
**UNIVERSITI PUTRA MALAYSIA**

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

**NURUL ASIKIN BINTI ABU BAKAR HAMZAH**

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**FPV 2016 24**

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**NURUL ASIKIN BINTI ABU BAKAR 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

Universiti Putra Malaysia,

Serdang, Selangor Darul Ehsan,

Malaysia

MARCH 2015

# 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.

# ACKNOWLEDGEMENT

First of all, I am gratefull to Allah S.W.T for establishing me to complete this Final Year Project.

I would like to express the deepest appreaciation to my supervisor, Prof. Dr. Abdul Aziz bin Saharee for the support, guidance and valuable suggestions throughout this project and not to forget to thank to my co-supervisors, Dr. Rozaihan Mansur and Dr. Siti Khairani Bejo for their guidance and support till the completion of this project.

I take this opportunity to record my sincere thanks to all staff of Bacteriology Laboratory (Dr. Mohd Sabri, En. Azri, Cik Krishnamah, Cik Adawiyah), Serology Laboratory, Veterinary Research Institute (En. Mazlan) and Taman Pertanian Universiti (TPU) for their helps and encouragement.

Finally, thanks to my family who always encourage me through out these years completing my degree. To everyone especially Aizuddin, Ezah and Zahidah who provided me with help, support, encouragement and valuable advice through out this project. Thank you so much.

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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**

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**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.

## 2.0 Literature Review

### 2.1 Aetiology agent

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causal agent of Johne's disease which belongs to the *M. avium* as the DNA of the bacteria is very closely related (Hayton, 2007). Therefore, this bacteria is considered as the same species with subspecies of paratuberculosis. It is characterized as a acid fast, aerobic rods, and has a length of about 1-2um. They stain uniformly, but longer forms may show stained. MAP have very slow and fastidious growth pattern which required about 12 to 16 weeks to produce visible colonies on the culture media (Stabel *et al*., 2004). MAP also is very resistant towards environmental degradation and it can survive in the water and slurry for more than a year and more than 47 months in soil (Hayton, 2007).

### 2.2 Pathogenesis

Primary route of infection is through ingestion of fecal material or colostrum. Once the bacteria is ingested, it will enter the intestinal wall through out the mucosa of the small intestine and slowly replicate within the macrophage and stimulate inflammatory and cellular responses. Since MAP is very slow in growth, it requires longer incubation period. It take several years to develop into extensive granulomatous inflammation in the terminal

small intestine that lead to malabsorption and protein leakage thus causing the animal to have chronic weight loss.

The main clinical signs showed by affected animals are diarrhea which develop slowly, starting from intermittent diarrhea to become progressively constant. Besides, they tend to have poor body condition eventhough they eat well. In subclinical case of Johne's disease, the number of bacteria that they shed are lesser compared to clinically affected animals.

## 2.3 Stages of MAP infection

According to Fecteau and Whitlock (2010) MAP infection can be divided into four stages. Stage I, is known as 'silent infection' that usually occur among calves, heifers, young stock and adult cattle. The disease start with ingestion of the organisms, and uptake by the macrophage within intestinal mucosa. Then, it replicates and followed by infection. In this stage, the infected animals may shed MAP in their faeces but below detection levels. However, if this cattle frequently being sampled, MAP may occasionally be detected by fecal culture but in low concentration. Meanwhile, it fail to detect antibody response in this stage.

In stage II, the animals appeared healthy but at the same time they tend to have adequate amount of MAP in the faeces which can be detected on bacteriological culture. At the same time, some of animal may show positive result for presence of antibody detection. Eventhough the animals showed

positive result in serology, it must be confirm by fecal culture as the serology test is less sensitive in detecting subclinical infection. Most of affected animals in this stage will contaminate the environment and become source of infection to other animals as MAP is shedding by these animals.

At stage III, the animals condition can be very emaciated and cachexia which caused by gradual weight loss and diarrhoea. However, the affected animals can still have normal appetite and normal vital signs. Milk productions also decreased and this stage usually occur after a recent stress. It may recover for the time being but it can relapse once the cattle become stressed. Here, they tend to shed a large number of bacteria and showed positive result on fecal culture. Serological test also will give the same result.

Lastly, Stage IV is where the affected cattle appeared very weak, emaciated and have chronic and profuse and persistent diarrhoea. Sometimes, they tend to have submandibular edema of bottle jaw. Once the diarrhoea become profuse, hypoprotenemia occurs and the animal's condition deteriorate rapidly. This will cause the animals to be sent for slaughter and sometimes the animals die due to dehydration and cachexia.

## 2.4 Diagnosis and treatment

According to Cousins *et al*., 2002, Johne's disease can be diagnosed based on MAP infection stages either clinical disease and subclinical infection. From both stages we can diagnose the disease either by serological

methods and identification of MAP. Besides, post-mortem also can be use as an aid of diagnosis from macroscopic and hispathological lesion and isolation of MAP. It is relatively easy when it comes to diagnose clinically affected animals but it differ in diagnosing subclinically infected animals as the results in subclinical infected animals may misinterpret the infection. This is because in subclinical animals, they tend to shed this bacteria intermittently and usually negative on serological methods. For serological tests that can be used to diagnose this disease such as agar gel immunodiffusion (AGID), enzyme-linked immuno assay (ELISA) and complement fixation test (CFT) are not very sensitive (Stabel, 1998). For CFT and AGID, both has high in specificity but low sensitivity but usually in CFT, it has been used for cattle and AGID in other non-cattle ruminants. Meanwhile, in ELISA it have high specificity and sensitivity but only can detect in clinical disease animals not in subclinical infection as the concentration antibody against MAP is below detection level.

Therefore, it is the best to use more that one method in order to diagnosing this disease as it compensate each other especially in detection of subclinical animals. For example, fecal culture can be use as a confirmatory test on ELISA positive animals in the herd which do not have any history regarding this disease. Since ELISA is less sensitive especially during detection in subclinical infection, fecal culture can compensate as it allowed the growth of this bacteria. Other than that, Ziehl-Neelsen acid fast staining

can be used but do not use it alone as it is able to stain other acid fast bacteria. The staining result need to be used with other serological test or fecal culture.

There is no specific treatment for this disease but this treatment is only to reduce the clinical signs and does not eliminate the infection from affected animals. Examples of antibiotic that can be use in MAP infection such as streptomycin, clofazimine and isoniazid. Besides, the treatment is very costly and the infection can flare up once the treatment is stopped. So, the most beneficial way is to practice test and cull policy in the herd.

## 2.5 Control

To control this disease in the herd is quite challenging as it is required long term and aggressive control programmes despite with high cost. Succesful prevention and control programmes depends on farmer's compliants, animal health authorities and livestock industries to work synergistically by understanding the epidemiology of this disease and how to use the current detection methods to diagnose and control this disease in the herd.

Based on the epidemiological of this disease, management is one of the most useful method to control this disease such as separation of newborn calves from the dam, feeding the calves with paratuberculosis-free colustrum and milk or use the milk replacer and provide good sanitation in the herd are among most important management tools to control this disease. Examples of

proper sanitation can be applied by thoroughly cleaning the udder and teats before milking as to avoid fecal contamination which can be a source to infection.

Other than that, farmer also can purchase the cattle from paratuberculosis-free herd and qurantine the all new incoming animals and test for this disease. Due to slow incubation period, it might require combination of diagnostic test to detect MAP among infected animals. Once the animals is positive, test-and-cull policy should be made in consultation with veterinarian.

In Malaysia, vaccination program is not being practiced but it can be considered. According to Manning and Collins, 2010, there are two type vaccines available which are killed and live vaccines. In United States, this vaccines is liscensed for calves that less than 30 days old. This disease only can reduced the severity of clinical signs but does not help to prevent the infection. Thus, it will not change the rate of cattle getting infection. Futhermore, a study in Netherland proved that farmer who changes in their management to prevent this disease are more succesful than those using vaccination as prevention.

## 2.6 Client education

According to DVS, it have been stated that Johne's disease is one of the important disease in Malaysia as it compromised animal's health and it is

subjected to eradication program which the animal that positive towards this disease will be slaughtered and farmers will get compensation from the goverment. If farmer suspect their cattle having this disease, report this problem to DVS and they will take action towards this problem.

Besides that, to avoid this problem farmer also can buy the cattle from herd that are free from this disease and practice proper sanitation management especially manure management to control this from happening.

## 3.0 Materials and Methods

### 3.1 Sampling

Sampling was done from 12$^{th}$ to 22$^{nd}$ January 2016 at Taman Pertanian Universiti (TPU). A total of 43 dairy cattle were used in this study which consisted of 14 young calves and 29 adult cattle. All the cattle were healthy during the time of sample collection. Fecal and blood samples were taken and tested.

For each dairy cattle, three fecal samples were collected for every 3 days by scrapping the mucosal wall of the rectum as this organisms resides within the intestinal wall. Meanwhile, for antibody detection, whole blood was obtained by jugular venipuncture by using venoject and plain tubes. Then, the samples were transported by using icebox for further processed in laboratory. Then, the blood was allowed to clot for few hours at room

temperature and centrifuged under 4000rpm for 5 minutes to separate the serum from the whole blood. Serum sample was collected using 1.5ml microcentrifuge tube and stored at -20ºC.

### 3.2 Ziehl-Neelsen acid fast staining

129 fecal samples were smeared on the glass slide and it was done under Biosafety Cabinet Level 3. Ziehl-Neelsen acid fast staining is one of technique used to detect MAP from fecal samples by red staining of the bacteria. This technique were used three types of solution to stain the acid fast bacteria such as concentrated Carbol Fushin, 3% acid alcohol and Loeffer's methylene blue.

### 3.3 Complement Fixation Test (CFT)

Forty three serum samples were analyzed by using CFT. This test depended on the antigen and antibody reaction that form an antigen-antibody complex. This mixture will become fixed when complement is added to it. Next, haemolytic system (consists of sheep red blood cells and haemolysin) was added to ensure that the complement either still in fixed condition or not. Two results can be obtained by this test which is positive or negative. In the positive result, there is no haemolysis reaction that can be observed in the well and complement become fixed with the antigen in the serum as we can see the complement is settle down at the bottom of the well like 'button' formation. Meanwhile, negative result will show haemolysis reaction of the

red blood cell that indicate that of the complement is free without being fix as the serum is free from antigen.

### 3.4 Data analysis

The prevalance of MAP infection was calculated by the number of positive samples for Ziehl-Neelsen acid fast stain and the number of positive samples for antibody against MAP from all samples tested. The result from both tests were calculated and analysed.

## 4.0 Results

### 4.1 Ziehl- Neelsen acid fast staining

From Ziehl-Neelsen acid fast stain, a total of 23 (17.8%) samples were positive which consisted of 3 (2.3%) samples from young calves and another 20 (15.5%) from adult cattle. Meanwhile, about 106 (82.2%) samples were negative, including 39 (30.2%) samples from young calves and 67 (51.9%) samples from adult cattle. Table 1 shows the percentage of positive and negative results of acid fast staining based on the age of the cattle.

| | | | result | | |
|---|---|---|---|---|---|
| | | | negative | positive | Total |
| Age | young | Count | 39 | 3 | 42 |
| | | % within age | 92.9% | 7.1% | 100.0% |
| | | % within result | 36.8% | 13.0% | 32.6% |
| | | % of Total | 30.2% | 2.3% | 32.6% |
| | adult | Count | 67 | 20 | 87 |
| | | % within age | 77.0% | 23.0% | 100.0% |
| | | % within result | 63.2% | 87.0% | 67.4% |
| | | % of Total | 51.9% | 15.5% | 67.4% |
| Total | | Count | 106 | 23 | 129 |
| | | % within age | 82.2% | 17.8% | 100.0% |
| | | % within result | 100.0% | 100.0% | 100.0% |
| | | % of Total | 82.2% | 17.8% | 100.0% |

**Table 1: Crosstabulation data of Ziehl- Neelsen acid fast staining based on age**

Assume $\alpha = 0.05$

$H_o$: There is no significant difference between young and adult in detection of MAP in Ziehl-Neelsen acid fast staining.

$H_a$: There is significant difference between young and adult in detection of MAP in Ziehl- Neelsen acid fast staining.

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 4.855[a] | 1 | .028 | | |
| Continuity Correction[b] | 3.833 | 1 | .050 | | |
| Likelihood Ratio | 5.525 | 1 | .019 | | |
| Fisher's Exact Test | | | | .029 | .021 |
| Linear-by-Linear Association | 4.817 | 1 | .028 | | |
| N of Valid Cases | 129 | | | | |

**Table 2: Fisher's Exact Test result**

From the Table 1 result, the calculated p value is below α (0.05), so $H_o$ can be rejected. Thus, we can conclude that there is a significant difference between the young and adult in detection of acid fast bacteria.

## 4.2 Complement Fixation Test (CFT)

From the tabulated data in Table 3, shows that among 43 serum samples that were diagnosed by using CFT, no animals were tested positive.

| RESULT CFT | NO. OF SAMPLES | PERCENTAGE (%) |
|---|---|---|
| Positive | 0 | 0% |
| Negative | 43 | 100% |

**Table 3: CFT result**

## 5.0 Discussion

The evidence of MAP infection in dairy cattle in TPU is unknown. All the animals in the studied area appeared healthy and there was no indication of any clinical signs that would suggest of MAP infection during sampling. This study is indicated to detect the prevalence of MAP infection among dairy cattle by detection of antigen by using Ziehl-Neelsen acid fast stain and also serological method which is Complement Fixation Test (CFT).

From Ziehl-Neelsen acid fast stain result showed, only 23 (17.8%) samples were positive among 129 samples tested which consisted of 3 (2.3%) from young calves and the remaining from adult cattle. Then, Fisher's Exact Test was calculated to see the significant from these two groups. From the calculated p value (0.029), it showed that there was significant difference between these two groups as the adult cattle tended to shed higher number of MAP in faeces. This is because the nature of the organisms itself is a slow growing bacteria. So, when the cattle was infected with MAP since young, the clicical signs do not often develop until 2 to 5 years of age.

Basically, this method is quick and low cost technique which can be used as a preliminary diagnosis (Collins, 2013). However, this technique is unable to differentiate between species of mycobacteria and does not provide species identification (OIE, 2014).It can give a false positive result because it is able to identify *Nocardia* and *Corynebacteria* as well. On top of that, it is

proven that this method is able to detect the presence of bacteria in 49.3% of samples from clinically affected cattle and another 19.3% for subclinical cases. Besides that, this technique also has the lowest detection rate. To make this result is more valid fecal culture can be carried out for isolation and identification of MAP but it take a longer time to incubate.

For the serological test, CFT was chosen as method the to detect the antibody against MAP. This test was the earliest serological test that was available for diagnosing Johne's disease and most frequently used in order to test for import and export of livestock (Rideout *et al*., 2003). According to Sockett *et al*., it have been reported that it's sensitivity and specificity as 38.4% and 99% respectively. A test with high specificity but low in sensitivity will result in many animals who are 'free' from disease can possibly have the disease. As a result, the animals become the carrier and contaminate the herd. So, another test must be carried out for further investigation.

In this study there was no positive result among 43 samples that were analysed. This result might be influenced by the stages of infection in MAP as the animals might be under Stage I or Stage II when the concentration of antibodies were assumed to be low to be detected. A study from Rice *et al*., 1958, proved that this serology test was not sufficiently sensitive to detect the small amounts of circulating antibodies because only 0.4% of animals were detected against MAP.

## 6.0 Conclusion and recommendation

From the results obtained, this study indicated that was no active MAP infection occuring among dairy cattle in TPU. However, this results were inconclusive because CFT have low sensitivity, thus it would produce a high number of false negative result in the herd. As a result, cattle which have the disease, will be diagnosed as non-diseased animals and they will become hidden threat as they tend to shed MAP in the faeces and contaminate the herd.

As a recommendation, fecal culture can be used to further diagnose MAP infection in dairy cattle as this technique is considered as gold standard to diagnose this disease despite being time-consuming as this technique required 12 to 16 weeks to culture MAP (Stabel *et al*., 2004).

## 7.0 References

Comparative Studies on the Validity of Ziehl-Neelsen Staining, Faecal Culture and a Commercially Available DNA-Probe® Testb in Detecting Mycobacterium paratuberculosis in Faeces from Cattle. *Journal of Veterinary Medicine, Series B*, *46*(2), 137-140.

Cousins, D. V., Condron, R. J., Eamens, G. J., Whittington, R. J., & De Lisle, G. W. (2002). Paratuberculosis (Johne's disease). *Australia and New Zealand Standard Diagnostic Procedures*, *1*, 1-21.

Fecteau, M. E., & Whitlock, R. H. (2010). 14 Paratuberculosis in Cattle.*Paratuberculosis: Organism, Disease, Control*, 144.

Haghkhah, M., Derakhshandeh, A., Jamshidi, R., Moghiseh, A., Karimaghaei, N., Ayaseh, M., & Mostafaei, M. (2015). Detection of Mycobacterium avium subspecies paratuberculosis infection in two different camel species by conventional and molecular techniques. In *Veterinary Research Forum* ,Vol. 6, No. 4, 337-341.

Hasonova, L., & Pavlik, I. (2006). Economic impact of paratuberculosis in dairy cattle herds: a review. *Veterinarni Medicina*, *51*(5), 193-211.

Hayton, A. J. (2007). Johne's disease. *Cattle Practice*, *15*(1), 79.

Larsen, A. B., & Kopecky, K. E. (1970). Mycobacterium paratuberculosis in reproductive organs and semen of bulls. *American Journal of Veterinary Research*, *31*, 255-258.

Manning, E. J., & Collins, M. T. (2010). 3 Epidemiology of Paratuberculosis.*Paratuberculosis: organism, disease, control*, 22.

Manning, E. J., & Collins, M. T. (2010). 1 History of Paratuberculosis. *Paratuberculosis: Organism, Disease, Control*, 2-3.

Manning, E. J., & Collins, M. T. (2010). Johne's Information Center: Control (University of Wisconsin- School of Veterinary Medicine). Retrieved from http://www.johnes.org/dairy/control.html on 13th March 2016

Mohan, A., Das, P., Kushwaha, N., Karthik, K., & Kiran Niranjan, A. (2013). Investigation on the status of Johne's disease based on agar gel immunodiffusion, ziehl-neelsen staining and nested PCR approach in two cattle farm. *Veterinary World*, *6*(10).

Paratuberculosis (Johne's disease). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 2.1.11 (NB: OIE Terrestrial Manual 2014). Retrieved from http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.11_PARATB.pdf on 8th January 2016

Rideout, B. A., Brown, S. T., Davis, W. C., Gay, J. M., Giannella, R. A., Hines, M. E., & Rouse, T. (2003). Diagnosis and control of Johne's disease. *Committee on Diagnosis and Control of Johne's Disease, National Research Council*, *244*.

Sockett, D. C., Conrad, T. A., Thomas, C. B., & Collins, M. T. (1992). Evaluation of four serological tests for bovine paratuberculosis. *Journal of clinical microbiology*, *30*(5), 1134-1139.

STABEL, J. (1998). Symposium: Biosecurity And Disease. *Journal of Dairy Science*, *81*(1), pp 283-288.

Stabel JR, Bosworth TL, Kirkbride TA, Forde RL, Whitlock RH, (2004). A simple, rapid, and effectivemethod for the extraction of *Mycobacterium paratuberculosis* DNA from fecal samples for polymerase chain reaction. J Vet Diagn Invest 16, 22.30

Zimmer, K., Drager, K. G., KLAWONN, K., & Hess, R. G. (1999). Contribution to the Diagnosis of Johne's Disease in Cattle.

## 8.0 Appendices

**Appendix 1- Ziehl-Neelsen acid fast stain**

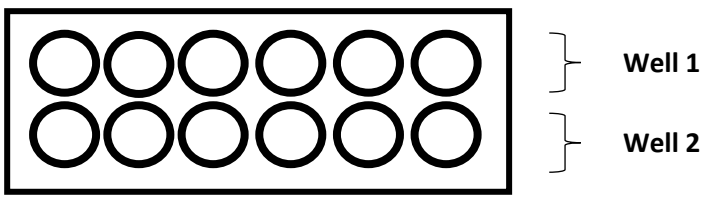
**Procedure:**

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
5. Add Loeffler's methylene blue for 1 minute
6. Wash with tap water and blot dry

**Appendix 2- Complement Fixation Test (CFT)**

**Procedure:**

1. Make a 1/10 dilution using 0.25% phenol saline of the test serum, positive serum and negative serum
2. Inactive in a water bath at 56°C for 30 minutes
3. Transfer 25µl into 2 wells of microplate
4. Set up the test below:



| COMPONENTS | WELL 1 | WELL 2 |
|---|---|---|
| Serum 1/10 | 25ql | 25ql |
| Antigen (1 unit) | 25ql | - |
| Saline | - | 25ql |
| Complement (1 unit) | 25ql | 25ql |

5. The test and control are incubate at 37°C for 1 hour
6. 50ql of haemolytic system is added into the well. Mix throughly and then incubate again for 15 minutes
7. Leave on the bench for button formation

**Reading:**

i. Both the test and anticomplementary wells must be read. The anticomplementary wells contain no antigen and there should be no fixation of complement, i.e. there should be complete haemolysis. If not the serum is anticomplementary

ii. Results are recorded as follows:

| | | | | |
|---|---|---|---|---|
| ++++ | = | Complete fixation | - | no haemolysis |
| +++ | = | 75% fixation | - | 25% haemolysis |
| ++ | = | 50% fixation | - | 50% haemolysis |
| + | = | 25% fixation | - | 75% haemolysis |
| - | = | No fixation | - | complete fixation |

**Interpretation**

Any serum showing 75% and more fixation (+++ and above) at a dilution of 1/10 is considered positive.