UNIVERSITI PUTRA MALAYSIA

SEROPREVALENCE OF MELIOIDOSIS AMONG SMALL RUMINANTS IN FOSTER FARM PROGRAMME OF FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA

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It is hereby certified that we have read this project paper entitled "Seroprevalence of Melioidosis Among Small Ruminants In Foster Farm Programme of Faculty of Veterinary Medicine, Universiti Putra Malaysia", by Thiviya Balakrishnan 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 – Final Year Project.

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DEDICATION

I would like to dedicate this project to my treasured parents,

Balakrishnan Sinniah and Saroja Krishnan,

my superstars: Ranjiny, Yasini and Shamini, my guardian angel: Jayanthi,

my loyal companions: Sakura, Enma Ai, Abby, Jimmy (I, II, IV) and Billy.

my well-wisher: Shanath Kumar, #teamspirit group and

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ABSTRAK

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

KADAR JANGKITAN MELIOIDOSIS DALAM KALANGAN RUMINAN KECIL DI BAWAH PROGRAM LADANG ANGKAT, FAKULTI PERUBATAN VETERINAR, UPM

Oleh

Thiviya Balakrishnan

2017

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Melioidosis adalah saprozoonosis, penyakit tropika disebabkan oleh *Burkholderia pseudomallei* iaitu sejenis saprofit tanah yang sentiasa ada disekeliling, bakteria fakultatif anaerob, tiada spora, dan basilus gram negatif yang mobil dengan pewarnaan dwikutub, bakteria oksidase positif. Penyakit ini boleh ditemui dalam kalangan haiwan ternakan seperti kambing biri-biri, kambing, khinzir dan spesis kurang terjejas seperti lembu, kerbau, kuda, rusa, anjing, kucing, primat, burung, ikan tropika, reptilia dan manusia. Ia merupakan satu kebimbangan kesihatan awam kerana ia adalah penyakit zoonotik dan penyakit ini juga adalah satu masalah kesihatan haiwan yang boleh

membawa kepada produktiviti haiwan yang kronik. Ladang-ladang yang terpilih adalah dalam kalangan Program Ladang Angkat Fakulti Perubatan Veterinar, UPM. Kajian ini dijalankan atas 100 kambing and 100 kambing biri-biri di mana sampel darah diambil dan diproses untuk ujian serologi iaitu *Complement Fixation Test* (*CFT*). *Complement Fixation Test* (*CFT*) dipilih sebagai penentuan kualitatif immunoglobulin G, antibodi IgG terhadap *Burkholderia pseudomallei*, antigen yang digunakan dalam ujian ini disediakan oleh Unit Serologi Institut Penyelidikan Veterinar Ipoh, Perak. Satu set soal selidik telah diberikan kepada setiap pemilik ladang untuk mengenal pasti faktor-faktor risiko yang berkaitan dengan Melioidosis. Data dianalisis berdasarkan kadar kelaziman. Daripada 100 sampel kambing, 1 sampel (1%) adalah positif bagi antibodi terhadap *Burkholderia pseudomallei* manakala semua sampel lain adalah diuji negatif (0%) untuk antibodi. Sampel yang diuji positif daripada kambing itu mempunyai 0% titik akhir di mana ia mempunyai skor 4 pembentukan butang lengkap. Semua keputusan negatif menunjukkan 100% titik akhir dengan lisis penuh, oleh itu tiada pembentukan butang. Di samping itu, analisis soal selidik itu mendedahkan bahawa semua bekalan air ladang untuk haiwan ternakan mereka dirawat, tetapi berbeza dari segi sistem pengurusan; Beberapa ladang mengamalkan pertanian semi-intensif dan intensif. Kesimpulannya, kadar jangkitan Melioidosis adalah sangat rendah dalam kalangan ruminan kecil di bawah Program Ladang Angkat, Fakulti Perubatan Veterinar, UPM.

Kata kunci: *Burkholderia pseudomallei*, Melioidosis, *Complement Fixation Test* (*CFT*), kadar jangkitan, kambing, biri-biri

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project

SEROPREVALENCE OF MELIOIDOSIS AMONG SMALL RUMINANTS IN FOSTER FARM PROGRAMME OF

FACULTY OF VETERINARY MEDICINE,

UPM

By

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2017

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Melioidosis is a saprozoonosis, tropical disease caused by *Burkholderia pseudomallei* which is a ubiquitous soil saprophyte, facultative anaerobic bacteria, non-spore forming, and motile Gram- negative bacillus with bipolar staining, oxidase positive bacteria. This disease can be commonly found in domesticated animals such as sheep, goats, pigs, and other affected species such as cattle, buffaloes, horses, deer, dogs, cats, primates, birds, tropical fish, reptiles and humans. It has public health concern as it is a zoonotic disease and the disease is also a significant animal health problem leading to chronic debility that reduces the productivity of animals. The study farms were selected from the Foster Farm Programme under Faculty of Veterinary Medicine,

UPM. In this study, 100 animals from each species comprises of caprine and ovine were sampled. Blood samples were taken and processed for serology test, Complement Fixation Test (CFT). Complement fixation test opted for qualitative determination of immunoglobulin G, IgG antibodies against *Burkholderia pseudomallei,* whereby the antigen used in this test is prepared by Serology Unit of Veterinary Research Institute Ipoh, Perak. A survey with a set of questionnaire was given to each farm to identify the risk factors related to melioidosis. The data was analysed based on the prevalence rate. Out of 100 goat samples, 1 sample (1%) was positive for antibodies against *Burkholderia pseudomallei* while all the sheep samples were negative (0%) for the antibody. The positive sample from the goat had 0% end point whereby it has a score of +4 of complete button formation. All the negative results shows 100% end point with full lysis, thus no button formation. In addition, analysis of the questionnaire revealed that all the farms supplies treated water to their farm animals except vary in management system; few practices semi-intensive and intensive farming. As a conclusion, the seroprevalence rate of melioidosis is very low among small ruminants under the Foster Farm Programme of Faculty of Veterinary Medicine, UPM.

Keywords: *Burkholderia pseudomallei*, Melioidosis, Complement Fixation Test (CFT), seroprevalence, goat, sheep

1.0 INTRODUCTION

Melioidosis is a saprozoonosis, tropical disease caused by *Burkholderia pseudomallei* which is a ubiquitous soil saprophyte, facultative anaerobic bacteria, non-spore forming, and motile gram negative bacillus with bipolar staining, oxidase positive bacteria which readily grows on routine culture media under aerobic condition ***(Allen, 2005)***. However, *B. pseudomallei* can multiply in the presence of nitrate or arginine under anaerobic condition ***(Yabuuchi et al., 1992)*** and survives in absence of nutrients in distilled water for several years ***(Wuthiekanun et al., 1995)***.

This disease can be commonly found in domesticated animals such as sheep, goats, pigs, and other affected species such as cattle, buffaloes, horses, deer, dogs, cats, primates, birds, tropical fish, reptiles and humans. It has public health concern as it is a zoonotic disease and the disease is also a significant animal health problem leading to chronic debility that reduce the productivity in animals and loss of valuable animal protein due to condemnation of carcasses at the abattoir ***(Ketterer et al., 1986; Choy et al., 2000)***.

The bacterium can be transmitted via percutaneous inoculation, open wound, ingestion or inhalation of pathogen from the contaminated environment (e.g., contaminated soil and surface water). Trans-placental infection has been reported in goats that results in abortion. Laboratory acquired infection and iatrogenic infection via contaminated antiseptics, injections, or other hospital or surgical equipment has been reported (***Merck Veterinary Manual, 10th Edition***).

Incubation period of melioidosis may vary, however, asymptomatic with presence of abscess may occur in goats, sheep and pigs. *B. pseudomallei* infection results in

suppurative or caseous lesions in lymph nodes or other organs. There are ranges of clinical signs including fever, anorexia and lymphadenopathy. In sheep and goats, lung abscesses and pneumonia are commonly found **(*Srikawkheaw, 2007*)**.

The gold standard method for melioidosis diagnosis is isolation and identification of the organism from lesions and discharges which may take 4 to 7 days for identification. The samples collected from animals for isolation are blood, nasal swab, wound exudates, pus or tissues. The organism is readily cultured on routine diagnostic media and a selective media. For immunological method, the most common approach is antibody detection as it is simple and requires minimal laboratory equipment. Effective serologic screening tests include complement fixation test (CFT) and indirect hemagglutination (IHA), Enzyme Link Immuno Serological Assay (ELISA) and immunofluorescence antibody test (IFA) ***(Sirisinha, 2000)***. Serology has always been used for detection *B. pseudomallei* of anti- *Burkholderia* antibodies in horses, goats and dairy cattle in veterinary diagnosis ***(Thomas et al., 1988)***.

In Malaysia, the disease was first reported in 1913 ***(Stanton, 1932)*** which continuously reported among animals and humans since then ***(Strauss et al., 1969; Puthucheary et al., 1992; Vadivelu et al., 1995; Norazah et al., 1996; How et al., 2005; Azizi et al., 2005; Puthucheary, 2009; Deris et al., 2010)***. The seroprevalence data on melioidosis in Malaysia in 2009/2010 was reported highest among sheep in Pahang while goat recorded highest in Selangor ***(Naama, 2011)***. There is no any screening up to date have been recorded on melioidosis status in small ruminant farms of Foster Farm Programme FPV, UPM. Therefore, this project was designed to obtain the current status of seroprevalence of melioidosis and the risk factor and its

association towards the seroprevalence of melioidosis among small ruminant farms in Foster Farm Programme of FPV, UPM.

2.0 LITERATURE REVIEW

2.1 *Burkholderia pseudomallei*

Burkholderia pseudomallei is visualized as Gram-negative, soil dwelling saprophytic motile bacillus, vacuolated, slender and has rounded ends; it is often described as having a "safety pin" appearance that causes melioidosis, an infectious disease that can infect humans and animals. It is a facultative anaerobic bacterium, non-spore forming, oxidase positive bacteria which readily grows on routine culture media under aerobic condition ***(Allen, 2005)***. Pathologist Alfred Whitmore and his assistant C. S. Krishnaswami first described melioidosis as a "glanders-like" disease among morphia addicts in Rangoon, Burma, in 1911 ***(Whitmore, 1913)***. In Malaysia, the disease was first reported in 1913 ***(Stanton, 1932)*** which continuously reported among animals and humans since then ***(Strauss et al., 1969; Puthucheary et al., 1992; Vadivelu et al., 1995; Norazah et al., 1996; How et al., 2005; Azizi et al., 2005; Puthucheary, 2009; Deris et al., 2010)***. It has public health concern as it is a zoonotic disease and the disease is also a significant animal health problem leading to chronic debility that reduce the productivity in animals and loss of valuable animal protein due to condemnation of carcasses at the abattoir ***(Ketterer et al., 1986; Choy et al., 2000)***.

2.2 Virulence of *B. pseudomallei*

Few virulence factors have been described in contribution of *B. pseudomallei* to its pathogenesis. The functions of capsule polysaccharides, lipopolysaccharides, T3SS, flagella, certain quorum-sensing (QS) molecules have been validated. Other factors such as pili, type 6 secretion system (T6SS) and regulatory genes has been studied but indicated a moderate to minor role in virulence ***(Adler et al., 2009).***

Individual strain virulence can be complex as the bacterium able to change its surface determinants and colony morphology. *B. pseudomallei* produces a glycocalyx polysaccharide capsule (biofilm) that, perhaps an important virulence determinant ***(Steinmetz et al., 1995)*** for the formation of microcolonies in a protective environment in which the organism is phenotypically altered, resulting in significant antibiotic resistance ***(Vorachit et al., 1993)***. It can survive within neutrophils and macrophages and resistant towards complement lysosomal defensins and cationic peptidases. Flagella and variable pili expression of *B. pseudomallei* have been demonstrated via electron microscopy study ***(Vorachit et al., 1995)***.

B. pseudomallei also produces proteases, lipases, lechithinase, catalase, perioxidase, superoxide dismutase, hemolysins, a cytotoxic exolipid, and a siderophore which makes it adapted to wide range of hosts. Secretory antigens such as proteases ***(Lee, 2000; Sexton, 1994)***, phospholipase C ***(Korbsrisate et al., 1999)***, and hemolysin, lecithinase, and lipase ***(Ashdown et al., 1990)*** are most likely secreted via the general secretory pathway (type II secretion system) ***(DeShazer et al., 1999)*** but the functions of these antigens are unclear. Type III secretion systems (TTSS) have been defined and bacterial products secreted which denoted as *Burkholderia* secretion apparatus (Bsa) includes BopE that assumed to result in cytoskeletal rearrangement that further facilitates host cell invasion ***(Stevens et al., 2003)***. Capsular polysaccharide (CPS) has significant role in environmental protection ***(Kanai et al., 1994)***, immune system evasion ***(Puthucheary et al., 1996)*** and epithelial cell attachment ***(Ahmed et al., 1999)*** by phagosomal environment protection ***(Pruksachartvuthi, 1990; Smith, 1987).***

Virulence factor	**Role(s)**	**Reference(s)**
Capsule (CPS)	Epithelial attachment; resistance to complement	Ahmed et al. (1999); Reckseidler-Zenteno et al. (2005)
Lipopolysaccharide (LPS)	Resistance to complement and defensins	DeShazer et al. (1998); Burtnick & Woods (1999)
Flagella	Motility	DeShazer et al. (1997)
Pili	Epithelial attachment; microcolony formation	Brown et al. (2002); Essex-Lopresti et al. (2005); Boddey et al. (2006)
Quorum sensing (QS)	Stationary phase gene regulation, including secreted enzymes and oxidative stress protein	Valade et al. (2004); Song et al. (2005); Lumjiaktase et al. (2006)
T3SS3 (Bsa)	Invasion and vacuolar escape	Stevens et al. (2002, 2003); Burtnick et al. (2008)
Morphotype switching	Alteration of surface determinants for in vivo phenotypic changes	Chantratita et al. (2007)

Table 1: Virulence factors and functions of *B. pseudomallei* (*Adler et al., 2009*)

2.3 Epidemiology

Melioidosis is considered endemic in Southeast Asia and Northern Australia, corresponding to the tropical areas within 20°N and 20°S ***(Currie et al., 2008)***.

However, massive outbreaks have occurred in Australia, an outbreak in sheep in 1949 in Winton, Northern Queensland (22ºS) and 159 cases of melioidosis in pigs over 3 years in the Burnett River region (25.5ºS), which were attributed to a contaminated water supply ***(Ketterer et al., 1986)***. The bacterium could be cultured from soil and surface water as it has been successfully isolated in Vietnam ***(Chambon, 1955)***. Rice paddies, rubber plantations and other cleared and cultivated areas have been recorded to have the highest isolation rates ***(Nachiangmai et al., 1985; Strauss et al. 1969)***.

Other factors that may influence environmental distribution include temperature, humidity, rainfall, ultra-violet exposure, soil composition, vegetation, fertilizers and soil disturbance such as excavation or ploughing ***(Inglis et al., 2001)***. Warm climates favour the persistence of *B. pseudomallei* in the environment, however when introduced to a non-endemic area the organism may persist for several years in soil with prolonged nutrient deficiency for up to 10 years ***(Wuthiekanun et al., 1995)***.

It is also capable of surviving antiseptic and detergent solutions ***(Sookpranee et al., 1989)***, acidic environments; pH 4.5 for up to 70 days ***(Dejsirilert et al., 1991)***, and a wide temperature range of 24º to 32°C and dehydration of soil water content of <10% for up to 70 days ***(Chen et al., 2003)*** but not exposure to UV light ***(Tong et al., 1996)***. Moist clay soils seem to be favored by the organism ***(Thomas et al., 1979)***, and irrigated areas have been shown to be favored by the organism in Malaysia and Thailand ***(Nachaingmai et al., 1985)***. The association between surface water and melioidosis is supported by the strong association with monsoonal rains ***(Chaowagul et al., 1989)***.

Melioidosis commonly affects and found in domesticated animals such as sheep, goats and pigs. Other species of animals that are rarely affected are cattle, buffaloes, horses, deer, dogs, cats, primates, birds, tropical fish, reptiles, rodents (in laboratory experiments) and humans.

2.4 Pathophysiology and Pathogenesis

The primary routes of *B. pseudomallei* infection are percutaneous inoculation, inhalation, or aspiration ***(Currie et al., 2000)*** which occurs at epithelial cell layer of abraded skin or mucosal surfaces and infection via ingestion also been described ***(Currie et al., 2001).*** Infected animals, may pass the agent in feces, spread via biting insects, as well as vertical transmission is possible, whereby pregnant goats experienced aborted and infected live born kids ***(Retnasabapathy 1966; Thomas et al. 1988a)***. Capsule which is a thin polysaccharide layer around the bacteria, results in attachment of *B. pseudomallei* to human pharyngeal epithelial cells ***(Ahmed et al., 1999).*** Certain strains have variable temperature at which epithelial attachment at its maximum and formation of pili-mediated microcolonies. The interactions between bacteria are enhanced but not for bacterial-host epithelial cell interactions ***(Brown et al., 2002; Boddey et al., 2006).*** Host actin cytoskeleton rearrangement is initiated by type 3 secretion system (T3SS3) that suggests more than one effector is involved in invasion ***(Stevens et al., 2003)***. *B. pseudomallei* can be observed primarily in vacuoles and later in the cytoplasm following cellular uptake ***(Harley et al., 1994, 1998),*** where bacterial replication takes place ***(Kespichayawattana et al., 2000).***

The activation of TLR2 and TLR4 by *B. pseudomallei* lipopolysaccharide (LPS) and flagella results in recruitment of the innate immune cells such as neutrophils,

macrophages and NK cells. *B. pseudomallei* can proliferate within phagocytes, including neutrophils, monocytes and macrophages without activating a bactericidal response ***(Pruksachartvuthi et al., 1990; Jones et al., 1996)*** where the proliferation of surviving bacteria eventually overpowers the macrophage ***(Nathan & Puthucheary, 2005).*** An enhanced killing of *B. pseudomallei* occurs if the macrophages are activated by interferon-g (IFN-g) ***(Miyagi et al., 1997).*** Chemical inhibitory studies suggest that reactive nitrogen intermediates (RNI) are responsible in macrophage-based *B. pseudomallei* killing ***(Miyagi et al., 1997).*** Reactive oxygen intermediates (ROI) activity is crucial for in vivo macrophage-based killing of *B. pseudomallei* as demonstrated in ROI-deficient mice laboratory studies where more mortality observed over RNI-deficient mice ***(Breitbach et al., 2006).***

T3SS3 plays a function in the evasion of autophagy, a catabolic pathway of one of the innate immune response component ***(Cullinane et al., 2008).*** The bacterium develope mechanisms to inhibit host innate immune responses, by release of ROI and stimulation of autophagy, resulting in increased intracellular survival and replication. Macrophage lysis or apoptosis may occur when abundant *B. pseudomallei* replication has taken place. *B. pseudomallei* extends to neighbouring cells via actin-mediated motility ***(Kespichayawattana et al., 2000; Breitbach et al., 2003)***. BimA, a *B. pseudomallei* autosecreted protein, is essential for actin-mediated intracellular motility that allows the bacteria to move effectively through both epithelial and macrophage cells while avoiding the host immune response, resulting in cell fusion and multinuclear giant cells (MNGC) formation ***(Kespichayawattana et al., 2000).***

The initial bacteremia presentation of melioidosis in goats and sheep is similar but infected sheep develop a severe febrile reaction accompanied by anorexia, lameness and thick, yellow exudate from the nose and eyes ***(Cottew, 1950)***, show signs of central nervous system (CNS) involvement, including lameness, walking in circles, nystagmus, blindness, hyperaesthesia and mild tetanic convulsions ***(Laws and Hall, 1963).*** Pneumonia with respiratory distress can be present. In rams, orchitis with testicular nodules can be seen. The clinical signs observed in goats include fever, anorexia, progressive emaciation, nasal discharge, coughing, salivation, lameness, paresis of the hind legs, severe mastitis, abortion ***(Lewis and Olds, 1952; Sutmőller et al., 1957; Shanta, 1960; Omar, 1963; Retnasabapathy, 1966; Ketterer and Bamford, 1967; Mohna and Arunasalam, 1976; Thomas et al., 1988; Van der Lugt and Henton, 1995)*** or CNS disorders ***(Omar, 1963).*** Apart from that, formation of abscesses and granulomas in superficial lymph nodes especially prescapular lymph nodes are commonly involved and contain greyish yellow, creamy pus ***(Sutmöller et al. 1957)***, lung, and other internal organs. Secondary spread can then occur via the lymphatic vessels, with bacteria probably carried within macrophages, or via the capillary vessels, with bacterial serum resistance mediated by capsule and LPS. As the *B. pseudomallei* infection progresses, the host mounts an adaptive immune response with T cells recruited in response to IFN-g production allowing for a CMI response, and B cells producing antibodies.

Necropsy findings in goats include raised nodules of the nasal septum and turbinates, frequent coalescing and irregular plaques formation. Testes and scrotal sac lesions been described ***(Omar, 1963; Fatimah et al., 1984)***, the heart and kidney are rarely affected ***(Omar, 1963)***. Sheep develop nodules in the lung, liver, spleen, limb

joints and spinal cord ***(Babjee and Nor Aidah, 1994)***. Ulcerated lesions and necrotic nodules are found in the nasal mucosa, on the septum and turbinate bones ***(Cottew, 1950)***. Microscopic lesions in the CNS in sheep and goat can be observed in the brain stem and spinal cord ***(Omar, 1963).*** Aortic aneurysms appear to be frequent in goats ***(Choy et al., 2000).***

2.5 Risk Factors

Studies have revealed that *B. pseudomallei* has highest yields from moist soils and pooled surface water ***(Nachiangmai et al., 1985).*** The relationship between surface water and melioidosis is correlated with monsoonal rains ***(Chaowagul et al., 1989)***. Thus, the management system in a farm that practices extensive, semi-intensive or integrated farming where the animals are exposed to soil or mud, and bushes, has higher chances of contracting the infection. Besides, the water source supplied to the animals also important as treated water supply has lower chances of spreading the organism than groundwater source or stagnant water.

2.6 Diagnosis

B. pseudomallei culturing is currently considered to be the gold standard test for melioidosis in animals. This organism may be found in various lesions including abscesses and wound exudates, milk, feces, throat swabs, blood, urine, and tissues collected at necropsy. It will grow on most media including blood agar. Selective media such as Ashdown's medium are often used in endemic regions. Ashdown's medium selects for gentamicin resistance, a characteristic of most *B. pseudomallei*; however, gentamicin-susceptible strains were recently reported to be common in some areas.

PCR and antigen detection tests (e.g., latex agglutination, immunofluorescence) are also used for identification. There have been multiple reports of the misidentification of *B. pseudomallei* by automated identification systems, it is also possible to mistake this organism for *Pseudomonas* or a contaminant during manual culture and identification. *B. pseudomallei* is a biosafety level 3 organism, and not all laboratories are equipped to safely culture and identify this organism. Other tests that can be used to identify *B. pseudomallei* in clinical samples include antigen detection assays such as direct immunofluorescence, latex agglutination or ELISAs, and PCR tests to detect nucleic acids. The specific tests available can differ between regions, and PCR does not appear to be widely used at present.

Serology is sometimes used to diagnose melioidosis in animals, but it is not considered to be definitive. Animals in endemic areas often have pre-existing titers to this organism. Some of the available serological tests include indirect hemagglutination, immunofluorescence and complement fixation. Environmental samples are sometimes taken from soil or water during outbreaks or case investigations ***(Iowa State University, 2016).***

2.7 Treatment

B. pseudomallei is susceptible to some antibiotics; however, this organism is intrinsically resistant to many drugs, including some that are commonly used to treat bacterial infections. Because relapses can occur when treatment is stopped, animals given antibiotics may need to be monitored afterward. Due to the cost of treatment, most of the infected animals are euthanized ***(Iowa State University, 2016).***

2.8 Prevention and Control

There is no licensed vaccine available for *B. pseudomalllei* for animal nor human. Water hygiene is crucial in endemic areas. Chlorination of water supplies, 2-6 mg/L, with pH under 6-7 prior to treatment can interrupt the infection chain ***(Thomas et al., 1981; Ketterer et al., 1986)***. Infected animals should be removed from the source of contamination ***(Choy et al., 2000)***. Besides, strict control of sewage disposal is essential to prevent the disease spread ***(Inglis et al., 2001).*** Carcasses that are infected for human consumption, should be condemned and destroyed, use of gloves and disinfection of knives in slaughter house is useful in prevention as it reduces contamination ***(Ketterer et al., 1986),*** milk pasteurization from infected animals is recommended ***(Choy et al., 2000).***

Potassium hypochlorite and cresol solutions can be used for disinfection. Infected animals in non-endemic areas should be culled as the bacterium needs a subclinically infected carrier animal to survive adverse climate, resistant to antibiotics, and possible zoonosis ***(Sprague et al., 2004)***. A routine environment collection for bacteriology will help in the disease surveillance and control. Treatment of soil with lime has been previously reported by ***Na-ngam et al. (2004)*** and ***Sommanustweechai et al. (2013)*** as control against melioidosis. Lime treatment alters the soil pH by increasing alkalinity which unfavourable for the survival of *B. pseudomallei*. Extreme acidity or alkalinity have been shown to adversely affect the survival of *B. pseudomallei* ***(Chen et al., 2003).***

2.9 Zoonotic cases in Malaysia

Far back in 1913, Stanton and Fletcher noticed animal cases at Institute of Medical Research of the Federated Malay States and thus published reports on subsequent human and animal cases in 1932. Both Peninsular and East Malaysia were involved and in 1992, there were 50 septicemic reviewed cases described ***(Puthucheary et al., 1992)*** in Kuala Lumpur and denoted a total of 85 cases from June 1976 to June 1991. In 1964 to 1966, a serosurvey conducted in Malaysia revealed that there was a 7.3% seropositivity (indirect hemagglutination assay [IHA] titers of >1:40), with the highest rates in recruits from Kedah and Sabah ***(Allen et al., 2005)***.

3.0 MATERIALS AND METHODS

3.1 Study design

A preliminary study was designed to investigate the seroprevalence of melioidosis among small ruminants which includes goats and sheep in Foster Farm Programme of Faculty of Veterinary Medicine, Universiti Putra Malaysia. Besides, study includes identification of the risk factors and its association to melioidosis in the respective foster farms.

3.2 Study population and sampling frame

The study farms were selected from the foster farms under the Large Animal Unit of Faculty of Veterinary Medicine, UPM. The study population comprised of small ruminant farms around Selangor and Negeri Sembilan area with unknown melioidosis screening results. The sampling frame consisted of 100 animals from each species comprises of caprine and ovine where by random-convenient sampling was done.

3.3 Study area

Small ruminant farms were particularly targeted for the study because sheep and goats were especially more susceptible to melioidosis ***(Choy et al., 2000)***. Five goat farms (Serdang, Dengkil, Hulu Langat, Sepang and Nilai) and 3 sheep farms (Serdang, Dengkil and Hulu Langat) were selected based on the species and the standing population.

3.4 Sample collection

Blood samples were collected from 5 goat farms and 3 sheep farms which sums to 100 animals respectively under Foster Farm Programme of Faculty of Veterinary Medicine, UPM. All the farms are located in Selangor and Negeri Sembilan (Farm A: Serdang, Farm B: Dengkil, Farm C: Hulu Langat, Farm D: Sepang, Farm E: Nilai).

Each goat and sheep were physically restrained, and the site of jugular venipuncture was disinfected using 70% alcohol swab. 21G, 1 inch vacutainer needle was used and the blood sample, approximately 3ml was collected in plain blood collection tube. All the samples were labelled accordingly and the samples were transported in an ice box to laboratory for further procedures.

In laboratory, serum separation was processed by centrifugation of the collected blood in a centrifuge machine with 3000 revolutions per minute (rpm) for 5 minutes. Then, the serum was drawn using an Eppendorf micropipette and transferred into 1.5ml Eppendorf tubes which were labelled accordingly. Duplicate of each serum sample was performed to be stored in two different temperatures which are -20°C and -80°C.

Questionnaire related to management, biosecurity and medical history of each farm was given to the farm owner or worker (Appendix A).

3.5 Diagnosis of *Burkholderia pseudomallei*

Complement fixation test (CFT) was the diagnostic test used in screening and monitoring of the levels of antibodies against *B. pseudomallei* in animals. The confirmation of diagnosis is by isolation and identification of the agent from biological

samples such as blood, pus and other exudates from suspected animals. Both tests are performed at the reference laboratory in VRI. CFT was preferred as screening test because of its high specificity in serodiagnosis of melioidosis ***(Thomas et al. 1988).*** The sensitivity of the CFT in for diagnosis of melioidosis in animals was estimated to be between 79.3 and 82.4% while its specificity was between 99.5 and 100%, which qualifies the test to be a good screening test ***(Thomas et al. 1988, 1990).*** On the other hand, the culture and identification method was the preferred confirmatory test because it remains the gold standard in the diagnosis of melioidosis ***(Inglis et al. 2006; Meumann et al. 2012).***

3.5.1 Complement Fixation Test

The primary materials required to run the test are test serum, one known negative serum, one known positive serum, diluent glucose normal saline (GNS), diluent and standard solutions; Melioidosis antigen, Melioidosis complement and hemolytic system.

Serum was inactivated at 63°C in water bath for 50 minutes. ¾ of row H was filled with inactivated serum. 25 µl diluent was added into wells A (control well to replace the Melioidosis antigen), C, D, E, F, and G. Titration was performed from well H to G, F, E, D, and C. Then, 25 µl of inactivated serum from well H was transferred into the well A (control well). Standardized 25 µl of Melioidosis antigen was added into wells C, D, E, F, and G. 25 µl of the Melioidosis complement was added to well A, C, D, E, F, and G. Then, incubate the plates at 37°C for first 15 minutes. Next, 25 µl of the haemolytic system was added to wells A (control well), C, D, E, F, and G. All the content in the plates were mixed using shaker and incubated at 37°C for another 15

minutes. All the plates were shaken and left to settle at room temperature for 2-3 hours before reading the result.

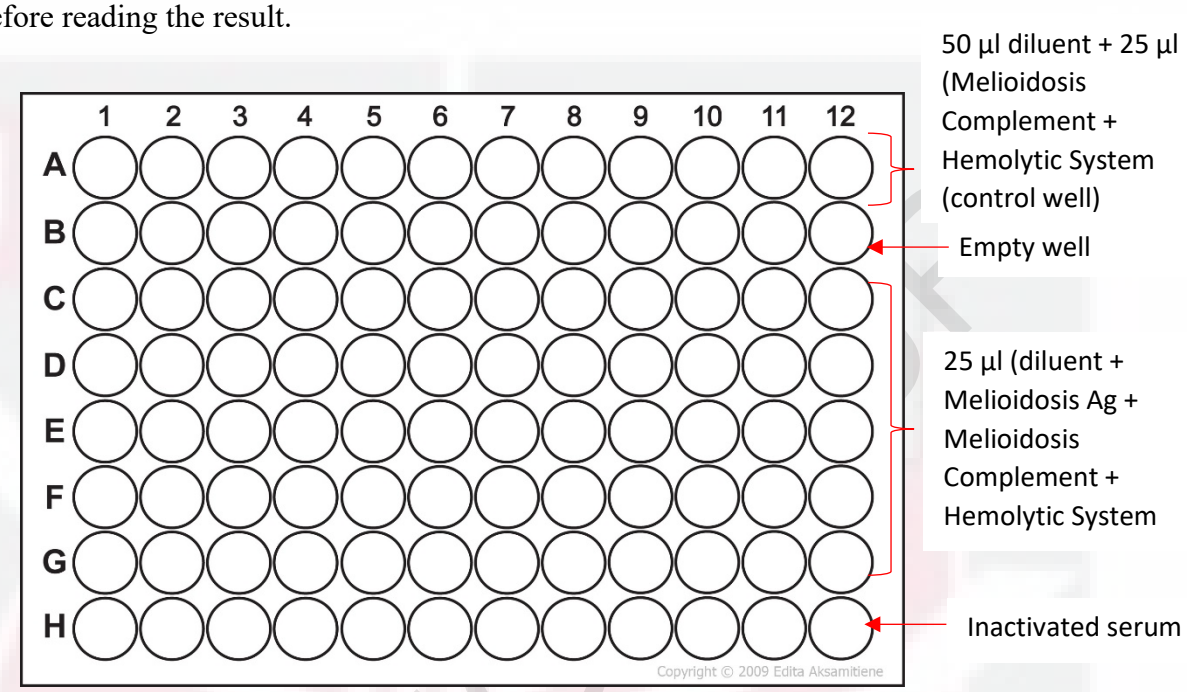


Figure 1: 96-well-plate illustration for Melioidosis Complement Fixation Test

Well No	Dilution
A	1/64
B	(empty)
C	1/32
D	1/16
E	1/8
F	1/4
G	1/2
H	Inactivated serum

Table 2: Titration for each row on the 96-well-plate

3.5.2 Interpretation of Result

Serum control well in Row A that contain no antigen should not give fixation of complement, therefore complete lysis shall occurred. If not, the serum can be considered as Anti-Complementary (A/C) if there is 50% lysis in the dilution of ¼. In positive well, there will be fixation of complement (button formation) while in negative well, the result shows no fixation (complete lysis, no button formation).

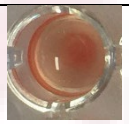
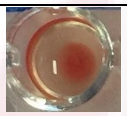
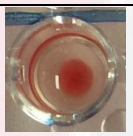
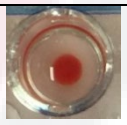
Lysis (%)	Score	Fixation
100	-	
75	+1	
50	+2	
25	+3	
0	+4	

Table 3: Complement fixation with the lysis percentage and the scoring

3.6 Questionnaire data

The data obtained from the questionnaire (Appendix A) were subjected to prevalence analysis to know the risk factors of the sampled farm contributing to melioidosis.

4.0 RESULTS

4.1 Interpretation of Complement Fixation Test

Complement Fixation Test (CFT) of the collected sera samples of goats and sheep revealed that there were positive and negative samples (Figure 2).

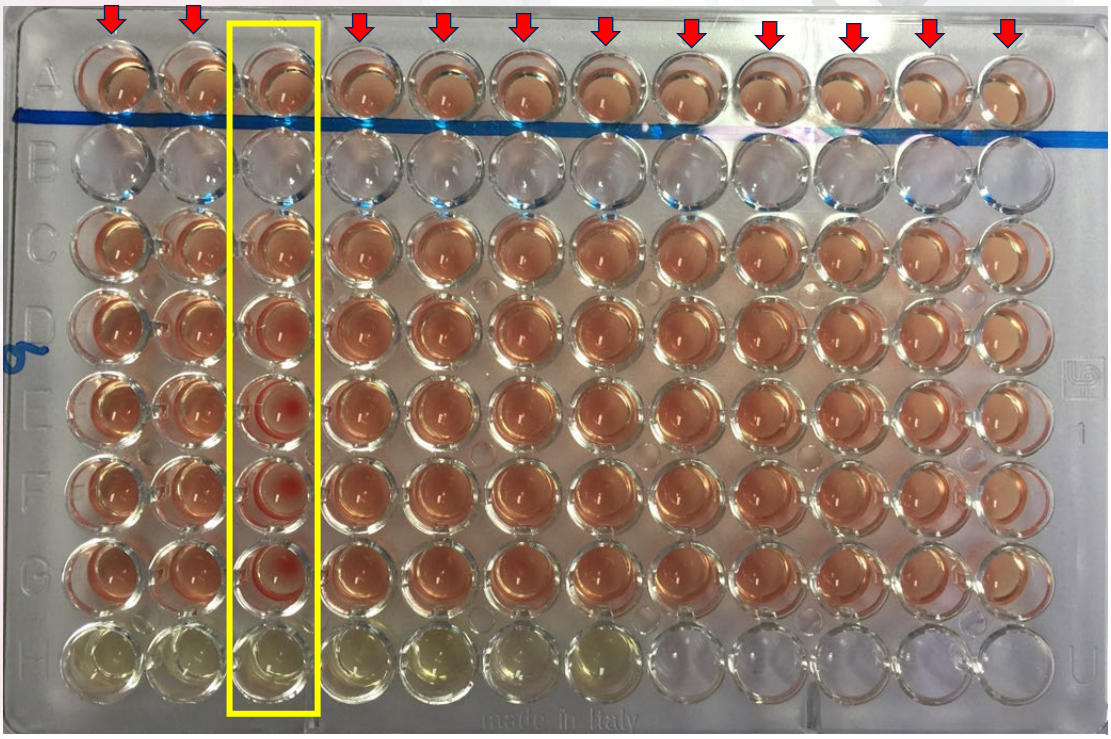


Figure 2: The Complement Fixation Test (CFT) plate shows a positive reaction (yellow box) of complete fixation (0% lysis) on the third row from the left at titration level of 1:2,1:4, and 1:8 indicative of presence of antibodies against *B. pseudomallei* in that individual. The rest of the columns (red arrow) shows negative reaction of zero fixation (100% lysis) indicative of absence of antibodies against *B. pseudomallei* in that respective individuals.

From 100 blood samples of goat, 1 sample (1%) was found to be positive for antibodies against *B. pseudomallei* while the rest 99 samples (99%) were negative.

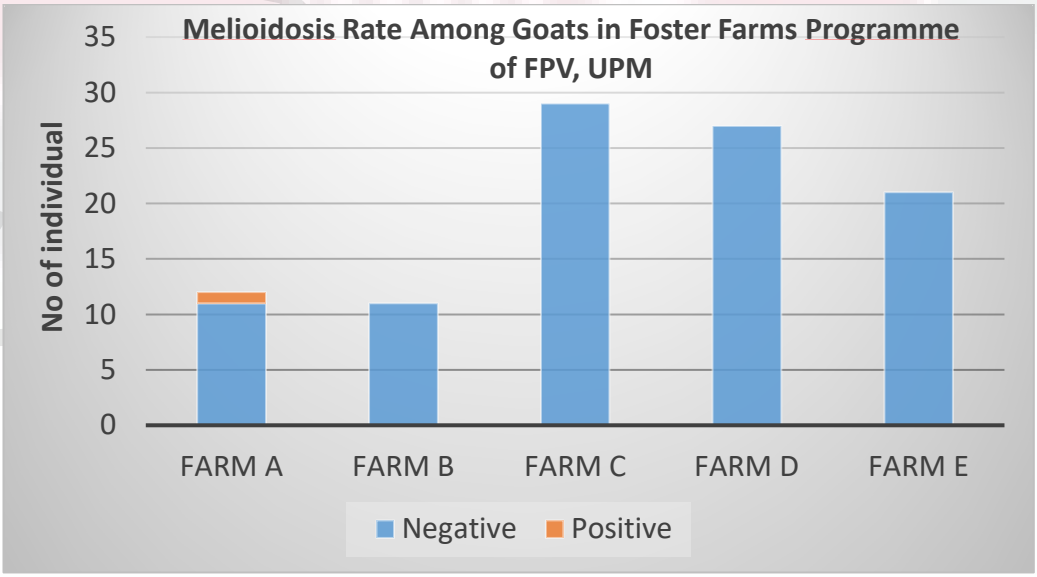
From 100 blood samples of sheep, all samples (100%) were negative antibodies against *B. pseudomallei*. The positive sample for *B. pseudomallei* was traced back to Farm A in Serdang. Based on the grading of complement fixation, the serum sample has a score of +4 where it had 0% lysis which means presence of complete complement fixation. Other negative samples were scored to be 100% lysis ***(VRI Serology Unit Test Manual, 2015)***.

4.2 Prevalence Rate of Melioidosis

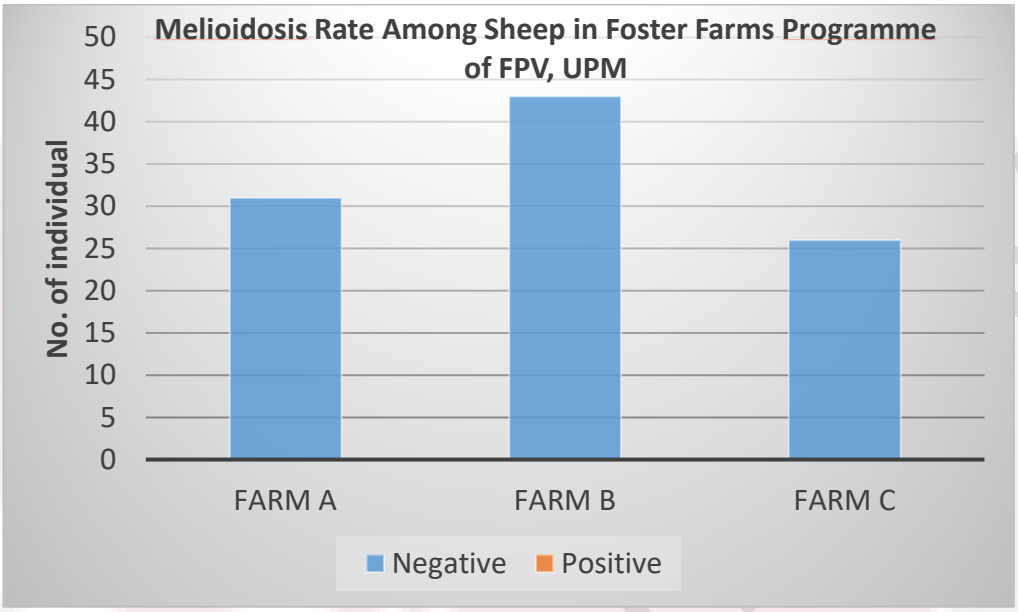
The prevalence rate was calculated based on the formula below:

$$\text{Prevalence Rate} = \frac{\textit{All new \& pre existing cases of a specific disease during a given time interval}}{\textit{Total population during the time period}} \text{ x } 100\%$$

There was 1% seroprevalence observed among 5 goat farms under the Foster Farm Programme of FPV which is from Farm A while zero seroprevalence observed among 3 sheep farms under the Foster Farm Programme of FPV.



Graph 1: The graph above shows the Melioidosis rate among goat population in 5 farms under the Foster Farm Programme of FPV, UPM which located within Selangor and Negeri Sembilan. 99 animals showed negative except 1 animal was positive for Meliodosis.



Graph 2: The graph above shows the Melioidosis rate among sheep population in 3 farms under the Foster Farm Programme of FPV, UPM which located within Selangor and Negeri Sembilan. All animals, 100 sheep showed negative for Melioidosis.

4.3 Questionnaire data

The answers obtained from the respondents which served as description of herd study were tabulated (Table 4). Based on the answers regarding the farm's management, the location of the goat study herds located 80% (4/5) in Selangor and 20% (1/5) in Negeri Sembilan while location of the sheep study herds located 100% (3/3) in Selangor. All of the farms were private owned goat and sheep farms; 40% (2/5) of the goat farms practice semi-intensive management system and 60% (3/5) practices intensive management system; 66.7% (2/3) of the sheep farms practice semi-intensive management system and 33.3% (1/3) practices intensive management system. All of the farms in this study were housed in raised floor with slatted flooring goat and sheep houses. 60% (3/5) of the sampled goat farms rear sheep and 40% (2/5)

do not rear goat alongside other animal species. 100% (3/3) of the sampled sheep farms rear goat that are housed side by side.

For the questions related to the biosecurity of the farm; 80% (4/5) goat farms do import animals from other countries such as Australia, and Indonesia while 20% (1/5) does not practice importation of animals; 100% (3/3) sheep farms do import animals from Australia and purchased locally. 80% (4/5) goat farms and 100% (3/3) sheep farms have isolation or quarantine facilities in their farms; they quarantine newly arrived or sick animals. 100% of goat and sheep farms clean their animal houses in daily basis and all farms do not use disinfectant when cleaning. All the farms cuts the grasses and clear nearby bushes regularly, once every 2 weeks. The farms do not practice soil liming at any point of time and supplies treated water to the animals. Besides, all the farms do not perform disease monitoring or screening annually or keeps good herd records.

For the previous farm medical history, 100% of both goat and sheep farms have respiratory related problems and worm problems. There is no specific record for melioidosis disease in the farms sampled. All the farms seek treatment from veterinarians from FPV, UPM but 80% also practices self-medication for their sick animals.

GOAT						
Name	Management	Water source	Bush/Grass Maintenance	Soil Liming	Importation	Results
Farm A	Semi-intensive	Treated water	/	-	/	+
Farm B	Semi-intensive	Treated water	/	-	/	-
Farm C	Intensive	Treated water	/	-	-	-
Farm D	Intensive	Treated water	/	-	/	-
Farm E	Intensive	Treated water	/	-	/	-
Total						100
SHEEP						
Farm A	Semi-intensive	Treated water	/	-	/	-
Farm B	Semi-intensive	Treated water	/	-	/	-
Farm C	Intensive	Treated water	/	-	/	-
Total						100

Table 4: Herd description based on questionnaire

5.0 DISCUSSION

From this study, only one positive sample was detected out of 100 samples (1%) negative for all samples (0%) for sheep sampled under Foster Farm Programme of Faculty of Veterinary Medicine (FPV), UPM. Complement fixation test (CFT) was performed to diagnose melioidosis among the small ruminants sampled at Veterinary Research Institute (VRI) Ipoh, which is a serological test against antibodies of *B. pseudomallei*. The significant result of this test is the score of the complement fixation to the antibodies present in the sera.

The findings of this study revealed that Farm A which located in Serdang revealed it has only 1% of seroprevalence rate among goats, where other sampled animals inclusive ovine are negative. These findings were in agreement with the hypothesis of the study where there will be low seroprevalence of melioidosis among small ruminants in Foster Farm Programme of FPV, UPM.

Malaysia is known to be one of the endemic countries for melioidosis disease outbreak worldwide as the organism is a natural ubiquitous soil-dwelling saprophyte bacterium. The climate in Malaysia is very favourable for the survival of the organism whereby the average humidity is 70-75%, average temperature is 27°C and average rainfall of 250 mm annually. *B. pseudomallei* can survive a wide temperature range of 24° to 32°C and dehydration of soil water content of <10% for up to 70 days ***(Chen et al., 2003)*** but not exposure to UV light ***(Tong et al., 1996)***. Epidemiological studies have defined an annual incidence rate in the Top End of the Northern Territory which were associated with two severe weather events and high annual rainfall ***(Currie et al., 2004)***.

For the findings from Farm A, only one blood sample was positive for melioidosis. Upon investigation on the farm practices, the farm manages a semi-intensive farm management system whereby the animals are let to graze at 10 am till 5 pm and the back to respective houses. This exposes the animals to soil and excess to mud and bushes. Moist clay soils seem to be favored by the organism ***(Thomas et al., 1979)***, and irrigated areas have been shown to be favored by the organism in Malaysia and Thailand ***(Nachiangmai et al., 1985)***. Treatment of soil with lime has been previously reported by ***Na-ngam et al. (2004)*** and ***Sommanustweechai et al. (2013)*** as control against melioidosis. Lime treatment alters the soil pH by increasing alkalinity which unfavourable for the survival of *B. pseudomallei*. Extreme acidity or alkalinity have been shown to adversely affect the survival of *B. pseudomallei* ***(Chen et al., 2003).***

The water source supplied to the animals are all treated water by the state government of Selangor and Negeri Sembilan which already been treated in water treatment plant, thus it reduces possibility of spread of disease compared to groundwater source or stagnant water. Chlorination of water supplies, 2-6 mg/L, with pH under 6-7 prior to treatment can interrupt the infection chain ***(Thomas et al., 1981; Ketterer et al., 1986)***. The farm does not practice soil liming as a routine biosecurity programmme.

Farm A also imports animal from endemic country which is Australia which could be a possibility for the positive reaction. Poor record keeping of the farm regarding purchase of animals and history of seller farm may contribute to this for introduction of infected to healthy animals. Disease monitoring involves a systematic series of investigations of a given population of animals to detect changes in the prevalence and

geographical distribution of disease which may involve testing samples of a population. Farm A has no past history of performing any health or disease screening. As stated in the present study by ***Jesse et al. (2015),*** only 54% of the farms has compliance to disease monitoring programme in the farm.

In overall, all the farm practices good feeding and sanitary management that may explains the low seroprevalence of melioidosis disease among the small ruminants. Satisfactory quarantine protocol of imported livestock in Malaysia and disease surveillance by the Department of Veterinary Services contribute to the controlled state of the disease. However, this may be also due to small and unequal sample size, random convenient sampling, and short duration of the study were involved to complete the project.

6.0 CONCLUSION AND RECOMMENDATION

As a conclusion, the seroprevalence rate of melioidosis disease among goats from selected farms under Foster Farm Programme of FPV, UPM is 1% while for sheep is 0%, which is a low occurrence rate. This disease should be considered as an emerging disease with high impact on animal and human. The detection of the agent or organism is a major challenge as it mimics other respiratory disease such as pneumonia.

Recommendations for the farmer are to isolate possibly sick animals away from the healthy animals, infected animals in non-endemic areas should be culled as the bacterium needs a subclinically infected carrier animal to survive adverse climate, resistant to antibiotics, and possible zoonosis ***(Sprague et al., 2004)***, milk from the infected animals has to be pasteurized before sold to consumers ***(Choy et al., 2000)***, critical review of the seller farm history especially from melioidosis endemic countries and to strengthen the farm biosecurity by performing annual disease screening, a routine environment collection for bacteriology will help in the disease surveillance and control. Treatment of soil with lime has been previously reported by ***Na-ngam et al. (2004)*** and ***Sommanustweechai et al. (2013)*** as control against melioidosis. Lime treatment alters the soil pH by increasing alkalinity which unfavourable for the survival of *B. pseudomallei*.

Recommendations for future research in melioidosis are to increase the sample size with equal sample size from each farm, longer duration of study with proper random sampling on the animals in different month or seasons, use the gold standard for diagnosis which is isolation and identification of *B. pseudomallei* from various

samples such as serum, soil and water. This is to ensure a better comparison of the disease in regards to the risk factors and true prevalence rate.

7.0 REFERENCES

Adler N. R. L., Govan B., cullinae M., Harper M., Adler B., Boyce J. D. 2009: The molecular and cellular basis of pathogenesis in melioidosis: how does *Burkholderia pseudomallei* cause disease? FEMS Microbiol Rev 33 (2009) 1079–1099.

Ahmed, K., H. D. Enciso, H. Masaki, M. Tao, A. Omori, P. Tharavichikul, and T. Nagatake. 1999. Attachment of *Burkholderia pseudomallei* to pharyngeal epithelial cells: a highly pathogenic bacteria with low attachment ability. Am. J. Trop. Med. Hyg. 60:90–93.

Allen, C. C., and Currie B. J. 2005. Melioidosis: Epidemiology, Pathophysiology and Management. American Society for Microbiology Apr. 2005, p. 383-416.

Ashdown, L. R., and J. M. Koehler. 1990. Production of hemolysin and other extracellular enzymes by clinical isolates of *Pseudomonas pseudomallei*. J. Clin. Microbiol. 28:2331–2334.

Azizi Z.A., Yahya M. and Lee S.K. 2005. Melioidosis and the vascular surgeon: Hospital Kuala Lumpur experience. Asian Journal of Surgery 28(4): 309-311.

Babjee, M. A., and A. R. Nor Aidah, 1994: Melioidosis in animals. In: Puthucheary, S. D., and Y. A. Malik (eds), Melioidosis: Prevailing Problems and Future Directions. pp. 112–122. SP-Muda Printing, Kuala Lumpur.

Boddey JA, Flegg CP, Day CJ, Beacham IR & Peak IR 2006. Temperature-regulated microcolony formation by *Burkholderia pseudomallei* requires pilA and enhances association with cultured human cells. Infect Immun 74: 5374–5381.

Breitbach K, Rottner K, Klocke S, Rohde M, Jenzora A,Wehland J & Steinmetz I 2003. Actin-based motility of *Burkholderia pseudomallei* involves the Arp 2/3 complex, but not N-WASP and Ena/VASP proteins. Cell Microbiol 5: 385 393.

Breitbach K, Klocke S, Tschernig T, van Rooijen N, Baumann U & Steinmetz I 2006. of inducible nitric oxide synthase and NADPH oxidase in early control of *Burkholderia pseudomallei* infection in mice. Infect Immun 74: 6300–6309.

Brown, N. F., J. A. Boddey, C. P. Flegg, and I. R. Beacham. 2002. Adherence of *Burkholderia pseudomallei* cells to cultured human epithelial cell lines is regulated by growth temperature. Infect. Immun. 70:974–980.

Chambon, L. 1955. Isolement du bacille de Whitmore a partir du milieu exterieur. Ann. Inst. Pasteur 89:229–235.

Chen, Y., Chen, S., Kao, C. and Chen, Y. 2003. Effects of soil pH, temperature and water content on the growth of *Burkholderia pseudomallei*. Folia Microbiol (Praha) 48, 253–256.

Choy, J. L., M. Mayo, A. Janmaat and B. J. Currie. 2000. Animal melioidosis in Australia. Acta Trop. 74: 153-158.

Cottew, G. S. 1950. Melioidosis in sheep in Queensland. A description of the causal organism. Aust. J. Exp. Biol. Med. Sci. 28:677–683.

Cullinane M, Gong L, Li X, Lazar-Adler N, Tra T, Wolvetang E, Prescott M, Boyce JD, Devenish RJ & Adler B (2008) Stimulation of autophagy suppresses the

intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines. Autophagy 4: 744–753.

Currie, B. J., D. A. Fisher, D. M. Howard, J. N. Burrow, D. Lo, S. Selva- Nayagam, N. M. Anstey, S. E. Huffam, P. L. Snelling, P. J. Marks, D. P. Stephens, G. D. Lum, S. P. Jacups, and V. L. Krause. 2000. Endemic melioidosis in tropical northern Australia: a 10-year prospective study and review of the literature. Clin. Infect. Dis. 31:981–986.

Currie, B. 2001. Environmental change, global warming and infectious diseases in northern Australia. Environ. Health 1:35–44.

Currie, B., J. and S. P. Jacups. 2003. Intensity of rainfall and severity of melioidosis, Australia. Emerg. Infect. Dis. 9: 1538-1542.

Currie, B. J., S. P. Jacups, A. C. Cheng, D. A. Fisher, N. M. Anstey, S. E. Huffam, and V. L. Krause. 2004. Melioidosis epidemiology and risk factors from a prospective whole-population study in northern Australia. Trop. Med. Int. Health 9:1167–1174.

Currie B.J., Dance D.A.B. and Cheng A.C. 2008. The global distribution of *Burkholderia pseudomallei* and melioidosis: an update. Transactions of the Royal Society of Tropical Medicine and Hygiene 102(Suppl. 1): S1-S4.

Dejsirilert, S., E. Kondo, D. Chiewslip, and K. Kanai. 1991. Growth and survival of *Pseudomonas pseudomallei* in acidic environments. Jpn. J. Med. Sci. Biol. 44:63–74.

Deris Z.Z., Hasan H. and Siti Suraiya M.N. 2010. Clinical characteristics and outcomes of bacteraemic melioidosis in a teaching hospital in a northeastern state of Malaysia: a five-year review. The Journal of Infection in Developing Countries 4(07): 430-435.

DeShazer, D., P. J. Brett, M. N. Burtnick, and D. E. Woods. 1999. Molecular characterization of genetic loci required for secretion of exoproducts in *Burkholderia pseudomallei*. J. Bacteriol. 181:4661–4664.

Fatimah, I., B. O. Ikede, and R. A. Mutalib, 1984: Granulomatous orchitis and periorchitis caused by *Pseudomonas pseudomallei* in a goat. Vet. Rec. 114, 67–68.

Harley VS, Dance DA, Tovey G & Drasar BS 1994. Interaction of *Pseudomonas pseudomallei* with macrophages. Biochem Soc T22: 88S.

Harley, V. S., D. A. Dance, B. S. Drasar, and G. Tovey. 1998. Effects of *Burkholderia pseudomallei* and other *Burkholderia* species on eukaryotic cells in tissue culture. Microbios 96:71–93.

How S., Ng K., Jamalludin A., Shah A. and Rathor Y. 2005. Melioidosis in Pahang, Malaysia. The Medical Journal of Malaysia 60(5): 606.

Inglis, T.J.J., Mee, B.J. and Chang, B.J. 2001. The environmental microbiology of melioidosis. Rev Med Microbiol 12, 13–20.

.

Inglis, T.J.J. and Sagripanti, J.L. 2006. Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. Appl Environ Microbiol 72, 6865–6875.

Iowa State University College of Veterinary Medicine 2016: Melioidosis. Retrieved from http://www.cfsph.iastate.edu/Factsheets/pdfs/melioidosis.pdf

Jesse F. F., Abdul M. R., Abdulnasir T., Eric L. T. C., Konto M., Muhammad A. S., Abdul A. S., Yusuf A. 2015: Survey of Goats Farmers' Compliance on Proper Herd Health Program Practices. International Journal of Livestock research. Vol 5(11) Nov 15.

Kanai, K., and E. Kondo. 1994. Recent advances in biomedical sciences of *Burkholderia pseudomallei* (basonym: *Pseudomonas pseudomallei*). Jpn. J. Med. Sci. Biol. 47:1–45.

Ketterer, P. J., and V. W. Bamford, 1967: A case of lameness in lambs in south Western Australia. Aust. Vet. J. 43, 79–80.

Ketterer, P. J., W. R. Webster, J. Shield, R. J. Arthur, P. J. Blackall and A. D. Thomas. 1986. Melioidosis in intensive piggeries in south eastern Queensland. Aust. Vet. J. 63: 146-149.

Kespichayawattana, W., S. Rattanachetkul, T. Wanun, P. Utaisincharoen, and S. Sirisinha. 2000. *Burkholderia pseudomallei* induces cell fusion and actin associated membrane protrusion: a possible mechanism for cell-tocell spreading. Infect. Immun. 68:5377–5384.

Korbsrisate, S., N. Suwanasai, A. Leelaporn, T. Ezaki, Y. Kawamura, and S. Sarasombath. 1999. Cloning and characterization of a nonhemolytic phospholipase C gene from *Burkholderia pseudomallei*. J. Clin. Microbiol. 37:3742–3745.

Laws, L., and W. T. K. Hall. 1963. Melioidosis in animals in north Queensland. 1. Incidence and pathology, with special reference to central nervous system lesions. Qeensland J. Agric. Sci. 20:499–513.

Lee, M., and Y. Liu. 2000. Sequencing and characterization of a novel serine metalloprotease from *Burkholderia pseudomallei.* FEMS Microbiol. Lett. 192:67–72.

Melioidosis. Generalized Condition, pages 602-604. The Merck Veterinary Manual, 10th Edition (2011) Published by Gary Zelko. ISBN 0-911910-93-X.

Meumann, E.M., Cheng, A.C., Ward, L. and Currie, B.J. 2012. Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. Clin Infect Dis 54, 362–369.

Miyagi K, Kawakami K & Saito A 1997. Role of reactive nitrogen and oxygen intermediates in gamma interferon-stimulated murine macrophage bactericidal activity against *Burkholderia pseudomallei*. Infect Immun 65: 4108–4113.

Mohna, S. S. S., and V. Arunasalam, 1976: Melioidosis in two goats. Mal. Vet. J. 6, 80–81.

Musa H.I., Hassan L., Rachmat R.F.N., Chandrawathani P., Zunita Z., Saleha A.A. 2012. Seroprevalence of Melioidosis among Livestock in Malaysia from 2000 2009.

Na-ngam, N., Angkititakul, S., Noimay, P. and Thamlikitkul, V. 2004. The effect of quicklime (calcium oxide) as an inhibitor of *Burkholderia pseudomallei.* TransR Soc Trop Med Hyg 98, 337–341.

Naama T., Khor S.K., Zainab Z., Mazlan L., Chin S.W., Azmaliza A.A., Nor Ashikin M.S., Amina Khadariah A.L., Surayani A.R., Vijaan G., Ramlan M.2010. Diagnosis of Leptospirosis, Brucellosis and Melioidosis Diseases in Human Conducted in VRI.

Naama T., Chin S.W., Mazlan L., Surayani A.R., Zainab Z., Aminah Khadariah A.L., Vijaan G., Azmaliza A.A., Nor Ashikin M.S., Nurul Fatiha A.S., Ramlan M. 2011. Melioidosis in Sheep and Goat: Diagnostic Cases in the Veterinary Research Institute from 2009 to 2010.

Nachiangmai, N., P. Patamasucon, B. Tipayamonthein, A. Kongpon and S. Nakaviroj. 1985. *Pseudomonas pseudomallei* in southern Thailand. Southeast Asian J. Trop. Med. Public Health 16: 83-87.

Nathan SA & Puthucheary SD. 2005. An electronmicroscopic study of the interaction of *Burkholderia pseudomallei* and human macrophages. Malays J Pathol 27: 3–7.

Omar, A. R. 1963. Pathology of melioidosis in pigs, goats and a horse. J. Comp. Pathol. 73:359–372.

Pruksachartvuthi, S., N. Aswapokee, and K. Thankerngpol. 1990. Survival of *Pseudomonas pseudomallei* in human phagocytes. J. Med. Microbiol. 31:109 114.

Puthucheary, S. D., N. Parasakthi, and M. K. Lee. 1992. Septicaemic melioidosis: a review of 50 cases from Malaysia. Trans. R. Soc. Trop. Med. Hyg. 86:683 685.

Puthucheary S.D., Vadivelu J., Gendeh G.S., Parasakthi N.1995 Serodiagnosis of Melioidosis in Malaysia. Singapore Medical Journal. Vol 36:299-302

Puthucheary S. 2009. Melioidosis in Malaysia. The Medical Journal of Malaysia 64(4): 266.

Rasana W. Sermswan, Phairat Royros, Nittaya Khakhum, Surasakdi W., Apichai T. 2015. Direct detection of *Burkholderia pseudomallei* and biological factors in soil.

Retnasabapathy, A., and P. G. Joseph, 1966: A case of melioidosis in a macaque monkey. Vet. Rec. 79, 72–73.

Sexton, M. M., A. L. Jones, W. Chaowagul, and D. E. Woods. 1994. Purification and characterization of a protease from *Pseudomonas pseudomallei*. Can. J. Microbiol. 40:903–910.

Shanta, S. C., 1960: A note on the isolation of *Pf. whitmori* from the large intestine of a goat. J. Mal. Vet. Med. Ass. 3, 35–38.

Sirisinha, S., N. Anuntagool, T. Dharakul, P. Ekpo, S. Wongratanacheewin, P. Naigowit, B. Petchclai, V. Thamlikitkul, Y. Suputtamonkol. 2000. Recent development in laboratory diagnosis of melioidosis. Acta Tropica. 74:235-245.

Smith, C., J. Allen, M. Embi, O. Othman, N. Razak, and G. Ismail. 1987. Human melioidosis; an emerging medical problem. MIRCEN J. 3:343–366.

Sommanustweechai, A., Kasantikul, T., Somsa, W., Wongratanacheewin, S., Sermswan, R.W., Kongmakee, P., Thomas, W., Kamolnorranath, S. et al. 2013. Environmental management procedures following fatal melioidosis in a captive chimpanzee (*Pan troglodytes*). J Zoo Wildl Med 44, 475–479.

Sookpranee, M., P. Lumbiganon, S. Puapermpoonsiri, A. Tattawasatra, and J. Nopwinyoovongs. 1989. Contamination of Savlon solution with *Pseudomonas pseudomallei* at Srinagarind Hospital., p. 211–213. *In* S. Punyagupta, T. Sirisanthana, and B. Stapatayavong (ed.), Melioidosis. Bangkok Medical Publisher, Bangkok, Thailand.

Sprague L. D., and Neubauer H. 2004: Melioidosis in Animals: A review on epizootiology, diagnosis and clinical presentation. J. Vet. Med. B 51, 305-320, Blackwell Verlag, Berlin.

Srikawkheaw, N. and Lawhavinit, O.-A. 2007. Detection of antibodies against melioidosis from animal sera in Thailand by indirect haemagglutination test. Kasetsart J Nat Sci 41, 81–85.

Stanton A.T. and Fletcher W. 1932. Melioidosis. Studies from the Institute for Medical Research, Federated Malay States.

Steinmetz, I., M. Rohde, and B. Brenneke. 1995. Purification and characterization of an exopolysaccharide of *Burkholderia* (*Pseudomonas*) *pseudomallei*. Infect. Immun. 63:3959–3965.

Stevens, M. P., A. Friebel, L. A. Taylor, M. W. Wood, P. J. Brown, W. D. Hardt, and E. E. Galyov. 2003. A *Burkholderia pseudomallei* type III secreted protein, BopE, facilitates bacterial invasion of epithelial cells and exhibits guanine nucleotide exchange factor activity. J. Bacteriol. 185:4992–4996.

Strauss J., Ellison D., Gan E., Jason S., Marcarelli J. and Rapmund G. 1969. Melioidosis in Malaysia. IV. Intensive ecological study of Carey Island, Selangor, for *Pseudomonas pseudomallei.*

Sutmoller, P., F. C. Kraneveld, and A. van der Schaaf. 1957. Melioidosis (pseudomalleus) in sheep, goats and pigs on Arubu (Netherland Antilles). J. Am. Vet Med. Assoc. 130:415–417.

Tong, S., S. Yang, Z. Lu, and W. He. 1996. Laboratory investigation of ecological factors influencing the environmental presence of *Burkholderia pseudomallei*. Microbiol. Immunol. 40:451–453.

Thomas, A. D., J. Forbes Faulkner, and M. Parker. 1979. Isolation of *Pseudomonas pseudomallei* from clay layers at defined depths. Am. J. Epidemiol. 110:515 521.

Thomas, A. D., J. H. Norton, J. C. Forbes-Faulknerand G. Woodland. 1981. Melioidosis in an intensive piggery. Aust. Vet. J. 57: 144-145.

Thomas, A. D., G. A. Spinks, T. L. D'Arcy, J. H. Norton, and K. F. Trueman. 1988. Evaluation of four serological tests for the diagnosis of caprine melioidosis. Aust. Vet. J. 65:261–264.

Thomas, A.D., Spinks, G.A., d'Arcym, T.L., and Hoffmann, D. 1990. Evaluation of a modified complement fixation test and an indirect hemagglutination test for the serodiagnosis of melioidosis in pigs. J. Clin. Microbiol. 28: 1874-1875.

Vadivelu J., Puthucheary S., Gendeh G. and Parasakthi N. 1995. Serodiagnosis of melioidosis in Malaysia. Singapore Medical Journal 36:299–302.

Van der Lugt, J. J., and M. M. Henton. 1995. Melioidosis in a goat. J. S. Afr. Vet. Assoc. 66:71–73.

Veterinary Research Institute, Malaysia, 2015: Veterinary Research Institute Serology Unit Test Manual: Complement Fixation Test

Vorachit, M., K. Lam, P. Jayanetra, and J. W. Costerton. 1993. Resistance of *Pseudomonas pseudomallei* growing as a biofilm on silastic discs to ceftazidime and co-trimoxazole. Antimicrob. Agents Chemother. 37:2000 2002.

Vorachit M, Lam K, Jayanetra P & Costerton JW 1995. Electron microscopy study of the mode of growth of *Pseudomonas pseudomallei* in vitro and in vivo. J Trop Med Hyg 98: 379–391.

Whitmore, A. and C. S. Krishnaswami.1912. An account of the underscribed infective disease occurring among the population of Rangoon. Indian Med. Gazette. 47: 262-267.

Whitmore, A. 1913. An account of a glanders-like disease occurring in Rangoon. J. Hyg. 13:1–34.

Wuthiekanun, V., M. D. Smith, D. A. Dance, and N. J. White. 1995. Isolation of *Pseudomonas pseudomallei* from soil in north-eastern Thailand. Trans. R. Soc. Trop. Med. Hyg. 89:41–43.

Wuthieknun, V., M. D. Smith and N. J. White. 1995. Survival of *Burkholderia pseudomallei* in the absence of nutrients. Trans. R. Soc. Trop. Med. Hyg. 89: 491.

Yabuuchi, E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki and M. Arakawa. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol. Immunol. 36: 1251-1275.

Yabuuchi, E., Kosako, Y., Arakawa, M., Hotta, H., and Yano, I. 1992. Identification of Oklahoma isolate as a strain of *Peudomonas pseudomallei*. Microbiol. Immunol. 36: 1239-1249

8.0 APPENDIX

Appendix A: Questionnaire related to management, biosecurity and medical history for each sampled farm

Farm's background			
Farm's Owner:			
Year of establishment:			
Type of farm (please tick):	[ ] Government	[ ] Small holder	[ ] Others:
Animal purposes (please tick):	[ ] Meat	[ ] Dairy	[ ] Others:
Breed of Goats:			
Farm's population:	Total: Male: Female: Young:		
Animal Performance in Farm			
General performance:	[ ] Fair	[ ] Good	[ ] Excellent
Housing Management			
Type of housing:	[ ] Raised		[ ] Others:
Management system:	[ ] Intensive [ ] Semi-intensive [ ] Extensive		
Farm Biosecurity and Disease Management and Control			
Any nearby farm:	[ ] Yes [ ] No		
Importation of Animals:	[ ] Yes [ ] No	Country of origin:	
Quarantine practice:	[ ] Yes [ ]No	Where:	
Water source:	[ ] Treated [ ]Groundwater [ ]Stagnant water		
Bush Cleaning:	[ ] Yes		[ ] No
Soil liming:	[ ] Yes		[ ] No
Previous disease outbreak:			