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REGULAR ARTICLES

Case–control study on risk factors associated with *Brucella Melitensis* in goat farms in Peninsular Malaysia

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Abstract Caprine brucellosis is a bacterial zoonotic infection affecting goats especially in developing countries all over the world. In Malaysia, the risk factors associated with this infection in farms have not been studied. A case–control study was carried out in goat farms in four states of Malaysia to elucidate the risk factors associated with the infection on the farms using structured questionnaires and face-to-face interviews. Results indicate that the introduction of new animals (OR= 5.25; 90 % CI=1.46, 18.88), younger age category of farms (OR=5.53; 90 % CI=1.09, 21.66), and farms with single breed of goats (OR=8.50; 90 % CI=1.27, 41.97) were significant risk factors for brucellosis. In order to control brucellosis or possibly eradicate it in goat farms, these factors need to be dealt with. Enforcing stringent importation protocols or complete ban of goat importation from brucellosis endemic countries will help reduce risk of introducing new infection into the country.

Keywords Case–control · *Brucella melitensis* · Risk factors · Goats · Farms · Malaysia

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Introduction

Brucellosis caused by *Brucella melitensis* occurs in many livestock farms all over the world. In goat farms, the infection may be detected using various serological tests (Rahman et al. 2013) but if left to persist in a farm, it can cause serious economic losses to the farmers in terms of loss of offspring, reduced milk production, increased kid mortality, market losses, and general loss in productivity (Sulima and Venkataraman 2010). Risk factors associated with brucellosis on goat farms vary from country to country and from region to region (Lopes et al. 2010). Some of the risk factors reported by various workers include the following: introduction of new animals into a herd, large herd size, rearing goats and sheep together on same farm, presence of other seropositive animals on the farm, breed of goats, importation of goats, age, pregnancy, and presence of does born outside the herd (Mikolon et al. 1998; Seleem et al. 2010; Islam et al. 2013).

Some of the reasons for the persistence of the infection in the farms in Malaysia are suspected to be an illegal trading of uncertified goats and low man power in the local veterinary authority to monitor the farmers and traders (Vu 2007; Aziz et al. 2012). It is also suspected that the large numbers of goats imported into the country from various countries is a major contributor to the persistence of brucellosis in the local goat farms (Aziz et al. 2012; Bamaiyi et al. 2014).

The risk factors of caprine brucellosis have been studied and reported from different countries of the world, but

information on the risk factors of caprine brucellosis in Malaysia is at best speculative even though the infections is prevalent in goat farms across Malaysia (Bamaiyi et al. 2012). This study was undertaken to determine the risk factors of caprine brucellosis in Peninsular Malaysia to enable effective or immediate control to be implemented if possible, from the modification of these disease determinants.

Materials and methods

Study location

Malaysia consists of 13 states and three federal territories and has a total land mass of 329,847 km^2 (127,350 mile^2). Malaysia is separated by the South China Sea into Peninsular Malaysia and East Malaysia (comprising of Sabah and Sarawak). Malaysia borders Thailand, Singapore, Indonesia, and Brunei and is located at 2° 30′ and 112° 30′ (Bunnell 2004; Anon 2012). Peninsular Malaysia comprised of 11 states and 2 federal territories. For the purpose of this study, four states were chosen in Peninsular Malaysia based on their *B. melitensis* seroprevalence level in the last 10 years (2000–2009) (Fig.1).

Study design

A case–control study was conducted between July and September 2011. A farm was considered positive if one or more goats within the herd were confirmed seropositive using the complement fixation test (CFT) by the Veterinary Research Institute, Ipoh, Malaysia and negative if all tested animals in the herd were negative. The CFT procedure was performed as described by the Office International des Epizooties (OIE) (OIE 2009), as prescribed by the protocol of the Department of Veterinary Services of Malaysia (DVS 2010). The CFT has a specificity of up to 100 % and sensitivity of up to 96 % (McCauley et al. 2007) and is a prescribed diagnostic method by the OIE.

Sample size calculation Sample size was calculated using OpenEpi epidemiologic statistics for public health software version 3.01 based on the method of Sullivan et al. (2009). At least 20 cases and 20 controls were required for the study. A total of 42 goat farms from four states in Peninsular Malaysia were included in the study which include Melaka, $n=12$ farms (cases, $n=7$; control, $n=5$) (28.6 %), Negeri Sembilan, $n=10$ farms (cases, $n=5$; control, $n=5$) (23.8 %), Pulau Pinang, $n=10$ farms (cases, $n=5$; control, $n=5$) (23.8 %), and Selangor, $n=10$ farms (cases, $n=5$; control, $n=5$) (23.8 %). These four states were chosen based on the logistical convenience and the nationwide *B. melitensis* serosurveillance findings of the years 2000 to 2009 that shows them among the states with the highest reactor rates for brucellosis in goats. The farms were chosen among those that were still confirmed *B. melitensis* positive up to the year 2010 to ensure that the farms are still operating and that the information derived from these farms was current and reflective of the true situation.

Data collection

Twenty-two case herds and 20 control herds were used for the study. Letters were initially sent to the selected farms via the local postal service to get the farmers' approvals for visits. We then engaged the state local veterinary services departments and district levels veterinary authorities to facilitate farm visits and to communicate with the farmers. During each visit, the farmers were introduced to the objectives of the study and were asked to sign a consent form for an interview. The enrolment into the study was based on the willingness of the farmers. Farmers were assured of the confidentiality of the

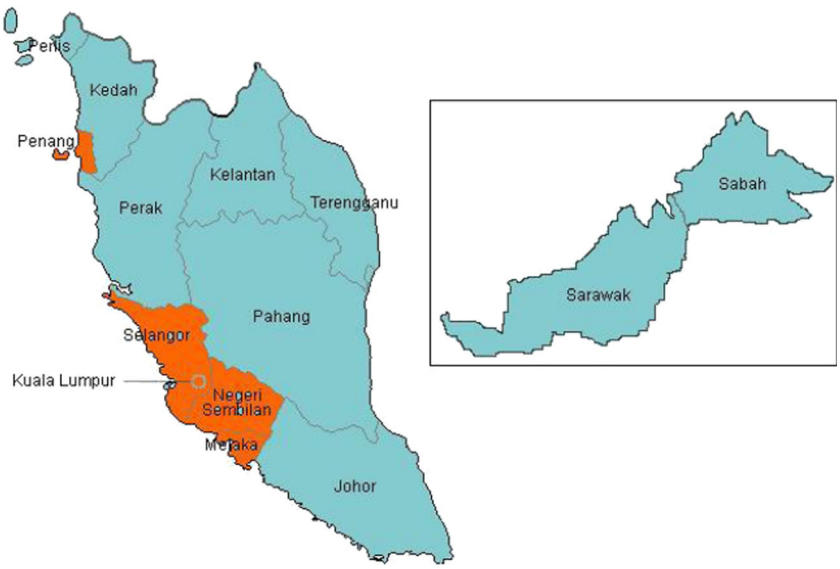

Fig. 1 Map of Malaysia showing study states in *orange* (adapted from Google maps)

Table 1 Univariate analysis of farm-level risk factors for caprine brucellosis in Selangor, Negeri Sembilan, Melaka, and Pulau Pinang states of Malaysia

Variables	Total	Case		Control		*P*	OR	90 % CI
	N	%	*N*	%	*N*			
Number of staff								
<5	34	52.94	18	47.06	16	0.881	1.125	0.309, 4.100
≥5	8	50.00	4	50.00	4			Reference
Herd size								
≥330	16	68.75	11	31.25	5	0.101	3.000	0.997, 9.026
<330	26	42.31	11	57.69	15			Reference
No. of breeds of goats								
Single breed	11	72.73	8	27.27	3	0.126	3.238	0.917, 11.438
More than one breed	31	45.16	14	54.84	17			Reference
Purpose of keeping goats								
Multipurpose	16	56.25	9	43.75	7	0.694	1.286	0.450, 3.676
Meat/breeding only	26	50.00	13	50.00	13			Reference
Location to another farm								
Near another farm (mean dist., 4,184 m)	32	53.13	17	46.87	15	0.863	1.133	0.344, 3.734
Far from another farm (>4,184 m)	10	50.00	5	50.00	5			Reference
Farm management system								
Intensive	23	56.52	13	43.48	10	0.555	1.444	0.518, 4.024
Semi-intensive	19	47.37	9	52.63	10			Reference
Species of animals in neighboring farms								
Ruminants	32	53.17	17	46.87	15	0.863	1.133	0.344, 3.734
Nonruminants	10	50.00	5	50.00	5			Reference
Separating pregnant does								
Do not separate	13	30.77	4	69.23	9	0.067	0.272	0.084, 0.877
Separate	29	62.07	18	37.93	11			Reference
Use of gloves								
Use bare hands	16	50.00	8	50.00	8	0.809	0.857	0.301, 2.441
Use gloves	26	53.85	14	46.15	12			Reference
Abortion in the past 6 months								
Yes	26	46.15	12	53.85	14	0.306	0.514	0.177, 1.496
No	16	62.50	10	37.50	6			Reference
Knowledge of transmission of brucellosis								
No	8	37.50	3	62.50	5	0.355	0.474	0.125, 1.789
Yes	34	55.88	19	44.12	15			Reference
Goats mixing with other farms' goats								
Yes	8	37.50	3	62.50	5	0.582	0.632	0.160, 2.497
No	34	55.88	19	44.12	15			Reference
Abortion in neighboring farms								
Yes	8	37.50	3	62.50	5	0.355	0.474	0.125, 1.789
No	34	55.88	19	44.12	15			Reference
Introduction of new animals								
Yes	22	63.64	14	36.36	8	0.129	2.625	0.922, 7.475
No	20	40.00	8	60.00	12			Reference
Source of new animals								
Import	17	47.06	8	52.94	9	0.570	0.698	0.247, 1.973
Do not import (govt., other farmers)	25	56.00	14	44.00	11			Reference
Allow unauthorized visitors in farm								
Yes	15	46.67	7	53.33	8	0.581	0.700	0.242, 2.027

Table 1 (continued)

Variables	Total	Case		Control		*P*	OR	90 % CI
	N	%	*N*	%	*N*			
No	27	55.56	15	44.44	12			Reference
Washing facility before farm entry								
No	23	47.83	11	52.17	12	0.516	0.667	0.239, 1.863
Yes	19	57.89	11	42.11	8			Reference
Protective covering								
No	27	51.85	14	48.15	13	0.927	0.942	0.326, 2.723
Yes	15	53.33	8	46.67	7			Reference
Wildlife near farm								
Yes	26	42.31	11	57.69	15	0.827	0.867	0.295, 2.546
No	16	68.75	11	31.25	5			Reference
Age category of farm								
<9 years old (young farms)	28	60.71	17	39.29	11	0.132	2.782	0.911, 8.497
≥9 years old (older farms)	14	35.71	5	64.29	9			Reference
Frequency of abortion								
Frequent (more than three times in 6 months)	10	60.00	6	40.00	4	0.582	1.500	0.447, 5.033
Not frequent (three times or less in 6 months)	32	50.00	16	50.00	16			Reference

OR odds ratio, *CI* confidence interval

research and encouraged to speak freely. Face-to-face interviews were conducted with the assistance of a research officer and one or two district veterinary officers on each farm using a structured questionnaire (available on request) in the local Malay language. The questions sought information on important farm-level variables such as herd size, breeds of goats kept, proximity to other farms, system of management of farm, number of employees, frequency of abortion on the farms, isolation of sick animals, consumption of raw milk produced on the farm, knowledge of brucellosis, importation of animals, use of protective covering, and presence of wildlife. The response of the farmers was transcribed into the structured questionnaire on each farm visited.

Data analysis

The variables were checked for collinearity and multicollinearity using SPSS version 20 (SPSS Inc., Chicago IL). Variables that show high correlation with each other were sorted by removing one of the variables. The variables were checked for multicollinearity using the variance inflation factor (VIF) and tolerance factor (TF). VIF values of greater than 10 or Tolerance less than 0.1 were considered the cut-off points (Field 2009) for the collinearity diagnostics. For continuous variables such as age and herd size, they were categorized using the mean age and mean herd size value as the cut-off point for the two categories created for the purpose of data analysis.

A univariate analysis was done on each of the variables studied, and variables with $P<0.3$ were recruited into the binary logistic regression analysis. The enter method was used to run the logistic regression analysis. Variables with higher $P>0.10$ were eliminated one at a time and the analysis rerun each time until all variables were significant at $P<0.1$. Several combinations of the variable were made in the binary logistic regression guided by the biological plausibility and the conditional statistical significance as revealed by the Wald and other tests. Only main effects were considered and the relevance of a variable was determined by the significance of its regression coefficient and quantified numerically by the odds ratio (OR). The final binary logistic regression model contained only all the variables significant at $P<0.1$.

Results

The age of the farms ranged from 1 year to 31 years with a mean of 8.45±6.82. The goat herds in the four states in Malaysia ranged in size from 15 to 1,444 goats with a mean number of goats of 330 per farm (SD=342).

Table 1 shows the results of the univariate analysis and descriptive analysis of 21 variables on the structured interviewer-administered questionnaires that were used to collect the data. At 90 % confidence level, herd size, number of breeds of goats, introduction of new animals, and age category of farm had OR>2. Table 2 shows the logistic regression

Table 2 Logistic regression model for the potential farm-level risk factors for caprine brucellosis in Selangor, Negeri Sembilan, Melaka, and Pulau Pinang states of Malaysia

Variables	*B*	S.E.	Wald	Sig.	OR	90 % CI for OR
Introduction of new animals						
Introduced new animals	1.657	0.778	4.532	0.033	5.245	1.458, 18.875
No new animals introduced	–	–	–	–	1.00	Reference
Age category of farms						
Young farms	1.711	0.830	4.249	0.039	5.532	1.088, 21.660
Old farms	–	–	–	–	1.00	Reference
Number of breeds of goats						
Single breed of goats	2.140	0.971	4.854	0.028	8.495	1.266, 41.966
Multiple breeds of goats	–	–	–	–	1.00	Reference

Overall model data: −2LL=46.867; Nagelkerke R^2 =0.314; Hosmer and Lemeshow chi-square=1.640; P=0.896; df=5

OR odds ratio, *CI* confidence interval

model with significant variables at 90 % confidence level. Table 3 shows the potential risks of the farmers based on information they gave about themselves which though not statistically significant may be suggestive of risks they are exposed to.

Discussion

One of the limitations of this study is the small sample size used which may not allow stronger conclusions to be made on the risk factors of caprine brucellosis and restricts the number of risk factors that were found statistically significant. Another limitation is the inability to take blood samples of goat farmers based on the brucellosis status of their farms in order to elucidate the association between the brucellosis status of goat farms and that of their owners. This restricts conclusive inferences on the human risk factors of the goat farms studied. But this study provides a clearer picture of risk factors for brucellosis in Malaysia and ways by which the infection can be controlled or eradicated.

From the binary logistic regression analysis, the practice of introducing new animals into herds places a farm at higher odds of brucellosis (Coelho et al. 2008; Aziz et al. 2012). This practice invariably will lead to the introduction of new infections if farms or geographical locations of the introduced animals was brucellosis endemic. The introduction of new animals into a herd increased the odds of brucellosis by 5.3 times. The finding agrees with others who have reported similar observations such as seen in Mexico where the likelihood of disease increased by 68.3 times in herds with goats brought from other states to the Mexicali Valley (Mikolon et al. 1998). In Portugal, the introduction of animals from other herds increased the odds of brucellosis 12.11 times (Coelho et al. 2007), and in Ethiopia, it was 1.2 times (Teklue et al. 2013).

The significantly higher odds of brucellosis in newer farms (<9 years) than in older farms (≥9 years) may be as a result of

Table 3 Potential risk of *Brucella melitensis* infection for the goat farmers

	Number of farms (%) (n=42)	Case farms (%) (n=22)	Control farms (%) (n=20)	*P*	OR	95 % CI
Consumed milk from goats						
Yes	24 (57.1)	11 (45.8)	13 (54.2)	0.3286	0.5385	0.1555, 1.8646
No	18 (42.9)	11 (61.1)	7 (38.9)	–	1.00	Reference
Family members consumed milk						
Yes	18 (42.9)	7 (38.9)	11 (61.1)	0.1334	0.3818	0.1086, 1.3425
No	24 (57.1)	15 (62.5)	9 (37.5)	–	–	Reference
How the goat milk is consumed						
Raw	18 (42.9)	11 (61.1)	13 (38.9)	0.3286	0.5385	0.1555, 1.8646
Boiled/pasteurized	24(57.1)	11 (57.1)	7 (42.9)	–	–	Reference

OR odds ratio, *CI* confidence interval

experience of farmers in the handling of infectious disease and preventive practices which are usually acquired over time. The older farms are most likely handled by older more experienced farmers who know the clinical signs of brucellosis and what preventive measures to adopt and may have been affected by this economically devastating infection in the past. It is also possible that newer farms are more likely to bring in new animals to further establish their farms thereby accidentally introduce infected animals into their flock. Coelho et al (2007) observed that farms in Portugal belonging to older farmers had decreased odds of brucellosis (older age was protective). The younger farms may suffer from difficulties arising from lack of experience in disease prevention and control methods which are very crucial in the prevention and control of brucellosis. Proven effective methods for control and prevention of brucellosis if not used will lead to infection and reinfection of farms with brucellosis (Refai 2002). In addition to this, our study indicated that many younger farms also have large herd sizes which have been associated with brucellosis (Coelho et al. 2007; Coelho et al. 2008; Seleem et al. 2010).

The higher odds of brucellosis in a single breed of goat farms may at first appear contradictory. But a closer look revealed that farms with single breed of goats mostly keep imported goats and managed them mostly intensively. These factors (importation and intensive management system) have been incriminated by previous workers to be risk factors for brucellosis (Refai 2002; Azevedo et al. 2009; Lopes et al. 2010).

The fact that many farmers still drink raw milk from their goats especially from farms seropositive for brucellosis is a point of concern from a public health point of view as drinking unpasteurized milk has been incriminated in many cases of brucellosis outbreaks and infections (Sofian et al. 2008; Bonfoh et al. 2012; Currò et al. 2012; Salata 2012). The findings of this study revealed many farmers still consume unpasteurized milk and also allow family members to freely drink raw milk from their goat farms despite disease risk.

Conclusion

Brucellosis is present in goat farms in Peninsular Malaysia. The risk factors for brucellosis in goat farms in Peninsula Malaysia include the following: introduction of new goats into the herd, age of the farm, and number of breeds of goats kept. In order to successfully control and possibly eradicate brucellosis from goats in Malaysia, closer attention must be paid to these variables. To be able to control brucellosis in countries like Malaysia, it is very important to create awareness of the infection among farmers as lack of awareness has been found responsible for persistent endemicity of the infection in some developing countries of the world (Adamu et al. 2012) and may be a similar scenario in Malaysia. Brucella melitensis infection is highly endemic in goats in many countries across southeast Asia, therefore importations of goats from these countries will increase the risk of introducing new infection into the local goat population. Hence, implementing and enforcing restrictive importation protocols or complete ban of importation from these countries would be prudent to reduce the risk of brucella infection and eventually achieve brucella disease minimization or freedom.

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Conflict of interest The authors declare that they have no conflict of interest.

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