

***EPIDEMIOLOGY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN
KELANTAN AND TERENGGANU, MALAYSIA***

ZARINA BINTI MOHAMED

FPV 2016 6



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KELANTAN AND TERENGGANU, MALAYSIA**

By

ZARINA BINTI MOHAMED

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the
Degree of Master of Veterinary Science**

March 2016

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Dedicated to
Nur Ain Hidayah
Mohamad Irfan Taufiq
Ahmad Taufiq Hakimi
Nur Amirah Hidayah
Nur Athirah Hidayah

May God bless them with wisdom and success

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

EPIDEMIOLOGY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN KELANTAN AND TERENGGANU, MALAYSIA

By

ZARINA BINTI MOHAMED

March 2016

Chair : Prof. Mohd. Zamri bin Saad, PhD

Faculty : Veterinary Medicine

Contagious Bovine Pleuropneumonia (CBPP) is a highly infectious disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* Small Colony (*MmmSC*). CBPP is one of the diseases recognized by OIE that needs to be controlled or eradicated through surveillance program. Therefore, the objectives of this study were to determine the seroprevalence and risk factors of CBPP in cattle and to isolate and detect the agent of CBPP from cattle in Kelantan and Terengganu. Data from National Serological Surveillance in 2013 and data from Kota Bharu Regional Veterinary Laboratory in 2011 to 2014 conducted by DVS were retrieved and analysed for seroprevalence and samples of nasal swab from farms and samples of organ from abattoirs were collected for mycoplasma isolation and detection of *MmmSC*. A total of 3,242 sera from 428 cattle farms were tested for CBPP using the competitive ELISA (c-ELISA) to detect specific antibodies. The overall animal-level seroprevalence of CBPP in nine states in Peninsular Malaysia between 2011 and 2014 was 8% (266/3,242) and the herd seroprevalence was 17% (75/428). The animal-level seroprevalence ranged between 5% (46/917) in Terengganu and 9% (220/2,325) in Kelantan whereas the herd prevalence ranged between 12% (22/210) in Terengganu and 24% (53/218) in Kelantan. Risk factors were identified in terms of farm location and animal characteristics such as farm location, age, sex, breeds and production system. A total of 204 nasal swabs from 18 sero-positive cattle herds in Kelantan were collected for isolation of *MmmSC*. One hundred and sixty three (163) lungs and mediastinal lymph nodes from cattle slaughtered in abattoirs in Kelantan were collected for isolation of *MmmSC*. Detection of *MmmSC* from broth and agar was by polymerase chain reaction (PCR) and detection of antigen from organs was by immunoperoxidase (IP). Out of the 326 samples of nasal swab, lung and mediastinal lymph node, 41 (12.5%) samples showed turbidity in the pleuropneumonia-like-organism (PPLO) broth and 15 (9.2%) of the lung and mediastinal lymph node samples had 'fried-egg colony' growth on the PPLO

agar. The 41 suspected cultures in broth and the 15 suspected colonies on agar were subjected to PCR but was negative. Nevertheless, 2 (1.2%) of the lung samples showed lesions suggestive of *MmmSC* infections, which included widening of the interlobular septa and hepatization of the lung parenchyma with mixture of oedematous fluid and fibrin. Similarly, no positive reaction was observed in any of these organ samples following immunoperoxidase staining.

In conclusion, the cattle in Kelantan and Terengganu were exposed to *MmmSC* due to seropositive of c-ELISA however *MmmSC* was not detected in any of the samples, indicating the absence of the organism in the cattle of Kelantan and Terengganu.

Keywords: CBPP, cattle, surveillance, seroprevalence, *MmmSC*

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

EPIDEMIOLOGI PLEUROPNEUMONIA BOVIN BERJANGKIT DI KELANTAN DAN TERENGGANU, MALAYSIA

Oleh

ZARINA BINTI MOHAMED

2016

Pengerusi : Prof. Mohd. Zamri bin Saad, PhD

Fakulti : Perubatan Veterinar

Pleuropneumonia Bovin Berjangkit (CBPP) adalah penyakit yang sangat berjangkit pada lembu yang disebabkan oleh *Mycoplasma mycoides subsp. mycoides* Small Colony (*MmmSC*). CBPP adalah salah satu penyakit yang diiktiraf oleh OIE sebagai penyakit yang perlu dikawal atau dihapuskan melalui sistem pengawasan (survalens). Oleh itu, tujuan kajian ini adalah untuk menentukan kadar seroprevalens dan faktor risiko bagi CBPP, serta pengasingan dan pengesanan agen CBPP daripada lembu. Data dari Pengawasan Serologi Kebangsaan pada tahun 2013 dan data dari Makmal Veterinar Wilayah Kota Bharu pada tahun 2011-2014 yang dijalankan oleh Jabatan Perkhidmatan Veterinar telah diambil dan dianalisis untuk seroprevalens dan sampel calitan hidung dari ladang-ladang dan sampel organ dari rumah penyembelihan telah dikumpulkan untuk pengasingan dan mengesan *MmmSC*. Sebanyak 3,242 serum daripada 428 ladang lembu telah diuji untuk CBPP menggunakan ELISA kompetitif (c-ELISA). Purata prevalens peringkat haiwan antara tahun 2011-2014 dalam sembilan negeri di Semenanjung Malaysia adalah 8% (266/3,242) dan prevalens gerompok adalah 17% (75/428). Seroprevalens peringkat haiwan adalah antara 5% (46/917) di Terengganu dan 9% (220/2,325) di Kelantan manakala prevalens gerompok adalah di antara 12% (22/210) di Terengganu dan 24% (53/218) di Kelantan. Faktor-faktor risiko telah dikenal pasti dari segi ciri-ciri ladang dan haiwan seperti lokasi ladang, umur, jantina, baka dan sistem pengeluaran. Sejumlah 204 calitan hidung daripada 18 ladang lembu positif di Kelantan telah diuji untuk pengasingan *MmmSC*. Satu ratus dan enam puluh tiga (163) sampel paru-paru dan nodus limfa mediastinal dari rumah penyembelihan di Kelantan telah dikumpulkan dan diuji untuk pengasingan *MmmSC*. Pengesanan *MmmSC* dari larutan (*broth*) dan agar adalah dengan tindak balas rantai polimerase (PCR) dan pengesanan antigen daripada organ-organ adalah dengan immunoperoxidase (IP). Daripada 326 sampel calitan hidung, tisu paru-paru dan tisu nodus limfa mediastinal, 41 (12.5%) sampel telah menunjukkan kekeruhan dalam cecair 'pleuropneumonia-like organism' (PPL0)

dan 15 (9.2%) sampel paru-paru dan nodus limfa mediastinal mempunyai pertumbuhan koloni seperti telur goreng di atas agar PPLO. Kesemua 41 sampel disyaki positif dalam bentuk cecair dan 15 sampel disyaki mempunyai koloni positif pada agar telah diuji menggunakan PCR tetapi tiada pengesanan diperolehi. Daripada 163 sampel paru-paru dan nodus limfa, dua (1.2%) paru-paru menunjukkan lesi menyerupai jangkitan *MmmSC*, iaitu pelebaran 'interlobular septa' serta 'hepatization' pada paru-paru dengan campuran cecair dan fibrin. Namun, tiada reaksi positif diperhatikan daripada mana-mana sampel setelah diwarnai immunoperoksida.

Kesimpulannya, lembu di Kelantan dan Terengganu terkena *MmmSC* kerana seropositif kepada c-ELISA bagaimanapun *MmmSC* tidak dikesan dalam mana-mana sampel, menunjukkan ketiadaan organisma dalam lembu di Kelantan dan Terengganu.

Kata kunci: CBPP, lembu, pengawasan, seroprevalens, *MmmSC*

ACKNOWLEDGEMENTS

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**In the Name of God,
Most Gracious and Most Merciful**

First and foremost, my humble gratitude to Almighty Allah for giving me strength, will power and patience to complete this task successfully. Alhamdulillah.

This thesis would not have been possible without the support and encouragement, both scientific and personal of many great people. I am deeply indebted to my main supervisor; Prof. Dr. Mohd. Zamri bin Saad who always believe in me from the beginning and always been very supportive and encouraging. No words can describe how grateful I am to have him as my mentor. Not forgetting, to my co-supervisor, Associate Prof. Latiffah binti Hassan and Dr. Shahrom bin Salisi for their constant encouragement, kind guidance and support in completing this thesis in a scientifically approved manner.

I owe my special thanks to the Malaysia Government for the scholarship which enabled me to complete this programme. I am indebted to the Department of Public Service (JPA), Ministry of Agriculture (MOA), Department of Veterinary Services (DVS), Malaysia for giving me an opportunity to pursue this study. My greatest gratitude goes to the Director of DVS Kelantan, Dr. Azri bin Adzhar and Dr. Norlida binti Othman, the Director of Kota Bharu Regional Veterinary Laboratory, DVS for inspiring me for this study, guiding me and facilitating the research work, logistics as well as operational. I sincerely thank Dr. Norina binti Lokman for guiding me and sharing her valuable knowledge on *Mycoplasma*.

I also like to express my sincere thanks to Dr. Norlida binti Othman for giving me freedom to work in the laboratory. Many thanks to Captain Mustafa bin Dollah, the Head of Kelantan State Enforcement Unit and Mr. Rizan, Eastern Zone Veterinary Inspection Unit for chalking out the sampling schedule and assistance provided. I heartily acknowledge the support extended by the staff in DVS Kelantan especially Dr. Rajeswary and Mr. Zaimi who made the proper arrangements for my sampling at field work. The patience, support and dedication of Enforcement Unit is highly admired, who has been of great help to me in the sampling procedure. My sincere thanks also go to laboratory staff and technicians of the Veterinary Research Institute at Ipoh, Perak, Regional Veterinary Laboratory Kota Bharu, Kelantan and Eastern Zone Veterinary Inspection Unit for creating a warm, friendly and supportive atmosphere which helped me to finish the research work, fruitfully. Without their support and

contribution this study would not be possible. I am highly obliged to Dr. Syarifah Asiah, Dr. Wan Norulhuda, Ms. Siti Zubaidah, Mr. Faisal, Mr. Che Mud, Mr. Mahazan, Mr. Halim and Mr. Haswadi and by heap of appreciation for them for providing me a pleasant working environment in the laboratories. I sincerely acknowledge the veterinary assistance of “Allahyarham” Mr. Saufi in the sampling work. May God grant peace to his soul!

I would also like to express my special thanks to the management and staff of the DVS ruminant slaughter house, and the owners of the private slaughter house, for their cooperation extended in the sampling process.

Finally, my heartfelt gratitude to my beloved parents, Mr. Mohamed bin Dollah and Mrs. Hamidah binti Yusoff, who always believed in me, stood beside me, giving me the inspiration, encouragement and support in everything until completion of this study. I am highly indebted to the support and sacrifice of my beloved children Nur Ain Hidayah, Mohamad Irfan Taufiq, Ahmad Taufiq Hakimi, Nur Amirah Hidayah, Nur Athirah Hidayah and my family members for their unwavering love, sacrifice, support and encouragement. Without their understanding, I would not have been able to complete this course.

Zarina binti Mohamed

I certify that a Thesis Examination Committee has met on 25 March 2016 to conduct the final examination of Zarina binti Mohamed on her thesis entitled "Epidemiology of Contagious Bovine Pleuropneumonia in Kelantan and Terengganu, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Abd. Wahid b Haron, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Saleha bt Abdul Aziz, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Mohd Mokhtar Arshad, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(External Examiner)



ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 June 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. The members of the Supervisory Committee were as follows:

Name of Chairperson, PhD

Professor Dr. Mohd. Zamri bin Saad
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Name of Member 1, PhD

Associate Professor Dr. Latiffah binti Hassan
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Name of Member 2, PhD

Dr. Mohd. Shahrom bin Salisi
Faculty of Veterinary Medicine
Universiti Putra Malaysia
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Committee:

Associate Professor Dr. Latiffah
binti Hassan, PhD

Signature:

Name of Member of
Supervisory
Committee:

Dr. Mohd. Shahrom bin Salisi,
PhD

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LIST OF ABBREVIATIONS

%	percentage
&	and
+ ve	positive
- ve	negative
µl	micro liter
°C	Degree Celsius
CBPP	Contagious bovine pleuropneumonia
c-ELISA	Competitive enzyme linked immunosorbent assay
CFT	Complement fixation test
CI	Confidence Interval
CL	Confidence Limits
DVS	Department of Veterinary Services
FAO	Food and Agricultural Organisation
g	gram
IgG	Immunoglobulin G
L	liter
mg	milligram
ml	milliliter
<i>MmmSC</i>	<i>Mycoplasma mycoides</i> subsp <i>mycoides</i> Small Colony
NaOH	Sodium hydroxide
OIE	<i>Office international des épizooties</i>
PAP	Peroxidase-antiperoxidase
PCR	Polymerase chain reaction
p-value	probability value
ppm	parts per million
RVL	Regional Veterinary Laboratory
χ^2	Chi- square

CHAPTER 1

INTRODUCTION

1.1 General Introduction

The Malaysian government aims to be 40% self-sustainable in beef by 2015 but the current local beef production can only fulfill about 30% of the demand (Jamaludin *et al.*, 2014). With increasing human population in Malaysia, there is a need to fulfill the local consumers demand for beef by live cattle to supplement the local Kedah-Kelantan (KK) breed, which is almost 85% of the total cattle population of in Malaysia (Johari and Yasmi, 2009). Among the cattle breeds that had been imported into Malaysia were Brahman, Hereford, Aberdeen Angus, Droughtmaster, Santa Gertrudis, Bali, Shorthorn and the Chinese Yellow Cattle (CYC) (Jamaludin *et al.*, 2014). They were subjected to the traditional production system (Jalaludin, 1998) where majority comprises smallholdings practicing extensive system and semi-intensive systems.

Following the importations of live cattle, the industry is prone to face new diseases such as the contagious bovine pleuropneumonia (CBPP). This is a significant disease, particularly among the African countries (FAO 2002, OIE 2014a). Based on the Malaysia Veterinary Protocol for CBPP (DVS, 2015), the seroprevalence rate for 2011 was 2.2 % and DVS aimed to reduce to 0.2 % due to the economic impact to farmers in term of reduction in meat and milk production and also may cause respiratory problem that can lead to death of the cattle.

Contagious bovine pleuropneumonia is a highly infectious acute, sub acute and chronic infection of cattle lungs and joints caused by *Mycoplasma mycoides* subsp. *mycoides* Small Colony (*MmmSC*) (OIE, 2014a; 2014b). The disease causes high production losses and is known to cause fatal respiratory disorder (Masiga *et al.*, 1996; FAO, 2002; OIE, 2014a). It spreads by inhalation of droplets from an infected animal, thus transmission needs close contact of up to 200 meters that usually occurs during animal movements (Masiga *et al.*, 1996; FAO, 2002; OIE 2014a; 2014b). The carrier animals are apparently healthy that can infect susceptible in-contact animals (FAO, 2002). Therefore, spread within and between herds occurs rapidly when the cattle mingled (Masiga *et al.*, 1996; FAO, 2002; OIE, 2014a; 2014b). The risk of CBPP transmission is through introduction of CBPP agent by trading the carrier animals and cattle movement from one herd, region or country (Masiga *et al.*, 1996; Msami, 2009).

Serological tests are used to study the epidemiology of any diseases because it is possible to detect latent infection and newly infected population (FAO, 2002; Schubert *et al.*, 2011; OIE, 2014a). The standard test described in the

OIE Terrestrial Manual, 2014 for screening the herds is the modified Campbell & Turner complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA).

For identification of the agent, culture media of heart-infusion broth or Bacto pleuropneumonia-like organisms (PPLO) broth can be used before being presumptively identified and confirmed by biochemical and immunological tests and polymerase chain reaction (PCR) (Bashiruddin *et al.*, 1994; FAO, 2002; Swai *et al.*, 2013; OIE 2014b). Lung lesions are also important to be examined histopathologically to detect the typical lesions of CBPP (Msami, 2009; Swai *et al.*, 2013) while the antigen can be detected by immunohistochemistry. Abattoir surveillance is important for estimating prevalence of CBPP and in order to estimate the carrier status of *MmmSC* among the infected cattle (Swai *et al.*, 2013).

1.2 Problem statement

The serological surveillance of CBPP in Malaysia began in 2011 and had revealed CBPP seroprevalence was 2.2 % (DVS 2015). Since then, there is increasing number of the animals with seropositive for CBPP (source: DVS database). At the moment, little is known about the epidemiology of CBPP in Malaysia. Since CBPP is an important disease in cattle (OIE 2014a; 2014b) that requires control or eradication, epidemiological study is deemed critical (Masiga *et al.*, 1996; Msami, 2009; Okaiyeto *et al.*, 2011; Swai *et al.*, 2013). Based on the OIE, the status of CBPP in 2013, Malaysia was stated as “confirmed infection but no clinical disease” reflecting the presence of serological positive animals indicating infection but without clinical signs or no isolation of *MmmSC*. However, there has not been a formal epidemiological study on this disease in Malaysia.

1.3 Significance of research

This study will determine the seroprevalence of CBPP in Peninsular Malaysia and also epidemiological status and risk factors of CBPP for cattle in Kelantan and Terengganu. This information is vital to ascertain the status of CBPP in the face of international requirement for reporting the disease and to provide evidence for disease control resource allocation at the national level. The findings from this study will:

1. For confirmation of CBPP status in Peninsular Malaysia
2. Assist the standardization of the laboratory, screening and disease control protocols.
3. Provide preliminary data and information for further study

1.4 Hypotheses

Seroprevalence of CBPP among cattle in Kelantan and Terengganu are relatively high with several risk factors influenced the occurrence of CBPP.

1.5 Objectives of the study

The specific objectives of the present study were to:

1. to determine the seroprevalence for CBPP of cattle in Peninsular Malaysia
2. to determine the seroprevalence and risk factors for CBPP of cattle in Kelantan and Terengganu
3. to determine the presence of *Mycoplasma mycoides* subspecies *mycoides* Small Colony (*MmmSC*) from cattle in Kelantan

1.6 Limitations of the study

1. the nasal swabs and organs for bacteriology were very fragiled (organism was fastidious) so need to transport direct to laboratory in cold box
2. the samples from slaughterhouses were limited due to less slaughtered bulls in the slaughterhouses
3. limited of time (at least 21 days for every culture passages) and funding for laboratory consumables for serology, bacteriology and immunohistopathology

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BIODATA OF STUDENT

Zarina binti Mohamed was born in Bachok, Kelantan. She completed her secondary education with Sijil Pelajaran Malaysia in Maktab Rendah Sains MARA (MRSM), Terendak, Malacca. In 1990, she pursued her studies in Diploma of Agriculture in the Universiti Pertanian Malaysia, Bintulu Campus, Sarawak and promoted to Degree of Doctor of Veterinary Medicine in the Faculty of Veterinary Medicine in the Universiti Pertanian Malaysia, Serdang Campus, Selangor. She completed the Degree of Doctor of Veterinary Medicine in 1996. Soon after graduation she being employ by Chareon Pokphand (CP) Ltd. Company in Senawang, Negeri Sembilan. After working for seven years in CP, she being employ by Department of Veterinary Services on October 2003 as Veterinary Officer and being located in Regional Veterinary Laboratory in Kota Bharu, Kelantan as Head of Virology Section for two years. In November 2005, she was being transfer to Section of Disease Control in DVS Headquarters, Putrajaya for 4 years. In June 2009, she once again being transfer to Regional Veterinary Laboratory in Kota Bharu, Kelantan and becomes the Head of Pathobiology Section which is including Microbiology Section, Pathology Section and Epidemiology Unit. In September 2013, she furthered to pursue Master of Veterinary Science majoring in Epidemiology in the Faculty of Veterinary Medicine in University Putra Malaysia, Serdang, Selangor under supervision of Professor Dr. Mohd. Zamri bin Saad.

PUBLICATIONS

Zarina, M., Zamri-Saad, M., Latiffah, H., Shahrom, M. S. and Norlida, O. (2016). Seroprevalence and Detection of Contagious Bovine Pleuropneumonia (CBPP) in Northeast States of Peninsular Malaysia. *Pertanika Journals of Tropical Agricultural Science*, 39 (2), 257 - 265