaluation for subsequent applications.

At the level of individual test performance, the LAMP assay published by Zhang et al., (2011) offered the highest diagnostic sensitivity and specificity, at 99.0% and 99.8%, respectively. These results are consistent with a recent comprehensive study (Mabe et al., 2022), where LAMP displayed 100% sensitivity and specificity in the detection of Brucella abortus infections in cattle, thus outperforming other nucleic acid—based tests. However, the heightened likelihood of crossover contamination leading to false-positive results restricts the widespread use of LAMP as a diagnostic test (Suleman et al., 2016). Furthermore, the reported LAMP assay also suffers a lack of specificity by virtue of its design, which was based on the MPT83 gene target. Without a significant number of studies to support its efficiency and reliability in the detection of *M. bovis* infections, its diagnostic accuracy could not be conclusively and reliably determined.

At the subgroup level, the sensitivity of the evaluated index test categories ranged between 60.2% and 70.1%, while the specificity was higher (61.6%-91.6%). The overall accuracy was higher for qPCR than for PCR and nested PCR, with a sensitivity value of 70.8% and a specificity value of 96.9%. However, there were limitations in the further identification of a qPCR assay from within the subgroup for recommendation in the specific detection of *M. bovis* infections, as one hundred percent of the included qPCR assays were designed based on the IS6110 insertion sequence, which, as discussed earlier, is present in all members of the MTB complex. Although the assay described by Costa et al., (2013) offers 98.1% sensitivity and 96.6% specificity, its development based on the IS6110 insertion element precludes its identification and recommendation as an accurate *M. bovis* diagnostic assay.

While the choice of index test and target gene are critical for test performance, sample choice is equally important. The development of lesions in lymph nodes makes them the preferred examination location for bovine TB infections, according to the 2016 publication on tuberculosis from the South African Department of Agriculture, Forestry, and Fisheries (DAFF, 2016). Our findings suggest that lymph nodes and lymph node aspirates are only moderately suitable (pooled sensitivity of 65.8%) for the diagnosis of bovine TB using molecular methods. Based on the meta-analysis, milk (pooled sensitivity of 90.9%) and tissue samples (pooled sensitivity of 88.7%) offer better alternatives for improving diagnostic accuracy (sensitivity). Each of these sample types, however, poses unique diagnostic challenges. Milk that is intended for human consumption is frequently combined from many sources. According to Hoffman et al., (2016), this method tends to dilute the pathogen and may reduce the sensitivity of nucleic acid—based amplification. Additionally, DNA extracted from milk may contain residual milk proteins or calcium ions that act as DNA amplification inhibitors. Thakur et al., (2012) suggested that bacilli may be shed intermittently in milk, presenting yet another challenge for the use of milk for diagnostic purposes. As a result, a culture of routine and continuous screening is required before questionable cattle can be sent for slaughter. Additionally, given that these conclusions are based on only two readily available studies, additional research is required to corroborate these findings. Nevertheless, milk is a latent medium for zoonotic tuberculosis, and in areas where milk is poorly pasteurized or not at all, humans may become infected with *M. bovis* (Tao et al., 2020). Thus, screening of milk samples is essential for food safety and managing zoonotic infections and is recommended for antemortem tests, as it is also a minimally invasive sample to obtain. On the other hand, although very useful for diagnostic purposes, tissue samples (including those from the kidneys, diaphragm, liver, and spleen) become impractical substitutes for testing live animals.

Both the veterinary and public health sectors will benefit from the complete eradication of bovine TB. However, this depends, among other things, on the prompt and correct identification of the pathogen responsible for the infection as well as the prompt evacuation of diseased cattle from a herd. This is where current diagnostic techniques fall short. The conclusive diagnosis of *M. bovis* is based on culture, which is time-consuming, insensitive, and impractical for field-based testing. Numerous countries have achieved eradication based on the WOAH-recommended TST, which has proven to be a reliable field-based diagnostic technique at the herd level (Bezos et al., 2014). The late onset of an immune response to TB, however, has a substantial impact on test sensitivity. Although novel tests with enhanced sensitivity and specificity must still be developed and validated for accurate detection of *M. bovis*, assays based on nucleic acid amplification may have high promise for the detection of bovine TB. Efforts toward their development will greatly benefit from paying attention to index test selection, target gene selection and clinical sample selection throughout the design and validation process.

5. Conclusion

Since biases in research design may produce unduly optimistic estimates of accuracy, it is necessary to rigorously assess published studies for diagnostic accuracy. Failing to do so could have a substantial impact on both the development and ensuing success of disease control programmes. Molecular diagnostic tests such as LAMP and qPCR have great potential in the diagnosis of bovine tuberculosis but lack specificity for the identification and distinction of *M. bovis* from *M. tuberculosis* due to the application of nonspecific gene targets during the design process. Consequently, we are currently unable to identify a diagnostic test that is reliable enough to diagnose *M. bovis* infections in cattle. Although needing further evaluation due to the high heterogeneity expressed in both sensitivity and specificity, the RD4 region is currently the only target identified by the literature and recent genome sequence data as specific for *M. bovis* identification and is thus a recommended target for future assay development in the ongoing search for specific *M. bovis* diagnostic tests. With a sufficiently accurate diagnostic test, the routine screening of milk samples will allow the prompt removal of diseased cattle from a herd and thus reduce the risk of zoonotic transmission of *M. bovis*.

Ethics approval

No animals were used in the study; therefore, ethical approval was not needed.

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CRediT authorship contribution statement

Lerato Mabe: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mpho Muthevhuli:** Data curation, Writing – review & editing. **Oriel Thekisoe:** Conceptualization, Writing – review & editing. **Essa Suleman:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome

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References

Algammal, A.M., Wahdan, A., Elhaig, M.M., 2019. Potential efficiency of conventional and advanced approaches used to detect Mycobacterium bovis in cattle. Microb. Pathog. 134, 103574 https://doi.org/10.1016/j.micpath.2019.103574.

Araújo, C.P., Osório, A.L.A.R., Jorge, K.S.G., Ramos, C.A.N., Filho, A.F.S., Vidal, C.E.S., Roxo, E., Nishibe, C., Almeida, N.F., Júnior, A.A.F., Silva, M.R., Neto, J.D.B., Cerqueira, V.D., Zumárraga, M.J., Araújo, F.R., 2014. Detection of Mycobacterium bovis in Bovine and Bubaline Tissues Using Nested-PCR for TbD1. PLoS One 9, e91023. https://doi.org/10.1371/journal.pone.0091023.

Bakshi, C.S., Shah, D.H., Verma, R., Singh, R.K., Malik, M., 2005. Rapid differentiation of Mycobacterium bovis and Mycobacterium tuberculosis based on a 12.7-kb fragment by a single tube multiplex-PCR. Vet. Microbiol. 109, 211–216. https://doi.org/10.1016/j.vetmic.2005.05.015.

Bezos, J., Casal, C., Romero, B., Schroeder, B., Hardegger, R., Raeber, A.J., López, L., Rueda, P., Domínguez, L., 2014. Current ante-mortem techniques for diagnosis of bovine tuberculosis. Res Vet. Sci. 97, S44–S52. https://doi.org/10.1016/j.rvsc.2014.04.002.

Bossuyt, P., Davenport, C., Deeks, Jon, Hyde, C., Leeflang, M., Scholten, R., 2013. Chapter 11:Interpreting results and drawing conclusions. In: Deeks, Jonathan, Bossuyt, P., Leeflang, M., Takwoingi, Y. (Eds.), Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy. The Cochrane Collaboration, pp. 1–31.

Chen, R., Bi, Y., Yang, G., Liu, Zhiling, Liu, Zhihui, Zeng, B., Tong, T., 2010. Development of a fluorescent microsphere-based multiplex assay for simultaneous rapid detection of mycobacterium tuberculosis complex and differentiation of M. tuberculosis and M. bovis in clinical samples. Diagn. Mol. Pathol. 19, 172–179. https://doi.org/10.1097/PDM.0b013e3181d8c241.

Clarke, C., Smith, K., Goldswain, S.J., Helm, C., Cooper, D.V., Kerr, T.J., Kleynhans, L., van Helden, P.D., Warren, R.M., Miller, M.A., Goosen, W.J., 2021. Novel molecular transport medium used in combination with Xpert MTB/RIF ultra provides rapid detection of Mycobacterium bovis in African buffaloes. Sci. Rep. 11, 7061. https://doi.org/10.1038/s41598-021-86682-5.

Comín, J., Otal, I., Samper, S., 2022. In-depth analysis of IS6110 genomic variability in the mycobacterium tuberculosis complex. Front. Microbiol. 13 https://doi.org/10.3389/fmicb.2022.767912.

Costa, P., Ferreira, A.S., Amaro, A., Albuquerque, T., Botelho, A., Couto, I., Cunha, M.V., Viveiros, M., Inácio, J., 2013. Enhanced Detection of Tuberculous Mycobacteria in Animal Tissues Using a Semi-Nested Probe-Based Real-Time PCR. PLoS One 8, e81337. https://doi.org/10.1371/journal.pone.0081337.

DAFF, 2016. Bovine tuberculosis manual. Pretoria.

de la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H., Clifton-Hadley, R.S., 2006. Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. Res Vet. Sci. 81, 190–210. https://doi.org/10.1016/j.rvsc.2005.11.005.

Deeks, J.J., Higgins, J.P., Altman, D.G., 2019. Analysing data and undertaking meta-analyses. Cochrane Handbook for Systematic Reviews of Interventions. Wiley, pp. 241–284. https://doi.org/10.1002/9781119536604.ch10.

Devi, K.R., Lee, L.J., Yan, L.T., Syafinaz, A.-N., Rosnah, I., Chin, V.K., 2021. Occupational exposure and challenges in tackling M. bovis at human–animal interface: a narrative review. Int. Arch. Occup. Environ. Health 94, 1147–1171. https://doi.org/10.1007/s00420-021-01677-z.

Downs, S.H., More, S.J., Goodchild, A.V., Whelan, A.O., Abernethy, D.A., Broughan, J. M., Cameron, A., Cook, A.J., Ricardo de la Rua-Domenech, R., Greiner, M., Gunn, J., Nuñez-Garcia, J., Rhodes, S., Rolfe, S., Sharp, M., Upton, P., Watson, E., Welsh, M., Woolliams, J.A., Clifton-Hadley, R.S., Parry, J.E., 2018a. Evaluation of the methodological quality of studies of the performance of diagnostic tests for bovine tuberculosis using QUADAS. Prev. Vet. Med 153, 108–116. https://doi.org/10.1016/j.prevetmed.2017.03.006.

Downs, S.H., Parry, J.E., Upton, P.A., Broughan, J.M., Goodchild, A.V., Nuñez-Garcia, J., Greiner, M., Abernethy, D.A., Cameron, A.R., Cook, A.J., de la Rua-Domenech, R., Gunn, J., Pritchard, E., Rhodes, S., Rolfe, S., Sharp, M., Vordermeier, H.M., Watson, E., Welsh, M., Whelan, A.O., Woolliams, J.A., More, S.J., Clifton-Hadley, R. S., 2018b. Methodology and preliminary results of a systematic literature review of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis. Prev. Vet. Med. 153, 117–126. https://doi.org/10.1016/j.prevetmed.2017.11.004.

Elsohaby, I., Mahmmod, Y.S., Mweu, M.M., Ahmed, H.A., El-Diasty, M.M., Elgedawy, A. A., Mahrous, E., El Hofy, F.I., 2020. Accuracy of PCR, mycobacterial culture and interferon-γ assays for detection of Mycobacterium bovis in blood and milk samples from Egyptian dairy cows using Bayesian modelling. Prev. Vet. Med. 181, 105054 https://doi.org/10.1016/j.prevetmed.2020.105054.

Farnham, M.W., Norby, B., Goldsmith, T.J., Wells, S.J., 2012. Meta-analysis of field studies on bovine tuberculosis skin tests in United States cattle herds. Prev. Vet. Med. 103, 234–242. https://doi.org/10.1016/j.prevetmed.2011.08.009.

Garbaccio, S.G., Garro, C.J., Delgado, F., Tejada, G.A., Eirin, M.E., Huertas, P.S., Leon, E. A., Zumárraga, M.J., 2019. Enzyme-linked immunosorbent assay as complement of intradermal skin test for the detection of mycobacterium bovis infection in cattle. Tuberculosis 117, 56–61. https://doi.org/10.1016/j.tube.2019.05.006.

Garnier, T., Eiglmeier, K., Camus, J.-C., Medina, N., Mansoor, H., Pryor, M., Duthoy, S., Grondin, S., Lacroix, C., Monsempe, C., Simon, S., Harris, B., Atkin, R., Doggett, J., Mayes, R., Keating, L., Wheeler, P.R., Parkhill, J., Barrell, B.G., Cole, S.T., Gordon, S. V., Hewinson, R.G., 2003. The complete genome sequence of Mycobacterium bovis. Proc. Natl. Acad. Sci. USA 100, 7877–7882. https://doi.org/10.1073/pnas.1130426100.

Guimaraes, A.M.S., Zimpel, C.K., 2020. Mycobacterium bovis: From Genotyping to Genome Sequencing. Microorganisms 8, 667. https://doi.org/10.3390/microorganisms8050667.

Higgins, J.P.T., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med 21, 1539–1558. https://doi.org/10.1002/sim.1186.

Hlokwe, T.M., Sutton, D., Page, P., Michel, A.L., 2016. Isolation and molecular characterization of Mycobacterium bovis causing pulmonary tuberculosis and epistaxis in a Thoroughbred horse. BMC Vet. Res 12, 179. https://doi.org/10.1186/s12917-016-0813-6.

Hoffman, T., Rock, K., Mugizi, D.R., Muradrasoli, S., Lindahl-Rajala, E., Erume, J., Magnusson, U., Lundkvist, Å., Boqvist, S., 2016. Molecular detection and characterization of Brucella species in raw informally marketed milk from Uganda. Infect. Ecol. Epidemiol. 6, 32442. https://doi.org/10.3402/iee.v6.32442.

Ibrahim, S., Abubakar, U., Danbirni, S., Usman, A., Ballah, F., Kudi, A., Lawson, L., Abdulrazak, H., Abdulkadir, I., 2016. Molecular identification of mycobacterium tuberculosis transmission between cattle and man: a case report. J. Microbiol Exp. 3, 84–86. https://doi.org/10.15406/jmen.2016.03.00091.

Kanipe, C., Palmer, M.V., 2020. Mycobacterium bovis and you: a comprehensive look at the bacteria, its similarities to Mycobacterium tuberculosis, and its relationship with human disease. Tuberculosis 125, 102006. https://doi.org/10.1016/j.tube.2020.102006.

Kapalamula, T.F., Thapa, J., Akapelwa, M.L., Hayashida, K., Gordon, S.V., Hang' ombe, B.M., Munyeme, M., Solo, E.S., Bwalya, P., Nyenje, M.E., Tamaru, A., Suzuki, Y., Nakajima, C., 2021. Development of a loop-mediated isothermal amplification (LAMP) method for specific detection of Mycobacterium bovis. PLoS Negl. Trop. Dis. 15, e0008996 https://doi.org/10.1371/journal.pntd.0008996.

Lesslie, I., 1960. Tuberculosis in attested herds caused by the human type tubercle bacillus. Vet. Rec. 72, 218–224.

Li, X., Xia, A., Xu, Z., Liu, J., Fu, S., Cao, Z., Shen, Y., Xie, Y., Meng, C., Chen, X., Jiao, X., 2022. Development and evaluation of a Mycobacterium bovis interferon-γ enzyme-linked immunospot (ELISpot) assay for detection of bovine tuberculosis. J. Dairy Sci. 105, 6021–6029. https://doi.org/10.3168/jds.2021-21301.

Lombard, J.E., Patton, E.A., Gibbons-Burgener, S.N., Klos, R.F., Tans-Kersten, J.L., Carlson, B.W., Keller, S.J., Pritschet, D.J., Rollo, S., Dutcher, T.V., Young, C.A., Hench, W.C., Thacker, T.C., Perea, C., Lehmkuhl, A.D., Robbe-Austerman, S., 2021. Human-to-Cattle Mycobacterium tuberculosis Complex Transmission in the United States. Front Vet. Sci. 8 https://doi.org/10.3389/fvets.2021.691192.

Mabe, L., Onyiche, T.E., Thekisoe, O., Suleman, E., 2022. Accuracy of molecular diagnostic methods for the detection of bovine brucellosis: A systematic review and meta-analysis. Vet. World 2151–2163. https://doi.org/10.14202/vetworld.2022.2151-2163.

Mugasa, C.M., Adams, E.R., Boer, K.R., Dyserinck, H.C., Büscher, P., Schallig, H.D.H.F., Leeflang, M.M.G., 2012. Diagnostic accuracy of molecular amplification tests for human african trypanosomiasis—systematic review. PLoS Negl. Trop. Dis. 6, e1438 https://doi.org/10.1371/journal.pntd.0001438.

Nuñez-Garcia, J., Downs, S.H., Parry, J.E., Abernethy, D.A., Broughan, J.M., Cameron, A. R., Cook, A.J., de la Rua-Domenech, R., Goodchild, A.V., Gunn, J., More, S.J., Rhodes, S., Rolfe, S., Sharp, M., Upton, P.A., Vordermeier, H.M., Watson, E., Welsh, M., Whelan, A.O., Woolliams, J.A., Clifton-Hadley, R.S., Greiner, M., 2018. Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. Prev. Vet. Med. 153, 94–107. https://doi.org/10.1016/j.prevetmed.2017.02.017.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ n71. https://doi.org/10.1136/bmj.n71.

Quan, Z., Haiming, T., Xiaoyao, C., Weifeng, Y., Hong, J., Hongfei, Z., 2016. Development of one-tube multiplex polymerase chain reaction (PCR) for detecting Mycobacterium bovis. J. Vet. Med. Sci. 78, 1873–1876. https://doi.org/10.1292/jvms.15-0216.

Reyes, A., Sandoval, A., Cubillos-Ruiz, A., Varley, K.E., Hernández-Neuta, I., Samper, S., Martín, C., García, M.J., Ritacco, V., López, L., Robledo, J., Zambrano, M.M., Mitra, R.D., Del Portillo, P., 2012. IS-seq: a novel high throughput survey of in vivo IS6110 transposition in multiple Mycobacterium tuberculosis genomes. BMC Genom. 13, 249. https://doi.org/10.1186/1471-2164-13-249.

Roy, A., Infantes-Lorenzo, J.A., de la Cruz, M.L., Domínguez, L., Álvarez, J., Bezos, J., 2020. Accuracy of tuberculosis diagnostic tests in small ruminants: A systematic review and meta-analysis. Prev. Vet. Med 182, 105102. https://doi.org/10.1016/j.prevetmed.2020.105102.

Ru, H., Liu, X., Lin, C., Yang, J., Chen, F., Sun, R., Zhang, L., Liu, J., 2017. The Impact of Genome Region of Difference 4 (RD4) on Mycobacterial Virulence and BCG Efficacy. Front Cell Infect. Microbiol 7. https://doi.org/10.3389/fcimb.2017.00239.

Sales, M.L., Fonseca Jr., A.A., Orzil, L., Alencar, A.P., Hodon, M.A., Issa, M.A., Soares Filho, P.M., Silva, M.R., Lage, A.P., Heinemann, M.B., 2014. Validation of two real-time PCRs targeting the PE-PGRS 20 gene and the region of difference 4 for the characterization of Mycobacterium bovis isolates. Genet. Mol. Res. 13, 4607–4616. https://doi.org/10.4238/2014.June.18.3.

Schiller, I., Oesch, B., Vordermeier, H.M., Palmer, M.V., Harris, B.N., Orloski, K.A., Buddle, B.M., Thacker, T.C., Lyashchenko, K.P., Waters, W.R., 2010. Bovine Tuberculosis: A Review of Current and Emerging Diagnostic Techniques in View of their Relevance for Disease Control and Eradication (no-no). Transbound. Emerg. Dis.. https://doi.org/10.1111/j.1865-1682.2010.01148.x.

Shah, D.H., Verma, R., Bakshi, C.S., Singh, R.K., 2002. A multiplex-PCR for the differentiation of Mycobacterium bovis and Mycobacterium tuberculosis. FEMS Microbiol Lett. 214, 39–43. https://doi.org/10.1111/j.1574-6968.2002.tb11322.x.

Sichewo, P.R., Etter, E.M.C., Michel, A.L., 2019. Prevalence of Mycobacterium bovis infection in traditionally managed cattle at the wildlife-livestock interface in South Africa in the absence of control measures. Vet. Res Commun. 43, 155–164. https://doi.org/10.1007/s11259-019-09756-w.

Sichewo, P.R., Hlokwe, T.M., Etter, E.M.C., Michel, A.L., 2020. Tracing cross species transmission of Mycobacterium bovis at the wildlife/livestock interface in South Africa. BMC Microbiol 20, 49. https://doi.org/10.1186/s12866-020-01736-4.

Sidhu, G.S., Narang, D., Filia, G., Singh, A., Singh, S.T., Chandra, M., Sharma, N.S., 2020. Real-Time PCR Detection of Mycobacterium bovis in Blood and Lymph Node Aspirates of Bovines Positive in Tuberculosis Screening Tests. Indian J. Anim. Res. https://doi.org/10.18805/ijar.B-3573.

Silva, M.R., Rocha, A. da S., Araújo, F.R., Fonseca-Júnior, A.A., Alencar, A.P. de, Suffys, P.N., Costa, R.R. da, Moreira, M.A.S., Guimarães, M.D.C., 2018. Risk factors for human Mycobacterium bovis infections in an urban area of Brazil. Mem. Inst. Oswaldo Cruz 113. https://doi.org/10.1590/0074-02760170445.

Srivastava, K., Chauhan, D.S., Gupta, P., Singh, H.B., Sharma, V.D., Yadav, V.S., Sreekumaran, Thakral, S.S., Dharamdheeran, J.S., Nigam, P., Prasad, H.K., Katoch, V.M., 2008. Isolation of Mycobacterium bovis & M. tuberculosis from cattle of some farms in north India–possible relevance in human health. Indian J. Med Res 128, 26–31.

Suleman, E., Mtshali, M.S., Lane, E., 2016. Investigation of false positives associated with loop-mediated isothermal amplification assays for detection of Toxoplasma gondii in archived tissue samples of captive felids. J. Vet. Diagn. Investig. 28, 536–542. https://doi.org/10.1177/1040638716659864.

Tao, Y., Yun, J., Wang, J., Xu, P., Li, C., Liu, H., Lan, Y., Pan, J., Du, W., 2020. High-performance detection of Mycobacterium bovis in milk using digital LAMP. Food Chem. 327, 126945 https://doi.org/10.1016/j.foodchem.2020.126945.

Taylor, G.M., Worth, D.R., Palmer, S., Jahans, K., Hewinson, R.G., 2007. Rapid detection of Mycobacterium bovis DNA in cattle lymph nodes with visible lesions using PCR. BMC Vet. Res 3, 12. https://doi.org/10.1186/1746-6148-3-12.

Thakur, A., Sharma, M., Katoch, V.C., Dhar, P., Katoch, R.C., 2012. Detection of Mycobacterium bovis and Mycobacterium tuberculosis from Cattle: Possible Public Health Relevance. Indian J. Microbiol 52, 289–291. https://doi.org/10.1007/s12088-011-0200-8.

Thierry, D., Brisson-Noël, A., Vincent-Lévy-Frébault, V., Nguyen, S., Guesdon, J.L., Gicquel, B., 1990. Characterization of a Mycobacterium tuberculosis insertion sequence, IS6110, and its application in diagnosis. J. Clin. Microbiol 28, 2668–2673. https://doi.org/10.1128/jcm.28.12.2668-2673.1990.

van Soolingen, D., de Haas, P.E., Hermans, P.W., Groenen, P.M., van Embden, J.D., 1993. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis. J. Clin. Microbiol 31, 1987–1995. https://doi.org/10.1128/jcm.31.8.1987-1995.1993.

Wallace, B.C., Dahabreh, I.J., Trikalinos, T.A., Lau, J., Trow, P., Schmid, C.H., 2012. Closing the Gap between Methodologists and End-Users: R as a Computational Back-End. J. Stat. Softw. 49 https://doi.org/10.18637/jss.v049.i05.

Wards, B., 1995. Detection of Mycobacterium bovis in tissues by polymerase chain reaction. Vet. Microbiol 43, 227–240. https://doi.org/10.1016/0378-1135(94)00096-F.

Westwood, M.E., Whiting, P.F., Kleijnen, J., 2005. How does study quality affect the results of a diagnostic meta-analysis? BMC Med Res Method. 5, 20. https://doi.org/10.1186/1471-2288-5-20.

Whiting, P.F., 2011. QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. Ann. Intern Med 155, 529. https://doi.org/10.7326/0003-4819-155-8-201110180-00009.

Woolford, A.J., Hewinson, R.G., Woodward, M., Dale, J.W., 2006. Sequence heterogeneity of an mpb70 gene analogue in Mycobacterium kansasii. FEMS Microbiol Lett. 148, 43–48. https://doi.org/10.1111/j.1574-6968.1997.tb10264.x.

Zahran, R.N., El Behiry, A., Marzouk, E., Askar, T., 2014. Comparison of LCD array and IS6110-PCR with conventional techniques for detection of Mycobacterium bovis isolated from Egyptian cattle and Buffaloes. Int. J. Mycobacteriol. 3, 197–204. https://doi.org/10.1016/j.ijmyco.2014.06.002.

Zhang, J., Zhang, G.-H., Yang, L., Huang, R., Zhang, Y., Jia, K., Yuan, W., Li, S.-J., 2011. Development of a loop-mediated isothermal amplification assay for the detection of Mycobacterium bovis. Vet. J. 187, 393–396. https://doi.org/10.1016/j.tvjl.2010.01.001.