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Strengthening the Monitoring and Surveillance System for Bovine Tuberculosis in Post Mortem Material from Slaughtered Cattle in Eswatini: A Review

B. N. Dlamini [a], C. Mudyanavana [b], S. Mdluli [b], V. E. Imbaytarwo-Chikosi [a], K. S. Ntshalintshali [a] and M. T. Masarirambi [a*]

[a] University of Eswatini, Luyengo, Eswatini.
[b] Ministry of Agriculture, Department of Veterinary and Livestock Services (DVLS), P. O. Box 4192, Manzini, M200, Eswatini.

Authors' contributions

This work was carried out in collaboration among all authors. Author BN developed the study concept, carried out literature review, wrote and revised manuscript. Authors CM and SM contributed towards development of the study idea, assisted in data compilation. Author MTM revised all the draft manuscripts. Authors VEI and KS assisted in the statistical analysis of the compiled data for the manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

The main objective of this review study was to examine the validity of test results generated by using the Ziehl-Neelsen smear microscopy as the sole diagnostic tool for the monitoring and surveillance of bovine tuberculosis in cattle slaughtered in Eswatini.
Methods: A retrospective analysis of available laboratory data of beef carcass condemnations for a study period from 2014 to 2018 was carried out. Literature was reviewed to find out potential sample preparation and concentration methods to improve the sensitivity of current Z-N smear microscopy.
Results: The limited literature reviewed in this study has briefly highlighted (i) the limitations of using the Z-N direct smear microscopy as a screening and confirmatory laboratory test for bovine

**Corresponding author: Email: mike@uniswa.sz;*

tuberculosis suspected carcasses, (ii) that the Z-N smear microscopy method may be inadequate for identifying and differentiating other acid fast bacilli co-existing in a *M. bovis* infection, (iii) that concentration of lymph node aspirate samples may improve the sensitivity of acid fast bacilli smear microscopy, (iv) that strategic deployment of ancillary laboratory tests, such as the Rapid diagnostic tests (RDT) and the Polymerase chain reaction test (PCR) alongside the Z-N smear microscopy may achieve an adequate level of diagnostic test performance.

Conclusion: In order to strengthen the surveillance and monitoring of bovine tuberculosis in the country, it is necessary to consider techniques for the concentration of bovine tuberculosis suspect lymph node samples, to use Rapid diagnostic test Kits in parallel to the Z-N direct smear microscopy.

Keywords: Bovine tuberculosis; Z-N stain; lymph node aspirate.

1. BACKGROUND

The Kingdom of Eswatini has access to the global beef export market. Due to various challenges the country has not been able to meet her allocated export quota [1]. This has gradually deprived the country of some valuable revenue in foreign exchange. The inability to meet the export quota of beef may indicate that the local beef industry should consider new strategies to improve local beef production capacity, particularly in terms of both quality and quantity. The slaughter of cattle for the global beef market provides a very important marketing avenue for Eswatini subsistence farmers. At present, it is estimated that 40% of the cattle slaughtered for export are sourced from subsistence farmers whilst the remaining 20% and 40% originates from commercial farmers and feedlot cattle operation, respectively [1, 2].

The current use of the modified Ziehl-Neelsen (Z-N) direct smear microscopy as the only diagnostic tool for a final diagnosis for bovine tuberculosis carcases is less sensitive and not specific to be relied on. The sensitivity (46%) and specificity (90%) of this method is lower than that of other bovine tuberculosis test methods [3, 4]. There are some noted challenges associated with the sensitivity and the specificity of the modified Z–N direct smear microscopy method. In addition, the Z-N direct smear Microscopy does not differentiate tuberculosis lesions from other lung pathologies [5]. The consequences of wrongly diagnosed and whole condemned beef carcases may indirectly contribute towards the country's failure to meet the allocated beef export quota.

Studies on the assessment of diagnostic tests performance for *M. bovis* have shown that there is currently no single test which will fulfil all the criteria necessary to identify all tuberculosis infected animals. A combination of diagnostic approaches could achieve an adequate level of diagnosis test performance [5, 6, 7]. The QuantiFERON test is deemed superior to the Tuberculin Skin Test for detecting latent TB infections [8, 9, 10].

The strategic deployment of ancillary in vitro tests alongside the primary skin tests has enhanced the detection of *M. bovis*-infected cattle and reduced the number of animals slaughtered as false positives. The intradermal tuberculin skin test is the primary screening test, and the in vitro gamma interferon assay is approved as the ancillary diagnostic tool [8, 11, 12].

The Ziehl–Neelsen stain method maybe the most suitable direct smear microscopy method for use in basically equipped laboratories found in developing countries. According to studies elsewhere, the concentration of lymph node aspirates for acid fast smear microscopy resulted in significantly higher sensitivity [13,14].

The Ziehl-Neelsen direct stain method lacks the specificity and cannot be used to distinguish between the various members of the family *Mycobacteriaceae* [15]. The phenomenon whereby there is co-existence of other pathogenic microorganisms that are a health hazard to consumers has been highlighted previously [5, 16].

This may indicate that the direct smear microscopy test results are not to be interpreted as results of a "stand-alone" test. The test results have to be interpreted after correlating with the relevant clinical findings and additional supplemental tests [5,8,16, 17].

Diagnostic tests based on antibody response alone show poor efficiency in the detection of TB-infected animals on an individual basis [5, 18, 19, 20]. The use of serology as ancillary tests in association with skin-testing has been found to improve the detection of *M. bovis*-infected cattle and reduce the unnecessary slaughter of false-positive reactors animals [7, 20].

Polymerase chain reaction (PCR) is a technique that is capable of targeting genetic material found only in *M. bovis* and not in other mycobacterial species [11, 21, 22]. The usefulness of using the PCR in parallel to other tests has previously been confirmed, more especially in cases of dubious reactions and presence of cross-reactivity with correlated antigenic determinants [21].

1.1 Diagnostic Test Evaluation

Sensitivity refers to what percentage of animals with the disease or infected and shows obvious clinical signs giving positive results. In practice, sensitivity can be influenced by a host of other factors including the test procedure, tuberculin potency, the stage of infection in the host, other inter-current infections and prevalence of cross-reacting organisms in the locality [19, 23, 24].

Specificity measures the proportion of true negative. This refers to the percentage of healthy bovines without disease or those not showing clinical signs and are correctly identified as not having TB [5, 25, 26].

In order to come up with a diagnosis on an animal, the decision is based on a number of factors illustrated in Fig. 1 [23, 27]. This illustration clearly identifies some of the uncertainties associated with routine diagnosis particularly multi-factorial causes of disease and the related impact of predisposing factors [24]. The need for new technology to be compared with an accepted "gold standard" that makes comparisons of sensitivity and specificity between different methods has become more of a reality [19, 28].

The specific objectives of this study were to carry out test performance evaluation and to highlight the existing base line trend of bovine tuberculosis in post mortem material from slaughter cattle in the country. As well as to investigate sample enrichment and preparation procedures that could increase the sensitivity of the Ziehl-Neelsen direct smear microscopy as a diagnostic tool for tuberculosis in Eswatini.

1.2 Meat Inspection and Sample Collection

The meat inspection at the beef export abattoir is guided and compliant to European Commission regulations. The unit is audited by FVO-DG (SANCO) Veterinary missions in order to ensure continued compliance and adherence to European Commission regulations. All bovine tuberculosis suspect lymph node specimen from the abattoir kill floor are examined using the modified Ziehl – Neelsen direct smear microscopy [1].

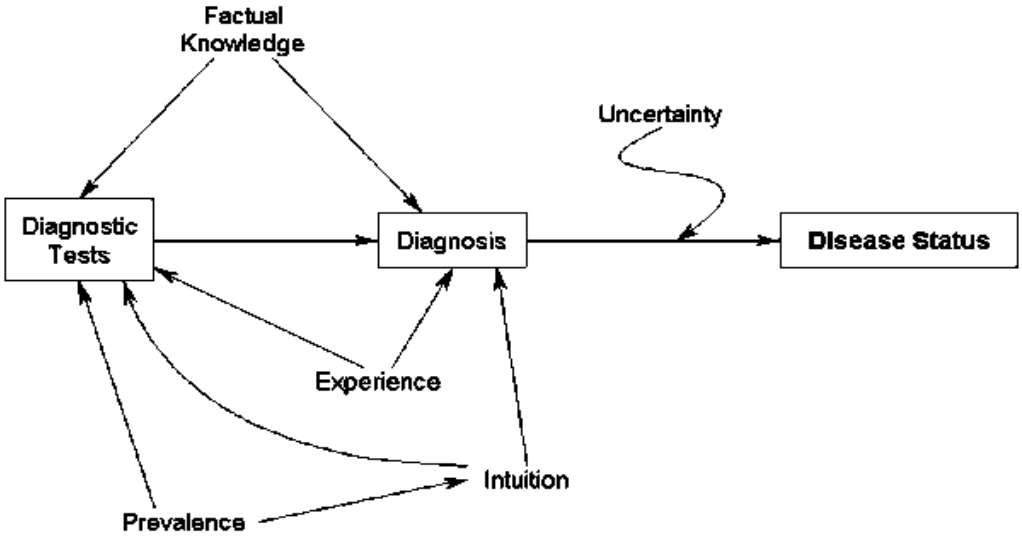

Fig. 1. Factors influencing veterinary diagnoses [18]

1.3 Laboratory Testing Using the Z-N Direct Smear Microscopy

Mycobacterium bovis can be demonstrated microscopically on direct smears from submitted samples. The acid fastness of *M. bovis* was demonstrated with the modified Ziehl–Neelsen stain. During post mortem examination of slaughtered carcasses, the presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristic histological lesions (caseous necrosis, mineralisation, epithelioid cells, multinucleated giant cells and macrophages) [9, 25, 26, 29]. For increasing sensitivity, concentration techniques that include centrifugation of the lymph node aspirate and pleural fluid samples may be used to increase the chances of coming up with a positive result [13, 14].

2. MATERIAL AND METHODS

2.1 Description of Study Site

The study was carried out using secondary data on cattle slaughter and tuberculosis (TB) diagnostic records obtained from Export Beef Abattoir of the Kingdom of Eswatini. This is the only abattoir that processes Eswatini beef for the export market. Its catchment covers the entire country. The Kingdom of Eswatini lies along - 26^{o}31'4.59" S latitude and 31^{o}27'46.69" E longitude. Because of the country's variable altitude, the winter diurnal temperatures range from 5^{o}C to 21^{o}C in the Highveld and from 13^{o}C and 25^{o}C in summer. In the Lowveld, diurnal temperatures range from 8^{o}C to 27^{o}C in winter and from 18^{o}C to 32^{o}C in summer. The country has annual average rainfall ranging from 400 mm in the low veld to 1,200 mm in the high veld. With permanent pastures constituting about 62% of all land use, livestock, and particularly cattle, farming is dominant among the smallholder farmers.

2.2 Meat Inspection and Sample Collection

All carcasses at the abattoir are routinely inspected according to the Food and Agricultural Organisation (FAO) manual on meat inspection for developing countries [30]. The meat is inspected for internal parasites, cysts and disease including tuberculosis (TB). Lymph nodes are collected from all carcases and tested for TB at an on-site Meat Hygiene laboratory. Tuberculosis (TB) is determined using the Z-N smear microscopy method [1].

2.3 The Z-N Direct Smear Microscopy

In the Meat Hygiene laboratory, a microscope is used to detect *Mycobacterium bovis,* the bacteria that causes bovine tuberculosis, from direct smears of lymph node samples collected from the abattoir. The process uses a modified Z-N smear to enhance the acid fastness of *M. bovis*. A presumptive diagnosis of *M. bovis*, and hence positive TB result, is assumed if the tissue has characteristic histological lesions (caseous necrosis, mineralisation, epithelial cells, multinucleated giant cells and macrophages) [9, 25, 26, 29].

2.4 Data Preparation

Data comprised of cattle slaughter, carcass condemnation and laboratory records on TB detection for the period 2013 to 2018. These records included geographical region, land tenure of the farm from which the cattle were raised, year and month of slaughter, total cattle slaughtered, total carcasses that turned positive for tuberculosis and their gender. Data were prepared for statistical analysis by generating two variables, %TB Incidence and TB occurrence (a binary variable).

i. The incidence of TB (% TB incidence) among all cattle brought for slaughter was calculated as:

$$\%TB\ Incidence = \frac{Number\ of\ TB\ cases\ per\ month}{Total\ cattle\ slaughter\ per\ month} x100$$

ii. A binary variable (TB occurrence) was created in which all months that had positive cases for TB were coded "1" and those that did not have positive cases were coded "0"

Months were classified into four seasons: Summer (November, December, January, February and March); Autumn (April and May) and Winter (June, July and August.

2.5 Data Analysis

Data were analysed using the Statistical Analysis System (SAS) Version 9.3 (SAS, 2012). Three analyses were carried out. (1) The incidence of TB in males and females was compared using

the PROC TTEST procedure for comparison of means for independent samples. (2) Trends in mean TB incidence by month of slaughter were generated using the PROC MEANS procedure of SAS and (3) The effect of region, year, tenure system and sex on TB occurrence were determined using a binary logistic regression equation with the PROC LOGISTIC procedure of SAS. The following model was used:

$$log\left[\frac{P}{1-P}\right] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_t X_t + \varepsilon$$

Where $\left[\frac{P}{1-P}\right]$ are the odds of getting positive TB result or not; $\boldsymbol{\beta_0}$ is the intercept, $\boldsymbol{\beta_1} to \boldsymbol{\beta_t}$ are the partial regression coefficients relating the geographical region, tenure system, year, season and sex to TB occurrence, $\boldsymbol{X_1}\ to\ \boldsymbol{X_t}$ are the independent factors (geographical region, tenure system, year of slaughter, month of slaughter and sex of animals) and ε are the random residuals.

3. RESULTS

3.1 Total Cases of TB

Table 1 shows the proportion of positive TB cases by year of slaughter of the animals. A total of 35,503 cattle were slaughtered at the export abattoir during the period under study. Of these, 1.4% tested positive for TB and were condemned.

Table 1. Total number of cattle slaughtered and corresponding whole carcasses condemned due to Bovine tuberculosis

Year of slaughter	Total cattle slaughtered	Number TB negative	Number TB positive	Proportion TB positive (%)
2013	8708	8635	73	0.83
2014	6343	6291	52	0.81
2015	9022	8938	84	0.93
2016	10541	10368	173	1.64
2017	5087	4999	88	1.73
2018	3510	3482	28	0.80
TOTAL	**35503**	**35005**	**498**	**1.40**

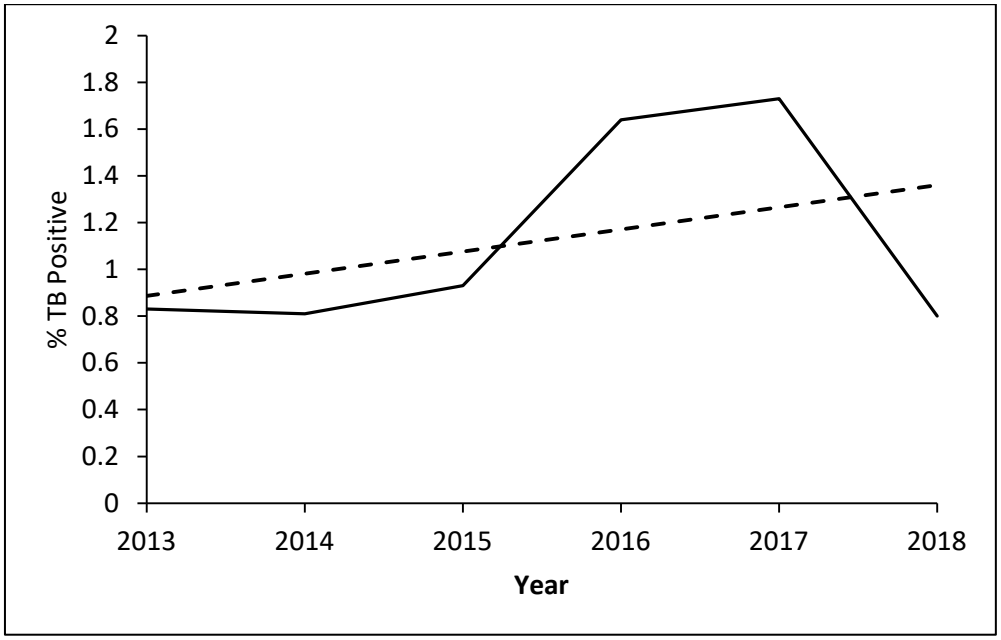

Fig. 2. Trend in incidence of TB at slaughter for the period 2013 - 2018

The trend in the proportion of cases positive for TB are shown in table 1. TB cases were particularly high during the years 2015 to 2017 but a general increase in TB cases over the years beginning 2013 was observed (Fig. 2).

3.2 Factors Influencing Occurrence of Bovine TB as a Binary Variable

Occurrence was expressed as a binary variable where months in which positive bovine TB cases were recorded were coded "1" and those months with no cases of bovine TB coded "0". Logistic regression analysis was then used to identify the factors that influence the occurrence of TB in Eswatini. The occurrence of bovine TB was significantly influenced by geographical region; year of slaughter; season of slaughter (P<0.05). However, occurrence of bovine TB was not influenced by the land tenure system and the sex of the animals (P>0.05). This implied that there were no differences in the occurrence of TB between cattle raised on Eswatini National Land and those on Title Deed land. Table 2 shows the odds ratios and the partial regression coefficient **(β)** obtained from the analysis.

From the table values of β, slaughtering cattle from region 2 and region 4 versus those in region 1 increased the log odds of getting bovine TB by 0.45 (P=0.0034) and 0.71 (P<0.05) correspondingly. Compared to 2013, cattle slaughtering cattle in 2014 lowered the log odds of getting bovine TB by 0.63 whilst slaughtering in 2016 increased the odds by 0.42 (P=0.0342). Slaughtering cattle in autumn and winter increased the log odds for getting bovine TB by 0.44 and 0.46 (P<0.05) respectively versus slaughtering in summer.

3.3 Incidence of Bovine TB by Sex of Animals

Incidence of bovine TB was computed and was expressed as a percentage. The incidence of bovine TB between bulls and cows across all regions was compared using the t-test for comparison of means. The mean incidence (%) of bovine TB in bulls was significantly higher than in cows (P=0.0089). Mean incidence of bovine in bulls and cows was 13.27% and 6.84% respectively.

4. DISCUSSION

This study had its own limitations, emanating from the estimation of bovine tuberculosis positive carcasses using recorded data derived only from the export abattoir.

The role of other factual elements that may require consideration in order to come up with a final diagnosis such as in case of total carcass condemnation has been elaborated [18].

A combination of laboratory tests may help in minimising any potential false-positives [19, 25, 26]. We are cognisant of the fact that some considerations on the cost implication and availability of resources should be taken into account to decide which laboratory test to adopt when strengthening the current diagnosis of bovine tuberculosis.

Table 2. Factors influencing occurrence of bovine TB (i.e., Binary variable)

Parameter	Class	β (s.e)	p-value for β	OR (95% CI)
Region	2	0.27 (0.152)	0.0809	1.31 (0.810 -2.129)
(Ref class = 1)	3	0.45 (0.153)	0.0034*	1.58 (0.971 - 2.562)
	4	- 0.71 (0.160)	<.0001*	0.50 (0.301 - 0.821)
Year	2014	- 0.63 (0.205)	0.0023*	0.67 (0.364 - 1.243)
(Ref class = 2013)	2015	0.34 (0.198)	0.0878	1.76 (0.966 - 3.213)
	2016	0.42 (0.198)	0.0342*	1.91 (1.048 - 3.485)
	2017	0.19 (0.196)	0.3396	1.52 (0.834 - 2.759)
	2018	- 0.09 (0.197)	0.6518	1.15 (0.632 - 2.095)
Season	Autumn	0.44 (0.180)	0.0139*	2.76 (1.664 - 4.569)
(Ref class = Summer	Winter	0.46 (0.157)	0.0035*	2.80 (1.799 - 4.360)
	Spring	- 0.33 (0.179)	0.0655	1.27 (0.772 - 2.102)
Tenure (Ref = ENL)	TDL	0.11 (0.089)	0.2200	1.24 (0.878 - 1.761)
Sex (Ref=male)	Female	0.23 (0.503)	0.6485	1.58 (0.220 - 11.355)

**Significant at P<0.05:*

Region 1= ; Region 2= ; Region 3= ; Region 4= ; ENL=Eswatini National Land; TDL=Title Deed land

The results of this study are not meant to challenge the current diagnosis for bovine tuberculosis and the performance of the current modified Z – N direct smear microscopy method as both a screening and confirmatory test. Our results provide some relevant information to guide the choice of potential laboratory tests for future use [31- 33]. An exercise, that could be useful as part of the global strategy for elimination of tuberculosis.

Post mortem carcass inspection involves checking for gross lesions that must be visible to the naked eye. Similarly, the literature reviewed in this study has indicated that the Z – N smear microscopy does not differentiate to other acid fast bacilli co-existing with the bovine *mycobacterium species*. Thus, this could be viewed as having some limitations in terms of diagnosing multifactorial disease causative agent such as bovine tuberculosis.

Diagnostic evaluation of the data generated by the secondary data using the modified Ziehl-Neelsen direct smear microscopy method could not be performed. Therefore, the approach used to collect the bovine tuberculosis data did not allow for an in depth diagnostic analysis [34, 28].

Total condemnation of the beef carcasses using the current test is an issue because it reduces the number of carcasses eligible for export. This makes it even harder to meet the allocated beef export quota. Some surveillance systems often lack the ability to monitor the human–animal interface for emergent pathogens. Therefore, identifying and ultimately addressing emergent cross-species infections will require a “One Health” approach in which resources from both veterinary and human health sections are pooled together. Evidently, there are “one health” activities between the relevant competent authorities. These entails bilateral meetings to deliberate on several agenda items aimed at improving public health security and promoting future collaborative efforts [2].

In terms of the results from the literature reviewed, the study has found that laboratory concentration techniques for collected lymph node specimen suspected to be infected with *M. bovis* were available and may be used to improve the sensitivity of acid fast bacilli smear microscopy for the diagnosis of bovine tuberculosis [12, 13, 14]. The disadvantages of using the Z-N direct smear microscopy as a screening and confirmatory laboratory test for bovine tuberculosis suspected cattle carcasses were pointed out [5, 6, 7], literature has been found that point to the fact that the Z-N smear microscopy method may be inadequate for identifying and differentiating other acid fast bacilli co-existing in a *M. bovis* infection [5, 15, 16]. The usefulness of using the PCR in parallel to other tests, its capability of targeting genetic material found only in *M. bovis* and not in other mycobacterial species has previously been confirmed [11, 21, 22].

Microscopic diagnosis has certain limitations, particularly in situations of or mixed infections [7]. This could be an indication for consideration of rapid diagnostic test kits (RDTs) [19, 34, 35].

5. CONCLUSION

The results of this study reveal that one way of strengthening the surveillance and monitoring of bovine tuberculosis would be to consider using rapid diagnostic tests (RDTs) and or polymerase chain reaction (PCR) as ancillary tests to the modified Z-N direct smear microscopy.

In this regard, improving the diagnostic efficiency of the current modified Z – N stain method at the slaughterhouse may positively influence the throughput of beef carcass meat generated and exported to the global meat market. Diagnosis using the microscope should not be completely abandoned, especially in a developing country. As much as a combination of the modified Z – N stain method/ RTD test looks more of a plausible solution to the identified challenges.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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