UPDATES ON BRUCELLOSIS IN MALAYSIA AND SOUTHEAST ASIA

BAMAIYI P.H.[1], HASSAN L.[1]*, KHAIRANI-BEJO S.[1] AND ZAINAL ABIDIN M.[2]

1 Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

2 Department of Agribusiness and Information System, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

* Corresponding author: latiffah@upm.edu.my

ABSTRACT. Brucellosis has been in South East Asia for many generations affecting both man and animals and responsible for massive economic losses. The threat to the economy and to public health from this zoonotic disease is so great to ignore. Over the years there have been many attempts to control and possibly eradicate this infection but these efforts have not fully yielded the desired results due to many factors mentioned in this paper. Eradication, though expensive, actually saves a lot of economic resources when properly implemented. In this review, the past and current situation of brucellosis in South East Asia is explored with particular reference to Malaysia and challenges to the full eradication of the infection are elucidated.

Keywords: Brucellosis, Southeast Asia, Malaysia, perspective, update, zoonoses

INTRODUCTION

Brucellosis is a zoonotic bacterial disease and a widespread zoonoses that affects man and various animals including cattle, buffaloes, sheep, goats, pigs, camels, dogs and in recent times, marine mammals (Renukaradhya *et al*., 2002; Minas, 2006). This disease was reported to have existed since 750 BC in Egypt (Seleem *et al*., 2010) and David Bruce (after whom the genus *Brucella* is named) is credited with isolation of *Brucella melitensis* (*Micrococcus melitensis*) on the island of Malta in 1887, therefore the disease has also been called Malta fever. It was also called goat fever (Time, 1928), undulant fever (Time, 1938) and cow, goat, pig and man fever (Time, 1929). Brucellosis was a devastating infection for thousands of British troops during the Crimean war (Time, 1938), mainly transmitted through infected milk from animals especially cattle and goats and was known to have no certain cure for many years following (Time, 1943).

Brucellosis has been reported in different regions of Africa, Europe, America and the middle East (Pappas *et al*., 2006). In Asia it has been reported in India (Ghodasara *et al*., 2010), Pakistan (Akhtar *et al*., 2010), China (Deqiu *et al*., 2002), Mongolia (Zinsstag *et al*., 2005), Sri Lanka (Priyantha, 2011) and other countries in Asia notably Iran and Saudia Arabia (Pappas *et al*., 2006; Seleem *et al*., 2010). The Southeast Asia has been plagued by many diseases in animals and humans (Kheng, 1966; Cameron *et al*., 1999) and these diseases have continued till contemporary times especially brucellosis (Seleem *et al*., 2010) which is still endemic in this region (Huynh *et al*., 2007).

In Malaysia there is generally a dearth of literature on brucellosis (Bahaman *et al*., 2007). The organism was first isolated in cattle (*Brucella abortus*) in 1950 after many animals in a cattle farm in Johor state tested serologically positive. Later the infection was found to be more widespread than was envisaged at first (Joseph, 1987). To control the infection, test and slaughter along with vaccination of calves was carried out which succeeded in controlling bovine brucellosis for some years. Unfortunately massive importation of cattle and other animals may have re-introduced the infection in cattle in 1965.

Brucellosis in pigs was first confirmed with isolation of *B. suis* from herds of swine in 1963 in Perak and Selangor (Joseph, 1987; Bahaman *et al*., 2007). In dogs, the infection was first reported with the first isolation of *B.canis* in 1982 (Joseph *et al*., 1983). In 1991, *B.ovis* was reported in sheep in Terengganu (Mahendran, 1991) while *B.melitensis* was also first reported in sheep in 1994 in Johor (Moktar *et al*., 1995). *B. melitensis* was reported prevalent among the goats and sheep in Malaysia (Bahaman *et al*., 2007; Al-Garadi *et al*., 2011a, 2011b) and molecular characterization of limited numbers of the isolates suggests that the isolates were closely related to other genotypes in the region (Bamaiyi *et al*., 2012).

Aetiology

Brucellae are Gram-negative facultative intracellular cocco-bacilli that are non-encapsulated, non-spore forming and non-motile belonging to the alpha-2 subdivision of the proteobacteria (Yanagi & Yamasato, 1993; Seleem *et al*., 2010). There are eight species of Brucella recognised that affect terrestrial animals which include: *B. abortus*, *B.melitensis*, *B. suis*, *B. neotomae*, *B. canis*, *B. ovis*, *B. microti* and *B. inopinata* (Verger *et al*., 1987; Scholz *et al*., 2008; Scholz *et al*., 2010). There are two others that affect marine mammals and they are: *B. ceti* and *B. pinnipedialis* (Foster *et al*., 2007). Complete genome sequences of *B. abortus*, *B.melitensis*, *B. suis*, *B. canis*, and *B. ovis* are available while many other strains are being sequenced. They are considered to be very similar in size and genetic make-up (Sriranganathan *et al*., 2009). *B. melitensis* is predominantly found in goats and is the most widely distributed of all the Brucella species. It is also the most pathogenic and virulent species for humans and affects almost all domestic animals and many wild animals (Benkirane, 2006). *B. abortus* is mainly found in cattle and buffaloes, *B. suis* in pigs, *B. ovis* in sheep and *B.canis* in dogs (Seleem *et al*., 2010).

Distribution

The disease is endemic in many countries and regions of the world (Seleem *et al*., 2010) except the following countries in which bovine brucellosis has been eradicated: Australia, Canada, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom (Kolar, 1984; Benkirane, 2006; Minas, 2006). New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Pappas *et al*., 2006). In the south east asian region which includes Myanmar (Burma), Singapore, Malaysia, Brunei, Thailand, Vietnam, Indonesia, Laos, Philippines and Cambodia, the disease is considered endemic even though a few countries sporadically report its occurrence (Manosuthi *et al*., 2004; Benkirane, 2006).

Spectrum and Range of Clinical Manifestations

The disease affects all ages and sexes of cattle, sheep, goats, dogs, camels and marine mammals. Humans who work closely with these animals are more likely to contract the infection (Deqiu *et al*., 2002; Jennings *et al*., 2007). The clinical manifestations of the disease vary in different animals depending on its sex. Among the pregnant females among livestock, the most common manifestation following infection is abortion. The principal strain affecting cattle incriminated in abortion is *B. abortus,* however cattle can be affected by *B. suis* and *B. melitensis* when they share pasture facilities with swine, sheep and goats (Kolar, 1984; Banai, 2002). As in cattle, brucellosis in goats, caused by *B. melitensis,* is characterized by late abortion, stillbirths, decreased fertility and low milk production. Similar manifestations are observed in swine and sheep. In males generally the disease can lead to epididymitis, orchitis, lameness, hind limb paralysis and occasionally in females metritis or abscesses occur (Wright *et al*., 1998). In humans, the most common symptoms of brucellosis include undulant fever in which the temperature can vary from 37.8 °C in the morning to 40.8 °C in the afternoon; night sweats with peculiar odor, chills and weakness. Common symptoms also include malaise, insomnia, hepatomegaly, splenomegaly, leukopenia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness and depression. Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be very diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis (Aygen *et al*., 2002). Spontaneous abortion especially in the first and second trimester can occur in pregnant women infected with *Brucella.*

The Host, Agent and Environmental Causal Factors of Brucellosis

The hosts are man, cattle, sheep, goats, swine, dogs, camels, buffalo, horse and marine mammals. The causative agents are *B. abortus* (cattle, man, buffalo, horse, camels, sheep and goats); *B.melitensis* (cattle, goats, sheep, and man); *B. suis* (swine); *B. canis* (dogs); *B. ceti* and *B.pinnipedialis* (marine mammals). Some of these agents like *B. melitensis* cause infection in several species (Boschiroli *et al*., 2001; Dobrean *et al*., 2002). The environmental causal factors include geographical area, contact with wildlife, herd size and breed type. Since *Brucella* spp. organisms can survive in water (at 20 °C for 2.5 months), wet soil (<7 days), manure (at 25 °C for 1 month), sharing infection through environmental contamination was likely both within and across animal species (Muma *et al*., 2007). In conditions of high humidity, low temperatures and no sunlight, *Brucella* organisms can remain viable for several months in water, aborted fetuses, manure, wool, hay, equipment and clothes (Al-Talafhah *et al*., 2003). Macro factors such as importation of animals and government policies play an important role in aiding the spread of brucellosis as well as the micro factors such as breed, herd size and individual factors of sex/gender and body immunity of individuals (Alavi-Shoushtari & Zeinali, 1995; Boschiroli *et al*., 2001; Aygen *et al*., 2002).

Economic Impact

Brucellosis is responsible for massive economic losses around the world especially in developing countries where accurate data are not available to truly assess the loss. Losses are usually due to culling of animals, abortion, infertility, reduced milk production, treatments costs of animals, vaccines, market losses, losses due to missed reproductive cycles, hospitalizations for human cases and administrative costs by governments in an attempt to control or eradicate the infection. In Latin America, annual economic losses was $600 million for bovine brucellosis and in the United States the eradication program spent $3.5 billion between 1934 and 1997 and loss due to reduced milk production in 1952 amounting to about $400 million (Acha & Szyfres, 2003; Sriranganathan *et al*., 2009). In assessing the economic impact of brucellosis in case of a bioterrorist attack, Kaufman *et al*. (1997) estimated it will have an economic impact of $477.7 million per 100,000 persons exposed.

In Malaysia, little study has been done on the economic impact but Joseph (1987) reported that the government of Malaysia spend RM450,000 in 1986 on the disease control and eradication administrative costs. Many other losses due to loss of foetus, decreased milk yield, infertility, interference with farrowing management and sequential seasonal calving, joint infections, weakling calves, disease in man and others could not be accounted for financially but are likely to run into millions of dollars annually.

Human Brucellosis

Brucellosis is one of more than 175 infectious diseases listed as zoonoses worldwide (Pappas *et al.*, 2006; Pappas, 2010). It is one of the most common laboratory acquired infections so laboratory workers must be careful with live cultures of *Brucella* (Seow *et al.*, 2009). Global travel has made its spread very easy and is listed as one of the diseases related to travel by the Centre for Disease Control (CDC) (CDC, 2009). The control of human brucellosis depends on its control in animals (Corbel, 1997). Currently there are no available vaccines for human use. However, efforts to develop human vaccine for brucellosis are on-going (Perkins *et al.*, 2010). Risk factors for brucellosis include presence of the disease among family member, consumption of unpasteurized milk, handling of aborted materials from infected animals with unprotected hands, keeping livestock, and living in houses in close proximity to other households (Sofian *et al.*, 2008; John *et al.*, 2010). Syria has the highest annual human incidence of brucellosis worldwide (1603 cases per million) with Mongolia coming second (Pappas *et al.*, 2006). In Southeast Asia, brucellosis has been reported in humans in Thailand (Manosuthi *et al.*, 2004), Singapore and Myanmar (Benkirane, 2006) and Indonesia (Berger, 2010b). Vietnam reported a seroprevalence of 14.8% in Binh Thuan Province, (Pappas *et al.*, 2006; Pappas, 2010). In Singapore, a 78-year old retired pig farmer died of brucellosis more than 20 years after active contact with animals was stopped (Paton *et al.*, 2001). Two Singaporeans contracted brucellosis in Saudi Arabia during Hajj from drinking unpasteurized camel's milk. No cases were reported for Brunei between 1997 and 2004 (Berger, 2010a).

In Malaysia, the prevalence has been reported to be 5.8% in hospital patients suspected to work closely with animals (Jama'ayah *et al.*, 2011) and up to 14.29% (Bamaiyi *et al.*, 2011) in occupationally exposed veterinary technical staff and farmers in different parts of Malaysia. More research is needed to determine the exact annual incidence rates of brucellosis in the human population in Malaysia.

Animal Brucellosis

Birds have been found to be seropositive for brucellosis and may play a role in the transmission of the infection as latent carriers travelling over wide distances. This may explain the difficulty experienced in tracing the exact origin of brucellosis outbreaks in some herds or flocks of animals (Kudi *et al.*, 1997). In Indonesia, brucellosis was detected in pigs with 22.3% positive in West Java and 14.9% positive in East Java (van der Giessen & Priadi, 1988) and is prevalent in cattle in different parts of Indonesia (Makka *et al.*, 1988). Brucellosis is endemic in Thailand (Subharngkasen, 1970) and is responsible for economic losses on farms (Kunavongrit *et al.*, 1980). Canine brucellosis has been reported in the Philippines (Wanke, 2004). The prevalence of brucellosis in animals in Malaysia varies from state and from species to species. An average national seroprevalence of 0.95% has been reported for the years 2000 to 2008 in goats (Bamaiyi *et al.*, 2010) and 5.0% and 1.6% for cattle and buffaloes respectively for years 2000 to 2009 (Anka *et al.*, 2010). Al-Garadi *et al.* (2011b) isolated four *B. melitensis* species from 300 vaginal samples taken from goats in Malaysia indicating the endemicity of the infection in goats in Malaysia. In a survey in dogs in the Klang valley, Malaysia, Khairani-Bejo *et al.* (2006) reported 6 serum positive samples out of 123 tested for brucellosis.

Brucellosis in wildlife

Wild animals that frequently enter the domestic animal population such as wild boars, deer and birds may play an important role in the epidemiology and sustaining of the disease in domestic animals (Pappas, 2010). Cases of transmission between wild boars and pigs and between cattle and elk have been established in some parts of the world (Pappas, 2010) and the situation is likely to be similar in the Southeast Asia with its vast population of wild life mixing with domestic livestock.

Diagnosis

The gold standard for the diagnosis of brucellosis remains the bacteriological isolation of the organism from cultures of tissues from infected animals such as the spleen, lymph nodes, foetuses or contents, uterine fluid, vaginal swabs, milk and in humans blood is often cultured (Poester *et al.*, 2010). Confirmatory diagnosis using serological techniques as recommended by the Food and Agriculture Organization (FAO) and the Office of International Epizootics (OIE) is usually done using the Complement Fixation Test (CFT) for many years (Klaus, 2002). This test detects specific antibody or antigen in the serum and is usually done using the method of Alton *et al.* (1988). For human samples it is normal to first screen blood sera with the Serum Agglutination Test and then use CFT or Coombs test for confirmation (Al Dahouk *et al.*, 2003).

In Malaysia and the Southeast Asia, CFT is usually used for confirmatory diagnosis. Many other tests are available and used such as Rose Bengal Plate Test (RBPT), Enzyme Linked Immuno-Sorbent Assay (ELISA), Brucella Coombs Test, Serum Agglutination Test (SAT), Milk Ring Test, 2-Mercaptoethanol Tube Agglutination Test, Buffered Antigen Plate Agglutination test (Gall & Nielsen, 2004; Khairani-Bejo *et al.*, 2006; Poester *et al.*, 2010). PCR has the potential for the future for routine diagnosis of brucellosis as it is relatively safe for laboratory workers and fast, in addition to being highly sensitive and specific (Al-Garadi *et al*., 2011a).

Prevention and Control

To control brucellosis in man it must first be controlled in animals (Zinsstag *et al*., 2007). Several methods are recommended by Alton *et al*. (1987), Seleem *et al*. (2010), and Ariza *et al*. (2007), to control and prevent brucellosis in a country. They are broadly divided into primary and secondary methods.

Primary Methods of Prevention

(i) Selection of a disease-free stock: As much as possible farmers should get animals that are free from brucellosis in order to minimize the probability of infecting their herd because one of the principal risk factors for brucellosis is introduction of new animals into the stock (Seleem *et al*., 2010; Chand & Chhabra, 2013). Vaccination: routine vaccination of the flock or herd is essential to boost their immunity against any form of disease challenge. *B. abortus strain 19* and the new strain *RB51* vaccine is available against *B. abortus* infection and Rev1 vaccine is available against *B. melitensis* (Banai, 2002; Renukaradhya *et al*., 2002; Schurig *et al*., 2002; Blasco, 2006). In Mexico 15 goats previously vaccinated with reduced-dose Rev-1 vaccine were still protected after 5 years of vaccination (Díaz-Aparicio *et al*., 2004). Care must be taken concerning Rev-1 vaccine as it can induce brucellosis in humans (Anon, 1993).

(ii) Culling: this is the act of destroying all animals that are positive to the infection. From screening conducted, animals that are positive can be eliminated from the herd or flock to stamp out the disease. Combining test and slaughter strategy with effective vaccination could potentially assist in the disease eradication (Minas, 2006). However, culling is very expensive for most developing countries and is not recommended in countries where caprine-level prevalence is already above 2% (Alton, 1987; McDermott & Arimi, 2002).

(iii) Avoid exposure to other herds or wild life: this is essential to prevent the animals contracting the infection it is necessary to avoid mixing with other herds which may be infected. Wild life should be avoided too since the disease is known to be maintained within the sylvatic cycle (Wright *et al*., 1998). This can be achieved by a more intensive system of managing the animals. In piggeries for example which are very prone to contact with wild boar protective fences should be built to minimize contact and mating with wild boars which may be carriers (Wu *et al*., 2012).

(iv) Resistant breeds: certain breeds of animals are suspected to be naturally resistant to brucellosis and the use of such breeds could prevent the infection, such as the Maltese breed of sheep which is very resistant to *B. melitensis* whereas the fat-tailed Awassi and kurdi breeds of sheep are highly susceptible to the pathogen (Alton, 1987).

Secondary Methods of Prevention

These methods are considered secondary because they deal with the disease that has already being introduced into the population instead of stopping it's entry in the first place. They are methods employed to control when the primary methods fail (Boschiroli *et al*., 2001; Muma *et al*., 2007).

(i) Treatment: these involve the use of drugs such as doxycycline combined with streptomycin for treatment of brucellosis in livestock after exposure. Doxycycline is also used in humans for treatment of brucellosis (Pappas *et al*., 2006)

(ii) Sanitation: proper disposal of wastes including aborted foetuses is paramount to controlling and preventing the infection (Taleski *et al*., 2002).

Eradication of Brucellosis

The eradication of brucellosis by the test and slaughter method is only recommended and feasible in countries where the individual animal prevalence is less than 2% (Alton, 1987). Where feasible the eradication of brucellosis though expensive saves $7 for every dollar spent on eradication of the infection (Acha & Szyfres, 2003). Southeast asian countries like the Philippines and Singapore had reported eradicating bovine brucellosis (Corbel, 1997) but at present brucellosis has re-emerged in these countries (Seleem *et al*., 2010).

Brucellosis infection require a holistic and coordinated approaches that can improve or control variables responsible for its re-emergence such as importation of infected animals, herd stocking density, hygiene, interactions with wild life and coordination of various health related disciplines to achieve eradication. The OIE requires a country to have intensive surveillance after eradication for at least 5 years to confirm that the agent is no longer in the population in order to achieve a disease-free status. One way to do this is to continue with the eradication policy that has been in existence. In addition to this, farmers should be adequately compensated and importation of goats from known endemic countries should be minimized or banned as there is at present not a single test that can detect the different stages of brucellosis and so the present screening test may still fail to detect positive animals (Herrera *et al*., 2011).

CONCLUSION

The Southeast Asian region is growing rapidly with an average annual income increasing from 4% to 8%, population from 2% to 3%, urbanization from 4% to 6% and meat consumption from 4% to 8% (Huynh *et al*., 2007). To support this growth this region has an estimated ruminant population of 21,247,586 but it is predicted to have 616,180 cases of brucellosis annually with 164 outbreaks reported in the year 2010 to World Animal Health Organization. It has a brucellosis prevalence of 2.9% with heavy economic losses (ILRI, 2012).

Brucellosis is likely to continue to be a global threat for years to come (Godfroid *et al*., 2005), but concerted efforts and political will power of various government agencies can facilitate the process of reducing the disease spread among animals and ultimately among the human population.

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