Original Article

Seroprevalence and risk factors of Q fever in small ruminant flocks in selected States of Peninsular Malaysia

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Abstract

Q fever is caused by *Coxiella burnetii*, an obligate intracellular parasite which causes abortion and reproductive disorders in domestic animals and febrile illness in man. Earlier studies have detected *C. burnetii,* including serum antibodies in humans, cattle, sheep and goats in Malaysia. However, the overall seroprevalence status and contributing factors of Q fever among small ruminants in Malaysia are still unexplained. This study was therefore conducted to investigate the seroprevalence and risk factors of Q fever among small ruminant flocks in Negeri Sembilan and Terengganu states. To determine the seroprevalence of Q fever among small ruminants, we collected blood samples from a total 272 sheep and goats across six smallholder farms in two states and tested all sera using commercial Sandwich-ELISA kit with a sensitivity of 100% and specificity of 99.6% for the direct detection of specific antibody against *Coxiella burnetii*. Our results revealed 12.1% (8.77-16.55) apparent prevalence and 11.8% (8.40-16.21) true prevalence of Q fever among individual sheep and goats in the two states. Univariable analysis revealed that states (X^2 =10.264: p=0.001), farms (X^2 =27.32: p=0.000), gender (X^2 =3.908: p=0.048), age (X^2 =12.845: p=0.000), breed (X^2 =13.435: p=0.004) and production (X^2 =8.992: p=0.003) of small ruminants were associated with their Q fever seropositive status. Multivariable logistic regression further revealed 3.972, 12.455 and 11.921 times more likelihood of Q fever in the young, the Barbados Black Belly sheep breed and the meat production animals. In conclusion, the seroprevalence Q-fever in Terengganu and Negeri Sembilan states is associated with the age, breed and the production purpose of small ruminants.

Keywords: *Coxiella burnetii*, Seroprevalence, Small ruminants, Malaysia, Q fever

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Received July 20, 2020.

Accepted September 22, 2020.

Introduction

The term Query fever (Q fever) was first used in 1937 by Edward Holbrook Derrick to describe a strange febrile illness among abattoir workers in Australia (Derrick, 1937). *Coxiella burnetii*, the agent of Q fever, is an obligatory gram-negative intracellular gamma *Proteobacteria*, measuring 0.2-0.4 by 0.4-1.0µm in size (Stein and Raoult, 1998; Maurin and Raoult, 1999). *C. burnetii* is a ubiquitous organism which has a complex life cycle involving arthropods, earthworms, cold-blooded and warm-blooded animals (Brooks *et al.,* 1986). Domestic ruminants are the primary sources of zoonotic infection, which is mainly transmitted by inhalation of aerosol through direct exposure to infected animals during assisted delivery or slaughter operations (Dupont *et al.,* 1995; Tissot-Dupont and Raoult, 2008; Robyn *et al.,* 2015). However, human infection may result from the ingestion of raw milk (Rodolakis, 2009) and rarely through contact with infected human secretions (Stein and Raoult, 1998). Moreover, arthropod-borne transmission involving various species of hard ticks is also essential in the epidemiology of zoonotic Q fever (Ghashghaei *et al.,* 2016).

Affected animals display the typical syndrome of abortion, premature delivery, stillbirth and weak offspring (APSW), and may persistently shed organisms in their body secretions and excretions (Agerholm, 2013). Q fever has a global distribution with sporadic cases and outbreaks reported in more than 50 countries, occurring at different rates in different geographical locations. For instance, a seroprevalence rate of 3.5% in goats and 2.1% in sheep has occurred in Thailand (Doung-Ngern *et al.,* 2017), 4.1 % have occurred in goats in Lao Peoples Democratic Republic (Burns *et al.,* 2018) and 4.4 % among slaughtered goats in India (Mohan *et al.,* 2017). On the other hand, reports from other countries show a higher prevalence with 10% among goats in Ghana (Johnson *et al.,* 2019), 12.6 % in mixed dairy cattle farms in Ecuador (Carbonero *et al.,* 2015), 18.6% among goats in Kenya (Browne *et al.,* 2017), 20 % among sheep in Turkey (Kennerman *et al.,* 2010), 21.4 % among dairy goats in the Netherlands (Schimmer *et al.,* 2011) and 55.1 % among dairy goats in Brazil (de Oliveira *et al.,* 2018).

Infectious diseases caused by bacteria, virus and parasites are common causes of morbidity and mortality (Jesse *et al.,* 2013, 2018; Mohammed *et al.,* 2016), reproductive failure and economic loss in small ruminant production in Malaysia (Chung *et al.,* 2019a; 2019b). A previous study in Malaysia reported 9.6% seroprevalence of *Coxiella burnetii* among the Orang Asli population (Khor *et al.,* 2018). A case of persistent febrile illness was also described in a farmer who handled the aborted foetus from a goat on a farm during an outbreak involving goats and cattle in three smallholder farms in Pahang (Rai *et al.,* 2011). Moreover, recent studies by Jing et al., (2017) detected *Coxiella burnetii* in specimens of hard tick species collected from ruminants, while Nurkunasegran *et al.,* (2017) isolated *Coxiella burnetii* in biological samples obtained from domestic livestock in Malaysia. These studies furnished preliminary data on the status of coxiellosis in animal and human populations and highlighted its economic and public health importance in Malaysia. However, there is a lack of published information on the seroprevalence status and risk factors of Q fever among smallholder sheep and goat flocks in Malaysia. We hypothesised that the seroprevalence of Q fever is unevenly distributed among individual animals and smallholder farms in the study areas. This study was therefore designed to investigate seroprevalence status and risk factors of Q fever in selected smallholder flocks in two states of Peninsula Malaysia.

Materials and Methods

Ethical approval and consent: The protocols used in this study were approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (UPM/IACUC/AUP-R041/2019). We also approached the smallholder farms through the Department of Veterinary Services in the respective states. Written informed consent was obtained from selected farmers who agreed to take part in this study before collecting samples and data for the study.

Study area and population: Negeri Sembilan is a state on the west coast of Peninsular Malaysia (2.8 2.7258 ^{o}N, 101.9424 ^{o}E), which is bordered by Selangor to the north, Pahang to the east, Melaka, and Johor to the south. Rubber, oil palm plantations, manufacturing and tourism are the main economic activities in addition to the growing livestock industry, which currently has 63,673 sheep and goats in smallholder farms across the state. Terengganu state is a tropical rainforest zone on the east coast of the Malaysian Peninsula (5.3117 ^{o}N, 103.1324 ^{o}E) which has an estimated 37,000 sheep and goats in smallholder farms within the rural localities.

Study population: Most of the sheep and goats examined in this study were farm animals kept by resource-poor individual smallholder farmers. The latter practised a semi-intensive management system that allows daytime grazing and provision for housing and a small amount of feed supplement at night. The indigenous Katjang was the most frequent, followed by the Jamnapari and Boer breed of goats while the Barbados Black Belly was the only sheep breed encountered in the study area. Meat production appears to be the significant reason for sheep and goat rearing in the study areas but some farmers also kept a few goats for milk production.

Study design and sampling: A cross-sectional survey design was implemented to simultaneously collect samples and data from small ruminant flocks in Negeri Sembilan and Terengganu. We calculated the minimum number of samples required to estimate the apparent and actual prevalence of Q fever according to (Thrusfield, 2005) based on the assumptions about large small ruminant population, expected prevalence of 89% (Jing *et al.,* 2017), 95% confidence interval (1.96^2) and 5% desired absolute precision (d=0.05). Accordingly, a total of 150 samples were needed for estimating the prevalence of Q fever in this study.

However, we randomly collected 272 blood samples from six small ruminant farms to increase the precision of our estimates. A total of 4 farms in Terengganu (A (n=40), B (n=40), C (n=55), and D (n=45)) and 2 farms in Negeri Sembilan (E (n=63) and F(n=29)) were included in the study based on the farmers consent to participate. During each farm visit, individual animals were routinely aged by dentition and their breed and gender determined by morphological characteristics. To collect blood, a trained assistant physically restrained each animal, and approximately 5ml of blood was withdrawn from the jugular vein using pre-labelled plain vacutainer tubes and transported to the laboratory in an icepack. The age, sex, breed, production purpose and management of each animal was noted on a sampling form and filed for risk analysis. After sample collection, animals were handed over to the farmer and released back into the flock.

Laboratory examination: We extracted serum from coagulated blood by centrifugation at 3000 revolutions for 20 minutes (Eppendorf® AG 22331, Hamburg Germany) and held the sera deeply frozen at -20 ^{0}C before the ELISA test. We used a commercial Sandwich-ELISA test kit with a sensitivity of 100% and specificity of 99.6% for the direct detection of specific antibody against Q-fever (Cat No. SL0048Gt) following the manufacturer's instructions (Sunlong Biotech Co., LTD, China). We measured the optical densities (OD) of test sera and standards at 450nm using an ELISA Microplate Reader (Tecan Sunrise®, Switzerland). The percentage of inhibition was calculated as 100 x [1-(Sample optical density/Negative control OD)], and OD values ≥ 1.00% were regarded as a positive result. In contrast, OD values ≤ 0.10% were considered a negative outcome.

Statistical analysis: The apparent prevalence and true prevalence of Q fever with the corresponding 95% confidence intervals were calculated using Epitools® software (Rogan and Gladen, 1978; Brown *et al*., 2001). We initially summarised the raw data in Microsoft® Excel Spreadsheet Program Version 2016 and imported the tabulated data in IBM® Statistical Package for Social Sciences (SPSS) software version 25.0 for further analysis. Univariable analysis was conducted to determine the association between the serological status of small ruminants and presumed risk factors using a Chi-square test at a 5% level of significance. The significant variables were further incorporated in a forward (stepwise) multivariable logistic regression model to filter the variables at a 5% level of significance, to determine the risk factors associated with Q fever seropositive status.

Results

Prevalence of Q fever among small ruminants: In total, 272 sera collected from 6 small ruminant farms in two states were tested by ELISA to determine the seroprevalence of Q fever among small ruminant flocks. Our results revealed an apparent prevalence of 12.1% (8.77-16.55) and a true prevalence of 11.8% (8.40-16.21) for Q fever among individual sheep and goats in the two states. Our results further revealed an apparent prevalence of 16.7 % (11.93-22.80) and a true prevalence of 16.3% (11.58-22.49) in Terengganu state. On the other hand, an apparent prevalence of 3.3% (1.12-9.15) and a true prevalence of 2.9% (0.72-8.79) was recorded in Negeri Sembilan state (Table 1).

Univariable analysis of risk factors: The univariable analysis between seropositive status of Q fever and presumed exposure factors of small ruminants revealed that states (X^2 =10.264: p=0.001), farms (X^2 =27.32: p=0.000), gender (X^2 =3.908: p=0.048), age (X^2 =12.845: p=0.000), breed (X^2 =13.435: p=0.004) and production (X^2 =8.992: p=0.003) were associated with the Q fever seropositive status of small ruminants (Table 2).

Multivariable analysis of risk factors: The multivariable analysis of significant exposure factors revealed that age, breed and production purpose of small ruminants were the risk factors associated with Q fever seropositivity in the study areas. Young animals were 3.972 times more likely to be seropositive (95% CI=1.51-10.49; p=0.005) than adults. Barbados Black Belly sheep were 12.455 more likely to be seropositive (95% CI=1.43-108.74; p=0.023) than Katjang and Boer goats. Similarly, small ruminants raised for meat production were 11.921 more likely to be seropositive than dairy animals (Table 3).

Discussion

The serological survey of Q-fever among small ruminants in selected farms in Terengganu and Negeri Sembilan revealed an apparent prevalence of 12.1% (8.77-16.55) and a true of 11.8% (8.40-16.21) which is similar to 12.2% prevalence of *Coxiella burnetii* detected in biological samples from sheep and goats in Malaysia (Nurkunasegran *et al*., 2017). Our result further agree with 12.6 % seroprevalence of Q fever recorded among ruminant herds in Ecuador (Carbonero *et al*., 2015) and 12.4% recorded among sheep and goat flocks in the Kingdom of Saudi Arabia (Mohammed *et al*., 2014). The presence of Q fever antibodies in Malaysian sheep and goat flocks indicates current or past exposure to *Coxiella burnetii*, which may be accompanied by immeasurable economic loss due to ill thrift and reproductive inefficiency due to abortions, stillbirths and dystocia (Johnson *et al*., 2019). The prevalence of Q fever in Malaysia may be due to environmental conditions that favour the biology of the tick vectors of *Coxiella burnetii*, which are abundant on small ruminants in Malaysia (Khoo et al., 2016; Jing et al., 2017; Nurkunasegran et al., 2017). Semi-intensive management of smallholder sheep and goats in Malaysia also allows limited grazing in the pastures around forests, which may increase the exposure of animals to the vectors.

On the other hand, the result of the present study is lower than 27.2% prevalence met in cattle and goat farms during an earlier outbreak that resulted in human infection in Malaysia (Rai *et al*., 2011). Our result also varies from other studies in Brazil, where 55.1% of goats were seropositive (de Oliveira *et al*., 2018), in Turkey where 20% of sheep were seropositive (Kennerman *et al*., 2010), in the Netherlands where

Schimmer *et al.,* (2011) reported 21.4% individual and 43.1% of herd-level seroprevalence, and in Kenya where Browne *et al.,* (2017) reported 18.6% prevalence. Also, contrary to our present result, lower prevalence rates have been reported in India, where two separate studies revealed 4.4 % and 7.0% (Mohan *et al.,* 2017; Keshavamurthy *et al.,* 2019). Likewise, Burns *et al.,* (2018) reported 4.1% among goats in Lao People's Democratic Republic, while Doung-Ngern *et al.,* (2017) showed a seroprevalence of 3.5% and 2.1% in goats and sheep in Thailand. The discrepancies in the occurrence of Q fever between results of the present study and past reports in Malaysia and elsewhere may be attributable to several factors influencing the epidemiology of *Coxiella burnetii*. Firstly, local differences in ecological conditions affect the bionomics, abundance and distribution of arthropod vectors and influence the role of ticks in disease transmission in different study areas (Ghashghaei *et al.,* 2016). Secondly, the availability of vertebrate reservoir hosts such as mice, cats and carnivores, which are unevenly distributed in different communities, may influence the exposure of animals to *Coxiella burnetii* and account for the observed differences in infection rates (Kazar, 2005). Thirdly, the difference in the prevalence of Q fever may be due to variations in laboratory diagnostic methods used in different studies (Wegdam-Blans *et al.,* 2012). Thus, the present study used a direct enzyme-linked immunosorbent assay technique, while other studies used the more sensitive immunofluorescent antibody technique (IFAT) and polymerase chain reaction (PCR).

Table 1 The apparent and true prevalence of Q fever among small ruminants in selected states of Malaysia

Variables	Number	Positive	Apparent (95% CI)	True (95% CI)
States				
Terengganu	180	30	16.7 % (11.93-22.80)	16.3% (11.58-22.49)
Negri Sembilan	92	03	3.3% (1.12-9.15)	2.9% (0.72-8.79)
Farms				
A	40	10	25.0% (14.19-40.19)	24.7% (13.84-39.95)
B	40	10	25.0% (14.19-40.19)	24.7% (13.84-39.95)
C	55	10	18.2% (10.19-30.33)	17.9% (9.83-30.05)
D	45	00	-	-
E	63	02	3.2% (0.87-10.86)	2.79% (0.48-10.50)
F	29	01	3.5% (0.61-17.18)	3.1% (-0.22-16.84)
Species				
Sheep	140	20	14.3% (9.44-21.04)	13.9% (9.08-20.72)
Goat	132	13	9.9% (5.85-16.12)	9.5% (5.47-15.78)
Sex				
Male	98	17	17.4% (11.12-26.04)	17.0% (10.77-25.74)
Female	174	16	9.2% (5.74-14.41)	8.8% (5.36-14.07)
Age				
Young	90	20	22.2% (14.87-31.85)	21.9% (14.53-31.57)
Adult	182	13	7.1% (4.22-11.84)	6.8% (3.84-11.48)
Breed				
SH-Barbados Black Belly	40	10	25.0% (14.19-40.19)	24.7% (13.84-39.95)
GT-Katjang	141	20	14.2% (9.37-20.90)	13.8% (9.01-20.58)
GT-Jamnapari	62	02	3.2% (0.89-11.02)	2.8% (0.49-10.66)
GT-Boer	29	01	3.5% (0.61-17.18)	3.1% (-0.22-16.84)
Production				
Meat	207	32	15.5% (11.17-21.01)	15.1% (10.81-20.69)
Dairy	65	01	1.5% (0.27-8.21)	1.1% (-0.32-7.84)
Management				
Semi-Intensive	187	25	13.4% (9.22-18.99)	13.0% (8.86-18.67)
Intensive	85	08	9.4% (4.85-17.49)	9.1% (4.46-17.16)
Overall	272	33	12.1% (8.77-16.55)	11.8% (8.40-16.21)

CI: confidence interval

Table 2 Univariable association between Q fever seropositivity and exposure factors in small ruminants.

Variables	Examined	Positive (%)	Negative (%)	X^2	*P*-value
States					
Terengganu	180	30 (16.7)	150 (83.3)	10.264	0.001*
Negri Sembilan	92	03 (3.3)	89 (96.7)		
Farms					
A	40	10 (25.0)	30 (75.0)	27.321	0.000*
B	40	10 (25.0)	30 (75.0)		
C	55	10 (18.2)	45 (81.8)		
D	45	0.0 (0.0)	45 (100)		
E	63	02 (3.2)	61 (96.8)		
F	29	01 (3.4)	28 (96.6)		
Species					
Sheep	140	20 (14.3)	120 (85.7)	1.255	0.263
Goat	132	13 (9.8)	119 (90.2)		
Sex					
Male	98	17 (17.3)	81 (82.7)	3.908	0.048*
Female	174	16 (9.2)	158 (90.8)		
Age					
Young	90	20 (22.2)	70 (77.8)	12.845	0.000*
Adult	182	13 (7.1)	169 (92.9)		
Breed					
SH-Barbados Black Belly	40	10 (25.0)	30 (75.0)	13.435	0.004*
GT-Katjang	141	20 (14.2)	121 (85.8)		
GT-Jamnapari	62	02 (3.2)	60 (96.8)		
GT-Boer	29	01 (3.4)	28 (96.6)		
Production					
Meat	207	32 (15.5)	175 (84.5)	8.992	0.003*
Dairy	65	01 (1.5)	64 (98.5)		
Management					
Semi-Intensive	187	25 (13.4)	162 (86.6)	0.858	0.354
Intensive	85	08 (9.4)	77 (90.6)		

*significant ($P<0.05$)

Table 3 Results of multivariable analysis showing risk factors for Q fever seropositivity.

Variables	B	SE	Wald	*P*-value	AOR	95% CI
Age (young)	1.379	0.495	7.756	0.005*	3.972	1.51-10.49
Breed (Sheep-Barbados Black Belly)	2.522	1.106	5.205	0.023*	12.455	1.43-108.74
Breed (Goat-Katjang)	1.744	1.073	2.642	0.104	5.718	0.698-46.82
Breed (Goat-Boer)	-0.360	1.261	0.081	0.776	0.698	0.059-8.27
Production (Meat)	2.478	1.064	5.426	0.020*	11.921	1.48-95.94

AOR=adjusted odds ratio, 95% CI=95% confidence interval, *P*-values with asterisk (*) are significant.

As expected, the seroprevalence rate of Q fever was significantly different among the six small ruminant farms sampled in this study which is similar to Rai *et al*. (2011), who reported differences in the seropositivity of goats and cattle examined on different farms during an outbreak of Q fever in Pahang. We attribute this finding to the observed differences in farm management practices such as housing, hygiene, biosecurity, vector control, prophylactic measures and breeding methods, which we have seen in the various farms under investigation. Variations in the prevalence of Q fever on different farms have been linked to differential exposure to the infectious agents in the environment (Maurin and Raoult, 1999; Tissot-Dupont and Raoult, 2008). The only farm with no case of Q fever in the present study kept merely the local Katjang goat breed, practised adequate hygiene and other biosecurity measures and sourced animals locally within the country.

The higher seroprevalence risk of Q fever among younger animals may be attributed to the increased risk of exposure to infection in the amniotic fluid, foetal membranes and vaginal discharge during pregnancy and parturition, and through the milk during lactation. Small ruminants are known to shed *C. burnetii* in their milk over several months to years and the organism may be present in the placenta and vaginal mucus over two years (Berri *et al*., 2000). The higher seroprevalence of Q-fever observed among males in this study is unlike the result of previous studies, which revealed higher seroprevalence among female animals due to increased exposure during service and pregnancy with localisation of bacteria in the foetus through the placenta (Sakhaee and Khalili, 2010). The higher seroprevalence of Q fever seen among males may be due to natural breeding practised in all the farms under investigation. During natural service, several males may be exposed to *Coxiella burnetii* in the vaginal mucous of one infected female. The higher risk of Q fever observed in Barbados Black Belly sheep relative to other goats agrees with the results of previous studies which reported different prevalence rates in cattle, sheep and goat breeds from the same environment (Mohammed *et al*., 2014; Doung-Ngern *et al*., 2017; Johnson *et al*., 2019). Species and breed differences in disease prevalence are linked with genetically controlled factors that determine host species susceptibility or resistance to specific pathogens (Thrusfield, 2005; Paul *et al*., 2016).

The prevalence of anti-Q-fever antibodies is associated with the age, breed and production purpose of smallholder sheep and goats in Terengganu and Negeri Sembilan states. This study provides preliminary data that serves as a baseline for further studies and the implementation of management and policy frameworks to contain the economic and zoonotic implications of Q fever in Malaysia.

Acknowledgements

The authors are incredibly grateful to the Director-General Veterinary Services Malaysia (DVS) and the Department of Veterinary Services Negeri Sembilan and Terengganu for their support in sample and data collection. We also appreciate the staff of the Faculty of Veterinary Medicine, Universiti Putra Malaysia, who were involved in the sampling and laboratory aspect of this study.

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