UNIVERSITI PUTRA MALAYSIA

MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS (MAP) INFECTION IN DAIRY CATLLE IN TAMAN PERTANIAN UNIVERSITI

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HAMZAH

A project paper submitted to Faculty of Veterinary Medicine, Universiti Putra Malaysia in partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

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CERTIFICATION

It is hereby that we have read this project paper entitle "*MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* (MAP) INFECTION IN DAIRY CATLLE IN TAMAN PERTANIAN UNIVERSITI" by Nurul Asikin binti Abu Bakar Hamzah and in our opinion it is satisfactory in terms of scope, quality, and presentation as partially fulfillment of requirement for the course VPD 4999 – Final Year Project

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DEDICATION

I would like to dedicate this thesis to my both beloved parents Abu Bakar Hamzah bin Haji Nafiah and Lasimah binti Wagini @ Wagino, my family members (Hannan, Atikah, Hanif) and also my other half, Mohamad Aizuddin bin Othman. Without their kindness, genorosity and encouragement I would able to finish this Final Year Project.

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LIST OF ABBREVIATIONS

MAP	*Mycobacterium avium* subspecies *paratuberculosis*
CFT	Complement Fixation Test
TPU	Taman Pertanian Universiti

ABSTRAK

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

Jangkitan*Mycobacterium avium* subspecies *paratuberculosis* (MAP) dalam lembu tenusu di Taman Pertanian Universiti (TPU)

Oleh

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Jangkitan MAP atau lebih dikenali sebagai penyakit Johne's merupakan salah satu penyakit amat serius dan kronik dalam ruminan kerana penyakit ini secara tidak langsung menyebabkan kerugian ekonomi kepada penternak. Kajian ini dijalankan untuk mengenalpasti kehadiran organisma MAP dalam najis dan antibodi dalam sampel serum yang telah diambil.

Sebanyak 129 sampel najis dan 43 sampel serum telah diambil daripada 43 ekor lembu tenusu dari Taman pertanian Universiti (TPU). Sampel tersebut kemudiannya diuji untuk mengenal pasti kehadiran antigen dan antibodi dengan menggunakan calitan pewarnaan Ziehl-Neelsen Complement Fixation Test (CFT). Keputusan daripada calitan pewarnaan Ziehl-Neelsen acid fast stain menunjukkan sebanyak 23 (17.8%) sampel daripada 129 sampel telah didapati positif. Manakala, tiada sampel positif dikesan berdasarkan ujian CFT ke atas sampel serum. Keputusan ini berkemungkinan dipengaruhi oleh tahap jangkitan oleh MAP yang dihidapi oleh haiwan tersebut sama ada pada Tahap I atau Tahap II dimana tahap kepekatan antibodi dan antigen terlalu rendah untuk dikesan. Konklusinya, tiada jangkitan aktif dikesan dalam gerompok yang diuji dan ujian serologi seperti CFT mempunyai kadar spesifik yang tinggi and kurang sensitif berbanding pewarnaan Ziehl-Neelsen. Kultur najis merupakan kaedah terbaik untuk mengenal pasti kehadiran MAP walaupon ianya memakan masa.

Kata kunci: MAP, Taman Pertanian Universiti, CFT, calitan pewarnaan Ziehl-Neelsen

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine, UPM in partial requirement of the course of VPD 4999 – Project

***Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in dairy cattle in Taman Pertanian Universiti (TPU)**

By

Nurul Asikin binti Abu Bakar Hamzah

2016

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MAP infection or also known as Johne's disease is viewed as one of the most serious and chronic bacterial diseases among ruminants that will indirectly cause economic losses to the farmers This study was conducted to determine the presence of MAP organisms in fecal and antibodies in the

serum samples. A total of 129 fecal samples and 43 serum samples were collected from 43 dairy cattle at Taman Pertanian Universiti (TPU) and the samples were used for detection of antigen and antibodies using Ziehl-Neelsen acid fast stain and Complement Fixation Test (CFT) respectively. Results from Ziehl-Neelsen acid fast stain technique revealed, 23 (17.8%) out of 129 samples were positive. Meanwhile, no positive result were obtained from CFT using serum samples. This result might be affected by the stages of infection in MAP as the animals might be under Stage I or Stage II where the concentration of antibodies and antigen were assumed to be too low to be detected. As a conclusion, no active infection was detected in the herd tested and serology tests such as CFT provide high specificity and low sensitivity compared to Ziehl-Neelsen stain. Fecal culture remains the best diagnostic method to confirm MAP despite being time-consuming.

Keywords: MAP, Taman Pertanian Universiti, CFT, Ziehl-Neelsen acid fast stain

1.0 Introduction

In 1895, paratuberculosis or Johne's disease which caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) was recognized by Johne and Frothingham. This disease occured among domestic ruminants such as cattle, sheep, goats, buffaloes and also wild ruminants (OIE, 2014). Commonly this infection is detected in ruminants, especially dairy cattle and a limited survey has revealed that bovine have a higher prevalance compared with other species (Manning & Collins, 2010). This disease is characterized by granulomatous lesion in the intestine that develop into chronic or intermittent diarrhea, emaciation and death (Stabel, 1998). MAP can be transmitted through fecal oral route by direct ingestion of the faeces from the infected animals or indirectly from fecal contaminated in colostrum, milk, water or feed (Manning & Collins, 2010). Other than that, it can also be transmitted vertically to the fetus (Larsen & Kopecky, 1970)and via colostrum from infected dam.

Once the animal has ingested, MAP survive and replicate within the macrophages which is located in the wall of intestines and in regional lymph node. As MAP is a slow- growing bacteria, it needs a longer time to incubate and usually can be detected during clinical stage of infection between 2 to 5 years (Stabel, 1998) where the clinical signs can be seen.

Animal will show chronic wasting syndrome with gradual loss of weight and diarrhea despite good appetite. As the disease progresses, there will be reduction in milk production in dairy cattle and increased cow-replacement cost thus affecting the level of productivity in herd (Hasonova & Pavlik, 2006).

There are various types of detection method available in order to diagnose Johne's disease, either serological or detection of the MAP itself. Some of the serological methods have a lack of sensitivity especially in detecting in the early stage of infection thus, their use have been restricted to diagnosing the suspected clinical case or to determine the status of infection in the herd (Mohan *et al.*, 2013). Futhermore, the difficulty in detecting the early stage of infection with the long incubation period of MAP in infected animal before the clinical signs develop may allow this disease to remain 'silent' and become problematic in the herd.

1.1 Rationale of study

Johne's disease is a chronic, infectious and wasting disease that affects dairy cattle in which it will affect the performance of animals and thus cause significant economic losses in the herd. For examples, reduction in milk production, increase the incidence of mastitis, altered the milk yield, increase the cost of replacement cows, poor feed conversion, increase susceptibility to disease in general and reduce reproductive efficiency (Hasonova and Pavilk, 2006). So, detection of MAP infection is very

important to determine the health status of the herd as this disease can affect the performance of dairy cattle. Once it has have been detected, prevention and control can be taken.

The reason why this study was carried out because there has been suspicious previous reports of MAP in TPU but no study have been done. Thus, this study acts as a medium to evaluate the health of the animals especially regarding this disease as this disease is listed under one of notifiable disease by DVS (Department of Veterinary Services).

1.2 Hypothesis and objectives of the study

MAP infection can be detected through serological methods such as CFT and Ziehl-Neelsen acid fast stain. Since, the prevalance of MAP infection among dairy cattle in TPU is unknown, therefore this study is:

1. To determine the presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the fecal of dairy cattle in TPU.
2. To determine the presence of antibody against *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the serum of dairy cattle in TPU.

standard to diagnose this disease despite being time-consuming as this technique required 12 to 16 weeks to culture MAP (Stabel *et al*., 2004).

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8.0 Appendices

Appendix 1- Ziehl-Neelsen acid fast stain

Procedure:

1. Flood the slide with concentrated carbol fuchsin. Heat for 5 minutes (do not boil)
2. Wash with tap water
3. Decolorize with 3% acid alcohol for 1 minute
4. Wash with tap water