STRENGTHENING THE MONITORING AND SURVEILLANCE SYSTEM FOR BOVINE TUBERCULOSIS IN POST MORTEM MATERIAL FROM SLAUGHTERED CATTLE IN ESWATINI.

Abstract:

Aim: The first objective of this study was to highlight the existing trend of bovine tuberculosis in post mortem material from slaughter cattle in the country. The second objective was to find out potential sample preparation and concentration methods to improve the sensitivity of the Ziehl-Neelsen direct smear microscopy as diagnostic tool for bovine tuberculosis in Eswatini.

Methods: A retrospective analysis of recorded laboratory data of annual beef carcass condemnations for a study period from 2014 to 2018 was carried out. Performed diagnostic evaluation of the modified Ziehl-Neelsen direct smear microscopy method. Literature was reviewed on the concentration techniques of tuberculosis suspect lymph node aspirate.

Results: The results of the study revealed that (i) the limitations of using the Z-N direct smear microscopy as a screening and confirmatory laboratory test for bovine tuberculosis suspected cattle carcasses, (ii) the Z-N stain method was not sensitive enough to identify and differentiate other acid fast bacilli co-existing in a *Mycobacteria bovis* infection in cattle, (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 and the polymerase chain reaction test alongside the Z-N direct smear microscopy may achieve an adequate level of diagnostic test performance.

Conclusion: The results of our study do not challenge the status quo as such. This is an attempt to offer an alternative perspective and constructive way to address the incidence of bovine tuberculosis cases. In order to strengthen the surveillance and monitoring of bovine tuberculosis in the

country, it is necessary to consider concentrating the laboratory samples and using the RDT and PCR in parallel to the Z-N direct smear microscopy.

Key words: Bovine tuberculosis, Z-N stain, lymph node aspirate.

1. Introduction

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]**.

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 Figure 1 **[23, 30]**. 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, 31]**.

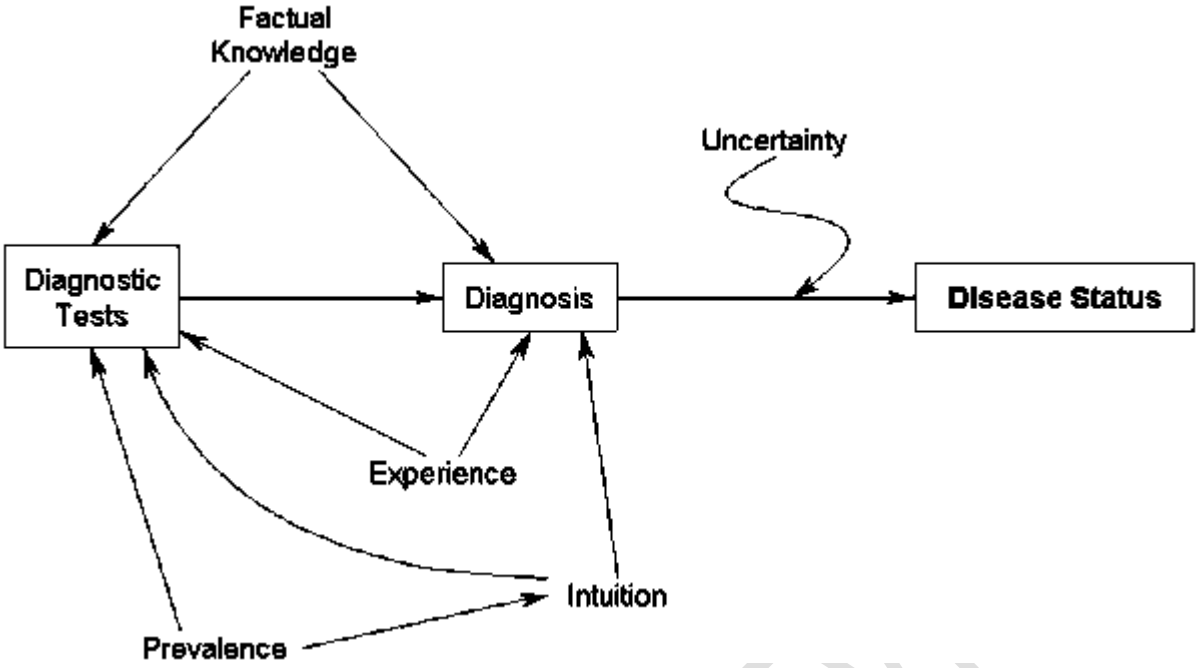

Figure 1: Factors influencing veterinary diagnoses **[18]**

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.

2.0 MATERIAL AND METHODS

In order to achieve the objectives of this study, the recorded slaughter data from 2013 to 2018 was examined. For data analysis Microsoft excel 2016 version was used to analyse the secondary data and to create the figures. Literature was reviewed in order to find out sample concentration techniques that could improve the sensitivity of the current Z – N direct smear microscopy.

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]**.

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, 27]**. 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]**.

3.0 RESULTS

During the study period 35,503 cattle were slaughtered, 1.4% of those slaughtered were totally condemned and 98.6% passed as fit for human consumption (Table 1, 2).

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

Year	**Slaughtered**	**Passed**	**Condemned**	**Proportion %**
2013	8,708	8,635	73	0.83
2014	6,343	6,291	52	0.81
2015	9,022	8,938	84	0.93
2016	10,541	10,368	173	1.64
2017	5,087	4,999	88	1.73
2018	3,510	3,482	28	1.64
TOTAL	**35,503**	**35,005**	**498**	**1.40**

The graphic outline of the current trend in the diagnosis of bovine carcass post mortem materials from slaughter cattle was illustrated by Figure 2.

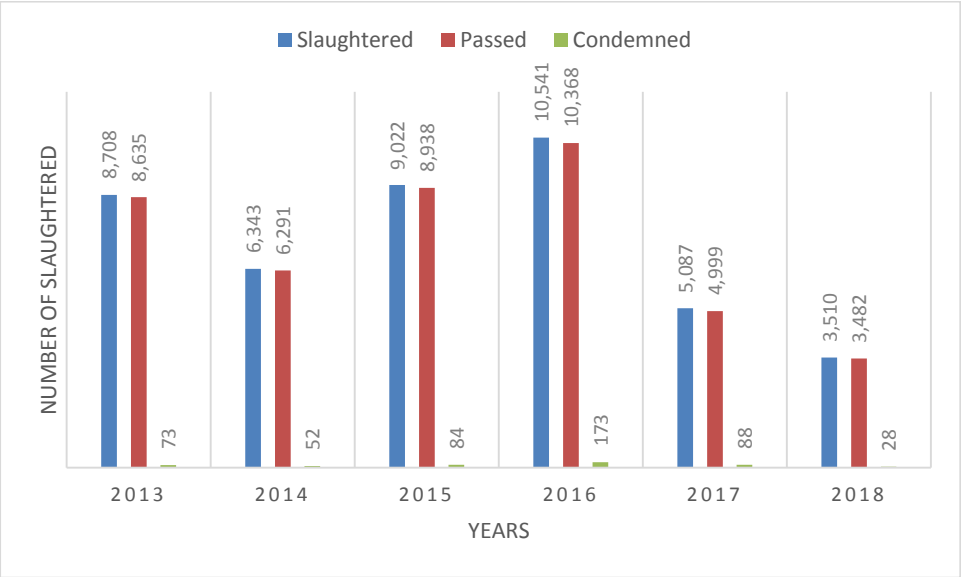

Figure 2: The current trend in whole carcass condemnations due to bovine tuberculosis from 2014 - 2018.

The incidence of bovine tuberculosis in the different categories per hundred thousand cattle is shown in Table 2.

Table 2: Total number of cattle slaughtered and corresponding whole carcasses condemned due to bovine tuberculosis.

Year	**Slaughtered**	**Condemned**	**per 100,000**
2013	8 708	73	838
2014	6 343	52	820
2015	9 022	84	931
2016	10 541	173	1641
2017	5 087	88	1730
2018	3 510	28	798
Total	**43 211**	**498**	**1152**

The current trend in terms of the incidence of bovine tuberculosis per hundred thousand whole carcass condemnations during the study period was shown by Figure 3.

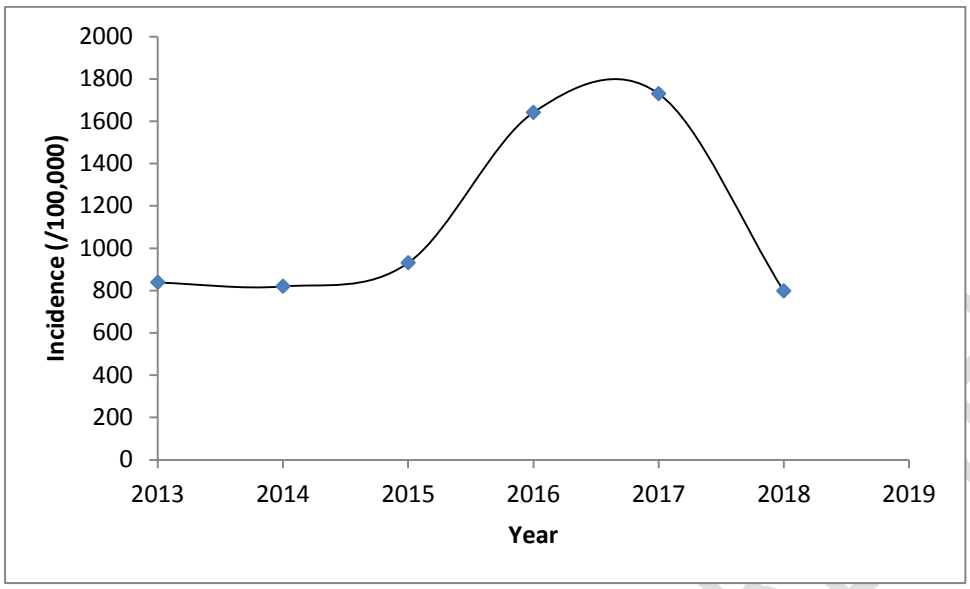

Figure 3: The current trend in whole carcass condemnation due to Bovine tuberculosis from 2014 - 2018.

The incidence of bovine tuberculosis in inspected carcasses in terms of the different animal categories slaughtered was found to occur mostly frequently in bulls. In terms of the cattle management system, cattle originating from subsistence farms showed more tuberculosis lesions than cattle sourced from privately owned farms (Table 3).

Table 3: Depicts Prevalence of Bovine tuberculosis in slaughtered cattle

Variable		**Inspected Carcass**	**BTb** +
Total cattle slaughter		35,503	498 (1.40)
Class	Heifers	654	10
	Cows	10,413	146
	Steers	11,426	160
	Bulls	20,738	291
Husbandry system	SNL	24,510	344 (1.40)
	TDL	15,201	213 (1.40)

The epidemiological tool used for diagnostic test evaluation of the Z-N direct smear microscopy as a valid measure of the true disease status of the suspected post mortem material was demonstrated in Table 4.

Table 4: Tuberculosis lesions diagnosed by visual meat inspection and those confirmed using the modified Z – N direct smear microscopy.

	Z – N Stain test result		
Tuberculosis infection	Test +	Test -	Total
True +	420	3442	3940
True -	584	30979	31563
Total	1082	34421	35503

4.0 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 the issue of 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.

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 **[32, 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.

The apparent prevalence of bovine tuberculosis was 11%, this being the false negative beef carcasses. In the present result, negative predictive value was in the higher side (98.15%). The diagnostic test results showed that the positive predictive value was low. This may be an indication that although the current modified Z – N smear microscopy test method could be useful as a laboratory tool for screening infected carcasses, it may not be sufficient on its own to be utilised in making a final confirmatory diagnosis because of the high false positive carcasses. Therefore, may not be relied on as the only basis for a decision for total condemnation **[28, 31]**.

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. Subsistence farmers may be discouraged with each downgraded carcass not sold to the lucrative global beef export market.

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]**.

Microscopic diagnosis has certain limitations, particularly in situations of low parasitaemia or mixed infections **[7]**. This could be an indication for consideration of rapid diagnostic test kits **[19, 28, 29]**.

Amongst the four classes of slaughtered cattle, bulls seem to be the most category that is routinely found to have bovine tuberculosis suspect lesions submitted to the laboratory for screening and confirmation. This indicates that communal cattle were mostly affected by bovine tuberculosis than in commercial cattle **[34]**.

5.0 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 some challenges exist.

6.0 RECOMMENDATIONS

6.1 The methods for the concentration of lymph node aspirate to improve the sensitivity of the Z-N direct smear microscopy could be further investigated **[13, 14]**.

6.2 The Z – N test results should not be interpreted as "stand-alone" test results. They have to be interpreted following due consideration of other ancillary laboratory tests.

6.3 Promotion and facilitation of an active serosurveillance system for bovine tuberculosis in the country.

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