UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF MORAXELLA OVIS INFECTION IN GOATS UNDER THE LADANG ANGKAT PROGRAMME OF UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

NAGACHANDRA RAO A/L GOPI NAIDU

PREVALENCE OF *Moraxella ovis* INFECTION IN GOATS UNDER THE LADANG ANGKAT PROGRAMME OF UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

NAGACHANDRA RAO A/L GOPI NAIDU

A project paper submitted to the
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

MARCH 2015

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Prevalence of *Moraxella ovis* infection in goats under the Ladang Angkat Programme of University Veterinary Hospital, Universiti Putra Malaysia and its economic impact" by Nagachandra Rao a/l Gopi Naidu 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.

_____

Prof Dr. Abdul Rahman Omar,
DVM (UPM), PHD (Cornell)
Lecturer
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Supervisor)

_____

Dr Faez Firdaus Jesse Abdullah
DVM (UPM), PHD (UPM)
Lecturer
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Co-Supervisor)

DEDICATION

This thesis is dedicated especially to:

- My supervisors for their untiring effort in guiding me throughout this FYP
- My family for their support and encouragement throughout my life
- Classmates of DVM 2015 for staying together with me through the thick and thin of our journey to become a veterinarian

Thank you all.

ACKNOWLEDGEMENT

Special thanks for those that had given their support and aid to the completion of this project paper

Prof Dr. Abdul Rahman Omar

Dr. Faez Firdaus Jesse Abdullah

Prof Dr. Abdul Aziz Saharee

Prof Dr. Mohd Ariff Omar

Prof. Dr. Abd Wahid Haron

Dr. Eric Lim Teik Chung

Dr. Abdinasir Yusuf Osman Ali

Dr Mohammed Konto

En Jefri Norsidin

En Nazim and Large Animal Ward Unit

Deva Darshini, Muhaimin, Hafizah, Hanani

Wan Syukri, Kushal, Hema, Ai Ling, Larry, Nana

DVM 2015

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRAK

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

KELAZIMAN JANGKITAN *Moraxella ovis* DI KALANGAN KAMBING DI LADANG-LADANG KAMBING DI BAWAH PROGRAM LADANG ANGKAT HOSPITAL VETERINAR HOSPITAL, UNIVERSITI PUTRA MALAYSIA

NAGACHANDRA RAO A/L GOPI NAIDU

FEBRUARY 2015

Penyelia: Prof Dr Abdul Rahman Omar

Penyelia bersama: Dr. Faez Firdaus Jesse Abdullah

Penyakit mata yang dikenali sebagai 'Infectious keratoconjunctivitis' adalah merupakan salah satu penyakit lazim di kambing dan merupakan salah satu faktor yang mempengaruhi produksi dalam industri ruminan kecil. Tujuan kajian ini adalah untuk mendapatkan kelazimanan jangkitan *Moraxella ovis* di kalangan kambing, impak ekonomi terhadap penyakit mata dan peranan lalat sebagai vektor bagi *Moraxella ovis* di kalangan ladang-ladang kambing di bawah program Ladang Angkat Hospital Veterinar Universiti. Sebanyak 60 ekor kambing telah dipilih secara rambang daripada 4 buah ladang (15 ekor kambing daripada setiap buah ladang) dan sampel mata telah diambil dengan mengunakan kapas pengesat. Pada masa yang sama, perangkap lalat

telah dipasang untuk mengumpul sampel lalat dan borang soal-selidik telah dikemukakan kepada penternak untuk mengetahui status penyakit mata di ladang mereka. Sampel mata dan sampel lalat telah dikulturkan di atas agar darah dan koloni yang telah tumbuh kemudiannya disaring menggunakan kaedah 'Gram staining' bagi menentukan koloni yang paling berkemungkinan. Koloni yang terpilih kemudiannya diuji menggunakan proses 'Polymerase Chain Reaction' (PCR) bagi menentukan sama ada koloni tersebut adalah *Moraxella ovis*. Daripada 60 ekor kambing yang diuji, 18 ekor (30%) kambing adalah positif bagi jangkitan *Moraxella ovis* dengan Ladang B yang mempunyai kadar kelazimanan yang paling tinggi dengan 6 ekor kambing positif daripada 15 ekor (40%) dan Ladang C mempunyai kadar kelazimanan yang terendah dengan 3 ekor kambing positif (20%). Kesemua sampel lalat yang telah diuji mendapat keputusan negatif untuk *Moraxella ovis*. Borang soal selidik menunjukkan bahawa 3 daripada 4 penternak (75.00%) mendapati bahawa penyakit mata merupakan salah satu masalah di ladang mereka tetapi bukan masalah yang utama. Purata peratus haiwan yang terjejas daripada penyakit mata setiap tahun adalah 16.50% dengan Ladang C yang mempunyai peratusan yang tertinggi dengan 50.00% dan Ladang D mempunyai peratusan yang terendah dengan 2.00%. Purata peratusan yang menunjukkan pengurusan sejurus selepas penyakit mata adalah 7.25% dengan Ladang C yang mempunyai peratusan yang tertinggi dengan 20% dan Ladang D mempunyai peratusan yang terendah pada 1.00%. 3 daripada 4 buah ladang (75.00%) menyatakan bahawa mereka menggunakan ubat sapu sahaja untuk merawat penyakit mata manakala yang lain turut menggunakan antibiotic sistemik selain daripada ubat sapu. Kesimpulannya,

kelazimanan jangkitan *Moraxella ovis* adalah 30% di kalangan ladang-ladang kambing di bawah program Ladang Angkat UVH, UPM dan 16.50% daripada kambing tersebut terjejas setiap tahun akibat penyakit mata dengan 7.25% menunjukkan pengurusan selepas dijangkit.

Kata kunci: Penyakit mata, *Moraxella ovis*, Polymerase Chain Reaction, Impak ekonomi

ABSTRACT

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

PREVALENCE OF *Moraxella ovis* INFECTION IN GOATS UNDER THE LADANG ANGKAT PROGRAMME OF UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

NAGACHANDRA RAO A/L GOPI NAIDU

FEBRUARY 2015

Supervisor: Prof Dr Abdul Rahman Omar

Co-supervisor: Dr. Faez Firdaus Jesse Abdullah

Infectious keratoconjunctivitis or pink eye disease is recognized as one of the common diseases affecting the small ruminant industry as well as an important factor affecting production in this industry. The purpose of this study is to know the prevalence of *Moraxella ovis* infection in goats, the economic impact of pink eye disease and the role of flies as a vector of *Moraxella ovis* in the goat farms under the Ladang Angkat Program of UVH, UPM. 60 goats were selected randomly from 4 farms (15 goats per farm) and subconjunctival swab samples were taken from the animals. At the same time, a fly trap was set and a questionnaire was posed to the owner regarding

the status of pink eye disease in their farm. Both the subconjuctival swabs and the fly samples were cultured on blood agar and the colonies were screened using Gram staining to isolate the most likely colonies. The pure isolates were then subjected for conventional Polymerase Chain Reaction (PCR) to detect for *Moraxella ovis.* From the 60 goat samples, 18 samples (30.00%) were positive for *Moraxella ovis* with Farm B had the highest prevalence where 6 goats were positive out of 15 (40.00%) and Farm C had the lowest prevalence where only 3 goats were positive(20.00%) for *Moraxella ovis*. Meanwhile, none of the fly samples were positive for *Moraxella ovis*. The questionnaire revealed that 3 out of 4 farmers (75.00%) opted that pink eye disease is a problem in the farm but it is not a priority or important disease in the farm. The mean percentage of animals affected annually was 16.50% according to the farmers with Farm C had the highest percentage of 50.00% and Farm D had the lowest of 2.00%. The mean percentage of goats that showed emaciation following clinical signs of pink eye disease were 7.25% with Farm C had the highest percentage of 20.00% and Farm D had the lowest percentage of 1%. For treatment, 3 out of 4 farms (75.00%) only used eye ointment or spray for treatment while the other farm opted for systemic antibiotic in addition to the eye ointment or spray. Therefore, we have concluded that the prevalence of *Moraxella ovis* infection among the goats in farms under the Ladang Angkat Program of UVH, UPM is 30.00% and the mean annual infection of pink eye is about 16.50% with 7.25% showing significant emaciation following the manifestation of clinical signs.

Keywords: Infectious keratoconjunctivitis, pink eye disease, *Moraxella ovis*, Polymerase Chain Reaction, Economic impact.

Chapter 1 : Introduction

Infectious keratoconjunctivitis or better known as pink eye disease is a term used to describe the combined inflammation of the cornea and the conjunctiva that are caused infectious bacteria that are highly contagious in goats (Schoenian, 2009). This disease will cause clinical signs such as cloudiness and redness of the cornea and the conjunctiva, hence, the name pink eye disease, with eye discharges that are serosanguinous originally and turns to mucopurulent and in severe cases, cause temporary or permanent blindness (Walker, 2007). This will lead to weight loss, reduced lactation and increase the cost of treatment in the animals if steps to control and treat the disease is not taken (Angelos, 2013). There are many predisposing factors such as age, breed, dusty condition, flies and so on for this disease.

There are many agents responsible for this disease in goats and they are *Chlamydophila pecorum*, bacteria from the *Mycoplasma spp* (esp. *Mycoplasma conjunctivae*), *Moraxella ovis, Colesiota ovis, Listeria monocytogenes* and *Acholeplasma oculi* (Angelos, 2013). In this study, the aim was to study the prevalence of *Moraxella ovis* in goats under the Ladang Angkat programme, Faculty of Veterinary Medicine, UPM.

Moraxella ovis is a Gram negative bacteria that are arranged in pairs with the adjacent sides flattened and the division in the two planes are right angle to each other . In some cases, tetrad formation may be observed and the bacteria are non-motile (Elad *et al*, 1988). After being cultured on blood agar for 48 hours, the colonies that are

observed appear about 5mm in diameter and they are greyish white with low convexity, appearing almost flat after longer incubation period.

Infectious keratoconjunctivitis is considered as one of the common disease affecting goats in Malaysia with most of the farmers being aware of the disease (Yusof, 2013). Despite this, there are no studies done previously is Malaysia regarding this disease with only some isolated case studies published previously. Therefore, this study was proposed to achieve the following objectives:

1) To determine the prevalence of *Moraxella ovis* infections in goats under the Ladang Angkat Program of University Veterinary Hospital, Universiti Putra Malaysia.
2) To determine the economic impact of pink eye disease in terms of production and treatment in the farms under the Ladang Angkat Program of University Veterinary Hospital, Universiti Putra Malaysia.
3) To determine the role of flies as a vector of *Moraxella ovis*.

Chapter 2 : Literature Review

2.1 Goat Population in Malaysia

As of 2013, there are 482,280 goats in Malaysia consisting of dairy, meat and breeder animals (dvs.gov, 2014). This is number is unable to meet the local demand, thus, making us highly depend on imported mutton to meet the market demand. In regards to this, the government had established many policies to boost our ruminant industry to reduce our dependency on importation. As shown by the gradual increase in our self-sufficiency level from 12.13% in 2010 to 13.45% in mutton production, the trend is that our small ruminant industry is growing gradually (dvs.gov, 2014).

2.2 Characteristics of *Moraxella ovis*

Moraxella ovis is a bacteria of the family Moraxellaceae and was originally known as *Branhamella ovis* (Elad *et al,* 1988**)**. It is a Gram-negative cocci bacteria that is arranged in pairs with the adjacent sides flattened. The pairs are divided in two planes at right angles to each other. Sometimes, tetrad formation may be observed with the bacteria being non-motile.

When cultured on blood agar at 37^{o}C for 48 hours, the bacteria will give us grayish white colonies about 2.5 mm in diameter with low convexity, almost flat after longer incubation periods(Elad *et al,* 1988**)**. The colonies usually are friable and are usually surrounded by narrow zone of clear hemolysis. In some cases, nonhemolytic variants

may arise spontaneously in the culture. The bacteria is strictly aerobic with no growth on MacConkey agar with some variable exceptions (ATCC)

The bacteria will give positive results for alkaline phosphatase, catalase, esterase, nitrate reduction and oxidase test and will give negative results for acid phosphatase, DN-ase, gelatin liquefaction, H_2S production, indole production, proteolysis on Loeffler slants, phenylalanine deaminase, Tween 80 hydrolysis & urease. No acid is produced from carbohydrates (Elad *et al*, 1988**)**.

Moraxella ovis is usually isolated from the conjunctiva of goats, sheep and cattle as well as from the upper respiratory sites in goats, sheep and horses and they are considered as a type of opportunistic bacteria that causes keratoconjunctivitis in small ruminants (Fraser & Gilmore, 1979). The geographic distribution of this bacteria is considered to be worldwide.

2.3 Infectious Keratoconjunctivitis in goats in Malaysia

Currently, there are no studies were carried out to know the prevalence of pink eye disease in Malaysia although it is widely acknowledged by most farmers as one of the common diseases affecting the small ruminant industry in our country (Yusof, 2013) with only some isolated case studies as reported by Jesse *et al*, 2014, leading to a huge information gap about this disease in our country.

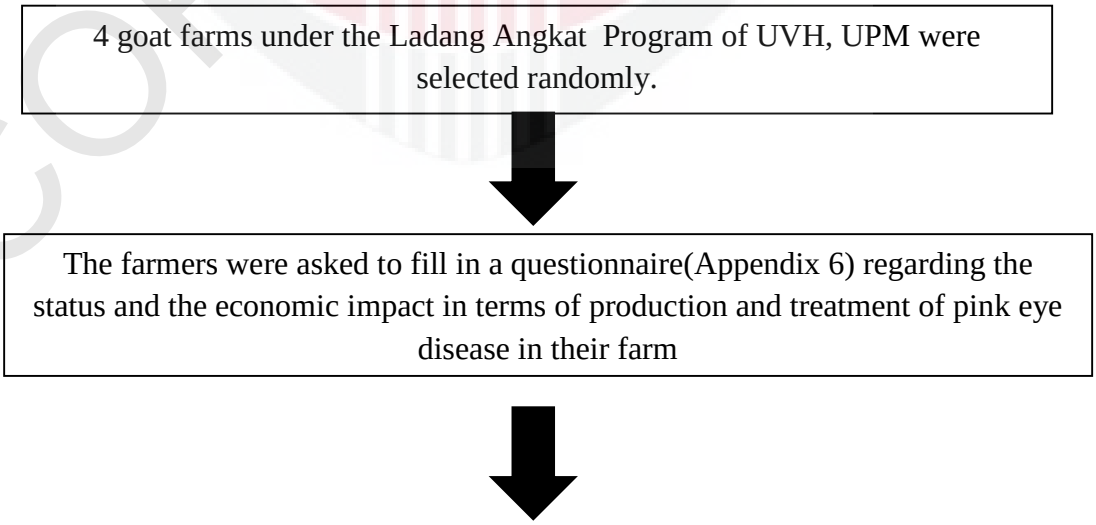
2.4 Flies and infectious keratoconjunctivitis

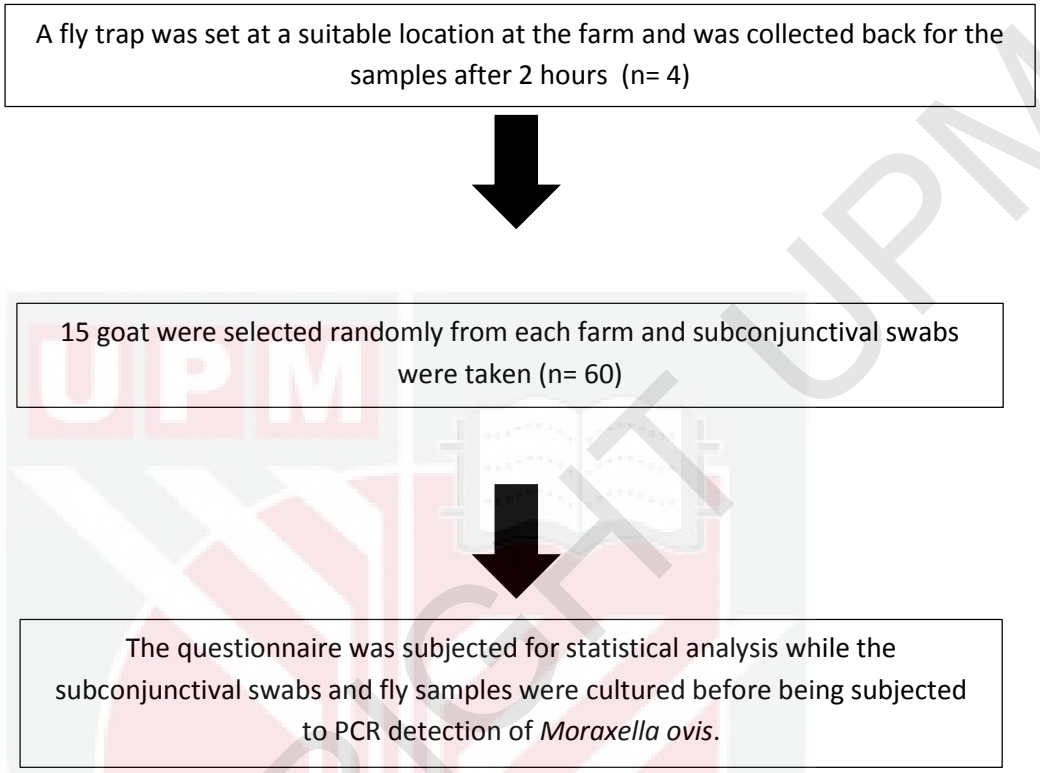
The incidence of infectious keratoconjunctivitis varies seasonally in temperate climates according to fly season with higher incidence during the fly season, causing them to be closely associated with the transmission of pink eye disease (Townsend, 2010). Flies can act as a vector for pink eye disease as an irritant of the eye that allows the invasion of bacterial agents(Schoenian, 2008**)**. While face flies and biting flies are more associated as a vector of pink eye disease, any type of fly can act as an irritant that leads to secondary bacterial infection(infonet-biovision.org, 2012**)**.

Chapter 3 : MATERIALS AND METHODS

3.1 Methodology

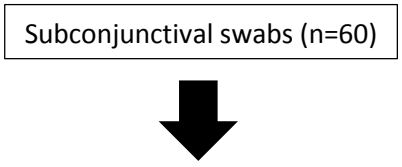
4 goat farms under the Ladang Angkat Programme of Faculty of Veterinary Medicine, UPM were selected randomly for our study. The farmers were asked to fill in a questionnaire regarding the status and the economic impact in terms of production and the treatment of pink eye disease in their farm. At the same time, an NZI flytrap was set at a suitable location on the farm and was collected back for fly samples after about 2 hours. 15 goats were then selected randomly from each of the farms and subconjunctival swab samples were collected. Both the fly samples and the subconjunctival swabs were cultured on blood agar before being subjected to conventional PCR assay for the detection of *Moraxella ovis*. The questionnaire, the fly samples and the subconjunctival swab samples were subjected to statistical analysis. [Figure 3.1.1: Flow chart describing the flow of the experiment]

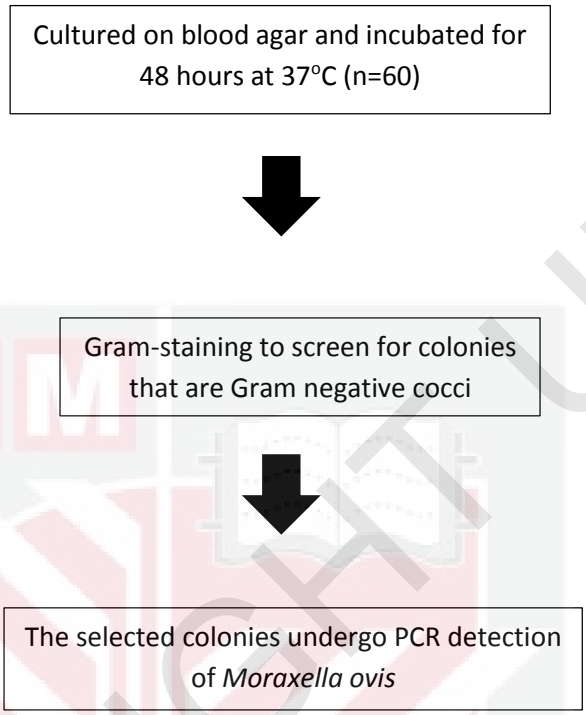




3.2 Subconjunctival Swabs

The subconjunctival swabs were cultured on blood agar for 48 hours at 37^{o}C aerobically to observe for the growth of bacteria. The resultant colonies were then gram stained to screen for the most likely colonies based on *Moraxella ovis*'s criterion of being Gram negative cocci occurring in pairs. All colonies that are Gram negative were then chosen to undergo conventional Polymerase Chain Reaction (PCR) detection for *Moraxella ovis*. [Figure 3.2.1: Flow chart describing the processing of subconjunctival swabs for the detection of *Moraxella ovis*]





3.3 Fly samples

The fly samples were crushed with a few drop of saline was placed in between two glass slides and the resultant mixture 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°C. The resulting colonies were then Gram-stained to screen for the most likely colonies based on the *Moraxella ovis*'s criterion of being Gram negative cocci occurring in pairs. All colonies that are Gram negative cocci are chosen for conventional Polymerase Chain Reaction (PCR) detection for *Moraxella ovis*.

3.4 DNA Extraction

DNA extraction was done using a modified DNAzol DNA extraction method. 2 µ*l* of the DNAzol was added to each PCR tube. Using the tip of a micropipette, one to two of

the selected colonies were scooped and the whole tip was left inside the PCR tube, making sure that the tip is in contact with the DNAzol. The tip was left for 10 to 15 minutes to allow for the DNAzol to work and breakdown the cells, leaving the DNA only. Caution should be taken to avoid leaving the tips inside the PCR tube for too short or too long as this will cause the lysis of the DNA, causing the results to be false negative.

3.5 *Moraxella ovis* Conventional Polymerase Chain Reaction Assay

Name	Sequence	Position	GenBank Accession No.
Ovi16S1F	5'-GAACGATGAGTATCCAGCTTGCT-3'	1-23	DQ647928
Ovis1849R	5'-CTCTTTACTTTGGTTAATTATTTTGTTGGA-3'	1849-20	DQ647928

Table 3.5.1 : The primers used in the *Moraxella ovis* conventional PCR assay.

A *Moraxella ovis* conventional PCR assay targeting the 16S rRNA gene that was modified from a *Moraxella spp* multiplex conventional PCR assay was used for the purpose of PCR detection of the colonies that was screened previously. The PCR was performed in a SENSOQUEST® Labcycler (thermocycler) 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 of the primers. The

cycling conditions were 95ºC for 5 minutes, followed by 35 cycles of 40 seconds at 95ºC, 40 seconds at 55ºC and 1 minute at 72ºC and finally extension at 72ºC for 7 minutes before cooled down indefinitely at 4ºC. The amplified PCR products were separated by gel electrophoresis on a 1% agarose gel at 90 V for 27 minutes visualized by UV irradiation using Flurosafe® DNA stain. The DNA extracted from a pure culture of *Moraxella ovis* obtained from the Bacteriology Lab of Faculty of Veterinary Medicine of UPM was used as the positive control while DNase/RNase free water was used as the negative control in each run of the *Moraxella ovis* conventional PCR assay.

3.6 Statistical Method

The data analysis for this study was done using the IBM SPSS Statistics 20 software. The P value was calculated using the Pearson Chi-Square Test to determine whether there was any significant difference in the prevalence from farm to farm. Meanwhile, Kruskal Wallis Test was used to determine whether there were any significant difference in the percentage of animals affected annually by pink eye disease as well as those showing signs of weight loss from farm to farm. Pearson Correlation test was used to see whether there was any significant correlation between the prevalence and the number animals affected by pink eye disease annually.

Chapter 4 : Results

4.1 Interpretation of PCR results based on gel electrophoresis

Below is one of the gel results viewed under UV after electrophoresis:

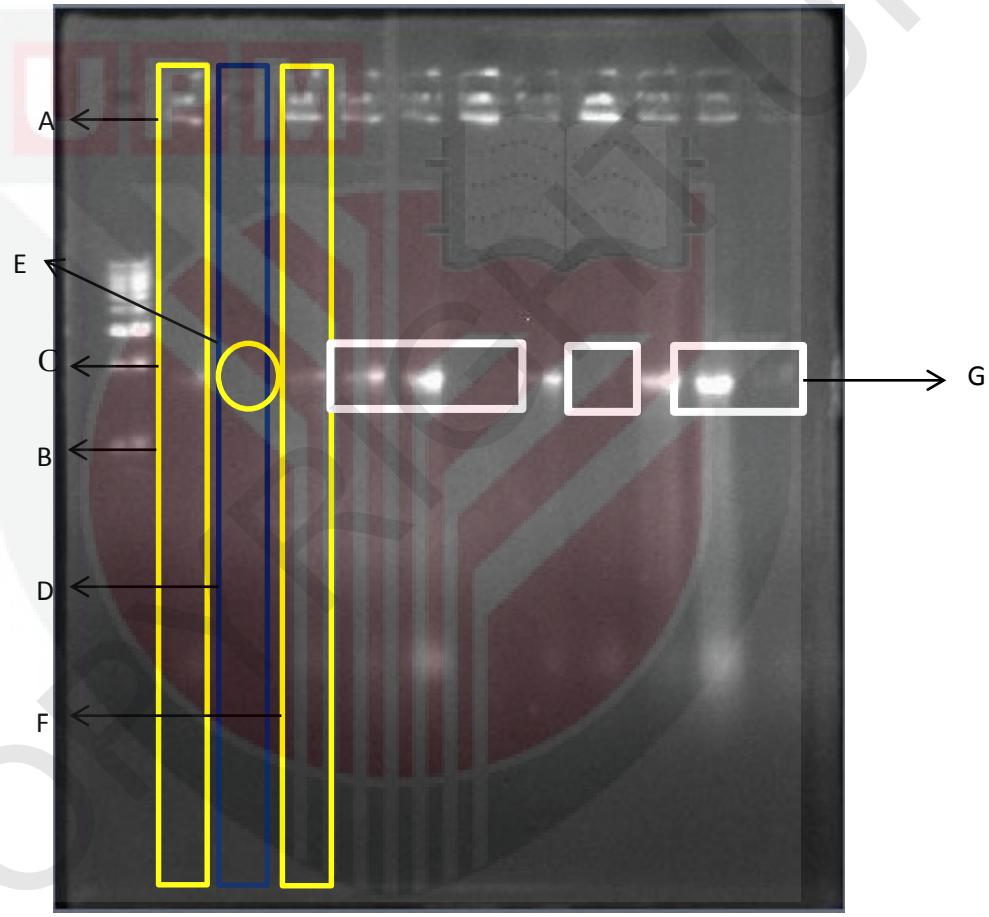


Figure 4.1.1: Gel electrophoresis results. A: The 10 kbp ladder, B: the 1kbp band, C: the 2kbp band, D:the positive control, E: the positive control band, F: the negative control, G: the positive results.

Based on the Figure 4.1.1, the result of the positive control is at 1849 bp as expected from Shoen *et al*, 2011.

4.2 Detection of *Moraxella ovis* (Subconjunctival swabs)

Farm B showed highest detection of *Moraxella ovis* (n=6), followed by Farm A (n=5), then Farm D (n=4) and the lowest detection is from Farm C (n=3).

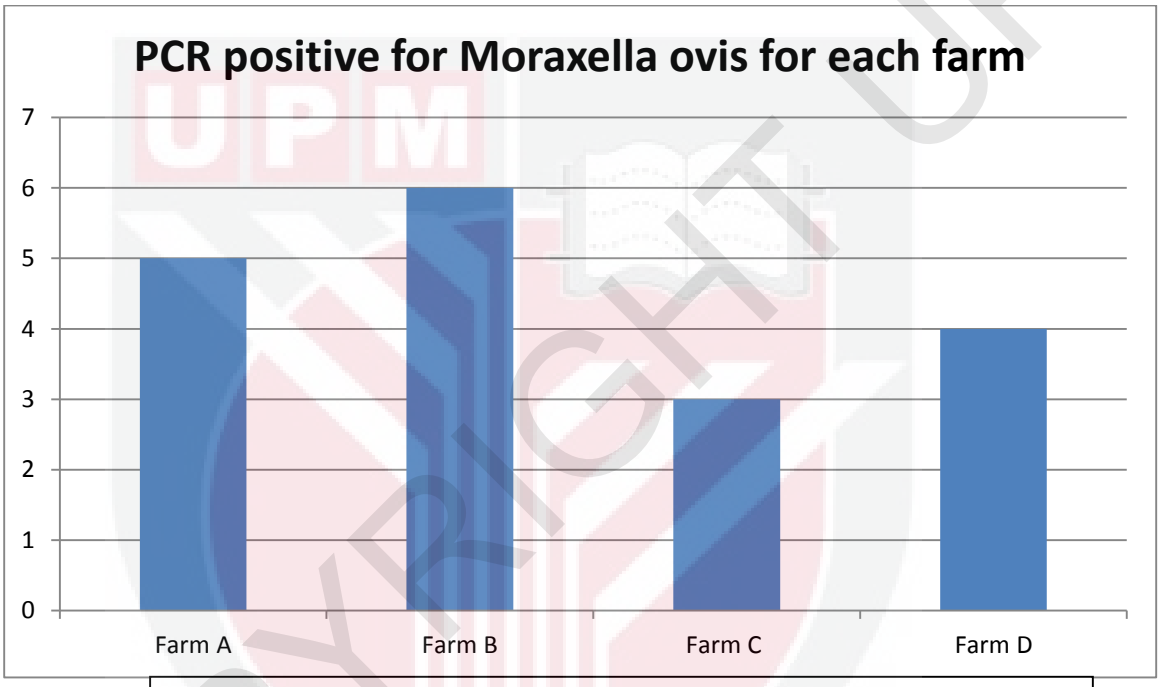


Figure 4.2.1: PCR positive for *Moraxella ovis* for each farm

From here, we can see that Farm B has the highest number of animals which are positive for *Moraxella* ovis while Farm C has the lowest.

From Appendix 1, the average prevalence of *Moraxella ovis* is 30% for all the 4 farms with Farm B having the highest prevalence at 40% and Farm C having the lowest prevalence at 20%. From Appendix 2, the p-value for the Pearson Chi-Square Test is

0.783> 0.05, therefore, there is no significant difference in the prevalence of *Moraxella ovis* among the farms.

4.3 Detection of *Moraxella ovis* (Fly samples)

Flies were managed to be trapped only from Farm B (n=3) and Farm C (n=2) where the species of flies that were captured was *Musca domestica* (house flies). There was no positive detection of *Moraxella ovis* for any of the tested samples.

Table 4.3.1: The number of fly samples obtained per farm

Farm	No of Flies	Positive detection
Farm A	0	0
Farm B	3	0
Farm C	2	0
Farm D	0	0
Total	5	0

We only managed to find fly samples which are house flies (*Musca domestica*) from farm B and C while the other two farms did not yield us with any fly samples due to a very good sanitation status and the limited time for collection of fly samples. None of the tested samples tested positive for *Moraxella ovis*.

4.4 Questionnaire

All the selected farms had a previous history of pink eye disease. From the questionnaire, 3 out of the 4 selected farms rate pink eye disease as a recognized problem in their farm but other problems had a higher priority while the other farm said that the pink eye disease occurs but it was not considered as a problem in their farm.

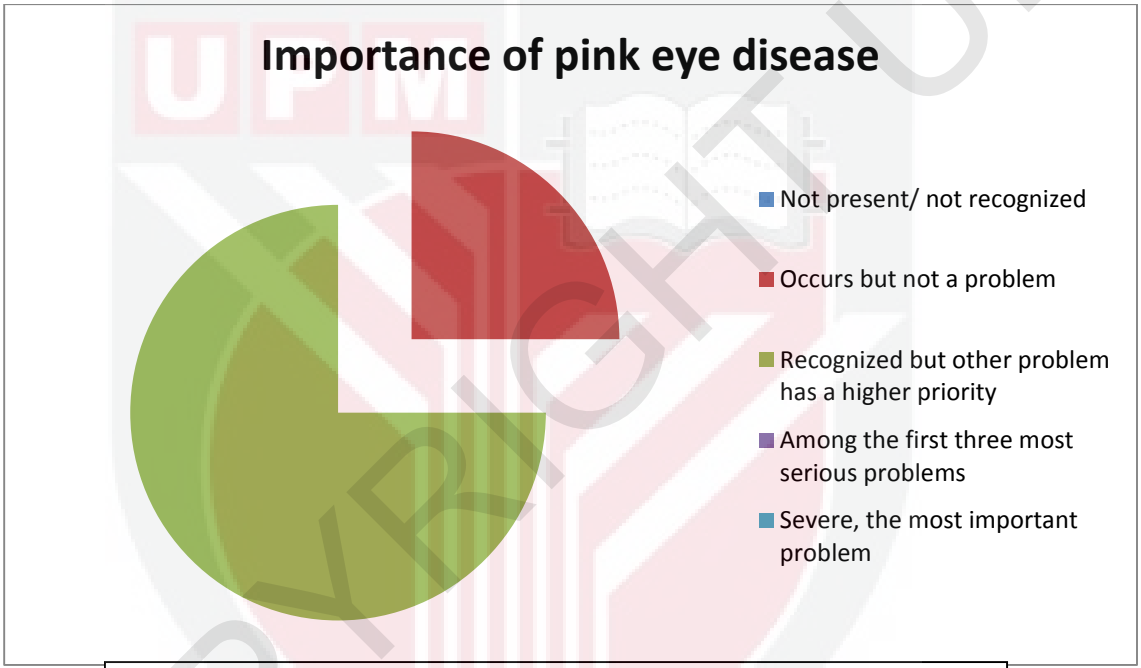


Figure 4.4.1: Pie chart showing the importance of pink eye disease in the farm

The mean percentage of animals affected annually by pink eye disease is 16.50% with Farm C having the highest percentage of animals affected annually at 50% and Farm D having the lowest percentage of animals affected annually at 2%. There were significant difference in the percentage of animals affected annually across the different farms based on Kruskal Wallis Test (P=0.000) in Appendix 3.

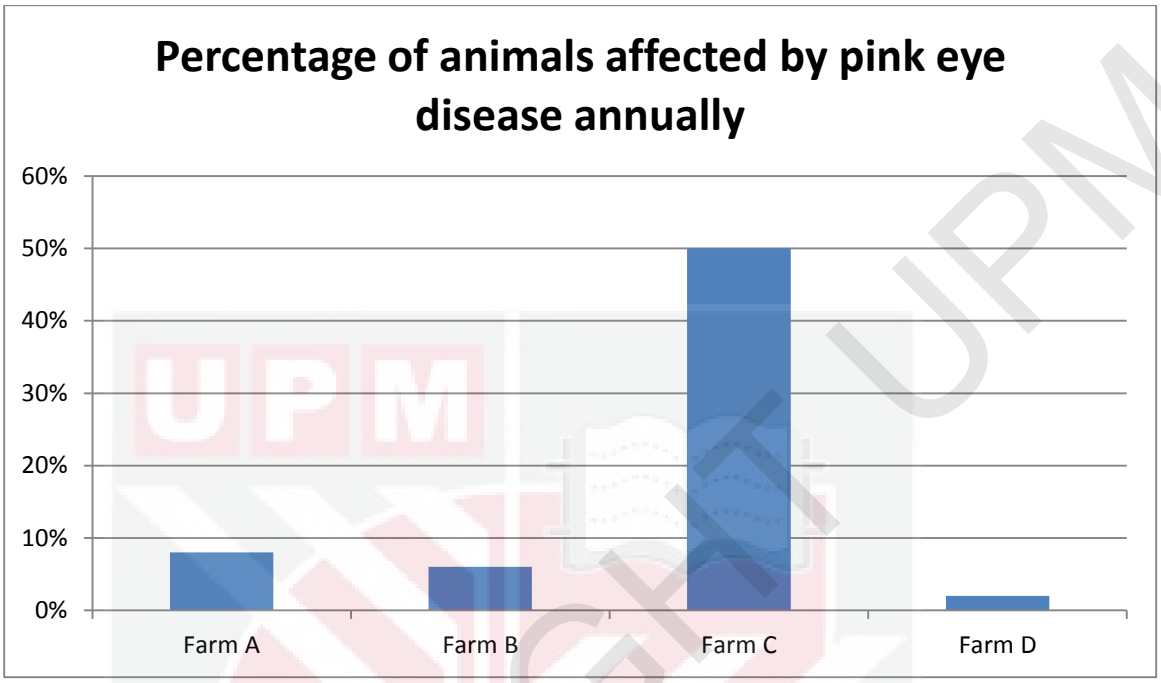


Figure 4.4.2: Percentage of animals affected by pink eye disease annually for each farm

The mean percentage of animals showing signs of weight loss after manifestation of clinical signs of pink eye disease is about 7.25% with, again, Farm C having the highest percentage of animals showing signs of weight loss at 20% and Farm D having the lowest at 1%. There was significant difference in the percentage of animals showing signs of weight loss after manifestation of clinical signs of pink eye disease across the farms (Appendix 4).

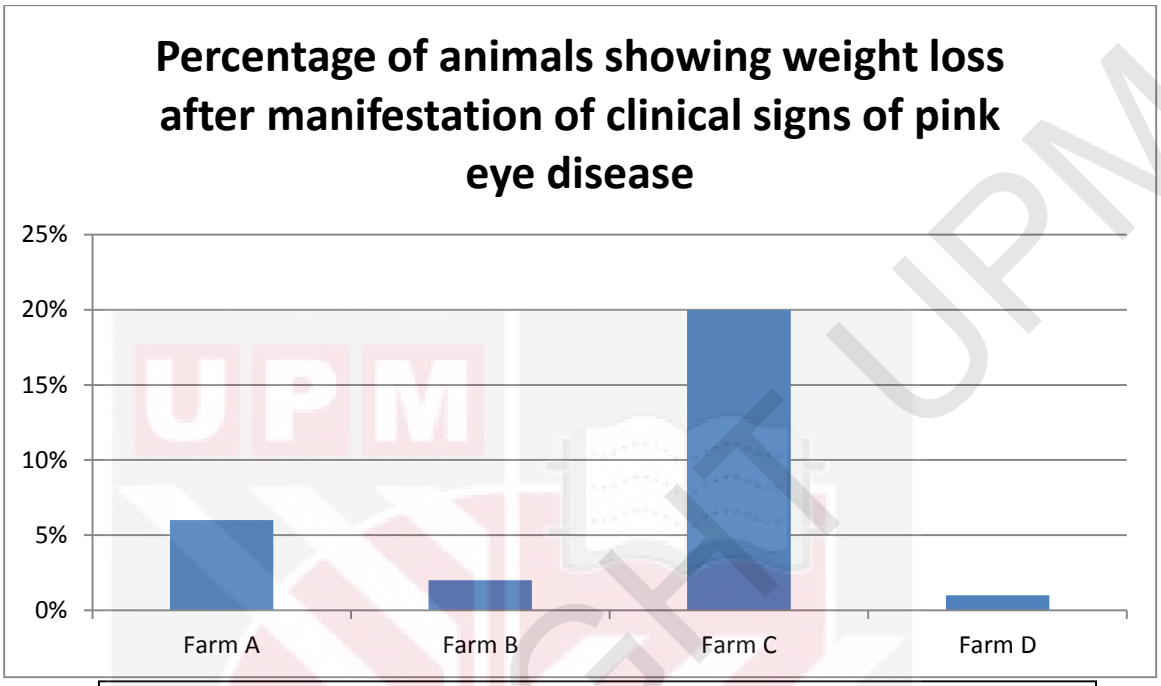


Figure 4.4.3: Percentage of animals showing signs of weight loss after manifestation of clinical signs of pink eye disease

From this study, there is no significant correlation between the prevalence of *Moraxella ovis* and the percentage of animals affected by pink eye disease in the farms (Appendix 5).

All the selected farms in this study uses antibiotic based eye ointment or spray to treat pink eye disease on their farm and only Farm C uses systemic antibiotics in addition to the eye ointment or spray.

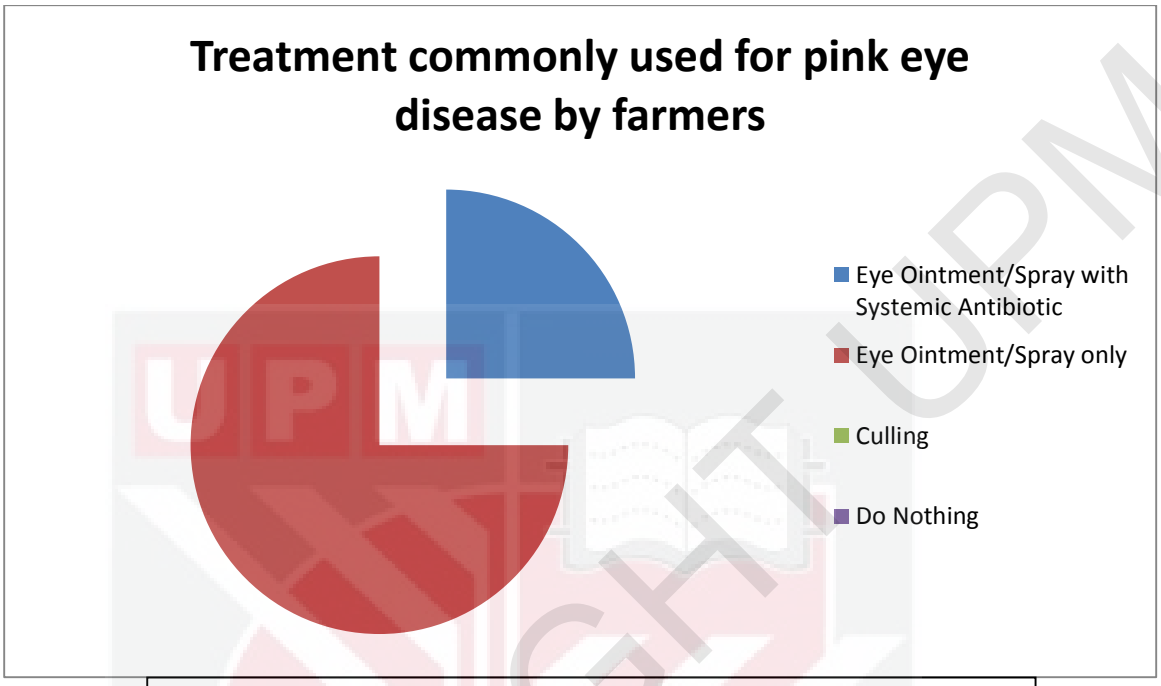


Figure 4.4.4: Treatments commonly used by farmers to treat pink eye disease on their farms

Chapter 5 : Discussion

The mean prevalence of *Moraxella ovis* in goats from the Ladang Angkat Programme is 30% with Farm B having the highest prevalence at 40% and Farm C having the lowest prevalence at 20%. From the result, this indicates that *Moraxella* ovis, is present in the herd regardless whether the animals are showing clinical signs or not. This is in agreement with the study done by Fraser & Gilmore , 1979 that had managed to isolate *Moraxella ovis* in sheep that are suffering from pink eye disease as well as those don't suffer from pink eye disease.

There were no fly samples positive for *Moraxella* ovis in this study. The results from this study is not in agreement with Townsend *et* al, 2010 that stated that flies play an important role in the transmission of *Moraxella* ovis. This may due to the small sample size, short sampling duration, climate during sampling and the sample farms were in good hygienic condition and practice proper sanitation. As for this study, we could not conclusively rule out flies as a vector for *Moraxella ovis.*

The mean percentage of animals affected by pink eye disease for all the farms is about 16.50% with Farm C having the highest percentage affected at 50.00% and Farm D having the lowest at 2.00%. The low percentage of animals showing clinical signs and the manifestation of weight loss in this study may be due to farmer compliance to the biosecurity protocol where the farmers in these selected farms practiced isolation of sick animals and prompt treatment measures to avoid the animals from suffering the disease chronically and in agreement with Whittier *et al*, 2006 which stated that prompt

treatment will save the animal and reduce the economic impact in the farm. As for Farm C, they employ systemic antibiotic as the farm is affected with the highest percentage of animals suffering from this disease, requiring a more aggressive treatment approach (Whittier *et al*, 2006), (Angelos, 2013), (Leite-Browning, 2015).

Chapter 6 : Conclusion

The prevalence of *Moraxella ovis* in the goats under the Ladang Angkat Programme of the Faculty of Veterinary Medicine, Universiti Putra Malaysia is about 30%. From the questionnaire, the mean percentage of goats affected by pink eye disease under Ladang Angkat programme is 16.50% with about 7.25% showing signs of weight loss. The role of flies as a vector of pink eye disease also cannot be effectively ruled out from this study due to the relatively small sample size and the other confounding factors in this study.

RECOMMENDATION

Further study should be done on the other agents responsible for pink eye disease in goats to better our understanding of this disease. A prolonged and in-depth study should be done to better evaluate the economic impact of pink eye disease. The fly sample size should be increased and tested for the various other agents to better understand the role of flies as a vector of pink eye disease. Many years of study and herculean effort will be needed to make an effective vaccine against infectious keratoconjunctivitis in goats due to the multitude number of agents responsible for this disease. Proper hygiene, fly control, proper biosecurity and prompt treatment should help to keep this disease in check.

REFERENCES

Abdullah, F. F. J. (2014). Stage II Keratoconjunctivitis in a Goat: A Case Report. Journal of Agriculture and Veterinary Science (IOSR-JAVS), Volume 7, Issue 1, Ver. IV (Feb. 2014), PP 16-18.

Angelos, J. A. (2013). The Merck Veterinary Manuals: An Overview of Infectious Keratoconjunctivitis. Retrieved on 11 November 2014 from http://www.merckmanuals.com/vet/eye_and_ear/infectious_keratoconjunctivitis/overview_of_infectious_keratoconjunctivitis.html.

Cerny, H.E. et al (2006). Effects of Moraxella (Branhamella) ovis Culture Filtrates on Bovine Erythrocytes, Peripheral Mononuclear Cells, and Corneal Epithelial cells. Journal of Clinical Microbiology, 2006 March; 44(3), (pg. 772-776).

dvs.gov.my, (2014). Bil Ternakan 2012. Retrieved on 23 February 2015 from http://www.dvs.gov.my/documents/10157/6e0f331d-600e-4627-a2d6-ec421a2dff3c.

dvs.gov.my, (2014). Kadar sara diri hasil ternakan 2004-2013. Retrieved on 23 February 2015 from http://www.dvs.gov.my/documents/10157/69177f95-9ec4-49d3-8d27-342b4d189bbe.

dvs.gov.my, (2014). Penggunaan hasil ternakan 2004-2013. Retrieved on 23 February 2015 from http://www.dvs.gov.my/documents/10157/17f4bb57-caf6-4d98-b300-124ec75c4eec.

Elad, D. *et al*, (1988). Moraxella ovis in cases of Infectious Bovine Keratoconjunctivitis (IBK) in Israel. Journal of Veterinary Medicine, B-35 (pg 431-434)

Fraser, J. Gilmore, N. J., (1979). The identification of Moraxella bovis and Neisseria ovis from cattle and sheep. Research in Veterinary Science; 27 (1): pg 127-130.

Infonet-biovision.org,. 'Infonet - Eye Problems'. N.p., 2012. Web. Retriened on 12 October 2014 from http://www.infonet-biovision.org/default/ct/659/animaldiseases.

Leite-Browning, M. L., (2004). South African Boer Goats: Keratoconjunctivitis (Pinkeye) in Goats. Retrieved on 10 March 2015 from http://www.sa-boergoats.com/ASP/Maria-Browning/Keratoconjunctivitis.asp.

Townsend, L., (2011). University of Kentucky, College of Agriculture: Face Flies and Pinkeye. Retrieved on 12 February 2015 from http://www2.ca.uky.edu/entomology/entfacts/ef510.asp .

Schoenian, S. (2009). Small Ruminant Info Sheet: Infectious keratoconjunctivitis (Pink Eye). Retrieved on 24 December 2014 from http://www.sheepandgoat.com/articles/pinkeye.html.

Shen, H.G., Gould, S., Kinyon, J., Opriesnig, T., O'Connor, A.M, (2011). Development and evaluationof a multiplex real-time PCR assay for the detection and differentiation of Moraxella bovis, Moraxella bovoculli and Moraxella ovis in pure culture isolates and lacrimal swabs collected from conventionally raised cattle. Journal of Applied Microbiology, ISSN 1367-5022 (pg. 1037-1043).

Walker, B. (2007). Pinkeye in Cattle. Retrieved on 14 February 2015 from http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0017/103904/pinkeye-in-cattle.pdf.

Whittier, W. D. (2006). Pinkeye in Beef Cattle. Retrieved on 11 February 2015 from http://pubs.ext.vt.edu/400/400-750/400-750.html.

APPENDICES

Appendix 1: Farm PCR Crosstabulation

			PCR		Total
			Positive	Negative	
Farm	Farm A	Count	5	10	15
		% within Farm	33.3%	66.7%	100%
		% of Total	8.3%	16.7%	25%
	Farm B	Count	6	9	15
		% within Farm	40.0%	60.0%	100%
		% of Total			25%
	Farm C	Count	3	12	15
		% within Farm	20.0%	80.0%	100%
		% of Total	5%	20.0%	25%
	Farm D	Count	4	11	15
		% within Farm	26.7%	73.3%	100%
		% of Total	6.7%	18.3%	25%
Total		Count	18	42	60
		% within Farm	30.0%	70.0%	100%
		% of Total	30.0%	70.0%	100%

Appendix 2: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	1.587	3	0.662	0.783		
Likelihood Ratio	1.608	3	0.657	0.783		
Fisher's Exact Ratio	1.623			0.783		
Linear-by-Linear Association	0.562	1	0.453	0.534	0.267	0.075
N of valid cases	60					

Appendix 3: Kruskal-Wallis Test for Percentage of Animals Affected by Pink Eye Disease Across the farms

	Percentage affected
Chi-Square	59.00
df	3
Asymp. Sig.	0.000
Exact Sig.	0.000
Point Probability	0.000

Appendix 4: Kruskal-Wallis Test for Percentage of Animals Showing Signs of Weight Loss after Manifestation of clinical signa of Pink Eye Disease Across the farms

	Percentage affected
Chi-Square	59.00
df	3
Asymp. Sig.	0.000
Exact Sig.	0.000
Point Probability	0.000

Appendix 5: Pearson Correlation table between the prevalence and the percentage of animals affected by pink eye disease annually

		PCR	Percentage affected
PCR	Pearson Correlation	1	0.118
	Sig. (2-tailed)		0.370
	N	60	60
Percentage Affected	Pearson Correlation	0.118	1
	Sig. (2-tailed)	0.370	
	N	60	60

Appendix 6: Questionnaire for the farmers regarding the status of pink eye disease on their farm.

FAKULTI PERUBATAN VETRINAR , UNIVERSITI PUTRA MALAYSIA, 43400, UPM SERDANG, SELANGOR, MALAYSIA

Farm ID/State/Date of Sample Collection:

Survey on the economic impact of Pink Eye Disease in goats Among the Ladang Angkat In UVH, FPV, up,

This survey is to be used to determine the economic impact of pink eye disease in goats in the Ladang Angkat of the University Veterinary Hospital, Faculty of Veterinary Medicine, UPM and the Veterinary Research Institute, Ipoh. This survey will take about 20 to 25 minutes to fill in. All the data obtained will be kept private and confidential and will only be used of the purpose of the study to complete the DVM students' final year project.

Farm's background:			
Farm's owner:			
Farm's address:			
Year of farm's established:			
Type of farm (please tick):	[ ] Government's owned farm	[ ] Small holder	[ ] Others(please specify):
Purpose of animals (please tick):	[ ] Meat	[ ] Dairy	[ ] Others(please specify):
Breed of goats:			
Farm's population:	Total: Female: Male: Young/kids:		
Performance of animals in farm:			
In general, how do you think the performance level of your goats? (please tick) [ ] Poor [ ] Fair [ ] Good [ ] Very Good [ ] Excellent			
No. of kidding per year:			

Range of age:	Youngest: Oldest:
Average Daily Gain (g/day):	
Market weight & age:	Market weight (kg): Market age:
Since last year, what is the percentage of your animals was:	Sold for cash: Slaughtered because of sickness: Exchanged but not sold for cash: Disposed of other reason: (Please specify reason):
Milk production (L/day):	

Pink Eye Disease Information:	
Please state if you had encountered any previous history of Pink Eye Disease in the farm?	
Do you recognize Pink Eye Disease as a problem distinct from other diseases?	[ ] Yes [ ] No
Do you perceive Pink Eye Disease as a problem in your herd?	[ ] Yes [ ] No
How important is Pink Eye Disease to your herd?	[ ] Severe, the most important problem [ ] Among the first three most serious [ ] Recognized, but other problem have high priority [ ]Occurs but not a problem [ ]Not present or not recognized
Number of clinical cases occurred in the farm for the year:	
Did the infected eye appear reddish pink with discharges and/or pus?	[ ] Yes [ ] No
Color of the eye discharge observed in the herd:	
How do you manage the eye infections of your goat herd?	[ ] Eye ointment/spray in addition to a systemic antibiotic [ ] Eye ointment/spray alone [ ] Do nothing [ ] Culling [ ] Others:……..
Do you separate animals with clinical signs of Pink Eye Disease from the herd?	[ ] Yes [ ] No

Do you use drugs to treat animals specifically with clinical signs of Pink Eye Disease?	[ ] Yes [ ] No
If NO, why did you not buy any drugs to treat the disease in your animals?	[ ] Because I could not afford to pay for the drugs I needed [ ] Because none of my animals got sick [ ] Because I don't know which drug to by [ ] Other reasons (please specify):…….
If YES, where do you normally buy your drugs from?	[ ] From the local government veterinary Assistant [ ] From the veterinary clinic in the nearest main town [ ] From a private trader [ ] From another sources not listed above (please specify): .....
Please indicate which of the following control measures are practiced on the farm:	[ ] Prompt removal of confirmed clinical cases [ ] Adequate separation from of newly born animals from adult [ ] Adequate hygiene and water supplies to housed animal [ ] Prevention of access to unknown animal

Data on Weight Loss:	
Do you observe emaciation as a problem in your herd?	[ ] Yes [ ] No
If YES, how many percentage shows signs of Pink Eye Disease before the emaciation?	

Data on Lactation:	
Do you observe any reduced milk production in your dairy goats?	[ ] Yes [ ] No
If YES, how many percentage shows signs of Pink Eye Disease before the reduction in the milk production?	