UNIVERSITI PUTRA MALAYSIA

SEROPREVALENCE OF Q FEVER AMONG DEER LIVESTOCK IN UPM DEER FARM (PPP UPM): PRELIMINARY STUDY

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CERTIFICATION

It is hereby certified that I have read this project paper entitled **"Seroprevalence of Q fever among Deer livestock in UPM Deer Farm (PPP UPM): Preliminary Study"** by Nur Hanis Nadhirah Binti Yosryhan and in my opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4901 – Project.

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DEDICATIONS

To the Almighty God for providing the health, strength, knowledge, wisdom, protection, and will to persevere and remain optimistic to complete this research.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 - Projek.

SEROPREVALENSI PENYAKIT DEMAM Q DALAM KALANGAN RUSA TERNAKAN DI LADANG RUSA UPM (PPP UPM): KAJIAN AWAL

Oleh

NUR HANIS NADHIRAH BINTI YOSRYHAN

2023

Pengerusi: Prof Ts Dr Faez Firdaus Jesse Abdullah

Penyakit demam Q (juga dikenali sebagai coxiellosis) disebabkan oleh *Coxiella burnetii*, sejenis bakteria gram-negatif intrasel yang tersebar luas dan berkaitan dengan penyakit zoonotik serta mengakibatkan gangguan dalam sistem pembiakan haiwan. Walaupun telah mempunyai banyak kajian seroprevalen *C. burnetii* pada rusa di seluruh dunia yang telah direkodkan, namun data mengenai kekerapan penyakit ini di kalangan rusa di Malaysia masih terhad atau tiada. Oleh itu, kajian awal ini bertujuan untuk menilai seroprevalen jangkitan *C. burnetii* dan mengenal pasti faktor risiko yang berkaitan di kalangan ternakan rusa (*Cervus timorensis*) di Malaysia. Jumlah keseluruhan sebanyak 92 buah sampel darah serum telah digunakan dalam kajian ini, yang terdiri daripada 36 sampel serum yang dikutip pada tahun 2023 (ternakan baru) dan tambahan 56 sampel arkib dari tahun 2017 (ternakan lama), dan kesemua sampel tersebut diperolehi daripada Ladang Ternakan Rusa UPM. Kesemua sampel serum ini disaring bagi penyakit demam Q dengan menggunakan kit ELISA komersial ID Screen ® Q fever Indirect Multi-species (IDvet) dengan kadar spesifikasi 99% dan sensitiviti 90%. Hasil kajian ini mendedahkan prevalens tampak keseluruhan sebanyak 14.1% (95% CI= 8.45 – 22.69) dan prevalens sebenar sebanyak 14.8% (95% CI = 8.37 - 24.37) bagi penyakit demam Q di kalangan rusa yang telah disampel dalam kajian ini. Analisis univariabel mendedahkan perbezaan statistik yang signifikan dalam seroprevalen mengikut kumpulan umur (X^2= 8.654, p = 0.013), dengan rusa dewasa menunjukkan kadar prevalens tampak tertinggi sebanyak 27.3% (95% CI= 15.07 – 44.22) dan prevalens sebenar sebanyak 29.5% (95% CI= 15.81 – 48.56) diikuti oleh rusa dewasa muda dengan prevalens tampak sebanyak 10.8% (95% CI= 4.29 – 24.71) dan prevalens sebenar sebanyak 11.02% (95% CI= 3.69 – 26.64) serta

rusa muda dengan prevalens tampak sebanyak 0.0% (95% CI= 0.00 – 14.87) dan prevalens sebenar sebanyak -1.12% (95% CI= -1.12 – 15.58). Selain itu, perbezaan juga diperhatikan antara kumpulan ternakan (X^2= 9.732, p = 0.002), dengan ternakan lama menunjukkan kadar prevalens tampak sebanyak 23.2% (95% CI= 14.10 – 35.77) dan prevalens sebenar sebanyak 25.0% (95% CI= 14.72 – 39.07) berbanding dengan ternakan baru dengan prevalens tampak sebanyak 0.0% (95% CI= 0.00 – 9.64) dan prevalens sebenar sebanyak -1.12% (95% CI= -1.12 – 9.71). Walau bagaimanapun, didapati bahawa tiada hubungan signifikan antara jangkitan demam Q dan jantina (X^2 = 2.189, p = 0.139). Analisis regresi logistik multivariabel selanjutnya menunjukkan bahawa kedua-dua umur dan kumpulan ternakan tidak berkaitan (p>0.05) dengan seropositiviti terhadap jangkitan penyakit demam Q. Setakat pengetahuan kami, kajian ini merupakan dokumentasi pertama mengenai mengenai antibodi serum terhadap *C. burnetii* di kalangan *Cervus timorensis* di Malaysia. Kajian lanjut diperlukan untuk mengasingkan dan mengkarakterisasikan strain *C. burnetii* yang tersebar di kalangan rusa di Malaysia.

Kata kunci: *Coxiella burnetii*; rusa; seroprevalen; penyakit demam Q

ABSTRACT

An abstract of the project presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901- Project.

SEROPREVALENCE OF Q FEVER AMONG DEER LIVESTOCK IN UPM DEER FARM (PPP UPM): PRELIMINARY STUDY

By

NUR HANIS NADHIRAH BINTI YOSRYHAN

2023

Supervisor: Prof Ts Dr Faez Firdaus Jesse Abdullah

Q fever (also known as coxiellosis) is caused by *Coxiella burnetii*, a widespread intracellular gram-negative bacterium associated with a zoonotic disease and reproductive disorders in animals. Although there have been extensive seroprevalence studies of *C. burnetii* infection in deer worldwide, data on its prevalence in Malaysia remain scarce. Thus, this preliminary study aims to assess the seroprevalence of *C. burnetii* infection and identify associated risk factors among deer (*Cervus timorensis*) livestock in Malaysia. A total of ninety-two serum blood samples were included in the study, comprising thirty-six serum samples collected in 2023 (new herd) and an additional fifty-six archived samples from 2017 (old herd), all sourced from UPM Deer Farm. The samples were screened using a commercial ID Screen® Q fever Indirect Multi-species (IDvet) kit with 99% specificity and 90% sensitivity. The result of this study has revealed an overall apparent prevalence of 14.1% (95% CI = 8.45 – 22.69) and a true prevalence of 14.8% (95% CI = 8.37 – 24.37) for Q fever among deer. The univariable analysis revealed statistically significant differences (X^2= 8.654, p = 0.013) in seroprevalence among age groups, with adult deer exhibiting the highest apparent seroprevalence rate of 27.3% (95% CI= 15.07 – 44.22) and a true prevalence of 29.5% (95% CI= 15.81 – 48.56) followed by young adult with an apparent prevalence of 10.8% (95% CI= 4.29 – 24.71) and a true prevalence of 11.02% (95% CI= 3.69 – 26.64) and young with an apparent prevalence of 0.0% (95% CI= 0.00 – 14.87) and a true prevalence of -1.12% (95% CI= -1.12 – 15.58). Additionally, differences were observed among herds (X^2 = 9.732, p = 0.002), with the old herd displaying an apparent prevalence rate of 23.2% (95% CI= 14.10 – 35.77) and a true prevalence of 25.0% (95% CI= 14.72 – 39.07) compared to the new herd with an apparent

prevalence of 0.0% (95% CI= 0.00 – 9.64) and a true prevalence of -1.12% (95% CI= -1.12 – 9.71). However, it was revealed that there is no significant association between Q fever infection and gender (X^2 = 2.189, p = 0.139). Multivariable logistic regression analysis further demonstrated that both age and herd were not associated (p>0.05) with seropositivity to *C. burnetii*. To the best of our knowledge, this study is the first documented evidence of serum antibodies towards *C. burnetii* among *Cervus timorensis* in Malaysia. Further studies are needed to isolate and characterise the strain of *C. burnetii* circulating among deer in Malaysia.

Keywords: *Coxiella burnetii*; deer; seroprevalence; Q fever

CHAPTER 1

INTRODUCTION

In recent years, zoonotic diseases have gained prominence as significant global health concerns. The emergence and spread of zoonotic diseases have sparked significant concerns worldwide. Among these diseases, Q fever, also known as coxiellosis, caused by the obligate intracellular bacterium *Coxiella burnetii*, stands as a formidable example. Q fever is a widely known zoonotic disease that causes reproductive disorders (Maurin & Raoult, 1999) and has a worldwide distribution, except in New Zealand and Antarctica (Eldin et al., 2017). This bacterium has been reported in various domestic and wildlife species, including humans, livestock, wild ruminants, and birds. *C. burnetii* is shed via urine, milk, feces, and birth products (Honarmand, 2012). The transmission of this bacterium occurs through inhalation of contaminated airborne or spore-like particles formed by the bacterium, vectors such as ticks and arthropods, vertical transmission, and also any secretions or excreta from infected animals (Ezatkhah et al., 2015). Animals infected with *C. burnetii* often show no apparent symptom, yet they can exhibit clinical signs such as abortions, stillbirths, metritis, infertility, and weak calves, causing significant economic losses to farmers (Marrie et al., 1996; Arricau-Bouvery & Rodolakis, 2005). However, this disease has raised particular concern due to its zoonotic potential and connection to public health as there is evidence of *C. burnetii* transmission in humans ranging from mild flu-like symptoms to severe cases and fatal complications (Angelakis & Raoult, 2010). In 1935, an outbreak of an undiagnosed febrile disease

emerged in an abattoir in Brisbane, Australia, proven to be a Q fever infection (Maurin & Raoult, 1999). In 2009, 2357 cases of humans infected with Q fever were recorded in the Netherlands (Guatteo et al., 2011).

Wildlife has been suggested to be a potential reservoir for Q fever (Madariaga, 2005). Therefore, in this study, our focus will be on deer, which are originally wild animals and progressively integrated into livestock farming systems. Their economic value, meat production potential, and adaptability to various ecological niches have made deer a valuable resource in the agro-industry. However, this integration brings potential risks from a One Health perspective, such as the transmission of this pathogen.

Many studies have demonstrated a high seroprevalence rate of *C. burnetii* infection among deer livestock worldwide. Research conducted by Ruiz-Fons et al. (2008) in southern and northern Spain recorded a seroprevalence of 5.6% in wild red deer, 15.4% in roe deer, 39% in cattle, and 40% in farmed red deer. Enright et al. (1971) stated that the seroprevalence rate of Q fever in deer in Mendocino County, California was 22%. In the Netherlands, Rijks et al. (2011) observed 43% of *C. burnetii*-positive deer in 2010, which shows a drastic increment from 2008 (11%) and 2009 (10%). The seropositivity rate of *C. burnetii* was 40% in farmed red deer in southern Spain (González-Barrio et al., 2015). A recent study by Voss et al. (2023) recorded 3.4% overall seroprevalence of *C. burnetii* in wild deer in eastern Australia. As a zoonotic disease, the transmission of Q fever from infected animals to humans underscores the importance of understanding the seroprevalence and epidemiology of this pathogen. In a research

investigation carried out by Bina et al. (2016) stated that the chronology of Q fever was first documented in Malaysia when the disease was diagnosed in a goat farm from Penang in the year of 2007.. A recent study in Malaysia by Jesse et al. (2020) reported that the prevalence of Q fever infection among goats and sheep in Malaysia is 12.1% . However, to date, no seroprevalence studies of Q fever infection among deer have been reported in Malaysia. Thus, a comprehensive study of serological data and risk factors is needed and therefore this preliminary seroprevalence study endeavors to shed light on the extent of *C. burnetii* or Q fever infection among deer livestock in UPM Deer Farm (PPP UPM). The results and findings of this preliminary study could be used as a benchmark to design a comprehensive study involving deer livestock related to prevalence of Q fever infection among deer livestock in Peninsular Malaysia.

The objectives of this study are as follows:

a. To determine the seroprevalence rate of *Coxiella burnetii* infection among deer from UPM Deer Farm (PPP UPM).

a. To identify potential contributing risk factors for *Coxiella burnetii* infection and its relation in deer from UPM Deer Farm (PPP UPM).

Hypotheses

a) H_0: There will be low or no seroprevalence of *Coxiella burnetii* or Q fever infection among deer from UPM Deer Farm (PPP UPM).

   H_1: There will be a high seroprevalence of *Coxiella burnetii* or Q fever infection among deer from UPM Deer Farm (PPP UPM).

b) H_0: There is no association between the seroprevalence of *Coxiella burnetii* or Q fever infection and gender, age, and herd among deer from UPM Deer Farm (PPP UPM).

   H_1: There is an association between the seroprevalence of *Coxiella burnetii* or Q fever infection and gender, age, and herd among deer from UPM Deer Farm (PPP UPM).

CHAPTER 2

LITERATURE REVIEW

2.1 Aetiological agent

Coxiella burnetii is a gram-negative bacterium that is obligately intracellular and exhibits a pleomorphic rod shape. Even though its membrane is similar to other Gram-negative bacteria, it does not readily stain using the Gram technique. Hence, Gimenez method is used to stain *C. burnetii* (Honarmand, 2012). Previously, *C. burnetii* was classified in the Rickettsiaceae family due to its biological similarity. However, based on the phylogenetic analyses mainly on the 16S rRNA sequence, it is proven that *C. burnetii* belongs to the gamma subdivision of the phylum Proteobacteria (Maurin & Raoult, 1999). Angelakis and Raoult (2010) revealed that the estimated size of the bacterium is about 0.2–0.4 μm wide and 0.4–1.0 μm long.

C. burnetii is highly infectious and persistent in the environment. This is due to its ability to form two distinctive morphological structures which is the non-replicating form, spore-like particles known as small cell variants (SCV) and replicating form known as the large cell variants (LCV) (McCaul & Williams, 1981). SCV can be found in the environment as it is able to withstand osmotic shock, oxidative stress, heat, pressure, and sonication (Amano et al., 1984) while remaining infectious (McCAUL & Williams, 1981).

C. burnetii exhibits antigenic variation, where the Phase I represents the natural virulent highly infectious phase found in animals, and the Phase II is only acquired after

serial passages in cell cultures and is avirulent (Andoh et al, 2007). The key distinction lies in the lipopolysaccharide (LPS) structure, where phase I exhibits smooth LPS, whereas phase II displays rough LPS (Angelakis & Raoult, 2010).

2.2 Mode of transmission

The reservoirs for this bacterium are widespread, which includes livestock, rodents, birds, ticks, pets and wildlife. Studies by Špitalská et al. (2018) reported evidence of *C. burnetii* detected in *Ixodes ricinus*, *Dermacentor reticulatus* and *Haemaphysalis inermis* ticks. This prompts consideration of ticks as potential vectors for the transmission of this bacterium, particularly among wild animals (Kazar, 2005; Celina & Cerný, 2022). However, ticks are not regarded as essential components in the natural cycle of *C. burnetii* infection in livestock (Angelakis & Raoult, 2010).

Transmission dynamics of *C. burnetii* can vary among different animal species and environments. Generally, *C. burnetii* is transmitted via inhalation. Although animals infected with *C.burnetii* often appear asymptomatic, they shed the bacterium in vaginal discharge, milk, urine, feces (Celina & Cerný, 2022) and birth products (Honarmand, 2021). Research conducted by Roest et al. (2012) demonstrated that the placentas of infected animals contain significant concentrations of *C. burnetii*. According to Arricau-Bouvery and Rodolakis (2005), the longest duration of *C. burnetii* shedding persists through milk in cows and goats, and through vaginal mucus in ewes. Moreover, there is evidence indicating that *C. burnetii* can multiply within amoebae, implying the possible involvement of these hosts in maintaining the bacterium's presence in the environment

(La Scola & Raoult, 2001). Furthermore, *C. burnetii* outbreaks have been found to be associated with cats experiencing abortions or delivering stillborn offspring (Malo et al., 2018), and similar associations have been observed in other animal species, where it has been linked to both premature births and abortions (Agerholm, 2013).

2.3 Pathogenicity and clinical signs

C. burnetii attacks the macrophages or monocytes of the host cells (Angelakis & Raoult, 2010). Currently, the mechanism of entry of *C. burnetii* into host cells in animals is poorly understood. However, a review has provided insights into the entry of *C. burnetii* into eukaryotic cells in humans. It reveals that *C. burnetii* phase I and phase II have different mechanisms in invading the host. Phase I enters macrophages through the CR3 receptor, internalizing within eukaryotic cells via the phagolysosomal pathway. On the other hand, phase II binds via a complex involving leukocyte response integrin and integrin-associated protein (Maurin & Raoult, 1999). Research found that the progression of Q fever infection into a chronic state is associated with impaired cell-mediated immunity (CMI), given its pivotal role in defending against the bacterium (Angelakis & Raoult, 2010). Another review also highlighted the association between genetic variability among *C. burnetii* strains and the occurrence of acute and chronic cases of Q fever infection in humans (Maurin & Raoult, 1999). However, recent studies revealed that the development of acute or chronic Q fever is contingent upon host-related factors (Honarmand, 2012).

Research has documented the pathogenicity of *C. burnetii* in both domestic animals (Celina & Cerný, 2022) and humans (Maurin & Raoult, 1999). Animals infected with *C. burnetii* infection often remain clinically asymptomatic (Angelakis & Raoult, 2010). However, during parturition, they may display reproductive disorders such as abortion, weak calves, stillbirths, infertility, and metritis, as *C. burnetii* primarily targets trophoblast cells of the placenta and mammary glands (Sánchez et al., 2006). For instance, a study reported evidence of goats infected with *C. burnetii*, showing inflammatory lesions in their placentas (Bildfell et al., 2000). Placentitis has also been observed in dama gazelles infected with *C. burnetii* (Lloyd et al., 2010). A study conducted by González-Barrio et al. (2013) has demonstrated that reproductive failure in deer following *C. burnetii* infection. However, a recent study (Sánchez et al., 2006) also noted the detection of *C. burnetii* in the lungs and liver of pregnant goats using PCR, which aligns with findings from another study in mice that showed evidence of pneumonia when administered intranasally and splenomegaly when administered intraperitoneally (Russell-Lodrigue et al., 2009).

2.4 Prevalence and distribution

The prevalence and distribution of Q fever can vary by region, influenced by factors such as the methodology employed and geographical location. A review by Guatteo et al. (2011) stated that the seroprevalence at both the animal and herd levels of any species appeared to be consistently around 15-20% in many countries.

A recent seroprevalence study conducted in eastern Australia revealed an overall seroprevalence rate of 3.4% in the wild deer population, employing a commercial indirect ELISA test. Specifically, *Cervus axis* and *Dama dama* exhibited seroprevalence rates of 6.5% (7/108) and 3.7% (7/188), respectively, while *Cervus timorensis* (0/56), sambar (0/53), and *Cervus elaphus* (0/8) showed no seroprevalence (Voss et al., 2022). This study further established a correlation between seroprevalence of Q fever and various risk factors, including age and gender. Among different age groups, adults (>2 years old) exhibited a seroprevalence of 2.9% (9/314), while yearlings (1-2 years old) displayed a higher seroprevalence rate of 5.0% (5/99). In terms of gender, 9 out of 206 females tested positive for antibodies against *C. burnetii*, resulting in a seroprevalence of 4.4%, whereas males had a lower seropositivity rate of 2.4% (5/207) against *C. burnetii* (Voss et al., 2022).

In Spain, a seroprevalence study of *C. burnetii* in sampled ruminants found rates of 15.4% (6/39) in roe deer, 5.6% (2/36) in wild red deer, and 40% (32/80) in farmed red deer. Fallow deer exhibited no seroprevalence in this study. The methodology utilized was the Immunofluorescence antibody assay (IFA) (Ruiz-Fons et al., 2008). A study conducted in China reported a seroprevalence rate of 12.3% (166/1347) in domestic sika deer, employing ELISA (Cong et al., 2015). In southern Spain, an overall seroprevalence rate of 36% (9/25) was observed in farmed red deer, using ELISA (González-Barrio et al., 2015). A separate study from California reported a seroprevalence of 22% (76/342) in black-tailed deer, employing the complement fixation test (CFT) (Enright et al., 1971). In Korea, a study found a seroprevalence of 1.4% (10/604) in wapiti and 0% (0/30) in sika

deer, utilizing ELISA (Jang et al., 2011). In New York, a prevalence study in white-tailed deer reported an overall seroprevalence rate of 14.5% (155/1069), employing the indirect microimmunofluorescence assay (IFA) (Kirchgessner et al., 2013). Finally, a prevalence study in wild Korean water deer in Korea detected antibodies in 9.18% (18/196) of samples and genomic material in 6.63% (13/196) of samples, using ELISA and RT-PCR, respectively (Shin et al., 2014).

Country	Reference	Study period	Sample	Species	Test	Number of tested animals	Prevalence
Eastern Australia	Voss et al., 2022	Nov 2017-Mac 2020	Serum	Cervus axis	ELISA	108	6.5%
				Dama dama		188	5.8%
				Cervus timorensis		56	0.0%
				Sambar		53	0.0%
				Cervus elaphus		8	0.0%
Spain	Ruiz-Fons et al., 2008	2004-2005	Serum	Red deer	IFA		
				Wild		36	15.4%
				Farmed		80	5.6%
				Wild roe deer		39	40.0%
				Wild fallow deer		13	0.0%
Northeastern China	Cong et al., 2015	2013-2014	Serum	Farmed sika deer	ELISA	1347	12.3%

Southern Spain	González-Barrioet al., 2015	2011	Serum	Farmed red deer	ELISA	25	36%
California	Enright et al., 1971	Jan 1965-Jan 1967	Serum	Black tailed deer	CFT	342	22%
Korea	Jang et al., 2011	Feb – Sept 2010	Serum	Sika deer *Cervus canadensis*	ELISA	30 604	0% 1.4%
New York	Kirchgessner et al., 2013	2009-2010	Serum	White tailed deer	FA	1069	14.5%
Korea	Shin et al., 2014	2010-2012	Serum	Wild korean water deer	ELISA RT-PCR	196	9.18% 6.63%

2.6 Diagnosis

Isolation of *C. burnetii* can be achieved through yolk sac of embryonated chicken eggs, mice, guinea pigs, and cell cultures (To et al., 1995) but it is not commonly practiced due to the meticulous handling requirements and the need for a biosafety level 3 laboratory (Spyridaki et al., 2002). Histological diagnosis of *C. burnetii* infection may

reveal lesions on the placenta, characterized by necrotic suppurative placentitis with inflammatory cell infiltration and trophoblast hypertrophy (Kersh et al., 2010). While *C. burnetii* infection lacks a pathognomonic lesion where a study by Norina et al. (2011) revealed the presence of a distinctive fibrin ring with a central space, often termed a 'doughnut granuloma,' in organs infected by *C. burnetii*, such as the lung, liver, and spleen. However, further confirmatory diagnostic tests are still necessary.

Immunohistochemistry (IHC) laboratory technique could detect the specific antigen-antibody reaction of *C. burnetii*, but its sensitivity to detect antigen in organs other than the placenta is low (Sánchez et al., 2006). However, a recent study by Norina et al. (2011) reported that IHC techniques are sensitive enough to detect the antigen in placenta, lung, and liver. For PCR technique it could detect *C. burnetii* in various biological samples (Berri et al., 2000), which includes the abortion material, placental tissue, feces, vaginal mucus, and milk samples, as recommended by OIE. However, PCR technique is only applicable for assessing tissue samples and carries a risk of false-positive results.

Therefore, the primary method for diagnosing Q fever relies on serological techniques, allowing the detection of antibodies within several weeks post-infection and differentiation between acute and chronic Q fever infections. For serological testing, ELISA, CFT, and IFA techniques are commonly used. However, compared to ELISA, the complement fixation test (CFT) exhibits lower sensitivity (Rousset et al., 2007) and is time-consuming, typically detecting seroconversion within 2-3 weeks, in contrast to ELISA and IFA, which yield results in an average of 10-15 days (Maurin & Raoult, 1999),

with a risk of false-negative results.ELISA screening can detect both phase I and phase II antibodies, with samples taken from milk and serum. Commercial ELISA kits are available for specific species and can be adapted for use with wild species after validation (González-Barrio et al., 2015).

2.6 Control and prevention

The control and prevention of Q fever infection in animals require a comprehensive set of measures aimed at minimizing risks of *C. burnetii* transmission.

Vaccines based on Phase I *C. burnetii* have been found to be more effective in providing protection compared to Phase II vaccines (Rodolakis, 2009). However, these vaccines do not appear to reduce the shedding of the bacterium in naturally infected animals. In fact, there is also evidence of animals vaccinated with Phase II, who were previously infected with *C. burnetii*, shedding the bacteria in milk for months (Schmeer et al., 1987). As a result, Q fever vaccination is not widely practiced.

In ruminants, antibiotic treatment typically involves administering two injections of oxytetracycline (20 mg per kg body weight) during the final month of gestation. However, this treatment may not completely prevent abortion and the shedding of *C. burnetii* during lambing (Angelakis & Raoult, 2010). Therefore, antibiotic treatment is not preferred due to the potential for antibiotic resistance to develop in the animal.

To minimize the risk of *C. burnetii* transmission, urgent measures should be taken. These include proper disposal of placenta, birth products, and manure, as well as quarantine of newly purchased animals and isolating pregnant animals showing signs of

reproductive disorders. Restricting the access of wild animals, rodents, and birds to the farm is also important. Other than that, stocking density and standard biosecurity measures also play a role in reducing the risk of pathogen transmission. As Q fever is a potential zoonosis, practicing hygienic measures within the farm is of utmost importance (Dhaka et al., 2020). Moreover, it is crucial to implement routine annual surveillance for Q fever in Malaysia and report any cases of *C. burnetii* to the relevant authorities. This proactive approach is essential for preventing the spread of Q fever infection, especially considering the numerous published reports have reported the presence of *C. burnetii* in the country, particularly among farmed animals (Bina et al., 2011; Jesse et al., 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical approval

The study received approval from the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia, with reference number UPM/IACUC/AUP-U042/2023 on July 31, 2023.

3.2 Methodology

For this study, UPM Deer Farm (PPP UPM), situated in Serdang, Selangor, Malaysia, within the geographical coordinates of approximately 2.982962 North latitude and 101.729190 East longitude, was selected as the study site. The study focused on the *Cervus timorensis* deer population at this farm, which was managed under a semi-intensive system and practiced natural breeding. The deer population on the farm was divided into three groups: young, adult, and docile deer. Fawns remained with their mothers in the adult group until they reached the weaning age, typically around 3 months old. The deer had access to grazing and received supplementary pellet feed.

Blood samples were collected from a total of 36 deer using a convenient sampling method. Additionally, this study incorporated 56 archived serum samples that were collected from deer in the year of 2017 of the same farm for routine herd health programme screening which resulted in a total of combined sample size of 92 samples.

3.3 Blood collection

Blood samples were collected from the deer population in the month of August 2023 for this study. Prior sampling, the deer were gathered in a dark room of Deer Dark House of UPM. The deer were manually restrained, and blood was collected through jugular venipuncture using a 21 G vacutainer with a plain (red) tube. Approximately 5 ml of whole blood was collected from each deer. The plain (red) tubes were carefully labeled with the respective deer's ID, and the collected blood samples were promptly transported to the laboratory in an ice box. Following the blood collection process, each deer was released from the dark room.

3.4 Serum sample preparation

The blood samples were centrifuged at 3000 rpm for 5 minutes. This step leads to effectively separating the serum from the whole blood. Subsequently, the serum was carefully extracted using a disposable pipette and stored in an Eppendorf tube at a temperature of -20 °C till further analyses.

3.5 Serological testing

The collected serum samples were subjected to the indirect ELISA kit to determine the seropositivity towards *Coxiella burnetii* or Q fever infection. The ELISA kit used for this study was ID Screen ® Q fever Indirect Multi-species (IDvet). The kit comprised negative and positive control, dilution buffers 2 and 3, 2 sets of coated 96-well ELISA microplate, wash solution, concentrated conjugate 10X, substrate solution, and stop solution. Single and multichannel pipettes, disposable pipette tips, distilled water, gloves,

wash bottles, and yellow bins were prepared before testing. Serum samples and reagents were brought to room temperature and homogenized by inversion shortly before testing.

The serum samples were tested for antibodies against *C. burnetii* as stated in the manufacturer's manual instruction. Then, an analysis of the ELISA microplate was performed immediately after adding a stop solution which stopped the reaction. The wavelength of the ELISA microplate absorbance reader (Infinite ® 200 PRO) was set at 450nm and the optical density (OD) was recorded. Results were expressed in S/P %, with the formula:

$$\text{S/P \%} = \frac{ODsamples - ODnc}{ODpc - ODnc} \text{ x } 100$$

Then, the OD values were imported into ID Soft™ software to calculate the S/P %. The samples with (S/P% > 50%) were interpreted as positive.

3.6 Risk factors data collection

The factors such as age and gender of the sampled deer were recorded, and the farm's condition was assessed through observations. Additionally, farm manager of UPM Deer Farm were interviewed to gather information about factors such as age, gender, and farm management practices that could potentially influence the presence of antibodies against *C. burnetii*.

3.7 Statistical methods

All the data obtained were summarised in a table in the Microsoft Excel spreadsheet programme (version 2019) and were further grouped in the form of class

intervals as a convenient means to analyse the data. Risk factors such as age, gender, and a herd of the sampled deer are identified as the independent variable, and seroprevalence to Q fever infection is identified as the dependent variable. Then, the data were analysed using the Chi-square test in the Statistical Package for Social Sciences (SPSS) Software version 25.0 to determine the relation between the seroprevalence of *C. burnetii* or Q fever infection (dependent variable) and the age, gender, or herd of deer (independent variable).

The Epitools statistical calculator was used to determine the 95% confidence interval (CI) of the sample proportion. A P-value ≤ 0.05 was deemed significant in all analyses. Then, all significant variables ($p \leq 0.05$) from the univariable analyses were utilised to calculate a multiple binary logistic regression analysis forward selection, to evaluate the contributing risk factors for seroprevalence of Q fever or *C. burnetii* infection in deer.

CHAPTER 4

RESULT

4.1 Result of serological test

Out of the 92 serum samples subjected to the indirect ELISA test kit for *C. burnetii*, 79 samples tested negative (S/P% ≤ 50%), while 13 samples tested positive (S/P% > 50%) based on the calculated values. Therefore, among the 92 serum samples examined, 13 were positive for *C. burnetii* or Q fever infection, resulting in an apparent overall prevalence of 14.1% (95% CI = 8.45 – 22.69) and a true prevalence of 14.8% (95% CI = 8.37 – 24.37).

Table 1: The apparent and true seroprevalence rates of *C. burnetii* or Q fever infection at UPM Deer Farm.

Variable	Categories	Tested	Positive	Apparent prevalence		True prevalence	
				Estimate (%)	95 % CI	Estimate (%)	95 % CI
Age	Young	22	0	0.0	0.00 – 14.87	-1.12	-1.12 – 15.58
	Young adult	37	4	10.8	4.29 – 24.71	11.02	3.69 – 26.64
	Adult	33	9	27.3	15.07 – 44.22	29.5	15.81 – 48.56
Gender	Male	22	1	4.5	0.81 – 21.80	4.0	-0.86 – 23.37
	Female	70	12	17.1	10.09 – 27.62	18.1	10.21 – 29.91
Herd	Old	56	13	23.2	14.10 – 35.77	25.0	14.72 – 39.07
	New	36	0	0.0	0.00 – 9.64	-1.12	-1.12 – 9.71
Total	All	92	13	14.1	8.45 – 22.69	14.8	8.37 – 24.37

4.2 Risk factors analysis

Analysis of risk factors includes age, gender, and the herd from which the samples were collected.

The deer population was categorised into three age groups: young (Age < 2), young adults (2 ≤ Age ≤ 4), and adults (Age > 4). Adult deer recorded higher seroprevalence across the age groups, with an apparent prevalence of 27.3% (95% CI= 15.07 – 44.22) and a true prevalence of 29.5% (95% CI= 15.81 – 48.56). In contrast, young adults exhibited a lower seroprevalence rate, with an apparent prevalence of 10.8% (95% CI= 4.29 – 24.71) and a true prevalence of 11.02% (95% CI= 3.69 – 26.64). Young deer, on the other hand, had an apparent prevalence of 0.0% (95% CI= 0.00 – 14.87) and a true prevalence of -1.12% (95% CI= -1.12 – 15.58).

Among the sampled female deer (n=70), 12 tested positive for *C. burnetii* or Q fever infection, whereas only 1 out of the 22 male deer tested positive. This analysis revealed that females had a higher prevalence rate, with an apparent prevalence of 17.1% (95% CI= 10.09 – 27.62) and a true prevalence of 18.1% (95% CI= 10.21 – 29.91), compared to male deer, who had an apparent prevalence of 4.5% (95% CI= 0.81 – 21.80) and a true prevalence of 4.0% (95% CI= -0.86 – 23.37).

The seroprevalence of *C. burnetii* or Q fever infection by herd indicated a higher prevalence rate in the old herd, with an apparent prevalence of 23.2% (95% CI= 14.10 – 35.77) and a true prevalence of 25.0% (95% CI= 14.72 – 39.07), whereas the new herd recorded an apparent prevalence of 0.0% (95% CI= 0.00 – 9.64) and a true prevalence of -1.12% (95% CI= -1.12 – 9.71).

The statistical analysis indicated an association between the seroprevalence of *C. burnetii* or Q fever infection and age ($\chi 2$= 8.654, p = 0.013) and herd ($\chi 2$= 9.732, p = 0.002). Consequently, the null hypothesis is rejected, signifying an association between seroprevalence with age and herd among deer at UPM Deer Farm (PPP UPM). However, for gender, the p-value exceeded the significance level ($\alpha = 0.05$), leading us to conclude that there is insufficient evidence to suggest an association between the seroprevalence of *C. burnetii* or Q fever infection and gender ($\chi 2$= 2.189, p = 0.139). Thus, the null hypothesis is accepted, indicating no association between seroprevalence and gender among deer at UPM Deer Farm (PPP UPM).

However, following multivariable logistic regression analysis (Table 3), the results reveal a non-significant model, with neither age nor herd exhibited an association with the seropositivity of *C. burnetii* or Q fever infection.

Table 2: Univariable analyses of the seroprevalence rate of *C. burnetii* or Q fever infection and its association with established risk factors.

Variables	Categories	Positive (%)	Negative (%)	95 % CI	$\chi 2$	*P*-value
Age	Young	0 (0.0)	22 (100.0)	0.00 – 14.87	8.654	0.013*
	Young adult	4 (10.8)	33 (89.2)	4.29 – 24.71		
	Adult	9 (27.3)	24 (72.7)	15.07 – 44.22		
Gender	Male	1 (4.5)	21 (95.5)	0.81 – 21.80	2.189	0.139
	Female	12 (17.1)	58 (82.9)	10.09 – 27.62		
Herd	Old	13 (23.2)	43 (76.8)	14.10 – 35.77	9.732	0.002*
	New	0 (0.0)	36 (100.0)	0.00 – 9.64		
Total	All	13 (14.1)	79 (85.9)	8.45 – 22.69	-	-

P- value with asterisk (*) is deemed significant (p <0.05)

Table 3: Multivariable analyses of the seroprevalence rate of *C. burnetii* or Q fever infection and its association with established risk factors.

Variables	Categories	B	S.E.	Wald	*P*- value	AOR	95% CI
Age	Young	-18.464	7306.186	0.000	0.998	0.000	-
	Young Adult	-0.383	0.686	0.312	0.576	0.681	0.177-2.617
Herd	New	-19.100	6165.452	0.000	0.998	0.000	-

CHAPTER 5
DISCUSSION

In this study, an apparent seroprevalence of *C. burnetii* or Q fever infection was found among deer livestock at UPM Deer Farm (PPP UPM), with a rate of 14.1% (95% CI = 8.45 – 22.69) and a corresponding true prevalence of 14.8% (95% CI = 8.37 – 24.37). This study employs the ID Screen® Q fever Indirect Multi-species (IDvet) kit, demonstrating a specificity of 99% and a sensitivity of 90%. Recent seroprevalence studies in cervids have reported varying rates, which may be attributed to multiple factors. In a seroprevalence study conducted by Cong et al. (2015), a seroprevalence of 12.3% was documented for farmed sika deer that utilized a commercial ELISA test kit, which demonstrated a sensitivity and specificity of 87% and 100%, respectively. A study by Kirchgessner et al. (2012) reported a seropositivity rate of 14.5% in wild white-tailed deer in New York, utilizing the immunofluorescence antibody assay (IFA). Similarly, another study by Ruiz-Fons et al. (2007) in Spain, which also employs IFA, found seropositivity rates of 15.4% in wild roe deer and 40% in farmed red deer.

The high seroprevalence of *C. burnetti* or Q fever infection suggests the presence of *C. burnetii* in the environment. Geographical factors, including air moisture, temperature, wind patterns, and soil conditions, can significantly influence the transmission and persistence of *C. burnetii* (Mori & Roest, 2018). Additionally, higher animal density (Nusinovici et al., 2015), as seen in the semi-intensive management system at the UPM Deer Farm, may elevate the risk of *C. burnetii* transmission, especially during

the calving season (Mori & Roest, 2018). Given that the calving season on the farm spans from October to January, it is crucial to monitor the health of the fawn and doe for early detection of any reproductive issues. The higher seroprevalence rate was observed in females of 17.1% (95% CI= 10.09 – 27.62) and a true prevalence of 18.1% (95% CI= 10.21 – 29.91), may be attributed to increased exposure during their service and pregnancy, as this farm practices natural breeding. Studies have also demonstrated the presence of *C. burnetii* in semen (Kruszewska & Tylewska-Wierzbanowska, 1997; Sakhaee & Khalili, 2010; Mangena et al., 2021). Moreover, adults exhibited the highest seropositivity against *C. burnetii* with 27.3% (95% CI= 15.07 – 44.22) and a true prevalence of 29.5% (95% CI= 15.81 – 48.56), likely due to repeated pathogen exposure with increasing age (McCaughey et al., 2010; Rizzo et al., 2016; Barlozzari et al., 2020). Furthermore, the farm's proximity to cattle and goat farms, known as reservoirs for *C. burnetii* (Roest et al., 2013; Jesse et al., 2020), raises concerns about possible indirect transmission through personnel, vehicles, and individuals entering and exiting the farm as there was lack of biosecurity control (Dhaka et al., 2020). The disinfectant used on the farm, Lindores-30 iodophore, may not effectively eliminate *C.burnetii*, which is resistant to common chemical disinfectants. Effective disinfection to inactivate the bacterium requires specific chemicals or exposure to heat for a certain period (Dalton et al., 2014). The presence of cats, potential reservoirs for the disease (Malo et al., 2018), further complicates pathogen control. Other than that, farm personnel have reported occasional rodent sightings in the food storage area, and rodents are potential reservoirs for *C. burnetti* or Q fever infection (Webster et al., 1995). Moreover, interviews have revealed

that the deer on this farm do not experience tick-related issues. Consequently, this eliminates the concern of tick-related transmission of *C. burnetii* to the deer, despite previous studies providing evidence of such transmission (Celina & Cerný, 2022). Furthermore, UPM Deer Farm regularly performs health checkups to screen for brucella and tuberculosis as part of its annual surveillance but does not perform screening for *C. burnetii*. In addition to these screenings, the farm administers FMD vaccines and Ivermectin. Although a notably higher seroprevalence rate of 23.2% (95% CI= 14.10 – 35.77) and a true prevalence of 25.0% (95% CI= 14.72 – 39.07) was observed in the old herd sampled in 2017, no abortion cases were reported by farm personnel. This information is not consistent with the assertion made by González-Barrio et al. (2013), indicating evidence of reproductive issues in deer due to *C. burnetii* infection. However, the high seropositivity observed could be attributed to the fact that it was past exposure to *C. burnetii*, as our study utilizes serological testing, which does not signify current active infection. Furthermore, the farm personnel emphasized that occasional abortion cases are associated with the rainy season and stress, with no significant concern. As a result, no additional samples were sent to the laboratory for further diagnostic testing, leaving the possibility of *C. burnetii*'s presence in the environment open.

Multivariable logistic regression analysis revealed no association between the occurrence of *C. burnetii* or Q fever antibodies and risk factors such as age, gender, and herd of the deer. This research serves as a foundation for future studies and the development of management and policy frameworks to address the economic and zoonotic consequences of Q fever in Malaysia. However, it is essential to note that this

study had a small sample size and only focused on one location (UPM Deer Farm), highlighting the need for further investigations into the seroprevalence of *C. burnetii* or Q fever and associated risk factors among deer livestock especially in Malaysia, as it poses a potential zoonotic.

CHAPTER 6

CONCLUSION

This study's findings lead us to the first documented evidence of serum antibodies towards *C. burnetii* among *Cervus timorensis* in Malaysia. The high seroprevalence indicates the susceptibility of *Cervus timorensis* deer to Q fever infection and suggests the presence of *C. burnetii* in the environment. However, there is currently insufficient evidence to establish an association between gender, age, and herd-related factors and *C. burnetii* or Q fever infection among deer livestock at UPM Deer Farm (PPP UPM). Further studies are needed to isolate and characterise the strain of *C. burnetii* circulating among deer in Malaysia.

CHAPTER 7

RECOMMENDATIONS

This study has limitations arising from its small sample size which focuses only on UPM Deer Farm, thus, the results may not be generalizable to the entire population in Malaysia, the absence of detailed clinical history data on deer with seropositivity against *C. burnetii* and the utilisation of single diagnostic tool which may lead to inaccurate estimates of seroprevalence. Therefore, it is advisable to consider conducting future research with a larger sample size with more sampling locations from different geographical areas in Malaysia, more comprehensive clinical history information on the deer, and utilise multiple testing methods to improve the accuracy of seroprevalence estimates. This approach will aid in identifying additional risk factors that may expose the deer to Q fever infection.

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APPENDICES

Table A: Chi Square Tests

Age x Q fever

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	8.654[a]	2	.013
Likelihood Ratio	10.927	2	.004
Linear-by-Linear Association	8.418	1	.004
N of Valid Cases	92		

a. 2 cells (3.33%) have expected count less than 5. The minimum expected count is 3.11.

Gender x Q fever

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.189[a]	1	.139		
Continuity Correction[b]	1.274	1	.259		
Likelihood Ratio	2.672	1	.102		
Fisher's Exact Test				.178	.126
Linear-by-Linear Association	2.165	1	.141		
N of Valid Cases	92				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.11

b. Computed only for a 2x2 table

Herd x Q fever

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	9.732[a]	1	.002		
Continuity Correction[b]	7.913	1	.005		
Likelihood Ratio	14.260	1	.000		
Fisher's Exact Test				.001	.001
Linear-by-Linear Association	9.627	1	.002		
N of Valid Cases	92				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.09

b. Computed only for a 2x2 table