UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS (IBK) AMONG DAIRY CATTLE IN 'LADANG ANGKAT' FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA

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It is hereby certified that we have read this project paper entitled "Prevalence Study Of Infectious Bovine Keratoconjunctivitis (IBK) Among Dairy Cattle In 'Ladang Angkat' Faculty Of Veterinary Medicine, UPM", by Deva Darshini Thinakaran and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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DEDICATION

GOD

FAMILY

Dad, Thinakaran Shanmugam

Mom, Bathma Devi Balan

Sister, Shamalee Thinakaran

Brother, Haans Raaj Thinakaran

Charlie

LECTURERS AND STAFF

All lecturers and sttafs in Faculty of Veterinary Medicine,

Universiti Putra Malaysia

FRIENDS

Devaprakash, Nithiyah, Nagachandra, Hafizah, Hanani, Muhaimin, Akmal, Diyana, Izdihar, Faizal

My batch mate, DVM 2010-2015

My friends from SMK(P) Kapar and SK Kapar

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LIST OF ABBREVATIONS

IBK	Infectious Bovine Keratoconjunctivitis
M.bovis	Moraxella bovis
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
PBS	Phosphate Buffer Saline
MgCl2	Magnesium chloride
UV	Ultra violet
Bp	Base pair

ABSTRAK

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

KAJIAN BERKENAAN KADAR JANGKITAN PENYAKIT RADANG MATA LEMBU MENULAR DI KALAGAN LEMBU TENUSU DARI LADANG ANGKAT, FAKULTI PERUBATAN VETERINAR, UPM

Oleh

DEVA DARSHINI THINAKARAN

2015

Penyelia: Dr. Faez Firdaus Jesse Abdullah

Penyelia bersama: Prof. Dr. Abdul Rahman Omar

Industri tenusu memainkan peranan yang penting dalam sektor pertanian negara kita. Kerajaan menyasarkan untuk meningkatkan pengeluaran susu tempatan kepada 120 juta liter dan mencapai tahap sara diri pada 8 % pada tahun 2020. Penyakit radang mata adalah salah satu penyakit yang boleh menyebabkan kesan ekonomi dalam industri ruminan. Penyakit ini disebabkan oleh bakteria *Moraxella bovis* bersama

dengan pelbagai faktor risiko yang lain seperti pembawa vector contohnya lalat dan kutu, factor persekitaran serta kecenderungan baka. Maklumat berkenaan penyakit ini amat terhad di Malaysia dan ini mendorong untuk tujuan kajian ini iaitu menyiasat kelaziman penyakit IBK di kalagan lading tenusu dari Program Ladang Angkat, Fakulti Perubatan Veterinar (FPV) faktor risiko, Sampel swab konjunktiva secara rawak daripada 50 lembu tenusu dari tiga Ladang Angkat, Fakulti Perubatan Veterinar (FPV) telah diambil dan kehadiran ejen penyakit dikenal pasti dengan penggunaan teknik tindak balas rantai polymerase (PCR). Hasil dari kajian ini mendapati bahawa tahap kezaliman penyakit radang mata ialah 2%. Walau bagaimanapun, ejen penyebab tidak dikenal pasti daripada lalat. Kajian ini juga menunjukkan bahawa tidak ada korelasi di antara penyakit dan factor risiko. Kesimpulannya, kelaziman IBK adalah rendah dan tidak menyebabkan kerugian pengeluaran lembu tenusu dalam 'Ladang Angkat', Fakulti Perubatan Veterinar.

Kata Kunci: Lembu Tenusu, penyakit radang mata menular, mata merah, *Moraxella bovis*, kadar jangkitan

ABSTRACT

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

PREVALENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS (IBK) AMONG DAIRY CATTLE IN 'LADANG ANGKAT' FACULTY OF VETERINARY MEDICINE, UPM

By

DEVA DARSHINI THINAKARAN

2015

Supervisor: Dr. Faez Firdaus Jesse Abdullah

Co-supervisor: Prof. Dr. Abdul Rahman Omar

Dairy industry plays an important role in our nations' agriculture sector. The government has targeted to increase the level of local milk production to 120 million litres and self-sufficiency at 8% by year 2020. Infectious bovine keratoconjunctivitis (IBK) is one of the major diseases in ruminants that may cause losses in economy. This disease is caused by *Moraxella bovis* bacteria and governed by multifactorial

factors such as vectors (flies, ticks and etc), environment and breed predisposition. impact is infectious bovine keratoconjunctivitis (IBK). There is limited information related to IBK disease among dairy cattle in Malaysia. Therefore this study was designed to determine the disease prevalence from dairy cattle in Program 'ladang angkat' Faculty of Veterinary Medicine, UPM. Sub conjunctival swabs were collected from 50 dairy cattle and the samples were subjected for isolation and identification of the causative agent using polymerase chain reaction (PCR) technique. The result from this study indicates prevalence rate of IBK among dairy cattle in Program 'ladang angkat' Faculty of Veterinary Medicine, UPM only 2%. There were no significant correlation between the disease and risk factors in these farms. In conclusion, IBK disease does not cause production loss in dairy farms of Program 'Ladang Angkat', Faculty of Veterinary Medicine, UPM.

Keywords: Dairy Cattle, infectious keratoconjunctivitis, pink eye, *Moraxella bovis*, prevalence

1.0 INTRODUCTION

Dairy industry in Malaysia is currently facing many challenges particularly in terms of production level. Total dairy production for 2013 in the country was 79.35 million litres (Department of Veterinary Services – Output of Livestock Products, 2013). The government is continuously providing aids and support to attain the target of 120 million litres and self-sufficiency at 8% by year 2020.

Disease outbreak is one of the main challenges faced by most of the dairy farms. Infectious bovine keratoconjunctivitis (IBK) is one of the examples of the disease that may cause production loss in dairy farms in many countries (Angelos, J. A. 2015)

IBK was first reported by Billings among cattle in Nebraska in the year 1889. (IBK) is a contagious disease that causes inflammation of the tissue lining of the eyelid, cornea and conjunctiva of the affected cattle (Alexander D, 2010) This disease is commonly known as pink eye, due to the early signs of swelling and redness of the conjunctiva that can either be unilateral or bilateral (Bradley R. et al., 1982) This infection may lead to development of ulcer that may eventually cause temporary or permanent blindness. The most common causative agent of IBK is *Moraxella bovis* (McConnel,C.S. et al., 2007), a gram negative aerobic bacteria. There are several factors contribute to the ability of *M.bovis* to cause infection. The factors are environmental factors, exposure to UV light, dust, trauma at ocular region, breed of cattle, and vectors such as flies (Brown and Adkins, 1972; Jeybal et al., 2013)

The morbidity of IBK disease can be high but the mortality of the affected animals due to this disease is considered low and affects the performance of the infected animals (Kizilkaya et al., 2013). In United States and Australia, this disease is considered as an economically important disease in animal production. Major economic losses are due to poor weight gain and inappetence as a result of ocular pain and visual impairment of the affected animals (Snowder, 2005).

There is limited information related to prevalence study of IBK in Malaysia (Abdullah et al., 2013). Therefore this study was designed to study the prevalence of IBK infection in selected dairy farms in Program 'Ladang Angkat' Faculty of Veterinary Medicine, UPM.

2.0 LITERATURE REVIEW

2.1 AETIOLOGICAL AGENT

Moraxella bovis is a member of the family Moraxellaceae (Pettersson B et al., 1998). They are Gram negative aerobes which appears short, plump, that measures 1.0 µm to 1.5µm by 1.5µm to 2.5µm which occurs in pairs or in small chain. The principle biochemical properties of this bacteria are gelatin liquefaction, oxidative, oxidase-positive, catalase-positive, non-motile and it neither reduces nitrates nor ferment carbohydrates and do not form indol (Fraser. et al., 1979) Traditionally,strains of *M.bovis* have been classified into seven non-cross-reacting serogroups (A–G) based on differences in antigenic determinants (Moore LJ et al.,). Prieto et al.,1999 have subdivided isolates of *M.bovis* by analyzing the variation in the lipopolysaccharides (LPS) types, outer membrane proteins (OMPs) profiles, and DNA fingerprints. When the three methods were used in combination, 15 distinct subgroups were identified. According to Angelos 2015, although the species will grow in non enriched media, their growth is enhanced by addition of blood or serum and in optimal temperature of 33-35^{o}C.This bacteria is also susceptible to desiccation and do not survive well away from animal host

2.2 VIRULENCE FACTOR

M.bovis exhibits several virulence factors,but only two are determinants are known to cause clinical disease and that includes the the presence of fimbriae (type IV pili) on the bacteria cell surface (Chandler et al., 1979), and the subsequent damage to the corneal epithelium is attributed to a repeats in the structural toxin (RTX) toxin which are better known as cytotoxin or hemolysin (Gray et al., 1995). In addition to this, other virulence factors that are studied includes a variety of hydrolytic enzymes that is important for tissue destruction including C4 esterase, C8 esterase - lipase, C14 lipase, phosphoamidase, phosphatase, leucine and valine amino peptidases and gelatinase (Frank and Gerber 1981), fibrinolysins (Nakazawa and Nemoto 1979a), proteins with hemolytic activity (Nakazawa and Nemoto 1979b), and cell detachment proteins (Marrion and Riley 2000) and filamentous hemagglutinin (Kakuda et al., 2006). Acquisition of iron through transferrin and lactoferrin-binding proteins may also contribute to virulence of *M.bovis* (Angelos et al., 2001)

2.3 TRANSMISSION

According to Brown et al., 1998 *M. bovis* is spread by direct contact, nasal and ocular discharges, and by mechanical vectors. The most important vector is considered to be the face fly *(Musca autumnalis).* The house fly *(Musca dornestica)* and barn fly *(Stomoxys calcitrans)* may also transport the organism. There is a positive correlation which exists between the number of flies per animal and *M.bovis*

infection. Disease prevalence will be reduced by rigorous fly control programs (Kopecky et al.,1986). Besides that, asymptomatic cattle may serve as carriers, and will harbour *M. bovis* in their nasal cavities for a period that may exceed one year. These carrier animals allow for the persistence of pinkeye at a particular site from year to year. (Postma et al., 2008)

2.4 RISK FACTORS

Since IBK is a multifactorial disease, there are several risk factors that is associated with the development of the disease. Outbreaks of IBK occur most frequently during the warmer months of the year (Thrift and Overfield 1974). During warmer climate, there will be prolonged exposure to UV radiation, increase in fly population and also increase in virulent *M.bovis* strain. Studies carried out by Pugh et al.,1986 have shown increase in solar radiation precedes the increase in infection rate and the onset of clinical signs of IBK. In their study, they have demonstrated that after UV radiation, there will be large number of degenerating corneal epithelial cells, epithelial defects and increased cell turnover that may enhance the ability of *M. bovis* to colonize the cornea.

According to Gerhardt et al., 1982, female face flies act both as a carrier and as an ocular irritant that allows establishment of the infection. Labellar movement during the fly feeds on the eye is the cause of ocular damage and increase of the eye secretions acts as a primary source of protein for egg development. The same study also showed that the bacteria can survive for up to 3days on the legs of the face fly.

Hence, after feeding on ocular discharges of infected animals, a fly may infect several other non-infected animals.

Studies done by Powe et al.,1992 shows that calves are generally more susceptible than older cattle. However, adults can be as severely affected when the herd has not previously exposed (Trout, 1985). Although all breeds may be affected, *Bos taurus* breeds are more susceptible than *Bos indicus* breeds (George, 1984). Hereford cattle had a significantly higher risk of IBK compared with other breeds (Frisch, 1975) because the lack of eyelid pigmentation in this particular breed and also the preference of the bacteria towards the tear solution of Hereford breed compared to other cattle breed.

Another contributing factor is irritation of the eye by dust, wind, tall grasses, weeds, or any other element creating mechanical injury to the eye which eventually increases the susceptibility of the cattle to IBK infection (Rodriquez, 2006)

2.5 CLINICAL SIGNS

According to Postmaet al., 2008, the clinical appearance, persistence of infection,and rate of disease progression varies from different animals. Disease may occur in either one or both eyes. The early signs of disease are copious watery ocular discharge, blepharospasm and photophobia, are the earliest indications of the disease. Generally, conjunctivitis precedes the keratitis. The conjunctiva appears to be hyperemic and edematous, and blepharitis may be present.

According to Whittier et al., 2009 clinical lesions of pink eye can be categorized into four stages according to their severity. Stage 1 is indicated when there is an excessive tearing and increased sensitivity to light which progresses to a small ulcer in the centre with the cornea which appears as a small white spot. Stage 2 indicated when the ulcer spreads across the cornea with the cornea becomes increasingly cloudy. Blood vessels from the outside portion of the cornea begin to grow across the cornea to help in healing process. Stage 3 is indicated when the ulcer has covered most all the cornea and inflammation continues to spread into the inner parts of the eye. The inside of the eye fills with fibrin that gives the eye a yellow appearance. Stage 4 becomes obvious when the ulcer completely extends through the cornea and the iris may protrude through the ulcer leading to permanent blindness

2.6 TREATMENT AND PREVENTION

According to Jesse et al., 2013 treatment for IBK can be treated according to the severity of the disease. Antibiotic eye spray can be used for early sign of disease meanwhile, systemic and sub conjunctiva injection of antibiotic is useful for the later stage of the disease.

Vaccination against IBK have been developed in some countries and this is derived from bacterin using pili from the causative organism. Cellular vaccine against IBK includes live, killed, whole cell or subunit vaccines (Funk et al., 2014)

Lastly, management of the farm plays a key role in preventing IBK. Farm hygiene, control of fly population and lower exposure to irritants may reduce the disease occurrence in the farm.

2.7 PCR DETECTION OF *M.Bovis*

PCR has become an important tool for research and clinical diagnosis. PCR method has been used in several studies to detect the causative agents of IBK (Giacometti et al, 1999). Recently, PCR system have been used to detect the presence of toxin genes, specific for *Moraxella bovis* (Angelos et al 2010). A study by Shen et al (2011) shows that multiplex real-time PCR assay was developed for the detection and differentiation of *M. bovis,M. bovoculi* and *M. ovis* in pure culture isolates and field lacrimal swabs.

3.0 MATERIALS AND METHOD

3.1 Sampling and culture

A total of three dairy cattle farm were selected under the 'ladangangkat' programme of Faculty of Veterinary Medicine, Universiti Putra Malaysia. Total of 50 animals were selected randomly from all the three farms. Sub conjunctival swabs from both eyes of each animal were obtained using sterile Amies transport swab (Labchem Sdn Bhd).

NZI fly traps were placed in all three dairy cattle farms for an average period of four hours in each farm. The fly traps used was a three dimensional design. The fly trap was placed in such way it faces the cattle house.

The collected sub conjunctival swab samples were cultured on blood agar and incubated for 48 hours at 37^{o}C aerobically. Meanwhile, the fly samples were crushed with a few drop of saline between two glass slides and the samples was swabbed with a sterile cotton swab and streaked onto a blood agar plate. The blood agar plate was then incubated for 48 hours at 37^{o}C.

After incubation, colonies were Gram stained and viewed under the light microscope. Colonies that were gram negative were chosen and sub cultured again on blood agar for 48 hours at 37^{o}C. These colonies were taken for identification using PCR technique.

To access the risk factors and economic impact related to IBK, a questionnaire was derived and answered by the farmers. Sample of questionnaire is attached in the appendix section.

3.2 DNA Extraction

DNA extraction for this study was done using DNAzol® reagent. Colonies from the culture were mixed into 2µl of this reagent and kept for a minimum period of 10 minutes in each PCR tube. Colonies were obtained using the micropipette tip and the tip was made sure to be in contact with the reagent for the maximum period of time to ensure cell breakdown.

3.3 PCR Condition

PCR was performed in at touchdown thermocycler(SENSOQUEST® Labcycler) in a total reaction volume of 50 µ*l* containing 42 µ*l* of distilled water (DNase/RNase free water), 2 µ*l* of PCR buffer (taq buffer), 2 µ*l* of $MgCl_2$, 0.5 µ*l* of dNTP and 0.5 µ*l* of each reverse and forward primers. The cycling conditions were 5 minutes at 95^0C,followed by 35 cycles of 40 s at 95^0C, 40 s at 55^0C and1 min at 72^0C and finally extension at 72^0C for 7 min minutes before cooled down indefinitely at 4^oC.

3.4 Primer Design

The primer for amplification of *M.bovis*was referred from Shen et al., 2011. The reverse primer used was (5-AGCTATAGACCCAATTTAACTTACGCTACT-3) and the forward primer used was (5-GAACGATGACTATCTAGCTTGCTAG

ATATG-3). This set of primer targets species specific regions 16S rRNA and the length of PCR product is 1541bp.

3.5 Agarose Gel Preparation

1% agarose gel was prepared using 1g of agarose gel powder that was poured into 100ml bijour bottle which then topped up with 1% TAE until 100ml . The mixture was then microwaved for about 3-5 minutes until all precipitate melts completely. Liquid form of the gel was poured into a suitable sized cast and was then left to cool down to for a period of 1 hour. After that, the wells were ready PCR loading and electrophoresis.

3.6 Electrophoresis

This process was done at 90 V for 27 minutes. Agarose gel was placed into the gel holder tank and submerged with 1% TAE buffer and make sure that the holding wells are near the negative terminal and the band will run towards the positive terminal. 100bp marker (Promega) was used. Lastly, the gel was placed under UV gel imaging capturing machine and the result was recorded

4.0 RESULTS

4.1 *M.bovis* detection from sub conjunctival swabs

Results in this study showed the prevalence of IBK in dairy cattle from 'LadangAngkat' farms, Faculty of Veterinary Medicine, University Putra Malaysia was 2%. There was only one sample positive out of 50 samples for *M.bovis*.(Table 1)

Table 1: Dairy cattle infection rate for infectious bovine keratoconjunctivitis (IBK) in each farm.

Farm	Number of sample (n)	Number of *M.bovis* isolated
Farm A	16	0
Farm B	18	1
Farm C	16	0
Total	50	1

4.2 *M.bovis* detection from fly samples

The infection rate of *M.bovis* form fly samples is 0%.

Table 2: Flies caught from dairy cattle farm of LadangAngkat, UPM

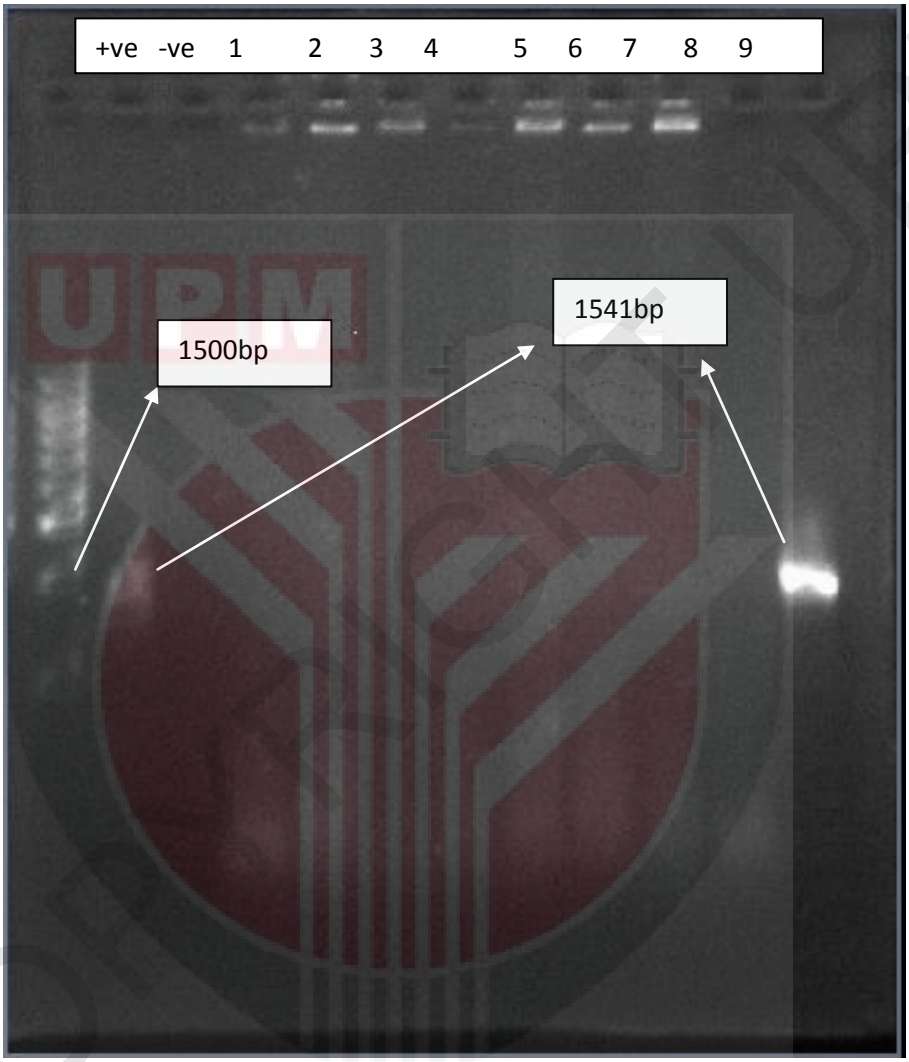
Farm A	Number of samples (n)	Detection of *M.bovis*
A	3	0
B	4	0
C	2	0
Total	9	0

4.3 Molecular findings

Random samples of 50 subconjunctival swabs were subjected to PCR detection. The principle for detecting positive of IBK agent which is *Moraxella bovis*is when the sample's lane forms the same bp size with the positive control which is 1541 bp (Figure 1)

Only one positive result for *M.bovis* that was obtained from farm B.There were no any positive result obtained from the fly samples.

Figure 1: PCR detection of *M.bovis* from sub conjunctival swab



4.4 Risk factors associated with IBK

Based on the questionnaires, there are several risk factors that are present in each farm. The risk factors are potential existence of carrier animal, fly population, age of cattle, breed of cattle, direct ultraviolet exposure, climate and potential mechanical injury. All 3 farms had previous history of eye disease but it is not so common according to farmer. This indicates there might be potential presence of carrier animal. There are presences of flies in all 3 farms but there is no any fly control programs that are practiced to eradicated the particular problem. According to one farm, this disease commonly occurs in younger calves compared to adult catlle. The breed from all 3 farms are cross breed which mainly comprise of Jersey cross. All 3 farms are managed semi intensively and the herd are exposed to direct sunlight during grazing period. One of the farmer stated that previous eye problem occurred during rainy season. All 3 farms are exposed to dust and there is possible occurrence of mechanical injury.

4.5 Economic impact associated with IBK

Based on the answers from the questionnaires, all three farmers stated that previous eye disease were treated with antibiotic eye spray and there were no production loss due to IBK.

5.0 DISCUSSION

5.1 IBK Prevalence

In Malaysia, there is only one reported clinical case of IBK in dairy cattlebut, there was no *M.bovis*recovered from the clinical case (Saharee et al., 2013). Farmers are not aware the importance of this particular disease as very least information available related to IBK and the potential consequences it may cause. In this study, the prevalence of IBK is only 2%. There were only one animal showed clinical sign of unilateral blindness and was positive for*M.bovis.* Absence of causative agent can be due to high herd immunity level, breed that comprise the cattle herd, satisfactory hygiene and sanitary practices, low exposure to direct sunlight, age of the sampled herd and also less exposure to eye irritation.

The low prevalence in this study is in agreement with other studies such as from Webber et al., 1981, in Missouri, United States stated that the average prevalence of IBK was 8% within the herd. According to Takale G, 1999 dairy cattle in different part of Arsi region, South Ethopia revealed prevalence of IBK was only 2.1 % which means 110 dairy cattle were positive out of 5221 samples. Chakrabarti et al., 2014, stated in Bihar, India the prevalence for IBK was only 1.91 % which means 54positive out of 2832 samples. Therefore, the low prevalence in this study is in agreement with other researches as stated above.

The absence of causative agent could be because there is no carrier animal and low vector population such as flies for this study. These findings also support the management of the farms are in good practise.

The low prevalence in this study can be contributed by samples from adult animals only as IBK is said to occur more in younger animals (Poweet al., 1992). Breed predisposition also could be a reason as the breed in the farms comprises mainly of Jersey – Friesian crossed and IBK tend to occur more in pure breed such as Hereford (O'Connor et al., 2012). In addition, *Bos indicus* and their crosses have been found to suffer lower incidence of IBK (Snowder et al., 2005) and in agreement with the result of this study.

All three farms are managed semi intensively and the herd is exposed to direct sunlight only in the morning in an average of 5 hours daily. This exposure may not be sufficient enough to cause significant damage to the cornea (Kopecky et al., 1980) that may contribute to low prevalence in this study.

CONCLUSION

The prevalence for *M. Bovis* in 'Ladang Angkat' dairy farms of Faculty of Veterinary Medicine UPM is 2%. There are presence of risk factors that may cause the disease and precautions must be taken to prevent the disease. Thereis no economic loss due to this disease in the particular farms. PCR method can be used as a diagnostic tool for infectious bovine keratoconjunctivitis (IBK) disease.

RECOMENDATION

There should be more study regarding pink eye disease in Malaysia and this should be carried out extensively through out all farms because once the outbreak occurs, it is difficult to contain and to sustain the economic loss that might occur as a consequence of this disease.

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APPENDICES

SOALAN BERKENAAN PENYAKIT 'PINK EYE'/ MATA MERAH DI KALANGAN LEMBU TENUSU

1. Pernahkan kejadian sakit merah berlaku di kalangan lembu tenusu di ladang ini? Ya/Tidak. Jika Ya, bila?(tahun)_____

2. Pernahkah lembu mengalami tanda-tanda klinikal seperti mata berair, buta, kemerahan, atau berwarna kelabu? Ya/tidak.
Jika ya, tanda apa yang paling ketara atau sering kali dilihat?
_____

3. Lembu kumpulan usia berapa yang sering kali mengalami tanda klinikal pada mata?
a) Anak lembu
b) Heifer
c) Dewasa

4) Apakah baka lembu yang sering mengalami sakit mata

_____

5)Adakah lembu-lembu tersebut terdedah kepada cahaya matahari secara langsung? Ya/Tidak

6)Bilakah selalu lembu mengalami sakit mata?
A)Musim hujan
B)Musim panas
C)Waktu jerebu
B)Sepanjang tahun / tidak kira waktu

7)Adakah lembu-lembu tersebut terdedah kepada habuk jerami atau benda-benda lain yang boleh mencederakan mata?
Ya/Tidak. Jika Ya, Sila nyatakan_____

8) Adakah anda ingin mengurangkan gejala sakit mata ini di kalangan lembu-lembu di ladang ini? Ya/Tidak. Jika Ya, nyatakan caranya

9) Adakah anda akan memberi suntikan pelalian(vaksin) terhadap penyakit ini jika ada?
Ya/Tidak

10)Berapa jumlah anggaran kerugian disebabkan penyakit mata di ladang ini? Termasuk jumlah produksi susu dan kos ubatan(RM)

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