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PATHOLOGY AND PATHOGENESIS OF Brucella melitensis INFECTION IN BUCKS

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By

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Brucellosis is an important disease of ruminants in many countries, including Malaysia. It is caused by *Brucella melitensis* leading to serious economic impact to goat farmers following abortions and stillbirths. The infection has not been thoroughly studied in bucks, particularly on the pathological changes and distribution of the organisms in the host. Furthermore, the efficacy of intracellular killing of *B. melitensis* by exposed bucks and the effectiveness of commonly used serological tests in identifying infected bucks need to be clarified. This study was conducted to observe the pathological changes and pathogenesis in bucks following experimental infection by *B. melitensis*.

Nine clinically healthy crossbred Jamnapari bucks of approximately 12 months old were used. The animals were confirmed as sero-negative for brucellosis following Rose Bengal Precipitation Test (RBPT) and Complement Fixation Test (CFT) tests. The selected bucks were divided into 3 equal groups. Groups 1 and 2 were infected intraconjunctival with 50 µl of an inoculum containing 10^9 cells/ml live local strain of *B. melitensis* and were sacrificed on days 7 and 14, respectively. Group 3 were similarly exposed to 50 µl normal saline before they were sacrificed on day 14. Serum samples for RBPT, CFT and ELISA, and conjunctival and prepuce swabs for bacterial isolation were collected at 3-day intervals. During post-mortem examination, the prescapular, submandibular and supramammary lymph nodes, the testis, epididymis, prepuce, seminal vesicle, bulbourethral gland, liver, spleen, conjunctiva and synovial membrane were collected for bacterial isolation and histopathology assessment.

Infected bucks developed mild pathological changes at 7 and 14 days post-infection (P.I) but did not demonstrate any clinical sign. There was no significant different ($p>0.05$) in the severity of pathological changes at days 7 and 14 post-infection. The histopathological lesions included necrotizing orchitis, epididymitis, seminal vesiculitis, hepatitis and phostitis. Nevertheless, immunoperoxidase positive reactions were observed in almost all organs that were sampled. The pathological findings proved that acute brucellosis led to mild histopathological changes even though the antigen was disseminated to all organs. *Brucella*

melitensis was not isolated from prepucial swabs that were collected between days 0 and 9 P.I. Later, isolations were successfully made from 66% of prepucial swabs on day 12 P.I and from 33% of the swabs on day 14 P.I. Isolations from the conjunctival swabs were successful on days 3, 12 and 14 P.I. Approximately 33% and 50% of the synovial membrane samples collected between days 7 and 14 P.I revealed positive isolations, and the synovial membrane was found to be the most suitable sample for isolations of *B. melitensis* in acutely infected bucks. Nevertheless, polymerase chain reaction (PCR) resulted in highest frequency of detection of *B. melitensis* and the most consistent results were observed in the testis (100% positive).

The *in vitro* assessments of phagocytosis and intracellular killing of *B. melitensis* were carried out using 6 healthy crossbred Jamnapari bucks of approximately 12 months of age. They were divided into 2 groups after the animals were tested with RBPT and CFT to ensure the brucellosis free status. The bucks of Group 1 were exposed subcutaneously with 2 ml inoculums containing 10^9 cells/ml of formalin-killed *B. melitensis.* The bucks of Group 2 were given 2ml sterile PBS as unexposed control group. Both groups were kept for 14 days before the neutrophil, macrophages and lymphocytes were harvested. The cells were then prepared as cell suspension containing 10^6 cells/ml in 200 µl in each individual chamber before 200 µl of an inoculum containing 10^7 cells/ml of live *B. melitensis* was introduced into the chambers. The extracellular Gram-positive bacterium, *Streptococcus agalactiae* and Gram-negative bacterium, *Pasteurella multocida* were used for comparison. The cells were then harvested at 0, 30, 60 and 120 minutes post-incubation and stained with Acridine orange and Crystal violet for viewing under fluorescent microscope to determine the phagocytosis index rate and intracellular killing index.

Phagocytosis activity by the neutrophils revealed no significant difference ($p>0.05$) between 30 and 60 min of incubation as well as between the two animal groups. However, rate of phagocytosis by neutrophils that were derived from exposed bucks was significantly ($p<0.05$) higher at 120 min. Subsequently, the neutrophils were able to kill 68% of the phagocytosed *B. melitensis*, which was significantly ($p<0.05$) lower than the two other extracellular bacteria. Similarly, macrophages from both groups showed no significant difference ($p>0.05$) in the phagocytosis activities at 30 and 60 min of incubation. However, at 120 min, macrophages that were derived from the exposed group demonstrated significantly ($p<0.05$) higher rate of phagocytosis. On the other hand, penetration of *B. melitensis* into lymphocytes of bucks revealed that *B. melitensis* was able to penetrate but was unable to survive long in the cells. The study proved the capability of *B. melitensis* to invade the lymphocytic cells, which enhanced movement of the organism within the body without triggering immunological response. Nevertheless, *B. melitensis* lacked replication capabilities in the lymphocytes.

In this study, sera from infected and uninfected bucks were processed to determine the antibody levels using ELISA and the two standard screening tests; the RBPT and the CFT. The RBPT and CFT assays provided negative results for all sera collected throughout the 14-day experiment. Meanwhile, ELISA revealed significantly ($p<0.05$) increased IgG level post-infection. However, the IgA levels in conjunctiva and prepuce showed fluctuating patterns

and peaked on day 6 P.I. Therefore, RBPT and CFT were found to be less useful for detection of acute brucellosis while ELISA would be a better test to be used for acute caprine brucellosis.

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PATOLOGI DAN PATOGENESIS JANGKITAN *Brucella melitensis* DALAM KAMBING JANTAN

Oleh

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Bruselosis merupakan penyakit ruminan yang penting di kebanyakan negara termasuk Malaysia. Masalah utama penyakit ini adalah keguguran pada kambing betina dan kematian anak kambing yang pasti memberi impak ekonomi yang serius kepada penternak. Selain itu, kambing jantan juga akan menghadapi masalah seperti radang sendi dan radang zakar yang boleh menyebabkan kemandulan jika tidak dirawat. Di Malaysia, masalah bruselosis kambing adalah disebabkan oleh *Brucella melitensis* dan dipercayai penyakit ini tersebar melalui makanan yang tercemar, transmisi melalui membran mukus dan hubungan seksual. Oleh itu, kajian ini dijalankan adalah untuk memahami perubahan patologi ke atas kambing jantan yang dijangkiti *B. melitensis*, corak penyebaran bakteria tersebut di dalam badan, respon imuniti terhadap jangkitan terutama proses fagosit oleh neutrofil, makrofaj dan kadar penembusan ke dalam limfosit, serta mengkaji keberkesanan ujian serologi yang sedia ada iaitu RBPT, CFT dan ELISA.

Sembilan ekor kambing jantan yang sihat, baka kacukan Jamnapari, berusia dalam lingkungan 12 bulan dan dibahagi kepada 3 kumpulan telah digunakan di dalam eksperimen ini. Semua kambing diuji dengan ujian RBPT dan CFT untuk membuktikan status bruselosis. Kumpulan 1 dan 2 telah diberi inokulasi sebanyak 50 µl 10^9 sel/ml *B. melitensis* hidup yang diperoleh dari wabak tempatan ke dalam setiap selaput mata, manakala kambing-kambing di dalam Kumpulan 3 diberi 50 µl salin normal ke dalam setiap mata sebagai kumpulan kawalan negatif. Sampel darah, sampel calitan selaput prepus dan selaput mata diambil pada setiap 3 hari untuk tujuan ujian RBPT, CFT dan ELISA manakala sampel calitan digunakan untuk ujian ELISA dan ujian pemencilan organism. Kumpulan 1 dikorbankan pada hari ke-7 selepas inokulasi manakala Kumpulan 2 dan 3 dikorbankan pada hari ke-14 selepas inokulasi. Semasa pos mortem, sampel nodus limfa dari preskapular, submandibular dan supramamari, testis, epididimis, selaput prepus, hati, limpa, selaput sinovial, selaput mata, kelenjar bulbouretral dan kelenjar seminal vesikel diambil untuk tujuan ujian pemencilan organisma dan pemeriksaan histologi.

Berikutan inokulasi *B. melitensis* ke mata kambing jantan, semua kambing tidak menunjukkan sebarang petanda klinikal walaupun pemeriksaan histopatologi menunjukkan

terdapat kesan sederhana. Jangkamasa jangkitan selama 7 dan 14 hari tidak menunjukkan sebarang perbezaan ketara ($p>0.05$). Lesi histopatologi termasuklah radang pada selaput prepus, zakar, kelenjar seminal dan hati. Pewarnaan imuno-peroksidas memberikan keputusan positif yang membuktikan kehadiran patogen tersebut di dalam semua organ yang diambil. Penemuan ini membuktikan bruselosis akut menyebabkan lesi histopatologi yang sederhana walaupun organism tersebut ditemui dalam setiap organ. Tambahan lagi, salur darah memberi pewarnaan imuno-peroksidas positif telah membuktikan bahawa *B. melitensis* merebak ke seluruh badan melalui saluran darah.

Pemencilan organisma daripada calitan selaput prepus yang diambil dari hari 0 hingga 9 menunjukkan keputusan negatif. Namun, calitan selaput prepus yang diambil pada hari ke 12 menunjukkan keputusan positif tertinggi iaitu sebanyak 66% dan pada hari ke 14 sebanyak 33%. Calitan mata memberi keputusan yang tidak konsisten di mana hanya pada hari ke 3, 12 dan 14 sahaja menunjukkan keputusan positif. Oleh itu, calitan selaput prepus merupakan sampel paling sesuai untuk diguna bagi pemencilan *B. melitensis*. Selaput sinovial pula menunjukkan keputusan positif tertinggi bagi kultur langsung dan terbukti sesuai untuk digunakan bagi proses pemencilan *B. melitensis*. Sementara itu, ekstrak DNA dan PCR menghasilkan keputusan positif bagi semua sampel. Ini sekali gus menunjukkan teknik tersebut sangat berguna untuk mengenalpasti patogen penyebab penyakit berikutan kaedah tersebut di dapati mempamerkan spesifikasi dan sensitiviti yang tinggi.

Kajian *in vitro* ke atas aktiviti fagositosis dan pembunuhan di dalam sel terhadap *B. melitensis* telah dijalankan dengan menggunakan 6 ekor kambing jantan yang sihat, baka kacukan Jamnapari, berusia dalam lingkungan 12 bulan kepada dibahagi kepada 2 kumpulan. Semua kambing diuji dengan ujian RBPT dan CFT untuk membuktikan status bruselosis. Kumpulan 1 telah diberi inokulasi 10^9 sel/ml *B. melitensis* mati yang dibunuh dengan formalin sebanyak 2 ml di bawah kulit. Kumpulan 2 diberi 2 ml PBS secara bawah kulit sebagai kumpulan kawalan negatif. Kedua-dua kumpulan dbiarkan selama 14 hari sebelum neutrofil, makrofaj dan limfosit diambil. Semasa ujian fagosit, 200 µl yang mengandungi 10^6 sel/ml neutrofil, makrofaj dan limfosit dicampurkan dengan 200 µl 10^7 sel/ml organisma *B. melitensis,* dan bakteria yang diguna sebagai kawalan negatif, *Streptococcus agalactiae* dan *Pasteurella multocida* secara berasingan. Proses inkubasi dijalankan mengikut tempoh waktu yang pelbagai iaitu 0, 30, 60 dan 120 minit. Kemudian, sel-sel tersebut diwarnakan dengan menggunakan Acridine orange and Crystal violet sebelum divisualisasikan melalui mikroskop fluresen. Indek fagosit dikira secara peratus sel yang mengandungi satu atau lebih bacteria daripada 100 sel yang dikira. Indek pembunuhan di dalam sel pula dikira secara peratus bakteria yang telah mati di dalam sel daripada jumlah keseluruhan bakteria yang difagosit oleh sel.

Kajian *in vitro* ke atas aktiviti fagosit oleh neutrofil, mendapati bahawa tiada perbezaan ketara ($p>0.05$) setelah inkubasi selama 30 dan 60 minit. Walaubagaimana pun, neutrofil yang diambil dari kumpulan terdedah menunjukkan perbezaan nyata ($p<0.05$) selepas 120 minit tempoh inkubasi. Keputusan ini adalah berikutan proses opsinisasi oleh antibodi yang terdapat di dalam serum haiwan terdedah. Aktiviti pembunuhan di dalam sel, tidak menunjukkan perbezaan nyata ($p>0.05$) di antara kedua-dua kumpulan bagi semua tempoh

inkubasi. Neutrofil hanya mampu membunuh sehingga 68% daripada *B. melitensis* yang difagositnya. Ini adalah kerana *B. melitensis* rintang dan boleh mengelak aktiviti bakteriasidal oleh neutrofil. Aktiviti fagosit *B. melitensis* oleh makrofaj juga tidak menunjukkan perbezaan ketara ($p>0.05$) di antara kedua-dua kumpulan setelah inkubasi selama 30 dan 60 minit. Walaubagaimana pun, pada minit ke 120, makrofaj dari kambing terdedah menunjukkan keupayaan fagositosis *B. melitensis* yang signifikan ($p<0.05$) berbanding kumpulan tidak terdedah. Hal ini adalah kerana proses opsinisasi yang dijalankan oleh serum yang membantu proses fagositosis. Kajian terhadap kadar penembusan oleh *B. melitensis* ke dalam sel limfosit mendapati bahawa patogen berjaya menembusi sel limfosit tetapi tidak boleh hidup lama di dalam sel. Ini membuktikan keupayaan *B. melitensis* untuk menjangkiti sel tanpa mencetus gerakbalas keimunan. Ketidakupayaan *B. melitensis* untuk hidup di dalam sel limfosit adalah disebakan kekurangan faktor replikasi yang diperlukan.

Sepanjang tempoh ujikaji, sampel serum diambil dan diproses menggunakan kaedah RBPT, CFT dan ELISA. Secara keseluruhannya, ujian konvensional; RBPT dan CFT menghasilkan keputusan negatif bagi semua sampel yang diambil sepanjang tempoh eksperimen. Manakala ujian ELISA menunjukkan paras IgG yang meningkat secara ketara ($p<0.05$) selepas terjangkit. Paras IgA dari selaput mata dan selaput prepus adalah tidak konsisten dan hanya berada di paras tertinggi pada hari ke 6 selepas terjangkit. Oleh itu, ujian RBPT dan CFT adalah kurang sesuai untuk digunakan dalam mengenalpasti jangkitan bruselosis akut, manakala ujian ELISA adalah berguna kerana keputusan yang diberikan adalah lebih spesifik.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µl	Micro liter
APC	Antigen presenting cell
BCV	*Brucella*-containing vacuoles
Bp	Base pair
CFT	Complement Fixation Test
CFU	Colony Forming Units (bacteria)
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
Dntp	Deoxynucleotide triphosphate
EDTA	Ethyenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fetal bovine serum
g	Gravitational force
H&E	Hematoxylin and Eosin
H_2S	Hydrogen sulfide
ICI	Inflammatory cells infiltration
IFN	Interferon
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin
In vitro	In an experimental situation outside the organism. Biological or chemical work done in the test tube rather than in living systems
IP	Immuno peroxidase staining
LAMP	Lysosomal-associated membrane protein
LN	lymph node
LPS	Lipopolysaccharide
M	Molar
MALT	Mucosa-associated lymphoid tissue
MHC	Major histocompatibility complex
NET	Neutrophilic extracellular trap
OD	Optical density
OMP	Outer membrane protein
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI	Post infection
RBPT	Rose Bengal Plate Test
RM	Malaysian Ringgit
RNA	Ribonucleic acid
RPMI	Rosewell Park Memorial Institute Medium
TBE	Tris-boric EDTA
Tc	T cytotoxic

Th	T helper
TLR	Toll like receptor
TNF	Tumor necrosis factor
U	unit
USD	United States Dollar
v/v	Volume per volume
Y	Gamma
α	Alpha

CHAPTER 1

INTRODUCTION

Caprine brucellosis is caused by *Brucella melitensis*, the most virulent *Brucella* species (Barbier *et al*., 2011). The disease is one of the major causes of reproductive related problems in goats and is becoming an important zoonotic infection in Malaysia (Bamaiyi *et al*., 2010). The disease is proved to be well distributed throughout the country and the trend of seroprevalence among animals is increasing every year (Bamaiyi *et al*., 2010). Although *B. melitensis* is known to infect goats and sheep, it can also infect other animal species such as cows, camels and buffalo (Blasco and Moriyon, 2010). Furthermore, *B. melitensis* is highly zoonotic and responsible for most of human brucellosis (Franco *et al*., 2007), which is presented with undulant fever, arthralgia, back pain and in chronic cases, abscess may developed in any organs such as liver, spinal cord, meninges and others (Hartady *et al.,* 2014).

Goat suffering from brucellosis will demonstrate a systemic clinical feature but prominently on the reproductive system. Infected does exhibit abortion, stillbirth, retained placenta, metritis or sub clinical mastitis. Infected bucks endure arthritis, orchitis and epididymitis (Eaglesome and Garcia, 1992; Xavier *et al.,* 2010). Most research activities were focussed on the reproductive organs of female animals infected with *B. abortus* rather than *B. melitensis* (Poester *et al*., 2006; Xavier *et al*., 2009). Infections are believed to occur by ingestion of particles contaminated by those animal excretion, consumption of contaminated colostrum or milk and the organism can be sexually transmitted although the rate of occurrence is low (SCAHAW, 2001). However, lesser histopathological studies have been conducted in bucks infected with brucellosis. Only several investigations were done exclusively to describe the lesions in male goats (Izadjoo *et al.*, 2008; Carvalho *et al*., 2012). In addition, immunoperoxidase (IP) technique is known to be an important tool to show the presence of antigen and its localization, thus it is recognized as the sensitive and specific test to detect *Brucella* antigen (Ilhan and Yener, 2008). Indeed, the technique has the capability to reveal the relationship of severity of the histopathology lesions and the antigen distribution in the tissues (Haritani *et al*., 1989).

The predilection sites of *B. melitensis* in female animals have been well documented (Keppie *et al*., 1965; SCAHAW, 2001). Nevertheless, few studies proved the predilection site of *B. melitensis* in male animals. Study by SCAHAW (2001) reported the localization of *B. melitensis* in the testis and epididymis, while *B. ovis* infection in sheep revealed localization in the epididymis rather in testis (Júnior *et al*., 2012). Isolation of the *Brucella* from any clinical specimens or post mortem samples is the gold standard for diagnosis of brucellosis (Lang *et al*., 1995). Blood culture is one of the suggestive methods to isolate the *Brucella* in canine (Carmichael and Kenney, 1970) and human cases (Colmenero *et al*., 2002). However, the procedure is unlikely to be used in other animals since the disease induces shorter bacteraemia as opposed to the canine and human brucellosis (Xavier *et al*., 2010). The

organs collected during post mortem should be handled with extra care to prevent exposure of the organism to the personnel involved. Direct isolation of *B. melitensis* is performed on selective media to enhance the growth and to ensure enough nutrient supply to the colonies (OIE, 2009). The polymerase chain reaction (PCR) technique is a powerful technique to be used since it is a specific and sensitive tool for identification. Furthermore, PCR technique is useful in identification of causative agent especially for any tedious microorganism such as *B. melitensis,* which required long incubation time. Thus, application of PCR in diagnosis of brucellosis may enhance the efficiency of the national control and eradication program.

The ability to cause persistent infection animals and humans is the unique characteristic of intracellular bacteria such as *B. melitensis* (Sangari and Aguero, 1996). The professional phagocytes, which comprised of neutrophils and macrophages are functioned to engulf, kill and disposal of pathogens (Lee *et al*., 2003). The capabilities of *Brucella* to replicate, to transmit to new host cells in intracellular environment and to avoid the immune detection make this pathogen to often be referred as 'Mr. Hides' (Gorvel, 2008). The main survival criteria in phagocytic cells is that *Brucella* incorporates itself into phagosomes after being engulfed and remains in the cells as a hiding site as well as a mechanism of transportation. In non-professional phagocyte cells, *Brucella* changes its method by residing itself in the endoplasmic reticulum (Arenas *et al*., 2000).

Neutrophils are the essential innate immune cell that quickly gathered at the site of infection with an important purpose; to ingest microbes and eventually kill them (Appelberg, 2006). Classically, it was thought that the main role of neutrophils in defensive system is to fight mainly the extracellular pathogens but recent study showed that the neutrophils also important in controlling the intracellular pathogens by initiate an adaptive immune system and bridging the neutrophil and macrophage cooperation to kill the intracellular bacteria (Appelberg, 2006). Correspondingly, it has been proved that the neutrophils are capable to response rapidly in order to phagocytize *Brucella* (Gallego and Lapena, 1990). It is important to realize that neutrophils are able to serve as transport medium for the engulfed pathogen to the lymphatic circulation with the purpose of enhancing the adaptive immune response in order to kill them (Abadie *et al.,* 2005; Maletto *et al.,* 2006). So far, however, there has been little discussion on the quantification of phagocytosis activity of neutrophils derived from bucks against *B. melitensis* except for the study done by Gallego and Lapena (1990).

The macrophages, also known as scavenger cells play an important role in defence mechanism. This professional phagocytic cell is known to be an important character in cellular immune system during battling the intracellular bacteria such as *Brucella*. Following ingestion, the activated macrophages induce bactericidal properties such as degradative hydrolytic enzyme, phagolysosomes acidification, cationic peptide and oxidative burst to kill *Brucella* (Gross *et al.,* 2004; Baldwin and Goenka, 2006). However, *Brucella* has a unique mechanism to prevent and resist the attack of these phagocytic cells (Köhler *et al*., 2002). Consequently, when these bactericidal properties failed to be executed, the macrophages trigger its own apoptosis process to prevent any intracellular replication, but unfortunately, *Brucella* is capable to prevent the host cell apoptosis, which resulted in persistence infection (Monack *et al*., 1997; Weinrauch and Zychlinsky, 1999). The preventive mechanism has been

demonstrated in *B. suis* infection in human macrophages (Gross *et al.,* 2000). Fortunately, *Brucella* also has its own weakness, which was proven by Dornand *et al.* (2002) who revealed that the survived *Brucella* was sensitive to the macrophages killing activity that was activated through Th1, and with the incorporation of IFN-α and cytotoxic T cells.

Besides phagocytic cells, *Brucella* can also invade other immune cells such as lymphocytes (Velásquez *et al*., 2012). It has been known that the only interaction between *Brucella* and T lymphocytes is through expression of Major Histocompatibility Complex Class II (MHC II), on toll-like receptor 2 (TLR2) (Barrionuevo *et al*., 2008). However, recent study proved the ability of *Brucella* to directly interfere with lymphocytes (Velásquez *et al.,* 2012). Back in 1981, Bratescu *et al*. (1981) provided evidence on the capability of *Brucella* to bind to the surface of B lymphocytes and suggested that the process might have some influence in pathogenesis of human brucellosis. The vast selection of invasion and preventive mode of *Brucella* make the eradication process by immune system harder than usual.

Caprine brucellosis remains endemic in many parts of the world except a few countries such as Canada, Australia, Cyprus, Finland, Denmark, United Kingdom, The Netherland, Norway, Sweden and New Zealand (Bamaiyani *et al*., 2012). In Malaysia, the caprine brucellosis was considered a re-emerging disease following extensive importation of goats into the country (Ibrahim *et al*., 1988; Zamri-Saad and Shafarin, 2007). Thus, the government has implemented a 'test and slaughter' policy in order to eradicate the disease. A comprehensive surveillance program was carried out to detect and monitor cases using the Rose Bengal Plate Test (RBPT) as screening test and the Complement Fixation Test (CFT) as the gold standard protocol for confirmation. Combination of these serological tests was required to reach a final diagnostic evaluation for brucellosis (de Oliveira *et al*., 2011).

Enzyme linked imunosorbent assay (ELISA) was considered as a meaningful tool to comprehend the current diagnostic tools in performing diagnostic activity for caprine brucellosis (García-Bocanegra *et al*., 2014). Although the gold standard for diagnosis of brucellosis is isolation and identification, serological test such as ELISA is an important routine serological test for brucellosis control and eradication program (EFSA, 2006). Efforts have been made to develop tests such as indirect ELISA, blocking ELISA and competitive ELISA that functioned in improving serological detection assay for caprine brucellosis (Minas *et al*., 2005; Garin-Bastuji *et al.,* 2006). According to OIE (2009), ELISA that used high content of smooth lipopolysaccharide as antigen produces better diagnostic result. In addition, the indirect ELISA produces more sensitive results, while competitive ELISA demonstrated similar sensitivity as other conventional serology tests including RBPT and CFT (OIE, 2009). On top of that, ELISA can be used to test desired immunoglobulin titration such as IgG, IgM and IgA. Ig G is an important antibody isotype found in the serum, which is widely used as indicative of immune status towards specific pathogen. Thus, elevation of IgG level with a combination of other immunoglobulin may help to indicate the chronicity of brucellosis (Lulu *et al*., 1988). On the other hand, IgM becomes one of the highest concentrations after IgG. It plays vital role in complement activation and opsonisation process, which play important roles in immunity response against brucellosis (Tizard, 2000). The IgA is an important defence mechanism at the mucosal surface against invading pathogens. Because of *B. melitensis* can

be transmitted through ingestion and mating activity, it is believed that the IgA level at the genital tract may reduce the presence of microorganism during shading (SCAHAW, 2001).

Problem statements

Generally, study and understanding on the pathology and disease development following *B. melitensis* infection in bucks are still lacking, particularly on the distribution of the organism within the host. Similarly, *B. melitensis* has been recognised as an intracellular bacterium that can survive intracellular killing. However, the phagocytosis and killing efficiencies by the neutrophils, macrophages and lymphocytes of exposed and unexposed bucks had never been studies and compared. Thus, understanding of these unique capabilities may improve the knowledge of the disease. Furthermore, the RBPT and CFT have been used in identifying goats naturally infected with *B. melitensis,* which usually chronic in nature. Their effectiveness in detecting acute infection must be studied to help in disease control.

Hypothesis

Consequently, the hypothesis for the study are:

1. bucks experimentally infected with *B. melitensis* at 14 days show significantly more severe lesions and more generalised distribution of the organism than at 7 days of infection
2. phagocytosis and intracellular killing activities by the phagocytic cells derived from exposed group are significantly higher than the non-exposed group
3. Rose Bengal Plate Test and Complement Fixation Test are capable to detect acute brucellosis

Objectives

Thus, this study was conducted with the following objectives:

1. to observe the pathological changes in bucks following experimental infection by *B. melitensis*
2. to determine the distribution of *B. melitensis* in the organs and tissues of bucks following experimental infection
3. to evaluate the phagocytosis and intracellular killing capability of neutrophils and macrophages derived from exposed and non-exposed bucks
4. to determine the penetration and survival capability of *B. melitensis* in lymphocytes of bucks
5. to evaluate the efficacy of commonly available diagnostic tests in detecting experimental caprine brucellosis

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