Seroprevalence and Associated Risk Factors of *Coxiella burnetti* in Small Ruminants in Southern States of Peninsular Malaysia

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Abstract

Q fever is caused by the bacteria *Coxiella burnetii*, a zoonotic disease that causes abortions and stillbirths in ruminants. The seroprevalence of Q fever in small ruminants, such as sheep and goats, can vary widely depending on geographical location, farming practices, and the prevalence of the disease in the area. The main objective of this study is to determine the prevalence of infectious reproductive diseases affecting the small ruminant population in the southern state of Peninsular Malaysia and its associated risk factors. The animals (n = 184), comprising 24 sheep and 160 goats, were from the states of Negeri Sembilan and Johor. Before sampling, a physical examination was conducted on the animal to establish its health status. An enzyme-linked immunosorbent assay was performed on the serum to detect the seroprevalence of infectious reproductive diseases in Q fever. Farm animal records and observations were designed to assess the risk factors associated with the prevalence of Q fever. The seroprevalence of Q fever in small ruminants was 2.7% (5/184). Male animals have a higher prevalence of Q fever at 3.63% (2/55) than female animals at 2.3% (3/129). Goats managed intensively were found to have a higher seroprevalence at 4.08% (2/49) than those managed semi-intensively at 2.17% (3/138). The state of origin factor was significantly associated with the seropositivity of Q fever. This study revealed the existence of low seroprevalence of Q fever among small ruminants in selected states and farms in Peninsular Malaysia. However, the low seroprevalence of Q fever suggests a persistent exposure to *C. burnetti*, which could present a public health threat and a substantial risk to the ruminant industry.

Keywords: enzyme-linked immunosorbent assay, goat, Q fever, sheep, zoonosis

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INTRODUCTION

Q Fever is one of the infectious reproductive diseases that infect ruminants and may have negative effects on reproductive performance. *Coxiella burnetii*, a small, pleomorphic rod, gram-negative Coccobacillus bacterium, is a causative agent of Query Fever (Q fever). The intracellular bacterium lives and reproduces inside cells. The pathogenicity and virulence of *C. burnetii* are associated with the type of strain, genetic characteristics, and plasmid groups (Eldin *et al.*, 2017). It is known for its robustness and ability to survive in harsh environmental conditions, such as extreme temperatures and the environment, for extended periods. In addition, the host factors such as pregnancy and health status (Amin *et al* 2022) also play an important role in the occurrence of the diseases.

It is a worldwide zoonotic infectious disease in which domestic animals such as cattle, sheep, and goats are the main reservoirs for the pathogen. In small ruminants, the infection may result in abortion or stillbirth, due to damage of the placenta tissue (Zarza *et al*., 2021). The disease has been described in most countries except New Zealand. Although it is an infectious zoonotic disease, most countries do not include it in the list of nationally notifiable diseases. In 2005, *C. burnetii* was detected for the first time in two dairy goat herds with abortion problems in the Netherlands (Bontje *et al*., 2016). Due to a large outbreak in the Netherlands in 2007–2009, the Q

fever gained attention worldwide (Pexara *et al*., 2018).

The World Organization for Animal Health (OIE) lists Q fever as a multiple-species disease category (Winter *et al*., 2021). A large range of animals can be infected by *C. burnetii*, especially domestic animals (sheep, goats, and cattle), wildlife, and arthropods. Q fever in small ruminants, such as sheep and goats, can manifest with various clinical signs. In domestic animals, *C. burnetii* infection is usually subclinical. The infected animals later develop the disease and present as late abortions and reproductive disorders (Bontje, 2016). In sheep, the common clinical signs were the delivery of live or dead lambs at the end of gestation with a large abortion wave (Bontje *et al*., 2016). A series of abortions, or an "abortion storm," can occur when a previously uninfected herd or flock becomes infected (Nugroho *et al*., 2021). The rate of abortion in sheep is approximately 5–20%, while in most cases in goats, the infection of *C. burnetii* is connected to abortion (Winter *et al*., 2021). In infected animals, *C. burnetii* replicates to high titers in the placenta and lactating mammary glands. Massive contamination of the placenta with *C. burnetii* may result in abortion and low fetal birth weight (Ullah *et al.*, 2022). In some cases, the susceptible pregnant female develops necrotizing placentitis. Signs of depression and anorexia have been reported in a few cases of goats infected with *C. burnetii* (Tabita, 2022).

Q fever transmission in small ruminants primarily occurs through direct contact with infected animals or bodily fluids. However, environmental contamination is also possible. The important transmission route of Q fever is by inhalation of aerosol or dust particles with the parturient fluids of infected animals. During the process of parturition, more than 109 bacteria are shed into the environment. *C. burnetii* is mostly being shed in the vaginal mucus of goats and the feces of sheep (Tagesu, 2019). Infected sheep shed *C. burnetii* in vaginal mucus, amniotic fluid, placenta, and lochia during the abortion only for one time as compared to goats, chronically infected goats may shed the bacteria for two consecutive pregnancies. When these materials dry out, the bacteria can become airborne and be inhaled by other animals or humans. Ticks may also play an important role as reservoirs for wild animals and spread bacteria through tick bites, which may be the predominant route of transmission between wild animals, such as rodents.

In Malaysia, Q fever is one of the 121 animal diseases listed as notifiable diseases under the Animal Act of 1953. It is a disease of public health concern in many parts of the world. The information on the status of seroprevalence of Q fever in Malaysia is limited. A study done by Khor *et al*. (2018) reported that 9.6% (n = 85/887) of the serum samples populations of Orang Asli (OA), Indigenous people, many of whom live within the forest fringe areas of Peninsular Malaysia, were positive for *C. burnetii*. Even though Q fever is not commonly tested for in Malaysian veterinary or hospital laboratories, it was discovered in 1988 during a serological survey in Sarawak, East Malaysia, among febrile patients in an Iban community (Bina *et al*., 2011). The detection of IgG antibodies against Q fever in small ruminants revealed the current or past infection because the IgG against Q fever may remain elevated for months or longer after the infection. It demonstrated the diseases present in animal herds and how they might infect humans, particularly those employees with direct contact with the animals. The main objective of this study was determined the prevalence and associated risk factors of Q fever diseases affecting the small ruminant population in Malaysia, which alerts the public to a re-emerging zoonotic disease concern.

MATERIALS AND METHODS

Ethical Approval

The Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM) (UPM/IACUC/AUP-R080/2022) has approved this research which involved animals, complies with ethical standards, and approved the use of animals in this research after the ethical considerations were met.

Study Period and Location

The sampling sites were farms selected from the Southern state of the Peninsular Malaysia, Johor and Negeri Sembilan from 2020 to 2022.

Animals and Experimental Design

A cross-sectional type of study was performed for this prevalence study on small ruminants i.e., sheep and goats. Male and female ruminants that were above 6 months old, considered sexually matured were carefully selected. Before sampling, a physical examination was conducted on the animal to establish its health status. It involved a visual inspection of the animals, checking for any signs of illness, such as unusual discharge, swelling, or abnormal behaviour. The animal was categorized as a healthy animal or an animal with infectious reproductive diseases before sampling. A total of 184 blood samples were taken from goats and sheep. Within each herd, blood samples were collected from both male and female animals whenever possible. For each animal sampled, the age, sex, and body score of the animal were recorded.

Blood Sampling and Processing

Approximately 5 mL of blood was drawn from the jugular vein, and sampling were performed once in each animal. The blood collected in the clotting activator tube was left to clot at room temperature for 30 minutes. The serum was separated from the whole blood by centrifugation at 1,500 ×g for 10 minutes (Khonmee *et al*., 2019), stored at 4°C for transfer, and stored at -80°C before being analyzed in batches.

Indirect Enzyme-Linked Immunosorbent Assay (iELISA)

The iELISA assay was performed using ID Screen® Q Fever Indirect Multi-species test kit (IDvet, Montpellier, France). The test kit detects antibodies against *C. burnetii* in serum. The sensitivity of the test kits was 85%, and the specificity was 97.6% for direct detection against *C. burnetii*. The wells were coated with phase I and II variants of *C. burnetii* antigens. The protocol was conducted based on manufacturer guidelines. In brief, an anti-multi-species horseradish peroxidase (HRP) conjugate was added to the microwells, which bind to the anti-phase I and II *C. burnetii* antibodies, forming an antigen-antibody-conjugate-HRP complex. After washing to eliminate the excess conjugate, the substrate solution of tetramethylbenzidine (TMB) was added. In the presence of antibodies, a blue solution appears, which becomes yellow after the addition of the stop solution. In contrast, the absence of antibodies has no coloration. Finally, the microplate was read at 450 nm using a microplate reader. The test was validated if the mean value of the positive control optical density ($OD_{PC)\ is}$ greater than 0.350, and the ratio of the mean values of the positive and negative controls (OD_{PC} and OD_{NC}) is greater than 3. The interpretation of the result for each sample performed by calculating the S/P percentage (SP%) using the sample and control values. Samples with an S/P percentage less than or equal to 40% were considered negative, and the samples with an S/P percentage greater than 40% and less than 50% were considered doubtful. The sample was considered positive if the S/P percentage was greater than or equal to 50% and less than 80%. If the S/P percentage was greater than 80, it was considered strongly positive.

Farm Animal Record and Observation

The history and direct observations of the farms were conducted to assess the risk factors associated with the prevalence of infectious reproductive diseases. Animal records were obtained from the farm, especially the reproductive records, which were usually translated to the parturition date of the animal. A datasheet was deployed to actively uptake data from farmers. Observations of areas, the distance of farms, and animals were taken into account to attribute the area's risk factors for diseases. The farm's practices, type of systems, feed used, and biosecurity were also noted.

Data Analysis

Data were subjected to statistical analyses using the statistical package IBM SPSS version

27. The prevalence of Q fever and its 95% confidence interval (95% CI) were calculated at the individual level. Readings from the ELISA of Q fever across different potential risk factors, such as species, farms, and health status, together with the reproductive parameter, were analyzed using univariable analysis. Descriptive statistics and analysis were done to analyze trends and patterns that were identified in this study. Farm and animal records and observations were analyzed to evaluate the qualitative and quantitative practices of the community on the farms that contribute to the disease risk factors.

RESULTS AND DISCUSSION

A total of 184 serum samples were included in this study to evaluate the seroprevalence of Q fever among small ruminants in the southern states of Peninsular Malaysia. In this study, five small ruminant farms in the states of Johor and Negeri Sembilan were selected. The result revealed that 2.7% (5/184) of the serum samples tested positive against *C. burnetii* antibodies (95% CI, 0.0117–0.062). Only Johor showed seropositivity, while the farms in Negeri Sembilan revealed no positive antibodies against *C. burnetii*. Antibodies against *C. burnetii* were found in both farms in Johor state, Johor 1 and Johor 2, with a prevalence of 4.84% (3/62) and 6.67% (2/30), respectively. There is a difference in the prevalence of Q fever in 2020 at 12.1% (Jesse *et al.,* 2020) compared to 2.7% in this study. The difference in prevalence could be due to the high prevalence of Q fever in states bordering a different country in the east-coast region of Malaysia, which is in Terengganu at 16.7%. Similarly, the prevalence of Q fever in Negeri Sembilan was low at 3.3%, which was comparable to this study, which showed zero prevalence. Comparing the seroprevalence of Q fever in different countries, Pakistan showed a seroprevalence of 11.3% (Amin *et al.,* 2022) and 13.22% in Turkey, both in small ruminants (Karagul *et al.,* 2019). This study suggests a low prevalence of Q fever in three states in Malaysia. However, the results suggest that Q fever is persistent in Malaysia, posing a threat to small ruminant farms in Malaysia.

At the animal level, only goats revealed positive results against the C. *burnetii* antibody with a prevalence of 3.1% (5/160). All sheep in our study were sampled from Negeri Sembilan and were negative for *C. burnetii* antibody (0/24). Unfortunately, no sheep sampled from Johor state had positive cases of Q fever in goats. In 2020, the prevalence of Q fever among small ruminants was recorded at 14.3% and 9.9% seropositive for sheep and goats, respectively, in selected states in Malaysia (Jesse *et al*., 2020). In Thailand, the seroprevalence of Q fever in small ruminants was 3.5% in goats and 2.1% in sheep (Doung-Ngern *et al*., 2017), which showed a comparable prevalence to this study. The seroprevalence of Q fever in Western Iran revealed 0.8% in sheep (Rahravani *et al*., 2022). In this study, the higher seroprevalence of Q fever found in Johor state compared to Negeri Sembilan state could be due to the higher number of goats in Johor state. Johor State recorded more than 35,731 goats in 2021, which increased to 36,426 goats in 2022. A higher susceptibility to Q fever in goats was also reported in small ruminant flocks in Punjab, Pakistan (Amin *et al*., 2022) and Hungary (Dobos *et al*., 2021) than in sheep.

From the positive goats, male goats revealed higher seroprevalence of Q fever as compared to female goats, with seroprevalence of 3.64% (2/55) and 2.33% (3/129), respectively. The result from this study aligned with a previous study done in Malaysia, which showed a higher seroprevalence of Q fever among male animals (Jesse *et al*., 2020). Natural breeding using selected male practices in all the farms in this study was associated with a higher seroprevalence of Q fever in male animals (Jesse *et al.,* 2020). In the current study, all the female goats that were positive for Q fever were not pregnant. Even though the infection of *C. burnet*i is related to pregnant animals, non-pregnant animals might play the role of persistent carriers of the pathogens in maintaining the disease in a herd (Bontje *et al.,* 2016). According to this finding, surveillance of Q fever in a herd is necessary to break the circulation of the disease.

The Boer-Katjang, Boer, and Savanna breeds of goats in this study showed positive Q fever. The seropositivity showed 6.67% (2/30) in Boer-Katjang goats, 7.14% (2/28) in Savannah goats, and 1.03% (1/97) in Boer goats. Due to the limitations of the data available, no significant findings on the breed of animals associated with the seroprevalence of Q fever were found in this study. However, the higher prevalence of Savannah goats suggests a higher susceptibility than Boer or Boer-Katjang goats. The breed differences and the genetic control factors play a major role in determining host resistance to specific pathogens and susceptibility (Doeschl-Wilson *et al.*, 2021).

In the current study, seropositivity of goats which were intensively reared were higher than semi-intensive at 4.08% and 2.17% respectively. In Italy, animals raised in high-density flocks had a risk of four times higher being infected compared to smaller flocks (<50 animals). This is due to the higher random contact between uninfected and infected animals (Villari *et al*., 2018). In addition, animals that were reared intensively and kept within the same place for a longer time have a higher exposure risk to the pathogen, especially in a flock with a higher density and poor hygiene. Bacteria are shed in birth material and aborted fetuses without proper gestation areas or pens, healthy animals can easily be exposed to the contaminated birth material and accelerate disease transmission. The data for seroprevalence of Q fever are presented according to state, farms, species, sex, reproductive status, breed and management type in Table 1.

As presented in Table 2, the only parameter that showed significant associations ($p < 0.05$) with the Q Fever seropositivity in small ruminants was the state that the samples were taken in Johor and Negeri Sembilan. Other variables, including farms, species, sex, reproduction status, breeds, and management systems, revealed no significant association with the p-value of 0.25, 0.38, 0.62, 0.75, 0.28, and 0.49, respectively. A study done by Rizzo *et al*. (2016) revealed the significant risk factors of seropositivity of Q fever among small ruminants encompass a flock size exceeding 12 animals (odds ratio (OR 4.2; 95% CI 2.6–6.7), contact with other flocks (OR 2.1; 95% CI 1.2–3.6), the presence of mixed flock types (OR 2.4; 95% CI 1.4–4.2), farms located in the western region (OR 2.4; 95% CI 1.4–4.2), and a history of infertility in the preceding year (OR 2.6; 95% CI 1.2–5.2). Other studies done in Malaysia recorded several factors that were found to be significantly associated with Q fever seropositivity, including states ($\chi^2 = 10.264$, $p = 0.001$), farms ($\chi^2 = 27.32$, $p = 0.000$), gender ($\chi^2 = 3.908$, $p = 0.048$), age ($\chi^2 = 12.845$, $p = 0.000$), breed ($\chi^2 = 13.435$, $p = 0.004$), and production type ($\chi^2 = 8.992$, $p = 0.003$) of small ruminants (Jesse *et al*., 2020). Similarly, in the current study, the state factor was significantly associated with the seropositivity of Q fever, while the other factors were not significantly associated. This might be due to the small sample size being analyzed in the current study.

The movement of animals has been identified as a well-acknowledged risk factor for the transmission of microorganisms (Schimmer *et al.*, 2014). This phenomenon underscores the potential for pathogens to spread between different locations as animals traverse various environments. From the current study, the farms of animals that tested positive for Q fever had a history of importing new animals within countries or from Africa for breeding in 2018–2020. This might be a strong reason why Q fever antibodies were detected at the farm. The importation of animals from other countries should be done strictly following the protocol set by the authority department to decrease the entry of unwanted diseases. The imported animals should be free from infectious diseases such as Q fever, and the farm on which the animals will be selected should have no recent history of infectious diseases. Additionally, the movement of animals through legal and illegal trade is an anthropogenic factor that can drive the emergence of novel infectious diseases (Rush *et al.*, 2021).

The burden of ectoparasites, especially ticks, may increase the transmission of Q fever on ruminant farms. Tick-borne disease *Haemaphysalis bispinosa* tested positive for *C. burnetti* bacteria collected from goat farms (Khoo *et al.*, 2016). Our results indicate that

animals managed intensively revealed a higher prevalence of Q fever infection than animals reared semi-intensively, suggesting management systems have an important role in modulating exposure risks.

Table 1. The seroprevalence of Q Fever according to varying factors in small ruminants in selected states of Peninsular Malaysia

Variable	Number of samples	Number positive	Apparent prevalence	95% CI	True prevalence	95% CI
State						
Johor	92	5	5.43	0.02–0.12	1.66	0.00–0.09
Negeri Sembilan	92	0	0.00	0.00–0.04	0.00	0.00–0.00
Farms						
Johor 1	62	3	4.84	0.02–0.13	0.97	0.00–0.11
Johor 2	30	2	6.67	0.02–0.21	3.08	0.00–0.20
Negeri Sembilan 1	35	0	0	0.00–0.10	0.00	0.00–0.07
Negeri Sembilan 2	38	0	0	0.00–0.10	0.00	0.00–0.06
Negeri Sembilan 3	19	0	0	0.00–0.17	0.00	0.00–0.15
Species						
Sheep	24	0	0	0.00–0.14	0.00	0.00–0.11
Goat	160	5	3.12	0.01–0.07	0.00	0.00–0.04
Sex						
Male	55	2	3.64	0.01–0.12	0.00	0.00–0.10
Female	129	3	2.33	0.01–0.07	0.00	0.00–0.03
Reproductive status						
Pregnant	14	0	0	0.00–0.22	0.00	0.00–0.20
Non-pregnant	94	3	3.19	0.01–0.09	0.00	0.00–0.06
Lactating	21	0	0	0.00–0.15	0.00	0.00–0.13
Adult male	55	2	3.64	0.01–0.12	0.00	0.00–0.10
Breed						
Boer-Katjang	30	2	6.67	0.019– 0.21	3.08	0.00–0.20
Katjang	5	0	0	0.00–0.43	0.00	0.00–0.46
Boer	97	1	1.03	0.00–0.06	0.00	0.00–0.019
Savannah	28	2	7.14	0.010– 0.23	3.63	0.00–0.22
White dorper	5	0	0	0.00–0.43	0.00	0.00–0.46
Malin	19	0	0	0.00–0.17	0.00	0.00–0.15
Management						
Semi-intensive	135	3	2.22	0.08–0.06	0.00	0.00–0.027
Intensive	49	2	4.08	0.01–0.14	0.09	0.00–0.11
Overall	184	5	2.72	0.01–0.06	0.00	0.00–0.03

Confidence interval (CI).

Table 2. Univariable association between seroprevalence of Q Fever and risk factors

Variable	Categories	N	Positive	%	p-value
State	Johor	92	5	5.43	0.023
	Negeri Sembilan	92	0	0.00	
Farms	Johor 1	30	2	6.67	0.249
	Johor 2	62	3	4.84	
	Negeri Sembilan 1	19	0	0.00	
	Negeri Sembilan 2	38	0	0.00	
	Negeri Sembilan 3	35	0	0.00	
Species	Sheep	24	0	0.00	0.380
	Goat	160	5	3.13	
Sex	Male	55	2	3.64	0.617
	Female	129	3	2.33	
Reproduction status	Pregnant	14	0	0.00	0.745
	Non-Pregnant	94	3	3.19	
	Lactating	21	0	0.00	
	Adult Male	55	2	3.64	
Breed	BoerKatjang	30	2	6.67	0.363
	Katjang	5	0	0.00	
	Boer	97	1	1.03	
	Savannah	28	2	7.14	
	White dorper	19	0	0.00	
	Malin	5	0	0.00	
Management	Semi-intensive	138	3	2.17	0.493
	Intensive	49	2	4.08	
Overall		184	5	2.72	

CONCLUSION

The presence of the *C. burnetti* antibody indicates a persistent circulating infection in the small ruminant population in the southern states of Malaysia. Due to the zoonotic potential of Q fever, increased focus on screening and eradicating this disease must be enhanced. Prevention of the risk of infectious reproductive diseases in the ruminant industry is important to uphold the food safety and food security agenda.

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AUTHORS' CONTRIBUTIONS

NAI: Formal analysis, writing of the original manuscript. WNF: Funding acquisition, administration, supervision, review and final editing. NHAR: Coordinating sampling, data analysis. ZAZA: Project conceptualisation, data analysis, manuscript direction. FFAJ: Oversee overall manuscript flow, research design, mentorship and expert reference. All authors have read, reviewed and approved of the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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