UNIVERSITI PUTRA MALAYSIA

GROSS AND HISTOPATHOLOGICAL CHANGES IN ORGANS OF CATTLE SEROPOSITIVE TO BOVINE TUBERCULOSIS

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Gross and Histopathological Changes in Organs of Cattle Seropositive to Bovine Tuberculosis", by Ain Izzati binti Abd Rashid and in my/our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD4901 - Project.

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DEDICATION

This thesis is especially dedicated to:

Allah S.W.T

the greatest who created beautiful creations

My source of strength

Abd Rashid Yasak, Zaiton Bahari

Muhammad Azri, Muhammad Hafiz, Aini Farhana

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PERUBAHAN MATAKASAR DAN HISTOPATOLOGI PADA ORGAN LEMBU SEROPOSITIF TUBERKULOSIS BOVIN

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Tuberkulosis bovin adalah penyakit yang disebabkan oleh bakteria *Mycobacterium bovis*. Ia merupakan penyakit berjangkit dimana jangkitan berlaku melalui saluran pernafasan atau pemakanan dan menghasilkan lesi kronik yang dikenali sebagai tuberkel bergranuloma. Tujuan kajian ini adalah untuk menghuraikan lesi matakasar dan histologi pada lembu positif tuberkulosis. Sejumlah 20 lembu tenusu dewasa yang seropositif dan 5 seronegatif telah dipilih secara rawak dari 6 ladang. Lembu yang positif adalah haiwan yang menunjukkan reaksi positif terhadap kedua-dua ujian, iaitu ujian gamma interferon (BOVIGAM®) dan tuberculin intrakulit. Haiwan positif disembelih dan pemeriksaan karkas dilakukan serta-merta bagi mengesan lesi matakasar, iaitu lesi tuberkel bergranuloma tuberkel dalam sistem pernafasan dan sistem pencernaan. Sistem pernafasan melibatkan paru-paru, nodus limfa mediastinum dan nodus limfa retrofaringial sementara sistem pencernaan melibatkan usus dan nodus limfa mesenterik. Setelah pemeriksaan matakasar, sampel organ tersebut diawet dalam larutan formalin 10%. Irisan histologi telah diwarnai dengan haematoxylin & eosin (H&E) untuk memeriksa lesi dan juga diwarnai dengan Ziehl-Neelsen bagi mengesan kehadiran organisma 'acid fast' di dalam tisu. Keputusan menunjukan perkaitan yang lemah antara ujian tuberculin intrakulit dengan kehadiran lesi tuberkel bergranuloma. Hanya 13 (65%) lembu positif menunjukkan lesi matakasar, histologi atau kedua-duanya. Lesi tersebut dijumpai dalam sistem pernafasan dan tiada lembu yang menunjukkan lesi tuberkel di sistem pencernaan. Walau bagaimanapun, pemeriksaan histologi menunjukkan perkaitan yang kuat

di antara lesi matakasar dan histologi ($p<0.05$; $r = 0.873$) di mana 92% reaktor dengan tuberkel matakasar juga menunjukkan lesi histologi bergranuloma. Pewarnaan Ziehl-Neelsen ke atas calitan organ menunjukkan kehadiran organisma 'acid fast' dalam 11 (55%) daripada 20 lembu positif. Sementara itu, organisma turut dikesan di dalam tisu paru-paru dan nodus limfa mediastinum yang melibatkan 5 (25%) haiwan. Perkaitan di antara pengesanan organisma daripada calitan organ dan tisu organ adalah ketara ($p<0.05$) pada tahap sederhana ($r = 0.56$). Selain itu, organisma ini seringkali dikesan di dalam lesi histologi bergranuloma tahap III dan IV dan ketara ($p<0.05$) pada tahap sederhana dalam tuberkel matakasar ($r = 0.53$). Kehadiran lesi mata kasar adalah ketara ($p<0.05$) pada tahap sederhana terhadap pengesanan organisma di dalam calitan organ ($r = 0.53$) dan tisu organ ($r = 0.48$). Kajian ini merumuskan bahawa keputusan seropositif sahaja dapat meningkatkan kadar positif palsu dalam mendiagnosis tuberkulosis. Oleh itu, ujian ante-mortem dan post-mortem merupakan elemen penting dalam mengesahkan diagnosis.

Kata Kunci: tuberkulosis bovin, gamma interferon (BOVIGAM®), ujian tuberculin intrakulit, perubahan matakasar dan histopatologi

ABSTRACT

GROSS AND HISTOPATHOLOGICAL CHANGES IN ORGANS OF CATTLE SEROPOSITIVE TO BOVINE TUBERCULOSIS

by

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2021

Supervisor: Prof. Dr. Mohd Zamri b Saad

Bovine tuberculosis is caused by *Mycobacterium bovis*. It is an infectious disease that is transmitted via the respiratory or oral route, and produces granulomatous tubercle, which is a typical chronic lesion. The aim of this study was to describe the pathological changes in dairy cattle seropositive to bovine tuberculosis. A total of 20 sero-positive and 5 sero-negative adult dairy cattle were randomly selected from 6 farms. All positive animals showed positive reactions to both γ-IFN (BOVIGAM®) and comparative intradermal tuberculin tests. The positive animals were culled by slaughtering and the carcasses were immediately examined for gross lesions, particularly the granulomatous tubercle lesions in two body systems, the respiratory and gastro-intestinal systems. The respiratory system involved the lungs, the mediastinal and retropharyngeal lymph nodes while the gastro-intestinal system involved the intestines and mesenteric lymph node. Following gross examination, samples of the organs were fixed in 10% buffered formalin and histological sections were stained with hematoxylin and eosin (H&E) to examine the lesions and were also stained with Ziehl-Neelsen staining to detect the presence of acid-fast bacilli in tissues. The results revealed a poor correlation between the comparative intradermal tuberculin test and the presence of granulomatous tubercle lesions. Only 13 (65%) positive animals had the lesion either grossly, histopathology or both. The lesions were found in the respiratory system and none of the cattle had gross tubercle lesion in the gastro-intestinal tract. However, histological

examination revealed a strong correlation between gross and histopathology lesion ($p<0.05$; $r = 0.873$) where 92% reactors with gross tubercle showed the evidence of granulomatous histopathology. Ziehl-Neelsen staining of organ smears revealed the presence of acid-fast bacilli in 11 (55%) of the 20 reactors while 5 (25%) were identified with the presence of acid-fast bacilli in the tissue sections of lungs and mediastinal lymph node. Hence, detection of the acid-fast bacilli in tissue section was moderate but significantly ($r = 0.56$; $p<0.05$) correlation with organ smear. Furthermore, the acid-fast bacilli were observed mainly in the stages III and IV of the granulomatous tubercles. The stage of granulomatous tubercle was significant ($p<0.05$) but at a moderate correlation with the presence of acid-fast bacilli in organ tissue ($r = 0.53$). The presence of gross tubercle lesion was moderate but significantly ($p<0.05$) correlated with the detection of acid-fast bacilli in organ smear ($r = 0.53$) and tissue ($r = 0.48$). Thus, this study concluded that relying on seropositive results alone could increase the occurrence of false positive animals. Hence, conducting a thorough ante-mortem and post-mortem tests are important in confirming cases of bovine tuberculosis.

Keywords: bovine tuberculosis, γ-IFN (BOVIGAM®), comparative intradermal tuberculin test, gross and histopathology

1 INTRODUCTION

Mycobacterium bovis is the aetiological agent for bovine tuberculosis. It is an aerobic Gram-positive, acid-fast bacterium with a slow growth rate of around 16 to 20-hour generation (Rodriguez-Moralez et al., 2014). The disease is transmitted via the respiratory or oral route, producing granulomatous tubercle, which is a consequence of the cell-mediated immune response against the organism (Pritchard, 1988). In order to diagnose bovine tuberculosis, few tests are needed, which include the ante-mortem tests and post-mortem examination. In fact, the percentage of true positive animals increases when more tests are used as well as involvement of both ante-mortem and post-mortem evaluations. Malaysia is currently trying to eradicate bovine tuberculosis through the test and culled of bovine tuberculosis positive animals as described in the "Protokol Veterinar Malaysia", 2011 (DVS, 2011).

Cattle that are infected with *M. bovis* usually develop chronic infection and remain subclinical for months to years before showing any clinical sign or typical tuberculosis lesions. Furthermore, infected animals are the silent source of infection to the herd (Rua-Domenech et al., 2006). As stated by Domingo et al. (2014), once the structure and function of the infected organs are severely damaged, infected animals start to show clinical signs. However, signs of bovine tuberculosis are not pathognomonic thus, detection of animals in early infection has to be efficient, employing sensitive immunodiagnostic tests (Rua-Domenech et al., 2006).

In Malaysia, herds are screened for tuberculosis with BOVIGAM® and confirmed with comparative intradermal tuberculin test. The efficacy of this combination of tests has not been determined in Malaysia. Hence, the aim of this study was to determine the efficacy of combination of BOVIGAM® and comparative intradermal tuberculin test in detecting cattle with tuberculosis. The confirmatory tests used in this study include gross and histopathology examinations.

2 LITERATURE REVIEW

The used of γ-IFN (BOVIGAM®) and comparative intradermal tuberculin test are to screen the possible positive bovine tuberculosis. However, a study has reported that comparative intradermal tuberculin test has low or moderate sensitivity that ranged between 50% and 64% (Nunez Garcia et al., 2018). This explains the inability of the intradermal skin test to detect infected cattle. The resulted wide range might be due to testing anergic cattle, poorly injected PPD, variance in PPD potency, loss of PPD potency over time, mistaken animal identification and operator error (Lepper et al., 1977). Besides that, the test performance also influenced by the test antigen formulation, the cut off positive point, stage of infection and cross reaction by environmental mycobacterium (Clegg et al., 2015). Hence, the combination of these tests with post-mortem and histopathology changes should be able to provide higher confidence in diagnosing cattle with tuberculosis, owing to the fact that histopathology demonstrates high sensitivity (93.4%) and high specificity (92.3%) in diagnosing bovine tuberculosis (Varello et al., 2008). Therefore, a tentative diagnosis for bovine tuberculosis is done by detection of gross lesions and the confidence could be increased following histopathology examination (Corner, 1994). Nevertheless, bacterial isolation is the definitive diagnosis to confirm that an animal is infected with *M. bovis* (Corner, 1994). Currently, none of the tests is able to provide precise determination of *M. bovis* infection status of a cattle (Rua-Domenech et al., 2006). To sum up, in order to properly diagnose a cattle or herd with bovine tuberculosis, combination of tests is important in provide adequate information and accurate diagnosis.

3 MATERIALS AND METHOD

3.1 Farms and animals

A total of 356 adult dairy cattle from 6 farms around Selangor were tested for tuberculosis. Two tests were conducted according to the protocol of OIE. Initially, the cattle were screened using 'BOVIGAM®' (Thermo Fischer Scientific, USA) test. Then, the positive

animals were further tested using comparative intradermal tuberculin skin test (Sanofi-Pasteur, USA) as the ancillary test. After 72 hours of intradermal injection, the difference in skin thickness were observed and recorded. Animals with skin thickness of >4mm are considered positive and were culled within 14 days under the supervision of the Department of Veterinary Services, Malaysia. This strategy is called the test and culled protocol.

In this study, a total of 20 positive and 5 negative available adult dairy cattle were randomly selected from 6 farms. The positive animals were those showing positive results for both BOVIGAM® and intradermal skin tests. All 25 selected animals were brought to the burial site and body condition score were recorded before slaughter. Field post-mortem was carried out immediately to examine for gross lesions and to collect the organ samples. The carcasses were immediately covered with lime and buried.

3.2 Sample collection and processing

The post-mortem examinations were focused mainly on the respiratory tract, particularly the lungs, and the mediastinal and retropharyngeal lymph nodes. At the same time the intestinal tract and the associated mesenteric lymph nodes were also been examined. The reason was that bovine tuberculosis infection usually occurs via either respiratory or digestive tract.

Following examination of the gross lesion, samples of the lungs, mediastinal, retropharyngeal and mesenteric lymoh nodes and the intestine were fixed in 10% buffered formalin for at least 24 hours. Then, the samples were embedded in paraffin wax and sectioned at 4µm before being stained with Hematoxylin & Eosin (H&E) and Ziehl-Neelsen (Varello et al., 2008). The slides were observed under light microscope. The H&E slides were examined to identify and determine the stage of granulomatous tubercle lesions while the Ziehl-Neelsen slides were examined to detect the presence of acid-fast bacilli in tissues under 100x

magnification. The detailed explanations with the examples for each stage of the granuloma are shown in Table 1 (Wangoo et al., 2005) and Figure 1

Table 1: The stages of granulomatous tubercle lesions observed in bovine tuberculosis (Wangoo et al., 2005)

Stage	Description
Stage 1	Irregular un-encapsulated clusters of epitheloid macrophages with interspersed lymphocytes and small number of neutrophils. Occasionally presence of the Langhan's giant cell. Necrosis is absence
Stage II	Partially or completely encapsulated by thin capsule which surround the epitheloid macrophages. Infiltration of lymphocytes, neutrophils and Langhan's giant cell usually present. Haemorrhage often notes and minimal necrotic area recognized
Stage III	Fully encapsulated with central necrotic area characterized by caseous and mineralized. Epitheloid macrophages admixed with Langhan's giant cell surrounded the necrotic areas. Macrophages mixed with lymphocytes clusters predominantly present at the peripheral zone and scattered neutrophils extended to the fibrous capsule
Stage IV	Presence of fully thick encapsulation, large, irregular, multicentric granuloma with prominent caseous necrosis. Greater part of the lesion is occupied by extensive island of mineralization. The necrotic area is surrounded by epitheloid macrophages and Langhan's giant cells with dense cluster of lymphocytes near peripheral fibrotic capsule.

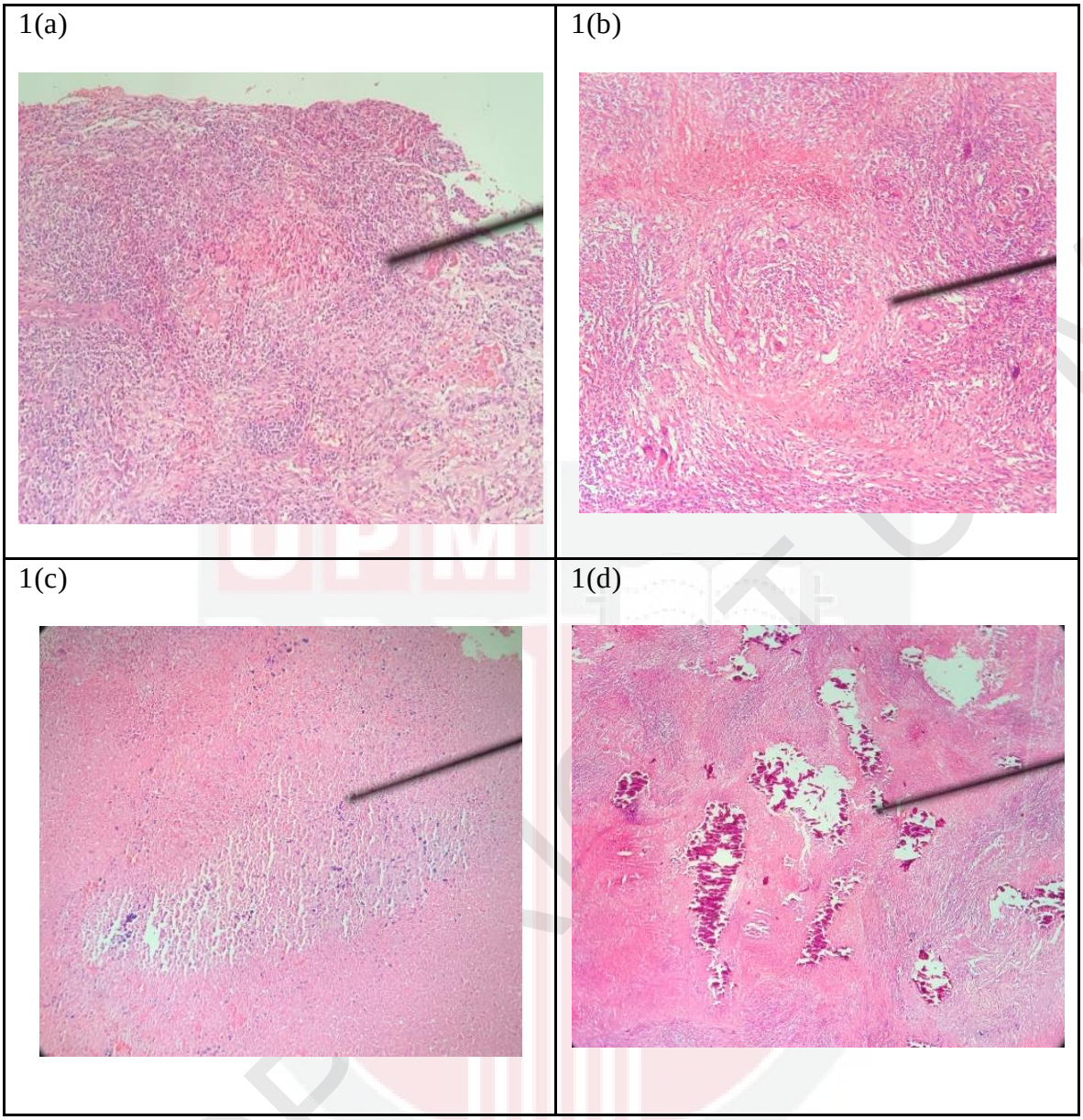

Fig. 1. Example of the four stages of granulomatous tubercle following H&E staining of lymph nodes of dairy cattle infected with *Mycobacterium sp*. (a) Stage I (Initial): irregular encapsulation, cluster of epitheloid macrophages with small number of neutrophils and Langhan's giant, necrosis absence. (b) Stage II (Solid granuloma): complete encapsulation, infiltration of lymphocytes and neutrophils, a greater number of Langhan's giant cells. (c) Stage III (Minimal necrosis): fully encapsulated with central necrotic area and surrounded with epitheloid macrophages, scattered neutrophils extended to fibrous capsule. (d) Stage IV (Necrosis & mineralization): thick encapsulation, large, irregular and multicentric granuloma with prominent caseous necrosis and surrounded with dense cluster of epitheloid macrophages and lymphocytes near peripheral fibrotic capsule.

3.3 Bacteriology

The same samples of lungs, intestine, mediastinal, retropharyngeal and mesenteric lymph nodes were aseptically collected by separately kept in small, sterile plastic bags for bacterial isolation. However, in this study, smears were prepared from the organ samples and stained with Ziehl-Neelsen to identify the presence of acid-fast organism.

3.4 Statistical Analysis

The correlation between ante-mortem test and each post-mortem examination was done using Pearson correlation for normally distributed data and Spearman's rho for non-parametric data in the Statistic Package for the Social Science (SPSS) data analysis. Hence, the correlation coefficient was used to identify the correlation strength (Table 2).

Table 2: Rule of thumb for interpreting correlation strength (Mukaka, 2012)

Correlation coefficient	Interpretation
0.90 to 1.00 (-0.90 to -1.00)	Very high positive (negative) correlation
0.70 to 0.90 (-0.70 to -0.90)	High positive (negative) correlation
0.50 to 0.70 (-0.50 to -0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (-0.30 to -0.50)	Low positive (negative) correlation
0.00 to 0.30 (0.00 to -0.30)	Negligible correlation

4 RESULT

Of the 356 dairy cattle tested with BOVIGAM® and comparative intradermal skin test, 38 (10.7%) were positive both tests, showing skin thickness >4mm that ranged between 4.08mm and 22.39mm. Of the 38 positive cattle, 20 were selected for pathological examination while another 5 control cattle showed skin measurement of <4mm. Table 3 shows the overall data recorded during ante-mortem and post-mortem examinations, which included the body condition score, gross lesions, comparative intradermal tuberculin skin measurement,

histopathology grading and bacteriology results. Among the reactor animals, only one (5%) had poor body condition score of 1.5 while the remaining animals had optimal body condition score ranging between 3.0 and 3.5.

Thirteen (65%) out of 20 reactors with positive skin thickness measurement of >4mm showed the presence of gross lesion in lung, mediastinal lymph node or/and retropharyngeal lymph node. In addition, the evidence of lesion histopathology can be found in 13 (65%) reactors. This conclude that the skin thickness measurement has poor correlation with the presence of lesion grossly and histopathology ($p>0.05$; $r = 0.290, 0.229$).

A total of 13 (65%) of the selected 20 reactors had granulomatous lesions in the respiratory tract. The gross lesion was absent from the gastro-intestinal tract. None of the 5 negative animals had tubercle lesions. Among the 13 reactors with gross lesion, 12 (92%) revealed histopathological lesions of tuberculosis granuloma thus, a strong correlation between gross and histopathology lesions ($p<0.05$; $r = 0.873$). Furthermore, 11 (85%) out of the 13 reactors with granuloma showed multi-stage granuloma in at least one of the organs of the respiratory tract. The most frequent stage of granuloma was stage IV with 15% frequency followed by stage I (14%), stage III (13%) and stage II with 7.6%. Approximately 53% of the stage IV granulomas were observed in the mediastinal lymph node while the remaining 47% were observed in the lungs. However, regardless of the stage of the granuloma, 12 (92%) reactors had histological granuloma lesion in the lungs, 10 (77%) reactors in the mediastinal lymph node, 2 (15%) reactors in the mesenteric and retropharyngeal and none in the intestine.

Table 3: Overall data for ante-mortem, post-mortem, histopathology and bacteriology

Animal		Intradermal Tuberculin Skin Test	Gross Lesion	Histology		Bacteriology
					ZN Histology	ZN Smear
ID	BCS	Skin Thickness, mm		Tissue and Lesion Grade (I-IV), H&E stain	AFB	
1	3.0	<4	-	-	NA	-
2	3.0	<4	-	-	NA	-
3	3.0	<4	-	-	NA	-
4	3.0	<4	-	-	NA	-
5	3.0	<4	-	-	NA	-
507	1.5	7.4	+ (M)	+ L (I), M (I-IV), MS (I)	-	-
510	3.5	5.1	+ (L, M)	+ L (IV), M (III-IV)	+ (L, M)	+ (L)
697	3.5	5.04	+ (L, M)	+ L (I-IV), M (III-IV)	+ (L, M)	+ (L, M, MS)
1553	3.0	22.39	+ (L, M)	+ L (III-IV), M (II-IV), MS (I-II)	+ (L, M)	+ (L, M)
2635	3.0	5.43	-	+ L (I)	-	+ (M)
2734	3.0	9.82	-	-	-	-
2736	3.0	13.59	-	-	-	-
2924	3.0	4.08	-	-	-	-
2925	3.0	9.57	+ (M)	+ M (II-III)	-	-
4222	3.5	9.29	-	-	-	+ (M, I, MS)
4281	3.0	4.69	+ (L, M)	+ L (I-IV), M (II-IV), RP (I)	-	+ (M)
4285	3.5	13.56	+ (L, M)	+ L (I-IV), M (I&IV)	-	-
5029	3.5	10.71	+ (L)	+ L (I-IV)	-	+ (I, MS)
6869	3.5	20.3	-	-	-	-
6870	2.5	9.18	+ (L, M, RP)	+ L (I-IV), M (I-IV)	+ (L)	+ (L, I, MS)
6888	3.5	4.6	+ (M)	+ L (I), M (I-IV)	-	+ (I, MS)
6893	3.5	9.4	+ (M)	-	-	-
6894	3.5	15.62	+ (L, M)	+ L (IV), M (IV)	-	+ (L, M, MS)
6899	3.5	11.1	+ (L)	+ L (II-III), RP (I)	+ (L)	+ (M)
8596	3.0	14.03	-	-	-	-

ID = animal individual number; BCS = body condition score.
L = lung; M = mediastinal lymph node; I = intestine; MS = mesenteric lymph node;
RP = retropharyngeal lymph node; ZN = Ziehl-Neelsen; AFB = acid fast bacilli.
NA = not analyzed; + = positive/presence; - = negative/absence.

Detection of acid-fast bacilli through Ziehl-Neelsen staining of histology slide and organ smears revealed that of the 20 reactors, 5 (25%) were identified with the presence of acid-fast bacilli in the tissue sections of lungs and mediastinal lymph node. The acid-fast bacilli were found in the Langhan's giant cells at the rate of 62.5% while 37.5% were found in the

caseous necrotic area. The stage of granulomatous tubercle was significant ($p<0.05$) but at a moderate correlation with the presence of acid-fast bacilli in organ tissue ($r = 0.53$). Hence, the acid-fast bacilli were observed mainly in the stage III and IV of the granulomatous tubercles. The presence of gross tubercle lesion was moderate but significantly ($p<0.05$) correlated with the detection of acid-fast bacilli in organ smear ($r = 0.53$) and tissue ($r = 0.48$). Ziehl-Neelsen staining of organ smears revealed that the presence of acid-fast bacilli in 11 (55%) of the 20 reactors, mostly in the mediastinal lymph node. Other than the mediastinal lymph node, the organism was occasionally observed in the mesenteric lymph node, lungs and intestine. Detection of the acid-fast bacilli in tissue section was moderate but significantly ($r = 0.56$; $p<0.05$) correlation with organ smears.

5 DISCUSSION

In this study, the gross and histopathology lesions, and the bacteria detection were evaluated from seropositive cattle to see of any relationship between the ante-mortem test and the post-mortem features. The results revealed that the most infected organ was lung, followed by the mediastinal, mesenteric and retropharyngeal lymph nodes. A study by Corner et al. (1990) revealed that the most frequently affected organ was the medial retropharyngeal lymph node, followed by the mediastinal lymph node, lungs and the least was the mesenteric lymph node. This explains that the frequency of organ affected is not specific and localization depends on the route of entry of *M. bovis*.

This study also revealed the poor correlation between comparative intradermal tuberculin skin test with gross lesions, indicating that positive skin test is not necessarily results in animal with gross lesion. This is in agreement with the study by Pollock and Neill (2002), who hypothesised that some cattle with positive skin tests may be latently infected due to the failure to detect tuberculous lesion or culture of *M. bovis* from several organ. Besides, Rua-

Domenech et al. (2006) strengthen the statement by reporting that the tuberculin skin test in bovines has notable problems of the specificity thus, resulting in false positive. Similarly, a study by Norby et al. (2004) that 6 infected cattle failed to show any gross lesion and this might be due to the infection at early stage or the role of host immune system in inhibiting the lesion growth.

Majority of the reactors had optimal body score condition although 65% were having tubercle lesions grossly and histologically. This is because, bovine tuberculosis is a chronic infection, where most of the affected animals only show the clinical signs once the affected organs are compromised. This could take months or even years and within that period, the affected animals live normally without loss of appetite and anorexic. Unfortunately, these infected animals act as carriers of *Mycobacterium* sp. and shed the pathogens to the herd in silence.

If the positive animals had granuloma grossly and histologically, they are likely to be diagnosed with bovine tuberculosis following a high degree of correlation between the gross and histopathology findings. Furthermore, the severity of granulomatous tubercle indicates the chronically infected animals. This is in agreement with Domingo et al. (2014) who stated that the initial small granuloma develops into large-sized and advanced granuloma over time and subsequently classified as chronic bovine tuberculosis, and these chronic lesions are commonly found in the lungs or in the mediastinal lymph node as observed in this study.

Most animals that showed granulomatous tubercle were usually showing multi-stage lesions with the involvement of more than one organ. This is in agreement with the previous report by Laisse et al. (2011), who concluded that multi-organ and multi-stage lesions are the result of repeated seeding by *M. bovis* in the organs over time. In this study, stage IV granulomatous tubercle was the most frequently observed, reflecting the level of chronicity of the disease. More damages were produced as the pathogens presence in the host for longer

period of time and more organ are affected via haematogenous or lymphatic system. Furthermore, localization of the lesion or granulomatous tubercle is related to the route of transmission (Domingo et al., 2014). Since large percentage of organs affected were lungs and mediastinal lymph nodes, the main route of transmission of *M. bovis* was likely through inhalation. However, the presence of histology lesion in mesenteric lymph node and *Mycobacterium* sp. in the intestinal smears suggest the possibility of transmission through oral route. In fact, Menzies and Niell (2000) reported that ingestion of *Mycobacterium* sp. from contaminated pasture, feed or water usually causes lesion in the mesenteric lymph node.

The presence of acid-fast bacilli in histology slides stained with Ziehl-Neelsen predominantly found in high stage of granulomatous tubercle (Stage II-IV) and can be seen in Langhan's giant cell or necrotic area. This finding is similar to the study done by Wangoo et al. (2005). Based on the study, in the early stage, the acid-fast bacilli were generally seen in macrophages or Langhan's giant cell and in advanced stage the organism is found within necrotic areas or sometimes even in mineralized debris. Lastly, the moderate presence of acid-fast bacilli in histology slides might be due to low survival rate of the organism in caseous environment and effect of granulomatous inflammation on bacterial structure as stated by Varello et al. (2008).

6 CONCLUSION

As a conclusion, seropositive animals indicate presence of bovine tuberculosis in the herd but lack the assurance of the individual animal infected with tuberculosis. Relying on seropositive result alone could increase the occurrence of false positive, when the result revealed that 25% of the reactors were negative following post-mortem examinations. Hence, a complete ante-mortem and post-mortem test results are crucial in confirming the diagnosis.

In a high prevalence area, the test and culled should be done routinely for detection of the positive bovine tuberculosis animals that could be the silent carriers.

7 RECOMMENDATION

The recommendation for this study is to further investigate and determine the species of the *Mycobacterium*. This is because, the cattle can be affected by different types of *Mycobacterium* and result in different clinical sign and post mortem and histopathology lesion. *Mycobacterium* species determination can be done by bacterial culture and isolation. Those methods are the gold standard in diagnosing cattle infected with bovine tuberculosis. By determining the species, the correlation between severity of lesion and species of *Mycobacterium* can be made.

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