Seroprevalence of Brucellosis among Farmers and Veterinary Technical Staff in Peninsular Malaysia
(Seroprevalens Bruselosis dalam Kalangan Peladang dan Kakitangan Teknikal Veterinar di Semenanjung Malaysia)


ABSTRACT
A study was conducted to determine the seroprevalence and risk factors associated with brucellosis in farmers, veterinary technical staff and other volunteers in Peninsular Malaysia using the One Health concept. A cross sectional study design was employed to sample a population of farmers and Veterinary technical staff on a volunteer basis. The Brucella Coombs test was used to elucidate the seroprevalence of brucellosis in the sampled population and the associated factors and predictors for brucellosis. The seroprevalence of brucellosis among farmers and non-farmers (veterinary technical staff and others) of 446 people studied was 1.35% (95% CI=0.28-2.42). Occupation, age and drinking unpasteurized milk were significant risk factors for brucellosis using multivariate logistic regression at 90% confidence level. The odds of having brucellosis increased by 7.19 times in farmers compared to non-farmers (90% CI=1.16, 44.71), it increased 7.16 times in individuals 40 years and below compared with those above 40 years old (90% CI=1.16, 44.41) and 4.45 times among those who drink unpasteurized milk compared to those who do not (90% CI= 1.03, 19.15). This study highlights the current state of human brucellosis in Malaysia among those groups that are occupationally at risk. A nationwide health education of the occupationally exposed population especially farmers may decrease the incidence of the infection and support the on-going eradication efforts of brucellosis in Malaysia.

Keywords: Brucellosis; farmers; Malaysia; seroprevalence; veterinary staff

INTRODUCTION
Brucellosis is caused by Gram-negative coccobacilli bacteria and is the most common zoonotic infection worldwide with an estimated 500,000 new cases annually (Pappas et al. 2006). The disease infects goats, sheep, cattle, buffaloes, dogs, camels, marine animals and man (Dawood 2008; Nagalingam et al. 2012). There are 10 different species of Brucella but the most pathogenic of all the species and common cause of brucellosis in humans is Brucella melitensis (Pappas 2010). The organism can be transmitted from animals to man through the consumption of unpasteurized milk, cheese, other dairy products and contact with contaminated animal tissues. Therefore, those individuals working closely with animals such as farmers and veterinary staff are considered at a higher risk of brucellosis due to their occupation (Bikas et al. 2003; Makita et al. 2010).

Brucellosis has been reported from goats and other animals in Malaysia (Bamaiyi et al. 2012; Khairani-Bejo et al. 2006; Shahaza et al. 2009) and the infection...
has continued to be persistent up to the present time (Bamaiyi et al. 2014). However, there have been limited reports on human brucellosis other than a few case reports (Jama’ayah et al. 2011; Sam et al. 2012). Even among those high risk individuals working closely with livestock, there is no information about the level of exposure that these individuals have had to the Brucella organisms. Since Brucella infection in the local livestock has been emerging in the past few years in Malaysia, it is worth investigating the serological evidence for the exposure to the Brucella organisms among workers with close contact to these animals. This study describes a cross-sectional study conducted among the veterinary technical staff and livestock farmers to determine the seroprevalence and risk factors of Brucella infection in the central states of Peninsular Malaysia. This study was undertaken as a collaborative effort between the Faculty of Veterinary Medicine Universiti Putra Malaysia (FPV, UPM), the Department of Veterinary Services Malaysia (DVS), Zoonosis Sector of the Ministry of Health Malaysia (MOH) and the Veterinary Research Institute Ipoh Malaysia (VRI), following series of discussions about the unknown public health impact of brucellosis among high risk or occupationally exposed individuals in Malaysia and as an example of the one-health concept approach in tackling zoonotic infections.

MATERIALS AND METHODS

STUDY AREA AND DATA COLLECTION

Malaysia consists of 13 states and three federal territories and has a total land mass of 329847 square km (127350 square miles). Malaysia is separated by the South China Sea into Peninsular Malaysia and Malaysian Borneo (comprising of Sabah and Sarawak). Malaysia borders Thailand, Singapore, Indonesia and Brunei and is located at 2° 30’ and 112° 30’ (Bunnell 2004; Tiong-Sa et al. 2001). Livestock farming is one of the preoccupations of many farmers and investors in Malaysia with the livestock sector contributing about 7.6% to the GDP of the Agriculture sector (Vu 2007). By the year 2010 about 545,682 goats; 134,408 sheep; 1,821,663 swine; 126,478 buffaloes and 912,230 cattle were present in the country mostly by importation from other countries (Anon 2011).

A set of structured closed-ended questionnaires (available on request) were administered via face to face interview by trained staff. The questionnaire sought information on the demography including age, gender, state, marital status, ethnicity, educational level, income and potential risk factors such as occupation, consumption of unpasteurized milk, type of animals kept, proximity to animal houses, cleaning of aborted foetus, assisting during parturition, knowledge of brucellosis transmission and symptoms of brucellosis. A set of structured closed-ended questionnaires (available on request) were administered via face to face interview by trained staff.

STUDY DESIGN AND SAMPLE SIZE DETERMINATION

States with relatively high seroprevalence rates for brucellosis in goats based on the analysis of the nationwide B. melitensis sero-surveillance information between 2000 and 2009 (available on request) were enrolled into the study. Three central states were identified namely Selangor, Negeri Sembilan and Pahang. A cross-sectional study was conducted independently within each selected state whereby farmers and veterinary technical staff of the Department of Veterinary Services of Malaysia were invited to participate in the study, in collaboration with the Ministry of Health Malaysia which was carrying out a routine surveillance for brucellosis, following an awareness seminar on brucellosis in livestock and its public health impact. This research was carried out in accordance with ethical procedure of the Ministry of Health Malaysia and the Department of Veterinary Services Malaysia joint committee. Informed consent of all volunteers was obtained. This research was funded by the Universiti Putra Malaysia, Department of Veterinary Services Malaysia and the Ministry of Health, Malaysia.

This study was performed as part of the routine screening by the Ministry of Health for emerging zoonotic infection therefore blood samples were collected by trained medical personnel of the ministry. Participation was completely voluntary and individuals were asked to sign an informed consent form before the study begun.

Sample size was calculated using OpenEpi Epidemiologic statistics for Public Health software version 3.01 based on Sullivan et al. (2009) using the following equation:

\[
\text{Sample size } n = \frac{[\text{DEFF}*\text{Np}(1-p)]}{[(\text{d}^2/2)^2 + \sigma^2(N-1)+p*(1-p)]},
\]

where \(n\) is the sample size; \(N\) is the total number of people in the study; \(p\) is the estimated prevalence of people with brucellosis in the states (0.5); \(d\) is the the precision or maximum acceptable error rate (0.01); \(\alpha\) is the probability of Type I error (0.05); and \(z\) is 1.96.

Based on this formula, at least 370 people were required for our study but up to 446 people were recruited into the study. Inclusion criteria were being a farmer or veterinary staff at the time of sampling, involvement in handling animals and willingness to be part of the study. Exclusion criteria were other professions and not being a farmer or Veterinary staff at the time of sampling.

SAMPLE COLLECTION

Participants from Selangor, Negeri Sembilan and Pahang states of Malaysia were invited to participate in the study by signing a written consent and filling of structured closed ended interviewer-administered questionnaires. The participants were sampled between June 2010 and August 2011 at the Department of Veterinary Services of the respective states and sometimes at home. Blood was collected by qualified medical personnel from the
cohen's 

and

SPSS

were considered in the multivariable analysis in accordance

in each case was chosen. Several combinations of factors

with significant multicollinearity only one of such group

using a Tolerance Factor (TF) of 0.1 which corresponds to

Independent variables were tested for multicollinearity

level using IBM SPSS version 20 (SPSS Inc, Chicago, IL).


dilutions normally ranging from 1:20 to 1:1280. The

suspending cell suspension with a standardized volume of twice serum

dilution with whole Brucella cell suspension was incubated for 24 h at 37°C and

agglutination at the bottom of the tube was seen visually. The highest serum dilution that showed more than 50% agglutination was considered the agglutination titre. These SAT tubes containing serum dilutions with whole B. abortus and B. melitensis cells as antigens and that were negative after incubation for 24 h were centrifuged at 3000 rev/min for 15 min, the supernatant was decanted and the cell pellet was resuspended and washed with 1 mL phosphate buffered saline using mechanical agitation. This washing was repeated three times. Then about 100 μL standardized antihuman globulin reagent (anti-IgG) was added to the final pelleting in each test tube. The pellet was re-suspended and incubated in a water bath at 37°C for 48 h. On examination agglutination was seen visually by using an agglutinoscope or on a slide under the microscope. The most important antigens available for the diagnosis of brucellosis are the smooth (S) Lipopolysaccharides (LPS) of the outer membrane and internal proteins. The Coombs test detects antibodies reacting against S-LPS which leads to agglutination and remains positive longer than other agglutination tests. The test was performed at the Veterinary Research Institute, Ipoh, Malaysia. All those who tested positive to brucellosis using the test were defined as cases.

**DATA ANALYSIS**

Data generated was first analysed using descriptive analysis with frequency tabulations of variables and then univariate logistic regression analysis of the variables studied. At this screening level, the significance of each factors was evaluated at α = 0.10. Variables that were considered important biologically were recruited into the multiple logistic regression model.

Logistic regression (enter method) was used to elucidate significant risk factors at 90% confidence level using IBM SPSS version 20 (SPSS Inc, Chicago, IL). Independent variables were tested for multicollinearity using a Tolerance Factor (TF) of 0.1 which corresponds to a Variance Inflation Factor (VIF) of 10 and among those with significant multicollinearity only one of such group in each case was chosen. Several combinations of factors were considered in the multivariable analysis in accordance with biological plausibility and the statistical significance of the univariate analysis by adding and removing variables until the best model is found. Only main effects were considered and the relevance of a variable was measured by the significance of its regression coefficient and quantified by the odds ratio (OR). Non-significant predictors were excluded from the logistic regression model.

All participants who were active in animal farming were categorized as farmers. All staffs in the veterinary department were considered as veterinary technical staff but all other individuals whose occupation was not indicated on the questionnaires were classified as others. The categories were further collapsed into two for easy analysis as farmers and non-farmers. The states were collapsed into two for the purpose of analysis as Selangor state and other states (Pahang and Negeri Sembilan).

The mean age of participants was used to categorize the respondents into two categories (younger being 40 years and less and older those above 40 years). Those with an income greater than RM2500 were categorized as high income earners and those with an income less than or equal to RM2500 were categorized as low income earners based on the consideration of the overall Malaysian economy and minimum income levels and purchasing power (Zulkifi & Bujang 2008). Majority of our respondents were the ethnic Malays. Other ethnicities such as Chinese, Indian, Orang Asli (the aborigines), Indonesian and foreigners who were in small numbers were collapsed into one category as others and compared to the Malays.

**RESULTS**

A total of 446 volunteers participated in this study. The mean age of all participants in the study was 40.62±13.11 (range: 15-80). Six (1.35%) individuals out of 446 were seropositive for brucellosis of which 5 (2.69%) were farmers, 1 (0.94%) was grouped as other individuals and none of the veterinary technical staff were positive (Table 1). Most of the farmers claimed to keep more than one animal at a time typically keeping goats, sheep and cattle and sometimes chicken so it was difficult to classify them based on animals they keep. The male respondents were in the majority (84.08%) compared to the females (15.92%). All those seropositive were males. Based on marital status 347 (77.80%) of the respondents were married while 99 (22.20%) were single. A total of 4 (1.15%) out of the married were seropositive for brucellosis, while 2 (2.02%) were singles.

Based on educational qualifications, 312 (69.96%) had secondary school education or below secondary school qualifications while 134 (30.04%) had above secondary school qualifications. A total of 4 (1.28%) were positive for brucellosis among those with secondary or below education level and 2 (1.49%) among those with above secondary school education. Based on monthly income, 69 (15.47%) had a monthly income of greater than RM2500 (high) and 377 (84.53%) had a monthly income below or equal to RM2500 (low). A total of 2 (2.90%) participants
in the high income category were positive for brucellosis compared to 4 (1.06%) positive in the low income category. Based on occupation, 186 (41.70%) of the respondents were farmers and 260 (58.30%) belong to the category of non-farmers (veterinary technical staff and others with unspecified occupation).

Most farmers, 251 (56.28%) had assisted livestock during parturition while 195 (43.72%) have not. Out of those who assisted livestock during parturition 5 (1.99%) were positive for brucellosis and those who did not only 1 (0.51%) were positive for brucellosis. A total of 185 (41.48%) have cleaned aborted materials previously and 261 (58.52%) have not. Among those who cleaned aborted materials 4 (2.16%) were positive for brucellosis and among those who have never cleaned aborted materials 2 (0.77%) were positive for brucellosis. Unpasteurized milk was consumed by 139 (31.17%) while 307 (68.83%) do not consume unpasteurized milk. Among those who consume unpasteurized milk 4 (2.88%) were positive for brucellosis and among those who do not consume unpasteurized milk 2 (0.65%) were positive for brucellosis. Among the participants, 135 (30.27%) lived adjacent to a livestock farm while the remaining 311 (69.73%) do not. Among those who live adjacent to a livestock farm, 3 (2.22%) were positive for brucellosis and among those who do not live adjacent to a livestock farm also 3 (0.96%) were positive for brucellosis. A total of 120 (26.91%) have had one or more symptoms related to brucellosis while 326 (73.09%) have not. Surprisingly none of those who claim to have brucellosis related symptoms were positive for brucellosis while the remaining total positive cases of 6 (1.84%) were among those who profess not to have any symptoms of brucellosis.

Based on knowledge of acquiring brucellosis as a zoonotic disease 144 (32.29%) knew they can get brucellosis while the remaining (67.71%) were ignorant. Among those who knew they could get brucellosis 2 (1.39%) were positive while among those who claimed not to know, 4 (1.32%) were positive. Based on age categories 214 (47.98%) were less than or equal to 40 years of age while 232 (52.02%) were greater than 40 years of age. Majority of the positive cases (5) were among people less than 40 years of age (2.34%) compared to only 1 (0.43%) positive for brucellosis among those older than 40 years of age. Based on race 83 (18.61%) of the respondents were Non-Malays (Chinese, Indian, Orang Asli, Indonesia and other foreigners) while 363 (81.39%) were Malays. Among the non-Malays 2 (2.41%) were positive for brucellosis compared to the Malays with 4 (1.10%) positive for brucellosis.

Univariate analysis revealed 2 significant variables at 90% confidence level (Table 2).

The multivariable logistic regression analysis showed that all of the predictors included into the model which are gender, marital status, educational level, monthly income, occupation, assisting during parturition, cleaning aborted materials, drinking unpasteurized milk, living adjacent goat farms, suffering any symptoms of brucellosis, knowledge about brucellosis, age, race and states; only occupation, age and drinking unpasteurized milk were considered significant risk factors at 90% confidence level in the final model. The logistic regression analysis showed that the odds of brucellosis increased 7.2 times (AOR 7.19, 90% CI=1.16, 44.71) in farmers compared with the non-farmers, about 7.2 times (AOR 7.17, 90% CI=1.16, 44.41) in younger individuals compared with the older and about 4.5 times (AOR 4.45, 90% CI= 1.03, 19.15) in those who drank unpasteurized milk compared with those who did not (Table 3).

**TABLE 1. Seroprevalence of human brucellosis in Selangor, Pahang and Negeri Sembilan, Malaysia**

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Total N</th>
<th>Seroprevalence n (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmers</td>
<td>186</td>
<td>5 (2.69)</td>
<td>1.19, 4.19</td>
</tr>
<tr>
<td>Vet. Technical Staff</td>
<td>154</td>
<td>0 (0.00)</td>
<td>0.0, 0.0</td>
</tr>
<tr>
<td>Others</td>
<td>106</td>
<td>1 (0.94)</td>
<td>0.04, 1.84</td>
</tr>
<tr>
<td>Total</td>
<td>446</td>
<td>6 (1.35)</td>
<td>0.28, 2.42</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Brucellosis is an occupationally-associated disease and reports from different parts of the world have shown that one of the high risk groups for brucellosis are livestock farmers (Ahmad et al. 1999; Bamaiyi 2016; El Sherbini et al. 2007; Jama’ayah et al. 2011). The findings in this study showed that farmers had a higher seroprevalence of brucellosis than veterinary technical staff which may due to the better preventive and precautionary measures usually taken by veterinary staff when handling animals based on their knowledge of routes of transmission of disease. The univariate analysis showed that Selangor, which can be considered more urban than the other states sampled (Negeri Sembilan and Pahang), had a higher seroprevalence of human brucellosis. Similar observations were made in Kampala, Uganda where a higher seroprevalence of brucellosis of 83.5% was reported from urban areas and it was concluded in that that living in an urban area was a risk factor for brucellosis in man (Makita et al. 2008). This higher seroprevalence level is made possible because of increased demands for milk and milk products which are considered good for human health.
### TABLE 2. Descriptive and Univariable analysis of risk factors for brucellosis in farmers and veterinary staff in Selangor, Pahang and Negeri Sembilan states of Malaysia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (N)</th>
<th>Positive (%) n</th>
<th>Negative (%) n</th>
<th>P-value</th>
<th>POR</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selangor</td>
<td>153</td>
<td>2.61</td>
<td>97.39</td>
<td>0.118</td>
<td>3.91</td>
<td>0.93, 16.39</td>
</tr>
<tr>
<td>Other states</td>
<td>293</td>
<td>0.68</td>
<td>99.32</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary and below</td>
<td>312</td>
<td>1.28</td>
<td>98.72</td>
<td>0.860</td>
<td>0.86</td>
<td>0.20, 3.60</td>
</tr>
<tr>
<td>Above secondary</td>
<td>134</td>
<td>1.49</td>
<td>98.51</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Monthly income</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High income (&gt;RM2500 monthly)</td>
<td>69</td>
<td>2.90</td>
<td>97.10</td>
<td>0.243</td>
<td>2.78</td>
<td>0.66, 11.76</td>
</tr>
<tr>
<td>Low income (RM2500 &amp; below)</td>
<td>377</td>
<td>1.06</td>
<td>98.94</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>186</td>
<td>2.69</td>
<td>97.31</td>
<td>0.074</td>
<td>7.16</td>
<td>1.17, 43.67</td>
</tr>
<tr>
<td>Non-Farmers</td>
<td>260</td>
<td>0.39</td>
<td>99.61</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Assist livestock during parturition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>251</td>
<td>1.99</td>
<td>98.01</td>
<td>0.212</td>
<td>3.94</td>
<td>0.65, 24.06</td>
</tr>
<tr>
<td>No</td>
<td>195</td>
<td>0.51</td>
<td>99.49</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Clean aborted materials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>185</td>
<td>2.16</td>
<td>97.84</td>
<td>0.228</td>
<td>2.86</td>
<td>0.68, 12.00</td>
</tr>
<tr>
<td>No</td>
<td>261</td>
<td>0.77</td>
<td>99.23</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Drink unpasteurized milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>139</td>
<td>2.88</td>
<td>97.12</td>
<td>0.084</td>
<td>4.52</td>
<td>1.08, 18.97</td>
</tr>
<tr>
<td>No</td>
<td>307</td>
<td>0.65</td>
<td>99.35</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

NB: POR= Prevalence Odds Ratio
<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (N)</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>P-value</th>
<th>POR</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live adjacent to livestock farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>135</td>
<td>2.22</td>
<td>97.98</td>
<td>0.303</td>
<td>2.33</td>
<td>0.60, 9.04</td>
</tr>
<tr>
<td>No</td>
<td>311</td>
<td>0.96</td>
<td>99.04</td>
<td>-</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Suffer any symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>120</td>
<td>0</td>
<td>100</td>
<td>0.996</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No</td>
<td>326</td>
<td>1.84</td>
<td>98.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Know you can get brucellosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>144</td>
<td>1.39</td>
<td>98.61</td>
<td>0.956</td>
<td>1.05</td>
<td>0.25, 4.40</td>
</tr>
<tr>
<td>No</td>
<td>302</td>
<td>1.32</td>
<td>98.68</td>
<td>-</td>
<td>1.0</td>
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</tr>
<tr>
<td>Age categories</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>≤ 40 years</td>
<td>214</td>
<td>2.34</td>
<td>97.66</td>
<td>0.120</td>
<td>5.53</td>
<td>0.91, 33.72</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>232</td>
<td>0.43</td>
<td>99.57</td>
<td>-</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ethnicity/Race</td>
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<td></td>
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</tr>
<tr>
<td>Non-Malays</td>
<td>83</td>
<td>2.41</td>
<td>97.59</td>
<td>0.354</td>
<td>2.25</td>
<td>0.53, 9.49</td>
</tr>
<tr>
<td>Malays</td>
<td>363</td>
<td>1.10</td>
<td>98.90</td>
<td>-</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

NB: POR = Prevalence Odds Ratio
Educational level appears not to affect the seroprevalence of brucellosis. We believe that having higher level of education will not ensure protection against brucellosis but having the right information (regardless of education level) about brucellosis as well as taking heed to all precautionary measures against brucellosis is very important to guard against the infection. Those who had some knowledge of brucellosis and how to protect themselves from it have less seroprevalence of brucellosis because knowledge of the mode of transmission is protective as it helps individuals to take necessary precautionary measures to protect themselves from being infected by the bacteria (Kozukeev et al. 2006).

The higher income category has higher seroprevalence of brucellosis than the lower income in the univariate analysis. Contrary to previous findings of no difference related to income (Al-Shamahy et al. 2000) it was found in this study that those with higher income had a higher odds of infection. We speculate that this may be due to the fact that the higher income farmers can afford to consume goat milk (which are relatively costly) rather than completely selling all milk produced in their farm. Farmers benefit greatly economically from selling dairy products of animals as a means of livelihood (Jelastopulu et al. 2008) and only the economically buoyant may afford to consume more of their products than sale for family subsistence and survival. Farmers in Malaysia, like any other developing countries, have modest income and live in rural areas. They usually keep goats for immediate financial needs and the milk is mostly consumed by the wealthier farmers and in more wealthier suburbs and estates (Yusuff 1985).

Most (83%) of the seropositive cases in this study were farmers who rear goats and other livestock and hence have a closer level of relationship with animals. Close contact with infected animals significantly increases the farmer’s risks for brucellosis (John et al. 2010). The non-farmers (veterinary technical staff, comprising of veterinarians and para-veterinary staff and others) who may or may not work with animals were less likely to be positive for brucellosis in our study. Non-farmers have less contact with infected animals and may be protected because of their increased knowledge of the mode of transmission of the infection thereby enabling them to take precautionary measures when handling animals. This agrees with a study from Kyrgyzstan who found that knowledge of the mode of transmission of brucellosis was a protective factor against infection (Kozukeev et al. 2006).

Most (84%) of the farmers in the study and respondents were males and all the positive cases were also males. The high number of male respondents and farmers may partially account for the reason for the positive cases being male. The Malaysian livestock industry like in many other developing countries is dominated by males (Alston 1995; Dixon 1982) and men form the majority of farmers in Malaysia because of the physical nature of farming requiring more strength and energy (Ahmad 1999; Noor & Dola 2011) exposing them to higher risks of brucellosis. In a study in Jordan the male gender was identified as a risk factor for brucellosis with odds of 2.5 (Al-Majali & Shorman 2009).

Singles had a slightly higher seroprevalence than the married. Though no specific reason may be given for this, it is suggested that singles usually fall within the age range of the young who are reported to be more susceptible to brucellosis (Young 2009) due to their risky behaviours such as adventurous nature and tendency to handle animals more closely than the married.

Those who assisted goats during parturition previously as expected showed a higher seroprevalence of brucellosis due to the reproduction-associated nature of this zoonotic infection. The univariate analysis showed that the odds of brucellosis were 3.9 times more in those who assisted animals during parturition compared to those who did not. Copious amounts of Brucellae are usually shed during parturition by infected goats. These goats may appear apparently healthy but are carriers of the organism thereby increasing the chances of infection greatly at the time of parturition (Garin-Bastuji et al. 2006). Assisting in parturition of such infected animals has on several cases been reported to lead to B. melitensis human infection (Earhart et al. 2009; Garin-Bastuji et al. 2006; Seleem et al. 2010). In addition, individuals who clean aborted babies also appear several times more likely to be positive for brucellosis (Earhart et al. 2009; Garin-Bastuji et al. 2006; Seleem et al. 2010).

### Table 4

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>Sig.</th>
<th>AOR</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>1.973</td>
<td>1.111</td>
<td>3.157</td>
<td>0.076</td>
<td>7.19</td>
<td>1.16, 44.71</td>
</tr>
<tr>
<td>Non-farmers</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Age categories</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Younger</td>
<td>1.970</td>
<td>1.109</td>
<td>3.156</td>
<td>0.076</td>
<td>7.17</td>
<td>1.16, 44.41</td>
</tr>
<tr>
<td>Older</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Drink unpasteurized milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.493</td>
<td>0.887</td>
<td>2.832</td>
<td>0.092</td>
<td>4.45</td>
<td>1.03, 19.15</td>
</tr>
<tr>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Overall model data: -2LL=51.949; Nagelkerke R square= 0.194; Hosmer and Lemeshow Chi-square= 3.965; P= 0.681; d.f.= 6

NB: AOR= Adjusted Odds Ratio; CI= Confidence Interval; Sig.=Significance (P-value)
materials were found to be at higher risk of infection with
brucellosis because aborted materials from brucellosis
infected animals contain high quantities of the infective
bacteria (Chahota et al. 2003; Yumuk & O’Callaghan
2012).

As expected, those who lived adjacent to goat farms
had a higher seroprevalence rate for brucellosis and were
more likely to come down with brucellosis because they
are more likely to handle the animals due to their close
proximity to the animals. Handling of animals and working
closely with them is a risk factor for brucellosis reported
by earlier works (Al-Shamahy et al. 2000; Hassanain &
Ahmed 2012; Meky et al. 2007; Sofian et al. 2008b).

Superficially, it may appear surprising that those who
claimed to have symptoms of brucellosis had a lower
seroprevalence rate or none compared to those who claimed
to have no symptoms that had a higher seroprevalence rate.
However the finding may be explained by the confusing
nature of the disease symptoms to many other diseases
that gave rise to symptoms such as headaches, fever, joint
pains and back pains which are symptoms also common
in other diseases such as malaria, dengue, Typhoid fever
(Lopes et al. 2010; Pappas et al. 2006; Seleem et al. 2010).
No specific reasons could be deduced for why the non-
Malay races had a higher seroprevalence than the Malays
but it is known that cultural differences and practices which
exist among different races and groups of people may
influence their rate of infection with brucellosis (John et al.
2010) as well as many other communicable diseases. It is
probable that the Malays took more hygienic precautions
that may have reduced their chances of infection due
to the prevailing culture of washing hands and feet and
other parts of the body often during a day for prayers and
may be less in the habit of consuming raw milk. Other
race, especially the traditional Chinese are known to
recommend consuming goats milk raw to preserve the
milks nutritional and medicinal properties which is said
to be the most complete food known to man (Memish &
Balkhy 2004; Soultravelers 2013; Zhang et al. 2009) in
spite of the apparent health risks that may be involved
(Angulo et al. 2009).

The multivariable logistic regression model analysis
showed that occupation, age categories and drinking
unpasteurized milk account for 19.4% of the variability
in the brucellosis infection in this study. Brucellosis is
a common occupational and recreational infection that
infects many people especially farmers in the Southeast
Asian region and many regions of the world and has been
in existence in this region for a long time (Kalimuddin et
al. 2010; Seow et al. 2009). In Thailand, most of those
affected were goat farmers similar to the Malaysian pattern
in this study and a rapid rise in the infection coincided with
the implementation of the government policy to promote
goat rearing (Danprachankul et al. 2011; Manosuthi et
al. 2004). In the years between 2004 and 2009 there
have been 35 reported cases of brucellosis in humans in
Thailand where it is a re-emerging disease (Danprachankul
et al. 2011). In other parts of Asia such as India, caprine
brucellosis is also associated with occupational hazards of
those who work closely with goats (Mantur & Amarnath
2008). Those who keep animals (farmers) are usually
more prone to brucellosis than other individuals (Earhart
et al. 2009). Brucellosis is very common (20.9%) among
farmers in Iran (Kassiri et al. 2013). These agree with the
findings of this study that shows higher odds of brucellosis
in farmers compared to non-farmers in Malaysia. It
appears that animal farming is a major risk factor for
brucellosis especially when farmers do not take necessary
precautionary measures of hygiene when handling animals
and their secretions.

Brucellosis can affect all ages but it is most common
in adolescents and young adults (Al-Khafaji 2003; Gur
et al. 2003). Consistent with the finding in the current study,
in the developing countries most brucellosis cases are in
the young and in developed countries it is mostly in adults
(Yagupsky 2011). The individuals 40 years and younger
had a higher odds of brucellosis than individuals older
than 40 years in our study. Other workers have associated
brucellosis with age (Al-Majali & Shorman 2009; Al-Sultan
et al. 2011). Higher risk for brucellosis were found more
in ages 11-30 in a study in Yemen which also reported less
brucellosis cases in humans older than 40 years (Al-Sultan
et al. 2011) and in Turkey, which has 18000 new cases of
brucellosis yearly (Arica et al. 2012), 63% of brucellosis
cases reported in humans were individuals between the ages
of 15 and 45 years (Gur et al. 2003). In Greece 52 cases
of childhood brucellosis in children aged between 0-14
years were reported in families of shepherds (Galanakis et
al. 1996). In our study it is suggested that because those
40 years and below are in their most active working and
adventurous years there is the tendency for them to have
more opportunities of contact with the source of infection
than those above 40 years who may be considered relatively
less active and less adventurous (Young 2009). The activity
and adventure of the younger individuals would imply
them trying out unpasteurized milk and having contact
with animals without adequate protective measures while
the older individuals may be more inclined to be more
cautious in trying such adventures. This age range may also
be a reflection of the magnitude of the socio-economic and
cultural impact of brucellosis as in some other countries
such as Turkey (Gur et al. 2003) that makes certain age
groups more prone to brucellosis infection, especially
adolescents and young adults.

The consumption of unpasteurized milk is linked to
higher odds of brucellosis and farmers who consumed
such milk in our analysis indicated odds of 4.5 times more
compared to those who consumed pasteurized milk. This
supports the assertion of previous workers in this field who
found that consumption of unpasteurized milk increases
the odds of brucellosis 3.7 times (Sofian et al. 2008a) and
up to 54.13 times (Earhart et al. 2009). Recently, there
have been reports of outbreaks of brucellosis originating
from Malaysia in Hong Kong due to the consumption of
unpasteurized milk, which underscores the seriousness
of this mode of transmission in the epidemiology of
brucellosis in Asia (Anonymous 2011). There have also been cases of cluster infection among some local people in Penang, Malaysia after consuming unpasteurized milk from an infected goat farm herd (Anonymous 2011).

Brucellosis is present with a low seroprevalence rate among occupationally exposed farmers in Malaysia. In rural Greece health education of the population reduced the incidence of brucellosis from 1.4/1000/year to 0.2/1000/year (Hadjichristodoulou et al. 1999) and same strategy can be applied in Malaysia.

LIMITATIONS OF THE STUDY
The low number of positive cases used for the logistic regression analysis may give rise to biases. Therefore, the results of this analysis should be interpreted with caution. The interpretation should take into cognisance the univariate analysis to have a balanced picture. The confidence level of 90% means our study has evidence at 0.10 Type I error which is not as strong as some studies that may have evidence at 0.05 Type I error, hence the need for caution when interpreting and drawing conclusions from our findings. However, in spite of the confidence level there is evidence of association of the risk factors with brucellosis. The occupation of some respondents is not indicated leading to classification among others which could be misleading as they must have been farmers or veterinary staff. This arose as an oversight on the side of trained personnel who administered the questionnaires. Due to these limitations, all interpretations should be made within the context of these limitations.

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P.H. Bamaiyi, L. Hassan* & S. Khairani-Bejo
Department of Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

M. ZainalAbidin
Department of Agribusiness and Information System
Faculty of Agriculture
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

A. Adzhar & N. Mokhtar
Department of Veterinary Services
62630 Putrajaya, Federal Territory
Malaysia

M. Ramlan & P. Chandrawathani
Veterinary Research Institute
31400 Ipoh, Perak Darul Ridzuan
Malaysia

N. Hamidah
Department of Veterinary Services
40630 Shah Alam, Selangor Darul Ehsan
Malaysia

N. Abdullah
Department of Veterinary Services
71770 Seremban, Negeri Sembilan Darul Khusus
Malaysia

A.M. Husna Maizura
Disease Control Division, Zoonosis Section
Ministry of Health
62590 Putrajaya, Federal Territory
Malaysia

P.H. Bamaiyi
Department of Public Health
School of Allied Health Sciences
Kampala International University Western Campus
Uganda

*Corresponding author; email: latiffah@upm.edu.my

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