



The Epidemiology and Immunopathophysiology of Brucellosis in Small Ruminant

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Abstract – *Brucella melitensis* is one of the major zoonotic pathogens worldwide with enormous economic losses as well as considerable human morbidity in endemic areas. The global burden of its incidence in both human and animal populations remains significantly at an alarming rate. The impact of the disease is even multidimensional in nature and not always well understood and significantly complicating effective policy response. The pathogenesis is complex and governed by several factors working together in synergistic manner. The evolutionarily developed diverse evasion strategy to avoid the host's innate and adaptive immunity is further worsening the situation. Until recently, lipopolysaccharide (LPS) remains the major virulent factor of *B. melitensis* and responsible for the mechanism by which the pathogen causes its deleterious effects. Mechanisms presiding to the colonization of the pregnant uterus in different animal species are still largely unknown. Information related to the epidemiology and immunopathophysiology is still scarce in the database and control programs are rarely implemented. Therapy is based on wide spectrum antibiotics with mysterious outcome. The pre-existing vaccines appear not promising. Thus, understating the biological behaviour of the disease becomes a fundamental issue. In this review, we highlight various key aspects of the disease with special reference to the epidemiology and the immunopathophysiology of the disease in sheep goat populations.

Keywords: *Brucella melitensis*, epidemiology, immunopathophysiology, small ruminant brucellosis, virulence factors, zoonosis

Introduction

Brucella melitensis is one of the major zoonotic pathogens with significant economic implications as well as considerable human morbidity in many countries across the globe. It is the main causative agent of small ruminant brucellosis (SRB) as it is also infectious to other species including cattle, buffalo and elk. The disease remains endemic and neglected in many regions of the world, with predominance in the Mediterranean Basin, Middle East, Africa, Latin America and central Asia (FAO, 2010). The global burden of its incidence in human and animal populations remains significantly at an alarming rate (Pappas, Papadimitriou, Akritidis, Christou, & Tsianos, 2006). The organism is facultative intracellular pathogen cocco-bacilli, non-spore-forming and non-capsulated with up to 3 biovars have been reported. These biovars differ biochemically by their pattern of metabolic activities. Other *Brucella* species that have been uncommonly associated with the disease in small ruminant including *B. ovis*. Brucellosis is an important zoonosis threatening public health in many countries of the world (Liu, Cao & Zhu, 2014). The risk of brucellosis is presumed to be high in nomadic pastoral societies, laboratory workers or

veterinarians where close and frequent contact between man and animals is part of the ecology. The disease affects wild and domestic mammals with special predominance in small ruminants and cattle causing abortion and reduced fertility. It is a notifiable and neglected disease with serious economic repercussions on both humans and animals (Ko & Splitter, 2003; Abernethy et al., 2011). The routes of infection for both humans and animals are similar of nature and include ingestion, inhalation, or through direct contact with the organism through a break in the skin (Corbel, 2006).

In small ruminants, most clinical infection is manifested as fertility-related issues. However, the most common symptoms are usually abortions during the trimester often followed by retained placenta, weak offspring and metritis which may result in temporary infertility. Others include drop in milk production due to the infection of the udder. Rams experience orchitis and epididymitis. In addition, animals with polyarthritis have been observed in endemic flocks (Corbel, 2006; Radostits, Gay, Hinchcliff, & Constable, 2007).

In spite of advances in treatment and prevention, *B. melitensis* still poses a major threat on public health worldwide. Understanding the pathogenicity and the mechanism by which the organism interact with their hosts to produce clinical manifestation becomes a fundamental issue. A key first step in this process is in understanding the epidemiology and immune-pathophysiology as well as the identification of the *Brucella* virulence factors that contribute to the replication and survival of the engulfed bacteria. These aspects are key elements for any research concerning the identification of future novel target for vaccine and drug development. Therefore, the overall objective of this study was to enhance our understanding on the epidemiology and the immunopathophysiological aspects of *B. melitensis* and its lipopolysaccharide which in return will contribute to the development of therapeutic drugs and vaccines in combating bacterial disease.

The organism

Historical Overview

Brucellosis is an ancient disease that dates back to the 5th plague of Egypt around 1600 BC. This is evidenced by recent examination of the ancient Egyptian bones that showed the presence of sacroiliitis and other osteoarticular lesions, which are common complications of brucellosis (Pappas & Papadimitriou, 2007). For many years, the disease remained a mystery and was confused with other vector-borne disease especially Malaria, until the zoonotic nature of the organism was later confirmed in 1905 by isolating it from goat's milk. The uncertainty, however, persisted where these animals were not significantly correlated to the source of infection since they did not show severe clinical features of the disease when inoculated with *Brucella* cultures. The discovery that healthy goats could act as reservoir of the disease has been termed one of the greatest advances ever made in the study of epidemiology (Seleem, Mohamed, Stephen M. Boyle & Nammalwar Sriranganathan. "Brucellosis: a re-emerging zoonosis." *Veterinary microbiology* 140.3 2010; Wyatt, 2005). Alice Evans, an American scientist who did landmark work on pathogenic bacteria in dairy products, confirmed the relationship between Bang's disease (*B. abortus*) and Malta fever (*B. melitensis*) and renamed the genus *Brucella* to honour David Bruce. The later work on *Brucella* was pivotal in gaining acceptance of the pasteurization process to prevent human brucellosis across the globe. The discovery of the *Brucella* in marine mammals in early 1990 has remarkably changed the concept of a land-based distribution of brucellosis and associated control measures (Seleem et al., 2010).

Bacteriological characteristics and taxonomy

Brucella species are gram-negative, facultative intracellular cocco-bacilli, non-spore-forming and non-capsulated. These organisms belong to the alpha-2 subdivision of the *Proteobacteria*, along with *Ochrobactrum*, *Rhizobium*, *Rhodobacter*, *Agrobacterium*, *Bartonella*, and *Rickettsia* (Yanagi & Yamasato, 1993). Information of conflicting nature persists whether there is one species, *B. melitensis*, with six biovars or six species (Moreno, Cloeckart & Moriyón, 2002; Foster, Osterman, Godfroid, Jacques & Cloeckart, 2010). The current scientific community, however, recognises nine separate species. Seven of them that affect terrestrial animals are: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, and *B. microti* (Scholz et al., 2008). Of these seven species, three of them are non-

zoonotic species *B. ovis* (sheep and goats) *B. neotomae* (desert wood rats), and *B. microti* (*Microtus arvalis*). The zoonotic species are *B. melitensis* (sheep and goats), *B. abortus* (cattle), *B. suis* (swine, reindeers and rodents), and *B. canis* (*canines*). In addition, disease in marine mammals has resulted in the proposition of new species called *B. maris*. Yet, phylogenetic differences have further led to dividing *B. maris* into *B. pinnipediae* (seals and otters) and *B. cetaceae* (porpoise and whale) (Moreno et al., 2002; Corbel, 2006; Foster et al., 2007).

Epidemiology

The person, place and time as well as the host, agent and environmental interactions of these organisms amongst livestock, wildlife and humans are variable across the globe based on a myriad of factors. Evidence of a risk factor and a successful intervention in one location does not necessarily mean that the same risk factor exists or that intervention will be successful if applied to another. This section highlights the *B. melitensis* infection in livestock populations, its current global trends and financial losses, the enzootic transmission cycles, the source and route of exposure as well as potential risk factors involved.

Brucella melitensis infection in livestock population

B. melitensis is the main etiological agent of brucellosis in sheep and goats. It is also the main agent responsible for human brucellosis, known as Malta fever (Alvarez et al., 2011). Cattle can also be infected by *B. melitensis* especially when animals are kept in close range and share grazing areas or facilities with infected sheep and goats. Abortion and infertility are the predominant clinical signs in small ruminants. Transmission of *B. melitensis* to human from contaminated milk of dairy cattle has been documented (Alvarez et al., 2011). Although there is a paucity of specific studies, it is recognized as a source of significant financial loss to both industries. Its incidence is very high especially in low-income countries. Infection in cattle has been successfully eradicated in many developed countries after significant investment and many years of vaccinating and culling. However, *B. melitensis* infection in sheep and goats has been traditionally neglected, because small ruminant production represents generally a low-income activity practiced by landless farmers from marginal rural areas in the developing nations.

Clinical signs

There is a great deal of variability of infection due to *B. melitensis* among species. Reproductive issues are predominantly involved in all infections, but the severity and the sex-association of the most severe reproductive issues vary based on the host species. Most clinical disease manifests as fertility-related issues in cattle and small ruminants. The incubation period varies and is influenced by the stage of gestation, exposure dose, and age as well as vaccination level. In sheep and goats, the infection is localized in the udder, uterus and uterine lymph nodes. The most common symptoms are usually abortions during the trimester often followed by retained placenta, weak offspring and metritis which may result in temporary infertility. Others include drop in milk production due to the infection of the udder. Rams experience orchitis and epididymitis. In addition, animals with polyarthritis have been observed in endemic flocks (Corbel, 2006; Radostits et al., 2007). Cattle infected with *B. melitensis* have the same pathogenesis of disease and, thus, the same clinical signs. *B. melitensis* is the causative agent of disease, cattle may not abort at all, but they will shed bacteria in their milk. When this occurs, human illness may be the only sign of disease in the cattle (Radostits et al., 2007). Similarly to sheep, infected cattle can develop arthritis and hygromas as well (Corbel, 2006). Bulls develop orchitis, epididymitis and seminal vesiculitis which may cause infertility and such animals could serve as the source of infection especially when the semen is used in artificial insemination (Acha & Szyfre, 2003).

Global distribution and economic impact

The geographical distribution of infections caused by *B. melitensis* is constantly changing, with new foci emerging or re-emerging. The epidemiology is complex and is governed by several factors that include sanitary, socioeconomic, and political reasons, together with increased international travel. New episodes of *B. melitensis* have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Pappas et al., 2006). The disease occurs worldwide

and thus is a significant contributor to disability-adjusted life years (DALYS) and financial loss associated with zoonotic disease. This includes Mediterranean countries (South and East Europe), North and East Africa, central and South America, Asia and the Middle East (Corbel, 2006). In some areas, *B. melitensis* remains undetected and yet that does not necessarily mean that these areas are free from the infection. Until recently, there are no reliable reports that it has ever been eradicated from small ruminants in any country (Robinson, 2003). Despite the scarcity in terms of estimates of the costs associated with brucellosis, the available data suggests that worldwide economic losses are extensive not only in animal production (reduced milk, abortion and delayed conception), but also in public health (cost of treatment and productivity loss) (Acha & Szyfres, 2003; Seleem et al., 2010).

Mode of transmission and route of exposure

B. melitensis infection is mainly contracted through contact with placenta, foetus, foetal fluids and vaginal discharges from infected animals. In human, it is considered a food borne disease or a disease related to occupational exposures. The routes of infection for both humans and animals are similar in nature and include ingestion, inhalation, or through direct contact of the organism through a break in the skin. The key feature of brucellosis as a zoonosis is that it is a pure zoonosis: a disease transferred only from animals to people. Human-to-human transmission has occurred, but is exceedingly rare. The main source of infection for the population is consumption of unpasteurized milk and milk products. The exposure of animal to *B. melitensis*, however, is the primary route of transmission and depends on the susceptibility of the animals and the amount of exposure to the infection. Other factors may also influence both the host susceptibility and exposure level. The oral route is the most common route of transmission and the primary method of horizontal spread of disease by which the organism reaches the susceptible host (Fugier, Pappas & Gorvel, 2007). Other routes of infection that include respiratory or conjunctival routes have also been described (Smits and Kadri, 2005; Corbel, 2006).

Source and level of exposure

The risk of brucellosis to susceptible animals after parturition or abortion of infected animals depend on factors such as survival of the organism in the environment, number of organism excreted and probability of susceptible animals exposed to the organism to establish disease. Other sources of infection are wild life and other domestic animals. Male infected shed the organism in semen and when use for artificial insemination (AI) may result in considerable risk of infection (Crawford, Huber, & Adams, 1990). Animal product and by-products, equipment and personnel and animal vectors have also been documented as a source of infection. The infection can spread via fomites including feed and water. In conditions of high humidity, low temperature, and no sunlight, the organism can remain viable for several months in water, aborted fetuses, manure, wool, hay, equipment and clothes. The microbes can resist drying, particularly when organic material is present and can survive in dust and soil (Abernethy et al., 2011)

Host Susceptibility

Age is one of the host factors significantly associated with brucellosis and studies have shown that *B. melitensis* infection causes disease only in adult females and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response (Al-Majali, Talafha, Ababneh & Ababneh, 2009). However, susceptibility increases after sexual maturity and especially with pregnancy. Similarly, sex was found to have a significant effect on the brucellosis occurrence. Infection has shown strong association with female compared to male animals. It was suggested that males are not important in the epidemiology of the disease, however, they may become infected (Crawford et al., 1990). Contradicting findings exist regarding the susceptibility of the species and different breeds to the infection. In certain areas, notably in Latin America, goats are more prone than sheep. In these areas, sheep are not significantly infected even when kept in close contact with goats. In many other areas the disease, however, is more important in sheep. Several reasons for this variation include the state of the animal, type of breed, herd size, management practices and species behaviour.

Immunopathophysiology

Pathogenesis of B. melitensis

The mechanism by which *B. melitensis* causes a variety of clinical manifestations across different organs and tissues of the exposed host have not been clearly described. However, most of these alterations were unequivocally ascribed to the augmentation of *Brucella* replication in the host. Like other intracellular pathogens, organisms of *B. melitensis* have complexity of escaping the natural killing mechanisms, and proliferate within macrophages and nonprofessional phagocytes. Moreover, survival in macrophages is considered to be responsible for the establishment of chronic infections; a microenvironment that allows the bacteria to escape the extracellular mechanisms of host defence system. Therefore, understanding the pathogenesis and immunopathophysiology of infections associated with *B. melitensis* infection remains a key centre for research.

Invasion of host cells

The pathogenesis of brucellosis is similar among livestock species and depends mainly on a myriad of factors, the host susceptibility and the agent virulence. Successful entry of *B. melitensis* into the host is the first stage of establishing infection and most small ruminant species become infected through the digestive tract, as ingestion is the primary method of horizontal spread of disease (Fugier et al., 2007). Upon entry, the organisms are ingested by phagocytes in the sub-mucosa during the inflammatory response which is often seen in lymphoid tissues and organs with reticuloendothelial tissues (Anderson, Meador & Cheville, 1986). The persistence of the bacteria in the phagocytic cells allows for bacterial replication in these cells. Replication leads to the release of the bacteria from the cells, thus resulting in a phase of bacteraemia (Ragan, 2002). The bacteraemia allows for the colonization of the bacteria in multiple tissues, but in livestock the bacteria is most frequently colonized in the lymphoid tissues, mammary gland and reproductive tract (Ragan, 2002). The localization of *Brucella* spp. in the reproductive tract leads to colonization of the chorionic trophoblast of the placenta in pregnant livestock. This affinity for the chorionic trophoblast is due to the presence of a steroid called erythritol, a substance present in allantoic fluids that stimulates the replication of *Brucella* spp. (Corbel, 2006). The stimulation of *Brucella* spp. seen in the presence of erythritol is due to the preferential use of erythritol by *Brucella* spp. as an energy and carbon source, even in the presence of glucose and other metabolites. The reason for the preferential use of erythritol is due to its ease of uptake by the bacteria, as compared to glucose. This makes erythritol more readily available to the bacteria for energy consumption (Anderson & Smith, 1965). The resulting placentitis caused by replicating bacteria results in ulceration of the chorioallantoic membrane while sparing the endometrium of the uterus. The resulting pathology leads to late gestation abortions in naively infected livestock (Enright, 1990; Radostits et al., 2007). Erythritol is not present in the human uterus and abortions associated with human infections are normally seen in the first trimester (Corbel, 2006). Other studies of experimental nature have also demonstrated that abortion and localization of the bacteria in the reproductive tract were observed in rodents with no detectable level of erythritol in their tissues (Keppie, Williams, Witt & Smith, 1965). The presence of erythritol, however, in the testes of male species leads to a localization of the *Brucella* spp. in their reproductive tracts with a resulting epididymitis and orchitis (Enright, 1990).

Intracellular survival

Intracellular survival of *B. melitensis* is dependent upon its ability to resist the acidified intraphagosomal environment and to inhibit phagosome-lysosome fusion (Wang, Qureshi, Soeurt & Splitter, 2001). These processes are triggered by the internalisation of *B. melitensis* that redirects intracellular trafficking, changing the normal maturation process of the phagosome and blocking fusion of *Brucella*-containing phagosomes with lysosomes (Gorvel & Moreno, 2002). It is believed that *Brucella* spp. traffics from a phagosome compartment towards the rough endoplasmic reticulum (RER) of the host cell, where the organism has an optimal environment for replication (Pizarro-Cerdá, Moreno & Gorvel, 2000). Early ultra-structural analysis of *Brucella*-infected cells has also identified the RER as the site of replication (Dettileux, Deyoe, & Cheville, 1990). *Brucella* has limited replication in the early compartments, and the RER is the only compartment that sustains optimal bacterial replication (Gorvel & Moreno, 2002). Intracellular replication of *B. melitensis* in trophoblastic cells is strongly influenced by the stage of gestation, with higher replication rates within trophoblasts in late gestation when the cells actively secrete steroid hormones (Samartino, Truax, & Enright, 1994). In trophoblasts, *B.*

melitensis induces steroid synthesis and modulates the metabolism of prostaglandin precursors, favouring bacterial growth (Anderson et al., 1986). In addition, hormonal changes take place in infected placentas, with increase levels of prostaglandin F₂ α , a decrease in progesterone, and an increase in oestrogen and cortisol. These changes mimic to some extent what happens during parturition (Gorvel & Moreno, 2002), and are likely to contribute to the abortion.

Major virulence factors

Brucella pathogenesis is governed by their ability to invade the host cell, intracellular survival and evasion of the immune system. Some of the virulence factors responsible for these mechanisms include predominantly lipopolysaccharide (LPS), followed by two-component system, type 4 secretion system and cyclic β 1, 2 glucans (C β P). In the context of *B. melitensis*, lipopolysaccharide (LPS) become significant as the most major virulent factor and thus is exclusively reviewed in the following section.

Lipopolysaccharide (LPS)

Studies on the pathogenicity and virulence factor of brucellosis suggest that the outer membrane of bacteria as the main component of virulence of the organism; the outer membrane contains lipopolysaccharide (LPS) which is the major virulent factor of *Brucella* organism. It contains specific non classical LPS as compared to other bacteria with classical LPS such as *Escherichia coli* (Lapaque, Moriyon, Moreno, & Gorvel, 2005). The presence of LPS in the outer membrane of the bacteria suggests its importance in the pathogenesis of brucellosis by controlling the immune response of the host from activating antigen-specific CD4 T cells. As the cellular response is important for controlling the invasion and proliferation of the pathogen in the host cell, the presence of LPS on the bacteria serves as a shield or barrier leading to impaired danger signal that alert the host response to the entry of pathogen (Lapaque et al., 2005; Carvalho Neta et al., 2010).

More specifically, LPS prevents the synthesis of immune mediators and have less potential to induce host release of inflammatory cytokines. This is due to its failure to be detected by Toll like Receptors (pathogen recognition receptors) of the innate immune system because of its low endotoxic properties (Lapaque et al., 2006). Through this mechanism, it prevents stimulation of the innate immune system that would otherwise facilitate the killing of the pathogens. Macrophages and dendritic cells are some of the target cells, which are well known for antigen presentation. In the same manner, smooth LPS is involved in the inhibition of apoptosis. Resistance to apoptosis of infected cells has been seen in the natural host. Moreover, *Brucellae* do not activate the alternative complement system and have relatively low endotoxicity. This makes them further poor inducers of some inflammatory cytokines. However, the nature of intracellular makes the bacteria evade the immune system of the natural host.

Immunological response

Little is documented about the immunological response due to *B. melitensis* infection in humans and natural hosts (Spink, 1956; Cotton, Buck, & Smith, 1933). Most of the published studies evaluated the disease extensively in small laboratory animals. Of particular interest, mice have been employed extensively as models in brucellosis research. Infection with *B. melitensis* usually results in the induction of both humoral and cell-mediated immune responses, but the magnitude and duration of these responses are affected by various factors including the virulence and dose of the strain, the route of inoculation physiological and immune status of the host. These aspects, however, are critical in the clearance of infection associated with *B. melitensis* and thus are highlighted in the following sections.

Humoral immune response

Serological response have been documented with a great deal of variation depending on several factors including the physiological status and the virulence of the strain to which the animals are naturally or experimentally exposed. For instance, invasion of the pregnant uterus can be expected to produce a large and persistent rise of antibodies, but this may be delayed until after abortion or parturition at the normal time. Invasion of the lactating udder causes a lesser serological response and localisation confined to a small number of lymph nodes may fail to stimulate any response. However, the pattern of the serological response in terms of immunoglobulin production has not been extensively studied in

sheep and goats, but available information suggests close similarity to that in cattle. Production of IgM normally precedes, within a week or two, by a predominance of IgG, with both isotypes falling to a low level in the more chronic stage of infection but with IgG predominating. The serological response is transient and sometimes missing in young sexually immature animals. Experimentation in the mouse model demonstrates that the relevant levels of anti-*Brucella* immunoglobulins are detectable only after the 2nd week of post-infection with relatively higher levels of IgG3 (High et al., 2007; Hort et al., 2003).

Cell mediated immunity (CMI)

Little is documented about the impact of *B. melitensis* on cytokine production. The extent of stimulation of these cytokines vary and dependent greatly on the method of measurement. The cytokines regarded as key players in Brucellosis research, which also, includes IL-12, IFN- γ , and TNF- α . However, the mechanisms of their induction in those cells have not been elucidated. Similarly, the role of IL-6 and IL-1 β has not been clearly delineated although extremely few studies are reported in the sera of susceptible mice during the second week of infection (Sathiyaseelan et al., 2006; Kim et al., 2005; Watanabe et al., 2008).

Hormonal alterations

A limited coverage exist regarding the impact of *B. melitensis* infection on reproductive hormones. There are no studies evaluated extensively on sex related hormonal response due to the infection in the natural host, but available information suggests close similarity to that in cattle infected with *Brucella abortus*. However, decrease in the levels of sex related hormones in mouse model have been reported (Jesse et al., 2015). In a later study, decreased levels of progesterone and oestrogen were detected in the sera of the mouse model with intermixed levels in terms of testosterone production in these models (Jesse et al., 2013).

Pathological changes

There is scarcity in terms of pathological changes in relation to *B. melitensis* infection in vivo settings. However, for ethical and practical reasons, most of the available studies have frequently employed mice as models in brucellosis research. In this species, manipulation of virulent strains of *Brucella spp* induces clinical and pathophysiological responses. The outcome of these patterns in mice mainly depends on the virulence and dose of the *Brucella* strain, the route of inoculation, and on the breed, genetic background, age, sex and physiological status of these models. The course of *Brucella* infection in mouse models becomes significant during three different stages; namely acute phase, chronic steady and chronic declining phase. Overall, these stages are characterized by significant inflammation and the appearance of the pathological lesions in the vital organs (Tobias, Cordes, & Schurig, 1993; Guilloteau et al., 2003; Hort et al., 2003). In particular, liver enlargement and splenomegaly is noticeable in brucellosis research (Cheville, Kunkle, Jensen, & Palmer, 1995; Murphy, Sathiyaseelan, Parent, Zou, & Baldwin, 2001).

Diagnosis

Serological tests

A myriad of testing modalities for *B. melitensis* in livestock have been widely employed. Unfortunately, definitive diagnosis of the disease is yet to be available despite many experimentation and studies that had taken place over the last two decades. However, the current methods or techniques for diagnosis include antigen and antibody detection as well as agent isolation through culture. These methods have different advantages and disadvantages in the laboratory and in their use in disease control and eradication programs.

In sheep and goats the prescribed tests are the buffered brucellosis antigen test and the complement fixation tests. The brucellin skin test and the fluorescence polarization assay are considered alternative tests. Rose Bengal Test (RBT) and the Buffered Plate Antigen Test (BPAT) are among the most commonly used tests (Nielson, 2002; Gall and Nielson, 2004). Another commonly used confirmatory test is the complement fixation test (CFT). It has high sensitivity and specificity of 97.1% and 100% respectively (Nielson et al., 2008). However, it is more complex and requires many laboratory facilities.

Others include fluorescence polarization assay (FPA) (Nielson, 2002; Gall and Nielson, 2004; Nielson et al., 2008); Competitive ELISA (Nielson, 2002; Gall and Nielson, 2004; Nielson et al., 2008).

Molecular methods

Polymerase Chain Reaction (PCR)

New techniques allowing identification and sometimes quick typing of *Brucella* at the genus, species and biovar levels have recently improved the diagnostic capacities. A number of these molecular methods have been developed and its applications ranges from diagnosis of the disease, and characterization of field strain for epidemiological purposes (Gopaul, Koylas, Smith, & Whatmore, 2008). Molecular typing of *Brucella* has also been used for epidemiological trace back in disease outbreaks and is an important component of disease eradication programmes.

However, PCR assays lack validation and improvement of specificity and sensitivity in comparison to other tests. Nevertheless, PCR techniques show a lower diagnostic sensitivity than culture methods, although their specificity is close to 100% (Bricker, 2002). The best results have so far been obtained by combining culture and PCR detection on clinical samples.

Molecular typing

Studies of genetic diversity of *Brucella* species have been performed using molecular typing. The multiplex AMOS PCR, named for its applicability to “*abortus, melitensis, ovis, suis*” species, is often used. The later allow discrimination between *Brucella* species and between vaccine and wild-type strains. They do not, however, allow discrimination among all the biovars of a given *Brucella* species (Bricker, 2004). Some techniques currently used include Pulse-field gel electrophoresis (PFGE), insertion sequence based typing, restriction fraction length polymorphism based approaches, genome-based typing approaches, tandem repeat based typing, multilocus sequencing (MLST) and single nucleotide polymorphism (SNP) typing. All these techniques have good capabilities in identifying *Brucella* diversity (Gopaul et al., 2008). In the near future, single-nucleotide polymorphisms, multilocus sequence analysis (MLSA), and Multiple-Locus Variable number tandem repeat Analysis (MLVA) will be used as routine typing techniques to allow the discrimination of strains within a given biovar, allowing molecular epidemiological analysis.

Strategies for disease control and eradication

Brucellosis is a disease of major economic and zoonotic importance. Thus, a strategy for its control in small ruminants is essential in endemic areas. The initial aim of the strategy is based on the reduction of infection in the animal population to such a level that the impact of the disease on human health as well as on animal health and production is minimized (Kaplan, 1966). Subsequent steps can include eradication from a region by test and slaughter and, following successful eradication, measures to prevent reintroduction of the disease. Control of a zoonosis is a general term that embraces all the measures designed to reduce the incidence and prevalence of a disease in a defined animal population. In the context of *B. melitensis* infection, control measures, however, include isolation of infected animal, followed by culling, periodic test of the herd at least 3 times a year, proper disposal of placenta and aborted fetus, and disinfection of contaminated farms (Dequ, Donglou & Jiming, 2002). Similarly, cooperation between public health authorities and livestock owners is an important component of brucellosis control.

Vaccination practically eliminates the clinical signs of brucellosis and is accompanied by a reduced contamination of the environment as well as exposure of the population at risk to the infectious agent (Nicoletti, 1993). Moreover, herd owners are more likely to accept vaccination as a method of control since they are accustomed to this form of disease control (Corbel, 2006). Effective vaccines have played an important role in reducing the incidence of brucellosis in many countries. Rev.1 vaccine is the most widely used vaccine for the prevention of brucellosis in small ruminants. This vaccine offers life-long immunity and thus minimizing the incidence of abortions in livestock population. The eradication of brucellosis in small ruminants in Malaysia means removing the pathogenic agent from the country. This necessitates a committed effort from the government as well as herd owners. Crucial factors of the success of any eradication programme are the implementation of an active surveillance system with

adequate laboratory support and the understanding and sharing of objective for eradication by the decision makers, farmers and all stakeholders. To keep the country free from the infection, prevention measures must be implemented to segregate an infectious organism from the animal population. In the long term, the eradication of brucellosis is more advantageous and economical when compared to control programme. So far, Malaysia has introduced almost all the measures highlighted above for the prevention, control and eradication programmes (Anka et al., 2013).

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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