



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

ZARINA BINTI MOHAMED

FPV 2016 6



**EPIDEMIOLOGY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN
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**

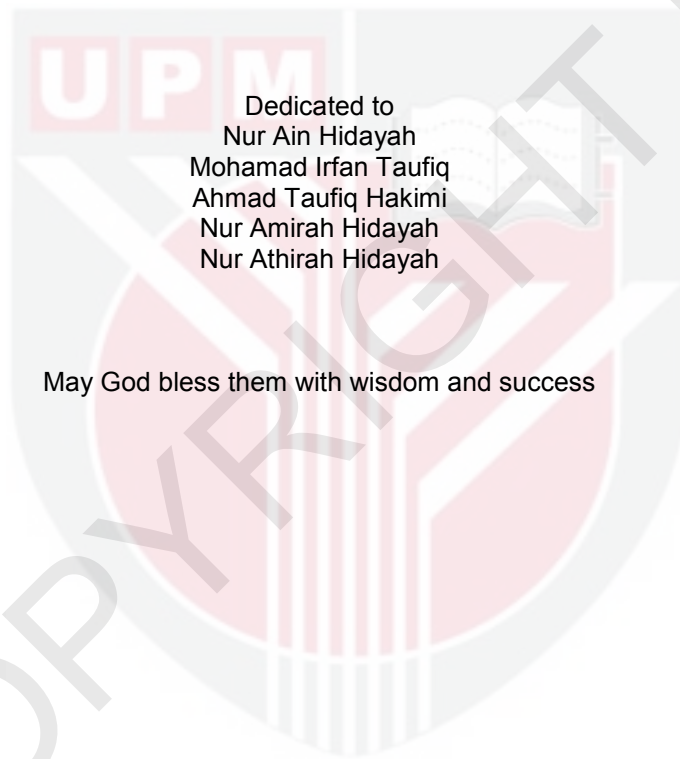
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Dedicated to
Nur Ain Hidayah
Mohamad Irfan Taufiq
Ahmad Taufiq Hakimi
Nur Amirah Hidayah
Nur Athirah Hidayah

May God bless them with wisdom and success

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment 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*



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ZARINA BINTI MOHAMED

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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' (PPLO)

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*

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Most Gracious and Most Merciful**

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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
(Member)

BUJANG B.K. HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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Signature: _____

Name of Chairman
of Supervisory
Committee:

Professor Dr. Mohd. Zamri bin
Saad, PhD

Signature: _____

Name of Member of
Supervisory
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
MmmSC	<i>Mycoplasma mycoides</i> subsp <i>mycoides</i> Small Colony
NaOH	Sodium hydroxide
OIE	Office international des épizooties
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 fragile (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

BIBLIOGRAPHY

- Abdo, E. M., Nicolet, J. & Frey, J. (2000). Antigenic and genetic characterization of lipoprotein LppQ from *Mycoplasma mycoides* subsp. *mycoides* SC. *Clinical and diagnostic laboratory immunology*, 7(4), 588-595.
- Ahmad, Z., Babar, S., Abbas, F., Awan, M. A., Abubakar, M., Attique, M. A., Hassan, Y., Rashid, N. & Ali, M. (2011). Identification and molecular characterization of *Mycoplasma* species from bovine lungs samples collected from Slaughter House, Quetta, Balochistan, Pakistan. *Pakistan Journal of life and Social Sciences*, 9, 91-97.
- Almeida, P. R., Andrade, C. P., Almeida, L. L., Oliveira, L. G., Castro, L. A., Zlotowski, P., Sérgio C. da Silva & Driemeier, D. (2012). Nested-PCR for the detection of *Mycoplasma hyopneumoniae* in bronchial alveolar swabs, frozen tissues and formalin-fixed paraffin-embedded swine lung samples: comparative evaluation with immunohistochemical findings and histological features. *Pesquisa Veterinária Brasileira*, 32(8), 715-720.
- Amanfu, W., Sediadie, S., Masupu, K. V., Benkirane, A., Geiger, R., & Thiaucourt, F. (1998). Field validation of a competitive enzyme-linked immunosorbent assay (c-ELISA) for the detection of contagious bovine pleuropneumonia in Botswana. *Revue D Elevage Et De Medicine Veterinaire Des Pays Tropicaux*, 51, 189-193.
- Amanfu, W., Sediadie, S., Masupu, K. V., Raborokgwe, M. V., Benkirane, A., Geiger, R., & Thiaucourt, F. (2000). Comparison between c-ELISA and CFT in detecting antibodies to *Mycoplasma mycoides mycoides* biotype SC in cattle affected by CBPP in Botswana. *Annals of the New York Academy of Sciences*, 916(1), 364-369.
- Amanfu, W. (2009). Contagious bovine pleuropneumonia (lungsickness) in Africa: historical overview: Onderstepoort and veterinary research in Africa. *Onderstepoort Journal of Veterinary Research*, 76(1), 13-17.
- Ameera M. Mohammed-Ahmed, Shallali A. A., Rania S. G., Faisal, M. H., Jala A., El Obaid. & Nour El Din T, M (2010). Identification of *Mycoplasma mycoides subsp mycoides* Sc (Small Colonies) types by PCR in the Sudan. *Sudan Journal of Veterinary Research*. 25: 9-14.
- Anon (2000). Programme for the control of contagious bovine pleuropneumonia (CBPP) in the Sale and Loliondo divisions. Ngorongoro District Council, Arusha, Tanzania, pp. 6
- Bamaiyi, P. H., & Wade, A. (2011). Molecular epidemiology of contagious bovine pleuropneumonia in Nigeria-A review. *Continental Journal of Veterinary Sciences*, 5(2), 1.

- Bashiruddin, J. B., Taylor, T. K., & Gould, A. R. (1994). A PCR-based test for the specific identification of *Mycoplasma mycoides* subspecies *mycoides* SC. *Journal of Veterinary Diagnostic Investigation*, 6(4), 428-434.
- Bednarek, D., Ayling, R. D., Nicholas, R. A. J., Dudek, K., & Szymańska-Czerwińska, M. (2012). A serological survey to determine the occurrence of respiratory *Mycoplasma* infections in the Polish cattle population. *Vet Rec*, 171(2), 45.
- Belli, P., Poumarat, F., Perrin, M., Longchambon, D., & Martel, J. L. (1990). Experimental reproduction of contagious bovine pleuropneumonia in a herd of cattle and goats: clinical and pathological aspects. *Contagious Bovine Pleuropneumonia. Commission of the European Communities, EUR*, 12065, 7-16.
- Black, S. R., Barker, I. K., Mehren, K. G., Crawshaw, G. J., Rosendal, S., Ruhnke, L., ... & Carman, P. S. (1988). An epizootic of *Mycoplasma ovipneumoniae* infection in captive Dall's sheep (*Ovis dalli*). *Journal of Wildlife Diseases*, 24(4), 627-635.
- Brandao, E. (1995). Isolation and identification of *Mycoplasma mycoides* subspecies *mycoides* SC strains in sheep and goats. *Veterinary record*, 136(4), 98-99.
- Brocchi, E., Gamba, D., Poumarat, F., Martel, J. L., & De Simone, F. (1993). Improvements in the diagnosis of contagious bovine pleuropneumonia through the use of monoclonal antibodies. *Revue scientifique et technique (International Office of Epizootics)*, 12(2), 559-570.
- Bürki, S., Frey, J., & Pilo, P. (2015). Virulence, persistence and dissemination of *Mycoplasma bovis*. *Veterinary microbiology*, 179(1), 15-22.
- Center for Food Security & Public Health (CFSPH) Fact Sheets. Contagious Bovine Pleuropneumonia at http://www.cfsph.iastate.edu/Factsheets/pdfs/contagious_bovine_pleuropneumonia.pdf 2008
- Cetinkaya, B., Ongor, H., Karahan, H. K., Lorenzon, S., & Thiaucourt, F. (2003). Abattoir-based survey of contagious bovine pleuropneumonia in cattle in Turkey. *Journal of the American Veterinary Medical Association*, 156, 1778-1781.
- Chazel, M., Tardy, F., Le Grand, D., Calavas, D., & Poumarat, F. (2010). Mycoplasmoses of ruminants in France: recent data from the national surveillance network. *BMC Veterinary Research*, 6(1), 32.
- Dahlia H., Tan L.J., Zarrahimah Z., Chandrawathani P., Ramlan M. (2011). Isolation of *Mycoplasma* species from Various Animal Hosts in Malaysia. *Malaysian Journal of Veterinary Research*. 2(1): 53-57

- Danbirni, S., Okaiyeto, S. O., Pewan, S. B., & Kudi, A. C. (2010). Concurrent Infection of contagious bovine pleuropneumonia and bovine tuberculosis in bunaji nomadic cows. *Research Journal of Animal Sciences*, 4(1), 23-25.
- Dassanayake, R. P., Shanthalingam, S., Herndon, C. N., Subramaniam, R., Lawrence, P. K., Bavananthasivam, J., E. Frances Cassirer, Gary J. Haldorson, William J. Foreyt, Fred R. Rurangirwa, Donald P. Knowles, Thomas E. Besser & Srikumaran, S. (2010). *Mycoplasma ovipneumoniae* can predispose bighorn sheep to fatal Mannheimia haemolytica pneumonia. *Veterinary microbiology*, 145(3), 354-359.
- Dedieu, L., Mady, V., & Lefevre, P. C. (1994). Development of a selective polymerase chain reaction assay for the detection of *Mycoplasma mycoides* subsp. *mycoides* SC (contagious bovine pleuropneumonia agent). *Veterinary microbiology*, 42(4), 327-339.
- Dedieu, L., Balcer-Rodrigues, V., Cisse, O., Diallo, M., & Niang, M. (2006). Characterisation of the lymph node immune response following *Mycoplasma mycoides* subsp. *mycoides* SC infection in cattle. *Veterinary research*, 37(4), 579-591.
- Department of Veterinary Services (DVS). Malaysia Veterinary Protocol for CBPP (2015) at www.dvs.gov.my
- Dienes, L., & Weinberger, H. J. (1951). The L forms of bacteria. *Bacteriological reviews*, 15(4), 245.
- Dupuy, V., Manso-Silván, L., Barbe, V., Thebault, P., Dordet-Frisoni, E., Citti, C Poumarat, F., Blanchard, A., Breton, M., Sirand-Pugnet, P. & Thiaucourt, F. (2012). Evolutionary history of contagious bovine pleuropneumonia using next generation sequencing of *Mycoplasma mycoides* subsp. *mycoides* "Small Colony".
- Egwu, G. O., Ameh, J. A., & Aliu, M. M. (1996). Detection of antibodies to *Mycoplasma bovis* in Nigeria cattle by Enzyme Linked Immunosorbent Assay [ELISA]. *Tropical Veterinary*, 14, 163-167.
- Enyaru, J. C. K., Biryomumaisho, S., Balyeidhusa, A. S. P., Ebong, C., Musoni, A., Manzi, M., Rutagwenda, T., Zimurinda, J., Asiimwe, T. & Gahakwa, D. (2012). Comparison of competitive ELISA, PCR and loop mediated isothermal amplification of mycoplasmal DNA in confirmatory diagnosis of an outbreak of contagious bovine pleuropneumonia in Eastern Rwanda. *International Journal of Animal Veterinary Advances*, 4(1), 22-28.
- Ezanno, P., & Lesnoff, M. (2009). A metapopulation model for the spread and persistence of contagious bovine pleuropneumonia (CBPP) in African sedentary mixed crop–livestock systems. *Journal of Theoretical Biology*, 256(4), 493-503.

- Ferronha, M. H., Nunes Petisca, L. J., Sousa Ferreira, H., Machado, M., Regalla, J., & Penha Goncalves, A. (1990). Detection of *Mycoplasma mycoides* subsp. *mycoides* immunoreactive sites in pulmonary tissue and sequestra of bovines with contagious pleuropneumonia. *Contagious Bovine Pleuropneumonia. J Regalla ed. Report EUR, 12065*, 17-25.
- Freundt, E. A., Erng, H. & Lemke, R. (1979). Identification of mycoplasmas. *Methods in Microbiology. Academic Press, London, 13*, 377-434.
- Food and Agriculture Organization of the United Nations (FAO) (1998). Report of the first meeting of the FAO/OIE/OAU-IBAR Consultative Group on Contagious Bovine Pleuropneumonia (CBPP) Rome, Italy 5-7 October 1998
- Food and Agriculture Organization of the United Nations (FAO) (2002). Animal Health Manual No. 13 (Rev. 1)
- FAO: Report of the 3rd Meeting of the FAO/OIE/OUA Expert Panel on CBPP, Khartoum, Sudan, Rome: FAO, 1967.
- Gedlu, M. (2004). Serological, clinical and participatory epidemiological survey of contagious bovine pleuropneumonia in Somali Region, Ethiopia. *Unpublished MSc thesis, Addis Ababa University*
- Gonçalves, R., Regalla, J., Nicolet, J., Frey, J., Nicholas, R., Bashiruddin, J., Paola de Santis & Gonçalves, A. P. (1998). Antigen heterogeneity among *Mycoplasma mycoides* subsp. *mycoides* SC isolates: discrimination of major surface proteins. *Veterinary microbiology*, 63(1), 13-28.
- Gorton, T. S., Barnett, M. M., Gull, T., French, R. A., Lu, Z., Kutish, G. F. & Geary, S. J. (2005). Development of real-time diagnostic assays specific for *Mycoplasma mycoides* subspecies *mycoides* Small Colony. *Veterinary microbiology*, 111(1), 51-58.
- Gourlay, R.N. & Howard, C.J. (1982). Respiratory mycoplasmosis. *Advances in veterinary science and comparative medicine Volume 26*, 289-332
- Gull T., Richard A. French, Timothy S. Gorton, Thomas G. Burrage, LeonProzesky, Steven J. Geary & L. Garry Adams (2013). Models of Contagious Bovine Pleuropneumonia: Evaluation of Two Novel Strains. *The Open Veterinary Science Journal*, 7, 23-33
- Hamsten, C., Neiman, M., Schwenk, J. M., Hamsten, M., March, J. B. & Persson, A. (2009). Recombinant surface proteomics as a tool to analyze humoral immune responses in bovines infected by *Mycoplasma mycoides* subsp. *mycoides* small colony type. *Molecular & Cellular Proteomics*, 8(11), 2544-2554.

- Haziroglu, R., Diker, K. S., Turkarslan, J., & Gulbahar, M. Y. (1996). Detection of *Mycoplasma ovipneumoniae* and *Pasteurella haemolytica* antigens by an immunoperoxidase technique in pneumonic ovine lungs. *Veterinary Pathology Online*, 33(1), 74-76.
- Hotzel, H., Sachse, K., & Pfützner, H. (1996). A PCR scheme for differentiation of organisms belonging to the *Mycoplasma mycoides* cluster. *Veterinary Microbiology*, 49(1), 31-43.
- Hudson, J. R. (1968). Contagious Bovine Pleuropneumonia: the keeping properties of the V5 vaccine used in Australia. *Australian Veterinary Journal*, 44(4), 123-129.
- Hudson, J. R. (1971). Contagious bovine pleuropneumonia. *FAO Agricultural Studies*, (86).
- Hudečková, R., & Sovjak, R. (2006). Strategy of the CBPP eradication in the western province of Zambia. *Agricultura Tropica Et Subtropica*, 39, 4.
- Huebschle, O. J., Ayling, R. D., Godinho, K., Lukhele, O., Tjipura-Zaire, G., Rowan, T. G., & Nicholas, R. A. (2006). Danofloxacin (Advocin™) reduces the spread of contagious bovine pleuropneumonia to healthy in-contact cattle. *Research in Veterinary Science*, 81(3), 304-309.
- Jalaludin, S., & Halim, R. A. (1998, June). Development of the livestock industry in Malaysia. In *Proceedings of the Regional Workshop on Area-Wide Integration of Crop-Livestock Activities*, (pp. 18-20).
- Jamaludin, M. H., Hassan, M. H., MR, A., & Zulhisyam, A. K. (2014). The Future of the Malaysian Beef Industry. *Journal of Tropical Resources and Sustainable Science*, 2(1), 23-29.
- Johari, J. A., & Jasmi, Y. (2009, August). Breeds and breeding program for beef production in Malaysia. In *Proceedings of the 8th Malaysia Congress on Genetics* (pp. 22-28).
- Joseph, P. G., Tan, L. J., Sivanandan, S. P., Jamnah, O., Cottew, G. S., & Yeats, F. (1988). *Mycoplasma* infections of animals in Malaysia with emphasis on the isolation and identification of the etiological agents. *Tropical Biomedicine*, 5, 167-177.
- Kassaye, D. & Molla, W. (2012). Seroprevalence of contagious bovine pleuropneumonia at export quarantine centers in and around Adama, Ethiopia. *Tropical Animal Health and Production*, 45(1), 275-279.
- Kilic, A., Kalender, H., Eroksuz, H., Muz, A. & Tasdemir, B. (2013). Identification by culture, PCR, and immunohistochemistry of mycoplasmas and their molecular typing in sheep and lamb lungs with pneumonia in Eastern Turkey. *Tropical Animal Health and Production*, 45(7), 1525-1531.

- Kumar, P., Roy, A., Bhandari, B. B. & Pal, B. C. (2011). Isolation, identification and molecular characterization of *Mycoplasma* isolates from goats of Gujarat State, India. *Veterinarski Arhiv*, 81(4), 443-458.
- Kusiluka, L. J. M., Ojeniyi, B., Friis, N. F., Kokotovic, B. & Ahrens, P. (2001). Molecular analysis of field strains of *Mycoplasma capricolum* subspecies *capripneumoniae* and *Mycoplasma mycoides* subspecies *mycoides*, small colony type isolated from goats in Tanzania. *Veterinary Microbiology*, 82(1), 27-37.
- Le Grand, D., Saras, E., Blond, D., Solsona, M., & Poumarat, F. (2004). Assessment of PCR for routine identification of species of the *Mycoplasma mycoides* cluster in ruminants. *Veterinary Research*, 35(6), 635-649.
- Le Goff, C. & Thiaucourt, F. (1998). A competitive ELISA for the specific diagnosis of contagious bovine pleuropneumonia (CBPP). *Veterinary Microbiology*, 60(2), 179-191.
- Lesnoff, M., Diedhiou, M.L., Laval, G., Bonnet, P., Workalemahu, A. & Kifle, D., (2002). Demographic parameters of domestic cattle in a contagious-bovine-pleuropneumonia infected area of Ethiopian highlands. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 55, 139-147.
- Lesnoff, M., Laval, G., Bonnet, P., Abdicho, S., Workalemahu, A., Kifle, D. Armelle Peyraud, A., Lancelot, R. & Thiaucourt, F. (2004). Within-herd spread of contagious bovine pleuropneumonia in Ethiopian highlands. *Preventive Veterinary Medicine*, 64(1), 27-40.
- Mair, G., Vilei, E. M., Wade, A., Frey, J., & Unger, H. (2013). Isothermal loop-mediated amplification (LAMP) for diagnosis of contagious bovine pleuro-pneumonia. *BMC Veterinary Research*, 9(1), 108.
- Manso-Silvan, L., Vilei, E. M., Sachse, K., Djordjevic, S. P., Thiaucourt, F. & Frey, J. (2009). *Mycoplasma leachii* sp. nov. as a new species designation for *Mycoplasma* sp. bovine group 7 of Leach, and reclassification of *Mycoplasma mycoides* subsp. *mycoides* LC as a serovar of *Mycoplasma mycoides* subsp. *capri*. *International Journal of Systematic and Evolutionary Microbiology*, 59(6), 1353-1358.
- March, J. B., Kerr, K. & Lema, B. (2003). Rapid detection of contagious bovine pleuropneumonia by a *Mycoplasma mycoides* subsp. *mycoides* SC capsular polysaccharide-specific antigen detection latex agglutination test. *Clinical and Diagnostic Laboratory Immunology*, 10(2), 233-240.
- Marobela-Raborokgwe, C., Ayling, R. & Bashiruddin, J. (2003). ELISA for detecting antibodies to *Mycoplasma mycoides* subspecies *mycoides* small colony (SC) in naturally infected cattle from the 1995 outbreak in Botswana. *Onderstepoort Journal of Veterinary Research*, 70, 21-27.
- Mariner, J. C., McDermott, J., Heesterbeek, J. A. P., Thomson, G. & Martin, S. W. (2006). A model of contagious bovine pleuropneumonia

transmission dynamics in East Africa. *Preventive Veterinary Medicine*, 73(1), 55-74.

- Masiga, W. N., Rurangirwa, F. R., Roberts, D. H., Kakoma, I. (1978). Contagious bovine pleuropneumonia: comparative efficacy trial of the (freeze-dried French T1 vaccine) and the T1 broth culture vaccine (Muguga). *Bulletin of Animal Health and Production in Africa. Bulletin Des Santé et Production Animales en Afrique*, 26(3), 216.
- Masiga, W. N., & Domenech, J. (1995). Overview and epidemiology of contagious bovine pleuropneumonia in Africa. *Revue Scientifique et Technique (International Office of Epizootics)*, 14(3), 611-630.
- Masiga, W. N., Domenech, J., & Windsor, R. S. (1996). Manifestation and epidemiology of contagious bovine pleuropneumonia in Africa. *Revue Scientifique et Technique (International Office of Epizootics)*, 15(4), 1283-1308.
- Matua, A. R. W., Ng'ang'a, Z., Kiara, H., Tere, C., Mbithi, F., Mwirigi, M., Marobella, R.C. & Sidiadie, S. (2006). The Prevalence of contagious bovine pleuropneumonia (CBPP) in cattle under different production systems in Kajiado District, Kenya. In *ISVEE 11: Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia, Theme* (p. 862).
- Matua-Alumira, R. W., Ng'ang'a, Z., Kiara, H., Matere, C., Mbithi, F., Mwirigi, M., Marobella-Raborogwe, C. & Sidiadie, S. (2011). Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 2006
- Mbengue Mb., Lo Ft., Diallo A M., Lo Mm., Diop M., Samb Y., Diouf M., Daffeh K. & Thiongane Y. (2013a). Re-emergence of pleuropneumonia contagious bovine in the Gambia. *American Journal of Research Communication*, 1 (3), 181-189
- Mbengue Mb., J. Sarr & Fall Massal (2013b). Sero - epidemiological studies on contagious bovine pleuropneumonia (CBPP) in Senegal. *American Journal of Research Communication*, 1(3), 190-199
- McAuliffe, L., Ellis, R. J., Ayling, R. D., & Nicholas, R. A. (2003). Differentiation of *Mycoplasma* species by 16S ribosomal DNA PCR and denaturing gradient gel electrophoresis fingerprinting. *Journal of Clinical Microbiology*, 41(10), 4844-4847.
- McAuliffe, L., Ellis, R. J., Lawes, J. R., Ayling, R. D., & Nicholas, R. A. (2005). 16S rDNA PCR and denaturing gradient gel electrophoresis; a single generic test for detecting and differentiating *Mycoplasma* species. *Journal of Medical Microbiology*, 54(8), 731-739.
- McAuliffe, L., Ayling, R. D., & Nicholas, R. A. (2007). Identification and characterization of variable-number tandem-repeat markers for the

- molecular epidemiological analysis of *Mycoplasma mycoides* subspecies *mycoides* SC. *FEMS Microbiology Letters*, 276(2), 181-188.
- Miles, K., Churchward, C. P., McAuliffe, L., Ayling, R. D., & Nicholas, R. A. (2006). Identification and differentiation of European and African/Australian strains of *Mycoplasma mycoides* subspecies *mycoides* small-colony type using polymerase chain reaction analysis. *Journal of Veterinary Diagnostic Investigation*, 18(2), 168-171.
- Miserez, R., Pilloud, T., Cheng, X., Nicolet, J., Griot, C., & Frey, J. (1997). Development of a sensitive nested PCR method for the specific detection of *Mycoplasma mycoides* subsp. *mycoides* SC. *Molecular and Cellular Probes*, 11(2), 103-111.
- Mitchell, J. D., McKellar, Q. A., McKeever, D. J., & Kaltenboeck, B. (2012). Pharmacodynamics of antimicrobials against *Mycoplasma mycoides mycoides* Small Colony, the causative agent of Contagious Bovine Pleuropneumonia. *PLoS ONE*, 7(8).
- Mizuki, H. (2001). In situ staining with DNA-binding fluorescent dye, Hoechst 33258, to detect microorganisms in the epithelial cells of oral leukoplakia. *Oral Oncology*, 37(6), 521-526.
- Msami (2009). Pathology of Contagious Bovine Pleuropneumonia: meat inspection perspective. Animal Disease Research Institute, Dar es Salaam. Paper presented at Meat Inspectors on CBPP recognition in Lake Zone held in Mwanza on 21 to 23 July 2009.
- Mondal, D., Pramanik, A. K., & Basak, D. K. (2004). Clinico-haematology and pathology of caprine mycoplasmal pneumonia in rain fed tropics of West Bengal. *Small Ruminant Research*, 51(3), 285-295.
- Muuka, G., Hang'ombe, B. M., Nalubamba, K. S., Kabilika, S., Mwambazi, L., & Muma, J. B. (2011). Comparison of complement fixation test, competitive ELISA and LppQ ELISA with post-mortem findings in the diagnosis of contagious bovine pleuropneumonia (CBPP). *Tropical Animal Health and Production*, 43(5), 1057-1062.
- Mtui-Malamsha, N. J. (2009). Contagious Bovine Pleuropneumonia (CBPP) in the Maasai ecosystem of south-western Kenya: Evaluation of seroprevalence, risk factors and vaccine safety and efficacy. Doctor of Philosophy thesis, College of Medicine and Veterinary Medicine the University of Edinburgh.
- Niang, M., Diallo, M., Cisse, O., Kone, M., Doucoure, M., Roth, J. A., Rodrigues, V.B. & Dedieu, L. (2006). Pulmonary and serum antibody responses elicited in zebu cattle experimentally infected with *Mycoplasma mycoides* subsp. *mycoides* SC by contact exposure. *Veterinary Research*, 37(5), 733-744.
- Nicholas, R. A. J., & Bashiruddin, J. B. (1995). *Mycoplasma mycoides* subspecies *mycoides* (small colony variant): the agent of contagious

bovine pleuropneumonia and member of the “*Mycoplasma mycoides* cluster”. *Journal of Comparative Pathology*, 113(1), 1-27.

- Nicholas, R. A. J. (2002). Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. *Small Ruminant Research*, 45(2), 145-149.
- Nicholas, R. A. J., Tijipura-Zaire, G., Mbulu, R. S., Scacchia, M., Mettler, F., Frey, J., Abusugra I. & Huebschle, O. J. B. (2004). An inactivated whole cell vaccine and LppQ subunit vaccine appear to exacerbate the effects of CBPP in adult cattle. In *Proceedings of the 3rd Meeting of the FAO-OIE-OAU/IBAR-IAEA Consultative Group on CBPP. Towards sustainable CBPP control programmes for Africa (Rome, 12–14 November 2003)*. FAO, Rome, Italy (pp. 91-97).
- Nicolet, J. (1996). Animal mycoplasmoses: a general introduction. *Revue Scientifique et Technique (International Office of Epizootics)*, 15(4), 1233-1240.
- Office International des Epizooties (OIE) (2008). Terrestrial Animal Health Code, Chapter 11.8.1, Office International des Epizooties, Paris, France
- Office International des Epizooties (OIE) (2014a). Terrestrial Animal Health Code, Chapter 11.8.1, Office International des Epizooties, Paris, France
- Office International des Epizooties (OIE) (2014b). Contagious bovine pleuropneumonia. In *Manuals of standards for diagnostic tests and vaccines*.
- Office International des Epizooties (OIE) (2015). WAHIS Interface (2015) at http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home
- Okaiyeto, S. O., Danbirni, S., Allam, L., Akam, E., Pewan, S. B., & Kudi, A. C. (2011). On-farm diagnosis of contagious bovine pleuropneumonia in nomadic herds using latex agglutination test (LAT). *Journal of Veterinary Medicine and Animal Health*, 3(5), 62-66.
- Onoviran, O., & Taylor-Robinson, D. (1979). Detection of antibody against *Mycoplasma mycoides* subsp. *mycoides* in cattle by an enzyme-linked immunosorbent assay. *The Veterinary Record*, 105(8), 165-166.
- Persson, A., Pettersson, B., Bölske, G., & Johansson, K. E. (1999). Diagnosis of contagious bovine pleuropneumonia by PCR–Laser-Induced Fluorescence and PCR-Restriction Endonuclease Analysis based on the 16S rRNA genes of *Mycoplasma mycoides* subsp. *Mycoides* SC. *Journal of Clinical Microbiology*, 37(12), 3815-3821.
- Pettersson, B., Leitner, T., Ronaghi, M., Bölske, G., Uhlen, M., & Johansson, K. E. (1996). Phylogeny of the *Mycoplasma mycoides* cluster as

- determined by sequence analysis of the 16S rRNA genes from the two rRNA operons. *Journal of Bacteriology*, 178(14), 4131-4142.
- Powell, D. A., & Clyde, W. A. (1975). Opsonin-reversible resistance of *Mycoplasma pneumoniae* to in vitro phagocytosis by alveolar macrophages. *Infection and Immunity*, 11(3), 540-550.
- Provost, A., Perreau, P., Breard, A., Le Goff, C., Martel, J. L., & Cottew, G. S. (1987). Contagious bovine pleuropneumonia. *Revue Scientifique et Technique de l'OIE (France)*.
- Radostits, O. M., Blood, D. C., & Gay, C. C. (1994). Diseases caused by *Mycoplasma* species. *Veterinary Medicine*, 8th ed. ELBS Govt. of UK, Bailliere Tindall, London, 908-925.
- Ramanoon, S. Z., Robertson, I. D., Edwards, J., Hassan, L., & Isa, K. M. (2013). Outbreaks of foot-and-mouth disease in Peninsular Malaysia from 2001 to 2007. *Tropical Animal Health and Production*, 45(2), 373-377.
- Rauf M., Z. I. Chaudhary, M. Younas, A. A. Anjum, H. M. Khan & M. A. Ali (2014). Detection of *Mycoplasma gallisepticum* in experimentally inoculated layer birds by immunohistochemistry. *The Journal of Animal & Plant Sciences*, 24(2), 385-391
- Regalla, J., Gonçalves, R., Niza Ribeiro, J., Duarte, L., & Penha Gonçalves, A. (1996). Use of immunoblotting test for carrier state definition in contagious bovine pleuropneumonia. *Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics*. COST, 826, 72-74.
- Rodwell, A. W., & Rodwell, E. S. (1978). Relationships between strains of *Mycoplasma mycoides* subsp. *mycoides* and *capri* studied by two-dimensional gel electrophoresis of cell proteins. *Journal of General Microbiology*, 109(2), 259-263.
- Rurangirwa, F. R., Shompole, P. S., Wambugu, A. N., & McGuire, T. C. (2000). Monoclonal antibody differentiation of *Mycoplasma mycoides* subsp. *mycoides* Small-Colony strains causing contagious bovine pleuropneumonia from less important Large-Colony strains. *Clinical and Diagnostic Laboratory Immunology*, 7(3), 519-521.
- Sadique U., Z. I. Chaudhry, M. Younas, A. A. Anjum, Z. U. Hassan, M. Idrees, M. Mushtaq, A. Sajid & S. M. Sabtain (2012). Molecular characterization of Contagious Caprine Pleuropneumonia (CCPP) in small ruminants of Khyber Pakhtunkhwa, Pakistan. *JAPS, Journal of Animal and Plant Sciences*, 22(2 Supplement), 33-37.
- Santini, F. G., Visaggio, M., Farinelli, G., Di Francesco, G., Guarducci, M., D'angelo, A. R. & Di Giannatale, E. (1992). Pulmonary sequestrum from *Mycoplasma mycoides* var. *mycoides* SC in a domestic buffalo:

Isolation, anatomo-histopathology and immuno histochemistry. *Veterinaria Italiana*, 4, 4-10.

- Scacchia, M., Tjipura-Zaire, G., Lelli, R., Sacchini, F., & Pini, A. (2010). Contagious bovine pleuropneumonia: humoral and pathological events in cattle infected by endotracheal intubation or by exposure to infected animals. *Veterinaria Italiana*, 47(4), 407-413.
- Scanziani, E., Paltrinieri, S., Boldini, M., Grieco, V., Monaci, C., Giusti, A. M., & Mandelli, G. (1997). Histological and immunohistochemical findings in thoracic lymph nodes of cattle with contagious bovine pleuropneumonia. *Journal of Comparative Pathology*, 117(2), 127-136.
- Scanziani, E. (1998). Immunohistochemical staining of fixed tissues. *Mycoplasma protocols* (pp. 133-140). Humana Press.
- Schnee, C., Heller, M., Jores, J., Tomaso, H., & Neubauer, H. (2011). Assessment of a novel multiplex real-time PCR assay for the detection of the CBPP agent *Mycoplasma mycoides* subsp. *mycoides* SC through experimental infection in cattle. *BMC Veterinary Research*, 7(1), 47.
- Schubert, E., Sachse, K., Jores, J., & Heller, M. (2011). Serological testing of cattle experimentally infected with *Mycoplasma mycoides* subsp. *mycoides* Small Colony using four different tests reveals a variety of seroconversion patterns. *BMC Veterinary Research*, 7(1), 72.
- Séry, A., Sidibé, C. A. K., Cissé, O., Diallo, M., Koné, M., Waret-Szkuta, A., Roger F., Thiaucourt F. & Niang, M. (2015). Seroprevalence of contagious bovine pleuropneumonia (CBPP) in Mali. *Tropical Animal Health and Production*, 47(2), 395-402.
- Sheehan, M., Cassidy, J. P., Brady, J., Ball, H., Doherty, M. L., Quinn, P. J., Nicholas R.A.J. & Markey, B. K. (2007). An aetiopathological study of chronic bronchopneumonia in lambs in Ireland. *The Veterinary Journal*, 173(3), 630-637.
- Srivastava, N. C., Thiaucourt, F., Singh, V. P., & Sunder, J. (2000). Isolation of *Mycoplasma mycoides* small colony type from contagious caprine pleuropneumonia in India. *Veterinary Record*, 147(18), 520-521.
- Sori, T., Zeleke, A., Gelaye, E., & Regassa, F. (2005). Isolation and Identification of *Mycoplasma mycoides* subspecies *mycoides* Small Colony bovine biotype in Eastern Ethiopia. *Journal of Applied Research in Veterinary Medicine*, 3(1), 30-34
- Stärk, K. D., Vicari, A., Kihm, U., & Nicolet, J. (1995). Surveillance of contagious bovine pleuropneumonia in Switzerland. *Revue Scientifique et Technique (International Office of Epizootics)*, 14(3), 621-629.

- Swai, E., Mwezimpaya, I., Ulicky, E., Mbise, A., & Moshy, W. (2013). An abattoir survey of contagious bovine pleuropneumonia lesions in slaughtered cattle in selected districts in Northern Tanzania. *Asian Pacific Journal of Tropical Biomedicine*, 3(4), 303-306.
- Taylor, G., & Howard, C. J. (1981). Protection of mice against *Mycoplasma pulmonis* infection using purified mouse immunoglobulins: comparison between protective effect and biological properties of immunoglobulin classes. *Immunology*, 43(3), 519.
- Taylor, T. K., Bashiruddin, J. B., & Gould, A. R. (1992). Application of a diagnostic DNA probe for the differentiation of the two types of *Mycoplasma mycoides* subspecies *mycoides*. *Research in Veterinary Science*, 53(2), 154-159.
- Teklu, T., Tesfay, T., Nirayo, T., Hailu, B., Wayu, S., & Atsbha, T. (2015). Epidemiological status of contagious bovine pleuropneumonia in Southern Zone of Tigray Regions, Northern Ethiopia. *Animal and Veterinary Sciences*, 3(1), 32-36
- Ter Laak, E. A. (1992). Contagious bovine pleuropneumonia a review. *Veterinary Quarterly*, 14(3), 104-110.
- Thiaucourt, F., Manso-Silvan, L., Salah, W., Barbe, V., Vacherie, B., Jacob, D., Breton, M., Dupuy, V., Lomenech A.M., Blanchard, A. & Sirand-Pugnet, P. (2011). *Mycoplasma mycoides*, from "mycoides Small Colony" to "capri". A microevolutionary perspective. *BioMedCentral genomics*, 12(1), 114.
- Thrusfield, M. (2013). *Veterinary Epidemiology*. Elsevier.
- Timenetsky, J., Santos, L. M., Buzinhani, M., & Mettifogo, E. (2006). Detection of multiple *Mycoplasma* infection in cell cultures by PCR. *Brazilian Journal of Medical and Biological Research*, 39(7), 907-914.
- Toma, B., Dufour, B., Sanaa, M., Benet, J. J., Moutou, F., Louza, A., & Ellis, P. (1999). *Applied veterinary epidemiology and the control of disease in populations*. Association pour l'Etude de l'Epidémiologie des Maladies Animales, 7 Avenue du Général de Gaulle.
- Transitional Federal Government of Somalia (TFG) (2009). Contagious Bovine Pleuro-Pneumonia, Contingency Plan. CBPP CP TFG.pdf
- Vilei, E. M., Abdo, E. M., Nicolet, J., Botelho, A., Gonçalves, R., & Frey, J. (2000). Genomic and antigenic differences between the European and African/Australian clusters of *Mycoplasma mycoides* subsp. *mycoides* SC. *Microbiology*, 146(2), 477-486.
- Walker, R. C. (1999). The fluoroquinolones. In *Mayo Clinic Proceedings* (Vol. 74, No. 10, pp. 1030-1037). Elsevier.

- Westberg, J., Persson, A., Holmberg, A., Goesmann, A., Lundeberg, J., Johansson, K. E., Petterson, B. & Uhlén, M. (2004). The genome sequence of *Mycoplasma mycoides* subsp. *mycoides* SC type strain PG1T, the causative agent of contagious bovine pleuropneumonia (CBPP). *Genome Research*, 14(2), 221-227.
- Windsor, R. S., & Boarer, C. D. H. (1972). A method for the rapid enumeration of *Mycoplasma* species growing in broth culture. *Journal of Applied Bacteriology*, 35(1), 37-42.
- Windsor, R. S., & Masiga, W. N. (1977). Investigations into the role of carrier animals in the spread of contagious bovine pleuropneumonia. *Research in veterinary science*, 23(2), 224-229.
- Windsor, R. S. (2000). The eradication of contagious bovine pleuropneumonia from south western Africa: a plan for action. *Annals of the New York Academy of Sciences*, 916(1), 326-332.
- Woubit, S., Lorenzon, S., Peyraud, A., Manso-Silvan, L., & Thiaucourt, F. (2004). A specific PCR for the identification of *Mycoplasma capricolum* subsp. *capripneumoniae*, the causative agent of contagious caprine pleuropneumonia (CCPP). *Veterinary Microbiology*, 104(1), 125-132.
- Yaya, A., Manso-Silván, L., Blanchard, A., & Thiaucourt, F. (2008). Genotyping of *Mycoplasma mycoides* subsp. *mycoides* SC by multilocus sequence analysis allows molecular epidemiology of contagious bovine pleuropneumonia. *Veterinary Research*, 39(2), 1-19.
- Yilmaz, F., Timurkaan, N., Kiliç, A., Kalender, H., & Kiliç, Ü. (2011). Detection of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* in chickens by immunohistochemical, PCR and culture methods. *Revue Méd. Vét*, 162(2), 79-86.
- Zaini, M.Z., Tan, L.J. & Hazliana, H. (2006). An update status of animal mycoplasmosis in Malaysia. Proceeding of the 18th Veterinary Association Malaysia Scientific Congress 2006. Seri Pacific Hotel, Kuala Lumpur.

PUBLICATIONS

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