UNIVERSITI PUTRA MALAYSIA

MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS DETECTION IN BEEF CATTLE IN TAMAN PERTANIAN UNIVERSITI, UPM

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It is hereby certified that I have read this project paper entitled "*Mycobacterium avium* subspecies *paratuberculosis* detection in beef cattle in Taman Pertanian Universiti, UPM", by Nur Farah Athirah binti Ismail. In my opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999- Project.

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DEDICATIONS

To the love of my life……

Abah and Mama,

Mama, for giving me a life to live, and love me unconditionally.

Abah, for giving me support and make me tougher from inside.

I am somebody now.

Love you both.

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Thank you,

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ABSTRAK

Abstrak daripada kertas kerja yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

Jangkitan *Mycobacterium avium* subspesies *paratuberculosis* pada lembu pedaging di Taman Pertanian Universiti, Universiti Putra Malaysia

oleh

Nur Farah Athirah binti Ismail

2016

Penyelia : Profesor Dr. Abdul Aziz Saharee

Mycobacterium avium subspesies *paratuberculosis* (MAP) adalah agen etiologi penyakit Johne yang dilihat sebagai penyakit bakteria usus kronik, menular dalam spesies ruminan. Penyakit yang menyebabkan kerugian besar kepada penternak ini diciri oleh cirit-birit kronik atau selang-seli, pengurangan berat badan progresif, dan penurunan pengeluaran. Kajian ini dijalankan untuk menentukan kewujudan antigen dan antibodi MAP, masing-masing dalam tinja dan serum lembu. Sejumlah 213 sampel tinja dan 71 serum dikumpul daripada 71 ekor lembu di Taman Pertanian Universiti (TPU), Universiti Putra Malaysia (UPM). Sampel ini diuji mengguna kaedah pewarna Ziehl-Neelsen kekal asid untuk pengesanan antigen dan kaedah ujian pengikatan komplemen (CFT) untuk antibodi. Pewarnaan Ziehl-Neelsen menunjukkan

60 (28.2%) daripada 213 sampel positif untuk antigen *M. avium*, manakala, CFT menunjukkan 3 (4.2%) daripada 71 sampel positif untuk antibodi. Walau bagaimanapun, MAP bukanlah satu-satunya organisma kekal asid; justeru, penentuan spesies organisma perlu disokong dengan kultur tinja. Kesimpulannya, jangkitan MAP wujud dalam gerompok lembu pedaging di TPU, UPM dengan wujudnya agen penyebab dan antibodi, masing-masing dalam sampel tinja dan serum lembu.

Kata kunci : *Mycobacterium avium* subspesies *paratuberculosis*, jangkitan, cirit-birit selang-seli, CFT, pewarna Ziehl-Neelsen kekal asid.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Project.

***Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in beef cattle in Taman Pertanian Universiti (TPU), UPM**

by

Nur Farah Athirah binti Ismail

2016

Supervisor : Prof. Dr. Abdul Aziz bin Saharee

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the aetiology agent causes Johne's disease which viewed as a chronic, contagious bacterial of intestinal tract in ruminant species. The infection that caused substantial losses to the farmer was characterized by chronic or intermittent diarrhoea, progressive weight loss, and decreased production. This study was conducted to determine the presence of MAP antigen and antibodies in the faeces and serum, respectively, in the cattle. A total of 213 faecal samples and 71 serum samples were collected from 71 cattle at Taman Pertanian Universiti (TPU), Universiti Putra Malaysia (UPM). These samples were tested using Ziehl-Neelsen acid-fast stain method for antigen detection and complement fixation test (CFT) for antibodies detection. Ziehl-Neelsen staining

revealed that 60 (28.2%) of 213 samples were positive for *M. avium* antigen while, CFT revealed 3 (4.2%) of 71 serum samples were positive for the antibody. However, MAP is not the only acid fast organism; thus the determination of species of the organism should supported by faecal culture. In conclusion, this study showed that there is MAP infection in the cattle herd at TPU, UPM, with the presence of antigen causing agent and antibodies in faecal and serum samples, respectively, of cattle.

Keywords : *Mycobacterium avium* subspecies *paratuberculosis,* bacterial disease, intermittent diarrhoea, CFT, Ziehl-Neelsen acid-fast stain

1.0 INTRODUCTION

Paratuberculosis or Johne's disease is a chronic contagious bacterial disease caused by causative organism called *Mycobacterium avium* subspecies *paratuberculosis (*MAP*)* which commonly affects domestic ruminants (cattle, sheep, goats and buffaloes) as well as wild ruminants (cervids) (Mercier, 2014). It was first observed by Drs Heinrich Albert Johne and Langdom Frothingham at Veterinary Pathology Unit, Dresden in 1895 (Manning and Collins, 2010). This organism causes granulomatous intestinal lesion and usually the disease is characterized by chronic or intermittent diarrhoea (Stabel, 1998), progressive weight loss, decreased production (milk and meat) and cause substantial losses (Hayton, 2007).

MAP can be transmitted and spreads by both horizontal and vertical means. It spreads through ingestion of MAP from contaminated environment (Mercier, 2014) most likely through faecal oral route, either by direct ingestion of faecal from infected animals or indirect ingestion of faecal contaminated colostrum, milk, water and feed (Manning and Collins, 2010). In addition, it can transmitted vertically from infected dam to foetus as conferred by Larson and Kopecky, 1970 and infection in calves is primarily due to ingestion of milk from infected dam or faecal contaminated milk (Mercier, 2014).

Once the MAP ingested, it survives and replicates within the macrophages in the intestine wall and the regional draining lymph nodes. Although subsequently phagocytised by macrophages, it will replicate slowly and stimulate inflammatory and cellular response as it is resistant to intracellular degradation (Hayton, 2007). The incubation period is long, up to 5 years. After this period, the animals will start to shed

this organisms in the faeces from low numbers and gradually will increase until the time of clinical onset although some will show intermittent shedding from the early course of the disease (Hayton, 2007), particularly the subclinical infection when the animals shed the organisms in the faeces while apparently looking normal with no clinical signs shown.

Clinical signs shown include intermittent to chronic diarrhoea, cachexia despite normal appetite while in advanced cases, they will show emaciation, lethargy, oedema and anaemia (Hayton, 2007). As the lesion start at the wall of intestine which will gradually develop to chronic granulomatous lesions thus causing protein leak and protein malabsorption syndrome leading to muscle wasting (Mercier, 2014). The economic impact of the disease in beef production is devastating particularly due to loss in production and treatment cost.

There are various types of detection method available for diagnosing Johne's disease, either by detection of the antigen in the faecal or tissue or serologically. Common serological methods used include complement fixation test (CFT), absorbed enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID) although their sensitivity and specificity is often based on the result of faecal culture. All tests lack accuracy and have difficulty to detect the *MAP* in subclinical infected animal.

1.1 Rationale of study

This disease is important as it can cause serious chronic disease which can spread to other animals within the same farm for the bacteria will be shed in the environment by the affected animals although not showing any clinical signs. Besides, there is a lack of information regarding this disease in Malaysia. But there are several cases of Johne's disease reported intermittently in Selangor. The results and knowledge obtained from this study may serve as a future reference in knowing the prevalence and improving the health protocol in the prevention of Johne's disease at TPU.

1.2 Hypothesis and objectives of the study

There were presence of the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) agent and antibody in faecal and serum tested respectively.

The prevalence of MAP infection in beef cattle at TPU is unknown, thus the objectives of this study are :

1. To determine the presence of *Mycobacterium avium subsp. paratuberculosis* (MAP) in the faecal of beef cattle at TPU, UPM.
2. To determine the presence of antibody against *Mycobacterium avium subsp. paratuberculosis* (MAP) in the serum of beef cattle at TPU, UPM.

2.0 LITERATURE REVIEW

2.1 Aetiology Agent

Mycobacterium avium subspecies *paratuberculosis* (MAP) which causes Johne's disease is characterized as small organism about 0.5 µm by 1-2 µm, rod shaped, an obligate intracellular pathogen and stained acid fast on Ziehl-Neelsen stain (Hermon-Taylor and El-Zaatari, 2004). *MAP* belongs to *Mycobacterium avium* as having DNA that is 99% similar to *M. avium*, thus considered as same species with subspecies of paratuberculosis (Hayton, 2007). It is an extremely slow growing organism and takes about 8-16 weeks to show visible colonies growth in the media culture besides having a long incubation period up to 5 years (Hayton, 2007). MAP is very resistant to degradation and may survive in contaminated soil and water for a year or more as well as occuring only in environment contaminated with faeces from infected animals (Manning and Collins, 2010).

2.2 Pathogenesis

Once the MAP is ingested, it will enter the intestinal wall through the mucosa of the small intestine since its primary target cells is the intestinal tract (Manning and Collins, 2010). There, since it is an obligate intracellular organism, it survives and replicates within the macrophages in the intestine wall and the draining mesenteric lymph nodes. Although subsequently phagocytized by the macrophage, it is resistant to intracellular degradation, some will replicate slowly and stimulate inflammatory and cellular response (Hayton, 2007). However, depending on the natural resistance of the

animal, the infection can be eliminated or remain infected as a carrier without showing clinical signs (Mercier, 2014).

Following the site of infection which is at the intestine wall, the early lesions will be confined to the intestine wall and mesenteric lymph nodes and later as the disease progresses the gross lesions will appear throughout the intestinal tract including the ileum, jejunum, terminal small intestine, caecum and colon as well as in the mesenteric lymph nodes. These lesions will gradually develop to granulomatous lesions causing the leakage of protein and protein malabsorption thus leading to muscle wasting and weight loss (Mercier, 2014).

Clinical signs shown include intermittent to chronic diarrhoea, cachexia despite normal appetite while in advanced cases will show emaciation, lethargy, oedema and anaemia (Hayton, 2007). Apart from that, the animals will start to shed this organisms in the faeces from low numbers and gradually increase until the time of clinical onset.

2.3 Stages of MAP Infection

There are several opinion regarding the stages of the paratuberculosis infection. Once, it was stated that animals became infected soon after birth and later progressed through the three stages (Sockett, Conrad, Thomas, and Collins, 1992). The first stage showed the animal being infected but not shedding the organisms in the faeces while the second stage stated that the animal showed no clinical signs but were shedding the organisms through the faeces. The third stage showed the animals being clinically ill with clinical signs as well as shedding the organism in the faeces.

The newer article state that there are four stages of infection as discussed in an article by Fecteau and Whitlock. The stages are divided depending on the severity of clinical signs, the potential of shedding organisms into the environment and the easier methods to detect the organisms. As for stage one, the animal will get the infection once the MAP enter and replicate in the intestinal mucosa after ingestion from contaminated environment. It does not show clinical signs and may shed the organisms into the environment, however the number of the organisms being shed is too low to be able to be detected by the current methods. It is also known as a 'silent' infection, usually occurs in calves, heifer, young stock and even adult cattle. The animals are said to be in stage two once the organism can be detected in the faeces. This is because the concentration of MAP is higher in the intestinal mucosa compared to stage one. Apart from that, the animals shed the organisms into the environment in the faeces in large numbers that it is able to be detected and this can be a source of infection in the farm. However, the infected animals still do not show any clinical signs despite shedding the organisms. In stage three, the infected animals are considered as clinically ill as clinical signs such as gradual weight loss and intermittent diarrhoea are seen despite normal appetite. The number of organisms shed in the faeces is much higher compared in stage two and mostly all are positive for faecal culture and serological methods. The animals are usually culled for weight loss and unresponsive diarrhoea. In stage four which is the last and severe stage of the disease, the animals appear weak, emaciated and have profuse diarrhoea. The infection progresses rapidly and later death will occur usually due to dehydration and cachexia (Fecteau and Whitlock, 2010).

2.4 Diagnosis of MAP infection

Johne's disease can be diagnosed by several methods and usually based on the clinical signs shown, immunological reaction and identification of the aetiology agent, MAP (Hayton, 2007). A number of laboratory tests can be used to diagnose the presence of this disease, include faecal smear, faecal and tissue culture, DNA probes using faeces and tissue, serology, necropsy and histology (Mercier, 2014). Necropsy is usually based on the finding of macroscopic lesion along the intestine and histopathological lesion and isolation of MAP. Many claimed that it was easier to diagnose the clinically affected animals compared to subclinical infected animals as the animals intermittently shed the organisms and often test negative in serological method in detecting antibody (Hayton, 2007).

Acid fast staining of the faeces or tissue is the most common diagnostic test used, however it has low sensitivity and specificity but it can be used to confirm clinical cases (Mercier, 2014). Commonly used serological tests include complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), and agar gel immunodiffusion (AGID) (OIE, 2014). In CFT, a research show that animal which was tested positive shed the highest number of organisms. However, due to lack of sensitivity, this test should not be used in diagnosing subclinical infection (Sockett, Conrad, Thomas, and Collins, 1992).

2.5 Treatment and Prevention

Currently, there is no specific treatment for the disease as the clinically infected animals are usually unresponsive towards any treatment (Mercier, 2014). However, supportive treatment such as fluid therapy and antibiotic to address the dehydration and diarrhoea is recommended and usually given to affected animals.

Prevention and control mainly involves good hygiene management, especially for manure management as the primary source of infection is ingestion of the aetiology agent either directly or indirectly from faeces contaminated environment. Since the young calves are very susceptible to this disease, proper calving management should be adopted including calving area that is free from others animal's manure to prevent contact with faeces from infected animals. As for the concern of transmission of the organisms through milk from the infected dam, the calf should be immediately separated soon after birth and given colostrum from another dam that is free from this disease. Apart from that, a good screening test for new animals before introduction in the herd. Moreover, any suspected or confirmed cases should be reported to the Department of Veterinary Services (DVS), for prompt test to be carried in order for the test and cull policy to be done (Mohd. Nor, Mustapa, Abu Hassan and Chang, 2003).

2.6 Client Education

Johne's disease is listed in the eradication programme where any positive animal will be slaughtered and compensation will be given to the farm owner. This is because of the significant impact from the disease that causes economical losses to the farmers due to reduced production of milk and treatment cost. Other than that, any suspected or positive cases should be reported to DVS as soon as possible which can help to determine the prevalence of the disease in the country (Mohd. Nor, Mustapa, Abu Hassan and Chang, 2003).

Since this disease is spread through faecal oral route, the farmer should focus in good sanitation and hygienic management especially in manure removal management.

3.0 MATERIALS AND METHODS

3.1 Sampling procedure

Sampling was done from 12th to 22nd January 2016 at Taman Pertanian Universiti (TPU). 34 female cattle of Kedah-Kelantan breed and 37 female cattle of Brangus breed with different age ranging from 22 months old to 13 years old were used in this study. All the cattle were apparently healthy during the time of sample collection. Both faecal and blood samples were taken and processed using Ziehl-Neelsen acid fast staining and Complement Fixation Test (CFT).

3.2 Ziehl-Neelsen Acid Fast Staining

Ziehl-Neelsen acid fast staining is the most common diagnostic method used to detect the MAP in the faeces and tissue. In this study, faecal sample was used in detecting the organism. Faecal samples from the 71 cattle were collected using rectal pinch technique per rectal by gently scrapping mucosal part of the rectum wall. A new and clean hand gloves were used during the time of collection. The samplings were done three times at 4 days apart from the same cattle. After each collection, the faecal samples kept in an ice box were transported to the bacteriology lab of veterinary faculty. The samples were processed soon after arrival. The faecal samples were smeared onto a new, clean glass slides using new cotton swabs and labelled according to the ID of the animals. This procedure was done inside the Biosafety Cabinet Level 3. The smeared slides were then slightly glided over a flame from the Bunsen burner to fix it.

There were six steps in acid fast staining procedure. Firstly, flood the smeared slides with concentrated carbol fuschin. Then, heat for five minutes over a flame but careful not to boil it. Secondly, wash the slides with tap water. Thirdly, decolorize the slides with 3% acid alcohol for one minute. After that, wash the slides again with tap water. Later, add Loeffer's methylene blue onto the slides for 1 minutes. And lastly, wash the slides with tap water and blot dry. The stained slides were examined under light microscope using 100x oil magnification. Total of 213 samples from 71 cattle were tested.

3.3 Serological method (Complement Fixation Test)

For complement fixation test, serum is needed to do the procedure. 71 blood samples were first collected in the plain tube taken from the jugular veins of the cattle. Pressure was put to the jugular vein to make it more visible. Then, alcohol swab was used before the insertion of the venoject needle. The plain tubes containing the blood were kept in an ice box and were transported to the bacteriology lab of veterinary faculty. The blood samples were allowed to clot under room temperature for a few hours and later centrifuged under speed of 4000 rpm for five minutes to get the serum separated from the whole blood. The serum samples were collected into 1.5ml labelled microcentrifuge tube and stored at -20^{0}C until ready for diagnostic test.

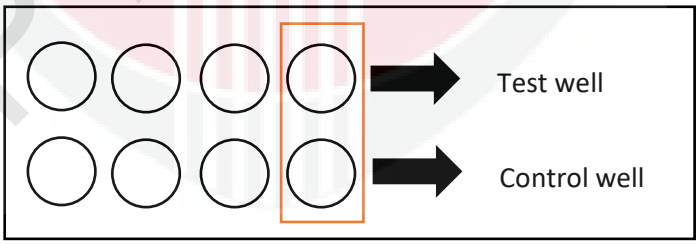
The serum samples were tested using a standard CFT procedure at Veterinary Research Institute. The procedure started with a 1:10 serum dilution using 0.25% phenol saline. Then inactivated at 56^{o}C for 30 minutes. Using a micropipette, 25µl was put into two well of microplate for each of the sample according to table 1. The

sample was divided into two which was tested and controlled as illustrated in diagram 1. The microplates were then incubated at 37ºC for one hour. After that, 50µl of haemolytic system (consist of sheep red blood cells and haemolysin) is added to all wells. It was mixed well and incubate at 37ºC for 15 minutes. This step was done twice. Then, mix it well again and left for button formation. The control is compulsory for the test.

Table 1 : Test procedure for CFT

Component	Well 1	Well 2
Serum 1:10 dilution	25µl	25µl
Antigen	25µl	-
Saline	-	25µl
Complement	25µl	25µl

Diagram 1 : Label of microplate well



This test mainly depended on the antigen and antibody reaction that formed an antigen-antibody complexes. Complement added will fix the mixture. Positive result will not show haemolysis reaction and the red blood cells will settle down and form a red button-like formation. While, negative result will show haemolysis reaction of the red blood cell indicating there is no antibody to react with the complement.

3.4 Data Analysis

The prevalence of MAP infection was calculated by the number of positive samples for Ziehl-Neelsen acid fast stain and number of positive samples for antibody against MAP from all the samples tested. The result from both tests were tabulated in Microsoft Excel 2010 and calculated and analysed using Chi-square statistical method using IBM Statistic 22.

4.0 RESULTS

4.1 Ziehl-Neelsen Acid Fast Staining

From 213 faecal samples tested, 28.2% were positive with the presence of acid fast organisms which consisted of 60 samples. From these samples, 46 samples were from Kedah-Kelantan (KK) breed and another 14 samples were from Brangus breed cattle. The other 71.8% were negative with 153 samples consisted of 56 samples of KK breed and 97 samples of Brangus breed. Table 2 below shows the result of the test according to the breed in a simple way.

Table 2 : Crosstabulation data result of acid fast stain based on breed.

Breed * Acid_fast Crosstabulation

			Acid_fast		
			positive	negative	Total
Breed	KK	Count	46	56	102
		% within Breed	45.1%	54.9%	100.0%
		% within Acid_fast	76.7%	36.6%	47.9%
		% of Total	21.6%	26.3%	47.9%
	Brangus	Count	14	97	111
		% within Breed	12.6%	87.4%	100.0%
		% within Acid_fast	23.3%	63.4%	52.1%
		% of Total	6.6%	45.5%	52.1%
Total		Count	60	153	213
		% within Breed	28.2%	71.8%	100.0%
		% within Acid_fast	100.0%	100.0%	100.0%
		% of Total	28.2%	71.8%	100.0%

From the result obtained, it showed that there were presence of MAP in the faeces of the beef cattle at TPU, UPM. Prevalence of the MAP infection in beef cattle TPU based on the acid fast staining result can be calculated. Prevalence of MAP infection

can be calculated by dividing the number of positives cases over total number of cases tested.

Prevalence of MAP infection within KK breed

Prevalence of MAP infection = 46 /102

= 0.4510

Prevalence of MAP infection within Brangus breed

Prevalence of MAP infection = 14/111

= 0.1262

Prevalence of MAP infection of total samples tested

Prevalence of MAP infection = 60/213

= 0.2817

The data result were also analysed by Chi-square method using SPSS to know the relation between breed and the frequencies of positive cases. As for that, null and hypothesis were created.

Assume $\alpha = 0.05$,

H_0 : There is no significant difference between breed and the frequencies of positive cases

H_A : There is significant difference between breed and the frequencies of positive cases

Table 3 : Chi-square test result of acid fast staining

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	27.723[a]	1	.000		
Continuity Correction[b]	26.141	1	.000		
Likelihood Ratio	28.727	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	27.593	1	.000		
N of Valid Cases	213				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 28.73.
b. Computed only for a 2x2 table

From the table 3 above, the tabulated p value is smaller than $\alpha = 0.05$, therefore we cannot accept the null hypothesis. Thus, it can be concluded that there is significant difference between breed and frequencies of the positive cases for acid fast staining.

4.2 Complement Fixation Test (CFT)

From 71 samples tested, only three cases were positive with 4.2%. One of the sample was from KK breed while the other two were of Brangus breed. The other 68 samples were negative with 95.8%. Table 4 below shows the tabulated data result of the test.

Table 4 : Crosstabulation data result of CFT based on breed

Breed * CFT Crosstabulation

			CFT		
			positive	negative	Total
Breed	KK	Count	1	33	34
		% within Breed	2.9%	97.1%	100.0%
		% within CFT	33.3%	48.5%	47.9%
		% of Total	1.4%	46.5%	47.9%
	Brangus	Count	2	35	37
		% within Breed	5.4%	94.6%	100.0%
		% within CFT	66.7%	51.5%	52.1%
		% of Total	2.8%	49.3%	52.1%
Total		Count	3	68	71
		% within Breed	4.2%	95.8%	100.0%
		% within CFT	100.0%	100.0%	100.0%
		% of Total	4.2%	95.8%	100.0%

From the result obtained, it showed that there were presence of antibody against MAP in the serum of the beef cattle at TPU, UPM. Prevalence of the MAP infection in beef cattle TPU based on the complement fixation test result can be calculated. Prevalence of MAP infection can be calculated by dividing the number of positives cases over total number of cases tested.

Prevalence of MAP infection within KK breed

Prevalence of MAP infection = 1/71

= 0.0141

Prevalence of MAP infection within Brangus breed

Prevalence of MAP infection = 2/71

= 0.0282

Prevalence of MAP infection of total samples tested

Prevalence of MAP infection = 3/71

= 0.0423

The data result was also analysed by Chi-square method using SPSS to know the relation between breed and the frequencies of positive cases. As for that, null and hypothesis were created.

Assume $\alpha = 0.05$,

H_0 : There is no significant difference between breed and the frequencies of positive cases

H_A : There is significant difference between breed and the frequencies of positive cases

Table 5 : Chi-square test result of CFT

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.266[a]	1	.606		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.272	1	.602		
Fisher's Exact Test				1.000	.532
Linear-by-Linear Association	.262	1	.609		
N of Valid Cases	71				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.44.

b. Computed only for a 2x2 table

From the table 5 above, the tabulated p value is bigger than $\alpha = 0.05$, therefore we accept the null hypothesis. Thus, it can be concluded that there is no significant difference between breed and frequencies of the positive cases for complement fixation test.

5.0 DISCUSSION

The result obtained from the test method done that is Ziehl-Neelsen acid fast staining of faecal smears reveals presence of acid fast staining organisms, most probably the organism that causes Johne's disease, MAP. However, since acid fast organisms does not only consists of MAP, it can be from *Nocardia sp* and *Corynebacteria sp*., thus it is not suggestive enough to diagnose as Johne's disease. Besides, this test method is low in sensitivity and specificity. Therefore, there is a need to be supported from other tests, particularly faecal culture where isolation and identification of the organism can be done. However, faecal culture can give definitive result as it has 100% specificity, despite being time consuming and a very difficult procedure as it is easily contaminated (Mercier, 2014).

Meanwhile, CFT test have a higher sensitivity and specificity compared to acid fast, thus has a give a better result for diagnosing. Moreover, it is a test to detect the antibody in the serum thus give more compromising diagnosis. Although it is the most common diagnostic methods used, it works well with clinically suspect animals thus cannot be used to accurately determine the prevalence of the disease in subclinical infection. In this however, the objectives are to detect the antigen MAP and antibody against MAP in the faeces and blood relatively, thus it is a cheap and an easy method to be used.

The different result obtained from both tests could be explained by the stages of MAP infection itself. Although the number of positive samples for MAP in CFT method were low compared to in acid fast staining method, it does not mean that there were low MAP infection in the herd. Based on the stages of MAP infection explained

before, the positive samples in acid fast staining could indicate that the animals were in subclinical infection stage where it would be detected through faecal but not serologically.

These subclinical infected animals may act as a source of infection since they will shed the MAP organisms into the environment through their faeces. In addition, the faecal-oral route is the main route of infection.

In term of management of this farm, these two breeds were let to grazing in the different paddock with rotational grazing system. However, they were using the same paddock despite rotational grazing system which may contribute to the spread of infection between these two breeds as the faeces from another breed may still available in the soil by the time the other breed came to graze.

Thus, based on the discussion and result from both tests, the farm should proceed with the faecal culture to confirm the infection truly is MAP infection. Then only, control and eradication can be carried out.

6.0 CONCLUSION AND RECOMMENDATION

In conclusion, this study indicates the presence of MAP infection among beef cattle at TPU based on the CFT and acid fast staining method. However, for acid fast staining method result, it should be supported with other diagnostic method to identify it as true MAP organism.

Since there is evidence of MAP infection in this farm, it is recommended that other diagnostic method should be carried out to confirm and determine the prevalence of MAP infection.

7.0 REFERENCES

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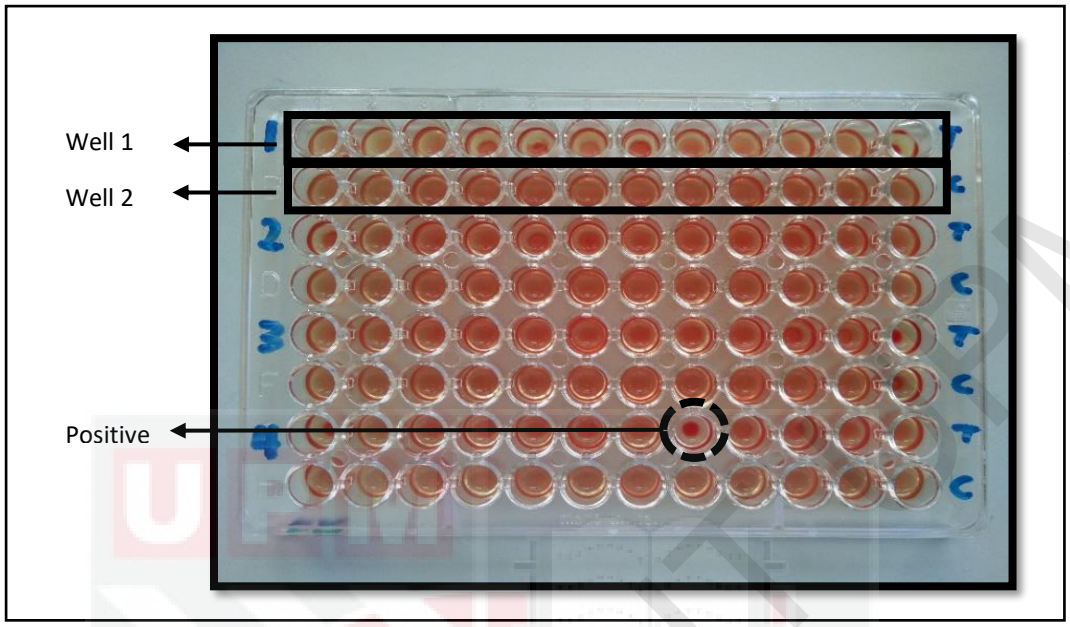
8.0 Appendix

ANIMAL ID	DOB	BREED	AGE	SEX	Acid Fast Staining			CFT
					1st	2nd	3rd	
K8511	02/04/2008	KK	Adult	Female	N	P	P	N
K0019	31/8/2010	KK	Adult	Female	P	N	N	N
K4538	14/8/2004	KK	Adult	Female	P	P	P	N
K4007	14/7/2014	KK	Young	Female	N	P	N	N
K3023	13/8/2013	KK	Adult	Female	P	P	P	N
K2029	13/8/2012	KK	Adult	Female	N	N	N	N
K6548	14/8/2006	KK	Adult	Female	P	N	P	N
K9502	01/02/2009	KK	Adult	Female	N	N	N	N
K3029	09/04/2013	KK	Adult	Female	P	P	N	N
K6525	30/2/2006	KK	Adult	Female	N	P	N	N
K9521	18/2/2009	KK	Adult	Female	P	P	P	N
K5515	16/4/2005	KK	Adult	Female	P	P	N	P
K7540	29/8/2007	KK	Adult	Female	N	N	N	N
K6503	25/1/2006	KK	Adult	Female	P	N	N	N
K5518	23/4/2005	KK	Adult	Female	N	N	N	N
K3007	29/5/2013	KK	Adult	Female	N	P	N	N
K1006	16/6/2011	KK	Adult	Female	P	P	N	N
K7516	21/2/2007	KK	Adult	Female	N	N	P	N
K9517	02/07/2009	KK	Adult	Female	N	P	P	N
K6518	16/3/2008	KK	Adult	Female	N	P	N	N
K8515	02/08/2008	KK	Adult	Female	N	P	P	N
K3026	25/8/2013	KK	Adult	Female	N	N	P	N
K6506	28/1/2006	KK	Adult	Female	N	P	P	N
K7517	22/2/2007	KK	Adult	Female	N	P	P	N
K6517	14/3/2006	KK	Adult	Female	N	P	N	N
K0050	25/11/2010	KK	Adult	Female	N	P	P	N
K7541	30/8/2007	KK	Adult	Female	N	P	P	N
K0030	09/10/2010	KK	Adult	Female	P	P	N	N
K2016	07/11/2012	KK	Adult	Female	P	P	N	N
K9507	01/12/2009	KK	Adult	Female	N	N	P	N
K3004	05/12/2013	KK	Adult	Female	P	P	P	N
K3011	13/7/2013	KK	Adult	Female	N	P	N	N
K8534	08/01/2008	KK	Adult	Female	N	P	N	N
K4004	07/08/2014	KK	Young	Female	P	P	N	N

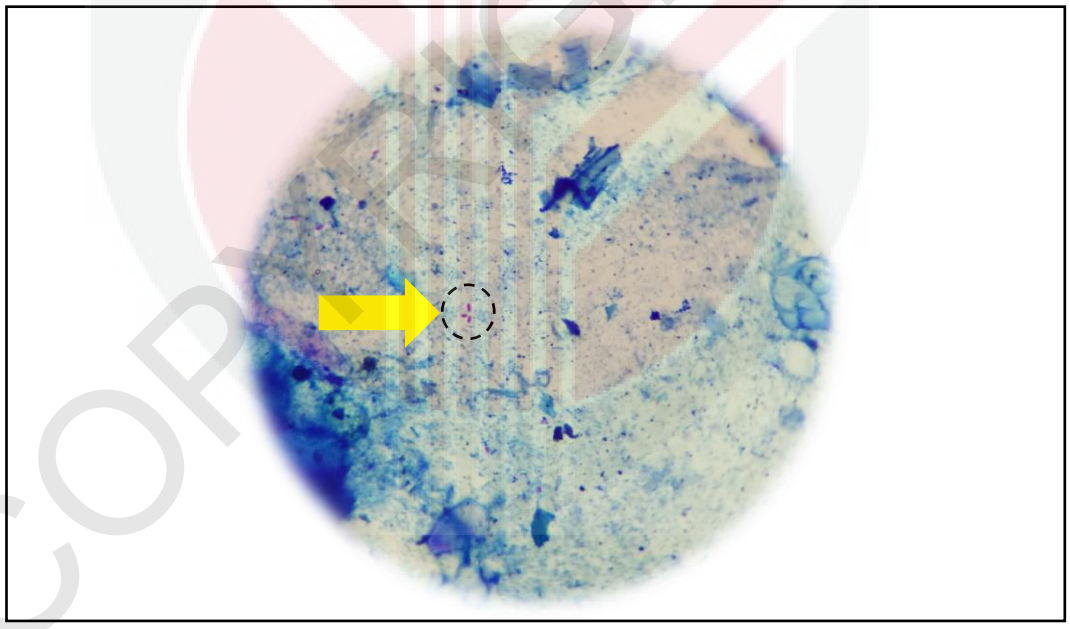
Appendix 1 : Data tabulation of Kedah-Kelantan cattle in TPU.

B230	11/11/2013	BRANGUS	Adult	Female	N	N	P	N
B215	26/7/2012	BRANGUS	Adult	Female	N	N	N	N
B316	18/8/2013	BRANGUS	Adult	Female	N	N	P	N
B104	18/6/2010	BRANGUS	Adult	Female	N	N	N	N
B702	05/01/2007	BRANGUS	Adult	Female	N	N	N	N
B608	15/6/2006	BRANGUS	Adult	Female	N	P	N	N
B720	14/8/2007	BRANGUS	Adult	Female	N	N	N	N
B313	08/02/2013	BRANGUS	Adult	Female	N	N	P	N
B218	01/01/2012	BRANGUS	Adult	Female	N	N	N	N
B717	08/07/2007	BRANGUS	Adult	Female	N	P	P	P
B103	16/6/2010	BRANGUS	Adult	Female	N	N	N	N
B125	24/9/2010	BRANGUS	Adult	Female	N	N	N	N
B201	01/01/2012	BRANGUS	Adult	Female	N	N	N	N
B613	26/7/2006	BRANGUS	Adult	Female	N	N	N	N
B325	20/9/2013	BRANGUS	Adult	Female	P	N	N	N
B719	14/8/2007	BRANGUS	Adult	Female	N	N	N	N
B303	07/12/2013	BRANGUS	Adult	Female	N	N	N	N
B305	15/7/2013	BRANGUS	Adult	Female	N	N	P	N
B711	23/5/2007	BRANGUS	Adult	Female	P	P	N	N
B320	24/8/2013	BRANGUS	Adult	Female	P	N	N	P
B317	18/8/2013	BRANGUS	Adult	Female	N	N	P	N
B114	08/10/2010	BRANGUS	Adult	Female	N	N	N	N
B126	26/9/2010	BRANGUS	Adult	Female	N	P	N	N
B402	16/7/2014	BRANGUS	Young	Female	N	N	N	N
B107	22/6/2010	BRANGUS	Adult	Female	P	N	P	N
B204	15/3/2012	BRANGUS	Adult	Female	N	N	N	N
B307	18/7/2013	BRANGUS	Adult	Female	N	N	N	N
B523	08/05/2013	BRANGUS	Adult	Female	N	N	N	N
B909	01/01/2009	BRANGUS	Adult	Female	N	N	P	N
B102	14/6/2010	BRANGUS	Adult	Female	N	N	N	N
B801	07/01/2008	BRANGUS	Adult	Female	N	N	N	N
B312	31/7/2013	BRANGUS	Adult	Female	N	N	N	N
B203	03/12/2012	BRANGUS	Adult	Female	N	P	P	N
BR02	07/04/2011	BRANGUS	Adult	Female	N	N	N	N
B808	15/5/2008	BRANGUS	Adult	Female	N	N	N	N
B326	10/01/2013	BRANGUS	Adult	Female	N	N	N	N
B803	07/01/2008	BRANGUS	Adult	Female	N	N	N	N

Appendix 2 : Data tabulation of Brangus cattle in TPU.



Appendix 3 : Interpretation of CFT result.



Appendix 4 : Acid fast stained organisms.