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Epidemiology and Economic Impact Assessment of Caprine Brucellosis in Penang State, Malaysia: A Retrospective Analysis

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Abstract: Brucella melitensis is a zoonotic pathogen affecting a wide range of hosts with economic significance in the animal industry worldwide. The disease is characterized by abortion, still birth, reduced milk production, weak foetus and infertility mainly in small ruminant populations. There is a wide distribution of the disease among domesticated species and the trends of the disease in goats have not yet been described in Penang state, Malaysia. In this study we describe the distribution, pattern and trend of B. melitensis in Penang state based on serological data obtained from nationwide B. melitensis serosurveillance activities in goat populations. A total of 13,724 goats were sampled within the period of study (2012-2014), of which 3.4% (95% CI; 3.35-3.45) goats tested positive for B. melitensis infection. The district of Seberang Perai Utara had the highest animal seroprevalence of 95.5% (95% CI; 94.2-96.8). The seroprevalence varied over type of the breeds and the period of study and generally increased in 2014. Seropositive animals clustered around Boer and Katjang breeds with the prevalence of 54% (95% CI; 51.8-56.2) and 38% (95% CI; 35.8-40.2), respectively. The months of March, August and September, illustrated the highest seroprevalences of 44 (95% CI; 41.54-46.46), 13% (95% CI; 10.8-15.2) and 14% (95% CI; 11.3-16.7), respectively. A noticeable variation was observed in annual-level economic loses, but the seroprevalence remained significantly high in 2014 (>80%). Considering the lack of information on the epidemiology of caprine brucellosis in peninsular Malaysia, this report could contribute to the ongoing area-wise national brucellosis eradication program.

Keywords: Epidemiology, Caprine Brucellosis, Distribution, Economic Losses, Penang, Malaysia

Introduction

Brucella melitensis organism remains as versatile as ever and continues to be one of the most fascinating microorganisms with multiple mysteries deeply buried in its genome (O’callaghan and Whatmore, 2011). B. melitensis, a Gram negative bacterial pathogen that presents varied clinical diseases in livestock, causes abortions during the trimester often followed by retained placenta, weak offspring and metritis in sheep, goats and cattle, orchitis, epididymitis and polyarthritis in rams, fever and focal forms with osteoarticular and genitourinary forms

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in humans and multi-systematic infections in camels, dogs and sporadic cases in horses, pigs and other wildlife species. Infections of *B. melitensis* are associated with a wide range of diseases, termed ‘brucellosis’. In some situations, *B. melitensis* also complicates pathogenesis of other diseases as secondary pathogen.

Infections related to *B. melitensis* occur worldwide and thus a significant contributor to Disability-Adjusted Life Years (DALYS) and loss associated with zoonotic disease (Osman et al., 2016). This includes Mediterranean countries (south and east Europe), north and east Africa, central and south America, Asia and the Middle East (Corbel, 2006). The disease has a high global index as an animal health constraint to poor farmers (Seleem et al., 2010; Samadi et al., 2011). Subsequently, several notable achievements in the realm of the organism’s characteristics, virulence, pathogenicity, diagnosis and development of vaccines have been reported (Doganay and Aygen, 2003; Corbel, 2006; Osman et al., 2016). *Brucella melitensis* is generally considered to involve infection through ingestion, inhalation and through direct contact with broken skin and often severely terminates in septicemia with isolation of the organism from multiple organs (Grilló et al., 2012; Díaz et al., 2013; Osman et al., 2017). Livestock farmers, veterinarians and laboratory workers are at risk of being affected by this organism due to their constant and close contact with animals and animal related materials (Samadi et al., 2011). In Malaysia, brucellosis has been occurring in the livestock population for many decades although at a relatively low prevalence compared to other countries in Asia have been reported (Bamaiyi et al., 2014). In Penang, *B. melitensis* surveillance for eradication purpose is still ongoing. However, there are no published reports to date on *B. melitensis* in goat farms in Penang and the works on the epidemiological aspects are very limited in Malaysia. Therefore, in this study, we describe the trends and pattern of caprine brucellosis between 2012 and 2014 from a retrospective analysis of data collected from a nationwide brucellosis active surveillance programme. We believe that information from this study provides insight on the epidemiology of caprine brucellosis in Penang, Malaysia and assist the authorities in improving their disease-control strategies.

**Materials and Methods**

**Study Area**

Malaysia (4.1936° N, 103.7249° E) is located in Southeast Asia and comprises East Malaysia (peninsular Malaysia) and West Malaysia (Sabah and Sarawak on Borneo Island). The two regions are separated by the South China Sea. Penang is located on the northwest coast of Peninsular Malaysia with an area of 651,784 square kilometers bordering Kedah to the north and the east and Perak to the south. Penang has an average rainfall of 340 mm of rain and experiences hot and humid weather throughout the year with two monsoon seasons; the north-east monsoon from November to March and the south-east monsoon from May to September (http://www.met.gov.my). Malaysia has a relatively small goat population size with estimated number of 482,280 heads (Malaysia: Livestock Population, 2009-2013).

**Data Sources**

Brucellosis serosurveillance activities were performed regularly by the state veterinary departments in Malaysia, as described in the Protokol Veterinar Malaysia Penyakit *Brucella*. The program allows for serological screening of goats at least twice a year. Once confirmed, all seroreactors must be culled in the government abattoir. Slaughter under the supervision of the veterinary officer is required to ensure compensation of culled goats. The livestock sampling and serological testing for the serosurveilance programme was performed via the state’s veterinary departments and its regional veterinary laboratories that are located at Penang, Malaysia. Accordingly, serum samples from goats were tested for evidence of *Brucella* antibodies using the Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT), using the protocols and guidelines described previously (Jacobson, 2004). The confirmatory diagnosis for *Brucella* antibodies using the CFT was performed at the Veterinary Research Institute (VRI), Ipoh. The study was approved to be conducted by the Department of Veterinary Services, Putrajaya Malaysia.

We obtained data generated from the serological testing for ovine brucellosis from the Epidemiology and Surveillance Unit at the Department of Veterinary Services (DVS), Putrajaya and the database at VRI, Ipoh, for years 2012 to 2014. These serosurveillance data have not been extensively analysed in the past. The data were compared and collated to improve their integrity. The data were thoroughly checked for accuracy in entry, coding and typing errors and repeated entry of a farm in the course of one year was eliminated to ensure that a herd or farm was not over represented in a given year of study. The information obtained from the data includes farm names and addresses, date of sampling, locations, breed, age range, number of animals tested and the number of animals within the tested herd.

**Data Analysis**

The data were managed and stored in a Microsoft Excel® (Microsoft Corporation) spreadsheet and frequency tables were used to calculate prevalence based on districts within Penang, year, herd, animal and breed. Seroprevalences rates over the 2 years were determined as the number of seropositive goats divided by the total
number of goats sampled and confidence intervals were calculated at a 95% level. The differences between/among proportions were tested using Chi-square and univariate logistic regression statistics. All statistical analyses were performed using SPSS (version 16, Chicago, IL) at a significance level of $\alpha = 0.05$ and the figures were plotted using Sigma Plot (version 11.0).

**Results**

A total of 13,724 goats were sampled within the period of study (2012-2014), of which 467 (3.4%) goats tested positive for *B. melitensis* infection (Fig. 1). The prevalence of the disease varied over year, months, districts and type of goat breeds. The prevalence of the disease by year was summarized in Fig. 2-4. The annual mean of seroprevalence level among goats for the period of study was 3.4% (95% CI; 3.35-3.45) with 2013 having the lowest (0.10%) and 2014 having the highest rate (2.6%) (Fig. 3 and 4, respectively). Among the 467 *Brucella melitensis* infected goats, majority of the seropositive animals clustered around Boer breeds followed by Katjang breeds with the prevalence of 54% (95% CI; 51.8-56.2) and 38% (95% CI; 35.8-40.2), respectively (Fig. 5). Sannen and Jamnapari breeds had the lowest seroprevalence with 6 and 2%, respectively (Fig. 5). Moreover, there was also variation in the prevalence of the disease over the sampled districts in Penang, Malaysia. Among the 467 positive cases, 446 cases (95.5%) were from Seberang Perai Utara (SPU) followed by Seberang Perai Tengah (SPT) with 14 cases (3%), Daerah Barat Daya (DBD) with 6 cases (1.3%), Seberang Perai Selatan (SPS) with one case (0.2%) and, in Daerah Timur Laut (DTL), there was no case of *B. melitensis* recorded (0%) (Fig. 6). Furthermore, the pattern of distribution of *B. melitensis* infection varied by months with March, August and September, depicted the highest seroprevalences of 44 (95% CI; 41.54-46.46), 13% (95% CI; 10.8-15.2) and 14% (95% CI; 11.3-16.7), respectively (Fig. 7). Overall, the number of goats that tested positive for *B. melitensis* infection fluctuated over the years. The highest percentage of goat positive for *B. melitensis* infection was 204 cases (44%) in March.
Fig. 3: Seroprevalence of goat brucellosis in 2013 in Penang, Malaysia

Fig. 4: Seroprevalence of goat brucellosis in 2014 in Penang, Malaysia
Fig. 5: Seroprevalence of goat brucellosis by breeds in Penang, Malaysia

Fig. 6: Seroprevalence of goat brucellosis in Districts of Penang, Malaysia from 2012 to 2014
The goats’ positive for *B. melitensis* infection showed decrease number of cases from April until June from 49 cases (10%) to 9 cases (2%), respectively. It slightly increased from July to September from 55 cases (12%) to 66 cases (14%) cases positive for *B. melitensis* infection. The percentage of goat positive for *B. melitensis* infection decreased from October to December from 8 cases (2%) to zero case (0%) (Fig. 7).

For the year 2012, the highest percentage of goat positive for *B. melitensis* infection was in September with 60 cases (Fig. 2). The goats positive for *B. melitensis* infection showed variation in number of cases from February until August from 9 to 2 cases. There were increased cases of positive *B. melitensis* infection from August to September rising from 9 to 60 cases. The number of goats’ positive for *B. melitensis* infection decreased from October to December from 2 to zero (Fig. 2).

For the year 2013, the highest number of goat positive for *B. melitensis* infection was in August with 6 cases (Fig. 3). The cases of goats positive with *B. melitensis* infection fluctuated from January until September from 6 to 1 cases. There were slightly increase in prevalence from July to August from 1 to 6 positive of *B. melitensis* infection. The percentage of goats’ positive for *B. melitensis* infection decreased from September to December from 2 to zero case (Fig. 3).

For the year 2014, the highest number of goat positive for *B. melitensis* infection was in March with 197 cases (Fig. 4). The cases of goats positive with *B. melitensis* infection fluctuated from January until October which ranged from 197 cases to 2 cases. There was a major increase from June until July from 6 to 48 cases positive for *B. melitensis* infection. It later decreased by 4 cases in the month of August. The number of goats positive for *B. melitensis* infection decreased between October and December from 6 cases to zero (Fig. 4).

### Economic Losses to Farmers Due to *B. melitensis* Infection

Economic loss to the goat farmers due to *B. melitensis* infection in Penang from 2012-2014 was calculated based on market value of slaughter and culled positive animal. The Malaysian government provides compensation for the goat farmer with a standard compensation value of RM5.60 per kg which only accounts for 23% of the value of the live weight of the animal.

In the year 2012, 97 goats were positive for *B. melitensis* infection at which the market value was approximately RM 65,960 but the compensation given by the DVS Penang was RM 29,550. Therefore, the economic losses in 2012 due to *B. melitensis* infection were RM 36,410 (Table 1). Similarly, in the year of 2013, 14 goats were positive for *B. melitensis* infection at which the market value was approximately RM 9,520 but the compensation given by the DVS Penang, Malaysia was RM 464. Therefore, the economic losses in 2013 due to *B. melitensis* infection were RM 9,056 (Table 1). The highest annual economic losses for the study period was observed in 2014. In the later year, 356 goats were positive for *B. melitensis* infection at which the market value was approximately RM 242,080 but the compensation given by the DVS Penang was RM 45,128 making the total of economic losses due to *B. melitensis* infection to a total of RM 196,952 (Table 1).
The total compensation given by government due to *B. melitensis* infection cases between 2012 and 2014 was estimated to RM 75,142 as the average market value of live goat is RM17 per kg within that time frame. Assuming that the average live weights of positive goats are 40 kg; therefore, the total current frame. Assuming that the average live weights of positive goats are 40 kg; therefore, the total current value should be RM 317,560. The calculated losses of the farmer due to *B. melitensis* infection would be approximately RM 242,418 (Table 1). The total economic losses were about 76.34%, however the government only compensated for 23.66% of the total value of the animals. This implies that the government compensated only 23.66% of the actual value of the animals culled in Penang during the study period.

### Discussion

The results showed that the prevalence of *B. melitensis* infection in Penang from year 2012 to 2014 was 3.4% which showed that the prevalence was considerably high when compared to the national prevalence which was 0.8%. According to a recent research paper published in Malaysia, the prevalence of *B. melitensis* infection in Penang was at 2.01%. The prevalence increased within one year by 1.39% from 2013 to 2014. The observed increase in the prevalence could be attributed to mass importation of goats from endemic countries (Zamri-Saad and Kamarudin, 2016). This is the major cause for the persistent infection of *B. melitensis* infection in Penang state, Malaysia. The increase in the prevalence of *B. melitensis* in this state was consistent with the increasing number of human brucellosis cases as a result of consumption of raw goats milk (Leong et al., 2015).

Recently, the number of small holders had dramatically increased over the last years as well as the importation of goats from neighboring countries (Bamaiyi et al., 2014). The increased number of small holders, however, is believed to be associated with the recent initiatives in which the Malaysian government promotes small holders to participate national economy reform. Moreover, the increased seroprevalence observed in Boer breed rather than indigenous species such as Katjang, Jamnapari and Sannen is strongly related to the importation of animals from other countries (Zamri-Saad and Kamarudin, 2016). Several factors influence the observed incidence of the disease and include the failure of quarantine management upon arrival of animals and also failure of herd health management of clinical and sub-clinical cases. The mass importation of Boer goats from endemic counties throughout the year became a major issue in Malaysia. Most of the farmers were rearing more Boer goats for meat purposes than any other breed. In addition, farmers preferred natural breeding rather than practicing Artificial Insemination (AI) as means of breeding which can contribute to the increasing number of *B. melitensis* cases in Malaysia.

The proportion of sero-reactor animals remained relatively high (95.5%) in Seberang Perai Utara (SPU). This phenomena reflects the geographical location as well as occupational factors where residents in this area are extensively involved in agricultural and livestock based industries. Moreover, large scale farms, sampled from this area, might contributed to the increased number of positive cases of *B. melitensis* infection. The number of goat’s population was two fold higher in SPU as compared to the other districts. We believed that the smaller numbers of farms sampled may not have given the correct picture as compared to other districts in Penang and may have contributed to the difference observed. It was also possible that the high seroprevalence of SPU was caused by its geographical area as it is mostly covered with agriculture land and also located at the border of Kedah DarulAman and Thailand which are areas known to be endemic for the disease. At the same time, we believed that the re-allocation of time and resources by the authorities to control other pandemics and re-emerging infections, notably the multiple outbreaks of FMD (Edwards, 2004; Wongsathapornchai et al., 2008), bird flu (H5N1) (Morrison et al., 2005) and swine flu (H1N1) (Goodwin et al., 2011) led to a substantial reduction in of nationwide brucellosis surveillance and control program. Previous studies have also reported that location, region or area have a significant correlation with brucellosis seropositivity, which, according to the authors, is attributable to management practices and other agro-ecological factors (Megersa et al., 2011). It is also possible that the difference was due to other confounding variables unaccounted for in this study.

Larger herd farmers are also more likely to be actively purchasing or introducing new animals into their herds thereby increasing the risk of acquiring *Brucella*

<table>
<thead>
<tr>
<th>Years</th>
<th>Description</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of goats positive for <em>B. melitensis</em></td>
<td>97.00</td>
<td>14.00</td>
<td>356.00</td>
<td>467.00</td>
</tr>
<tr>
<td></td>
<td>Market value (RM17/kg)</td>
<td>65,960.00</td>
<td>9,520.00</td>
<td>242,080.00</td>
<td>317,560.00</td>
</tr>
<tr>
<td></td>
<td>Compensation value (RM5.60/kg)</td>
<td>29,550.00</td>
<td>464.00</td>
<td>45,128.00</td>
<td>75,142.00</td>
</tr>
<tr>
<td></td>
<td>Total economic losses (RM)</td>
<td>36,410.00</td>
<td>9,056.00</td>
<td>196,952.00</td>
<td>242,418.00</td>
</tr>
</tbody>
</table>
melitensis seropositive animals. These infected goats could be carriers of the bacteria for lifetime and while shedding the organism, they may show little or no sign of infection (Al-Majali, 2005; Seleem et al., 2010).

The association of months of sampling with seropositivity to Brucella infection is in agreement with other previously published reports in which rainfall or season was reported to play a major role in the epidemiology of the disease (Muñoz et al., 2010).

The increase in number of positive cases of B. melitensis infection in March and September could be ascribed due to of multiple factors that include socio-economic, seasonal factors among other environmental conditions. Seasonal variation in the epidemiology of infectious diseases are common phenomenon in both temperate and tropical climates (Grassly, 2006). However, the mechanism of the change is poorly understood (Osman et al., 2016) and has been linked to the interaction of several intrinsic and extrinsic factors (Pascual, 2005; Altizer et al., 2006; Grassly, 2006). In the epidemiology of B. melitensis infection, seasonal factors have been reported to be associated with human brucellosis which, in most cases, coincide with the period of parturition among farm animals and, hence, increased exposure to farmers when attending to animals and consuming their milk (Corbel, 2006). This was also evidenced by recent outbreaks of human Brucellosis following consuming of raw goats’ milk in Penang, Malaysia (Leong et al., 2015).

In the analysis of economic loses, the observed pattern varied also over the study period with highest loses recorded in 2014 followed by 2012 and 2013, respectively. The total of financial loss was estimated to be around RM 242,418 during the study period.

We believe that the high economic losses reported in our study would provide awareness to the different sectors regarding this zoonotic pathogen, hence extreme measure could also be exercised. The losses to the farmer are usually from culling of positive animals with or without minimum compensation. However, we strongly believe that the figures of economic losses associated with Brucella infection are always underestimated due to the fact that most of the cases remains unreported. This was evidenced by the persistence nature of the infection and thus invalidating to arrive a definitive conclusion in terms of the economic losses due to this devastating disease.

Conclusion

In conclusion, the prevalence of goat brucellosis is significantly high among goats in Penang state, Malaysia. Breed, locality and seasonality are considered as potential risk factors for contracting brucellosis. This study would be expected to reduce the impact of the disease and its economic consequences on the livelihood of small holders and ultimately avoid a disease spread in human during a possible brucellosis outbreak. Further molecular characterization studies such as, complete bacterial genome sequencing and subsequent phylogeny would be necessary to determine the genetic lineages of Brucella strains circulating in infected hosts in Malaysia.

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Authors’ contribution

MAA performed the study. AYO, MLN and SNC designed the study and analyzed the data. MMA, RS, AAS and AAK helped the proof reading of the manuscript. All the authors shared in the drafting of the paper and all of them approved the paper.

Compliance with Ethical Standards

The authors declare that they have no competing interests. The result of this study does not reflect the opinion of the funding sources. All authors have read and approved the final version of this manuscript.

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