Short Communication

First serological evidence of *Coxiella burnetii* and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) among deer in an institutional farm in Malaysia

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Abstract

Q fever is a zoonotic disease caused by *Coxiella burnetii,* which is associated with reproductive disorders such as abortion and reproductive problems in ruminants, while paratuberculosis is an underdiagnosed disease caused by *Mycobacterium avium paratuberculosis* (MAP) which causes chronic wasting, low fertility, and mortality in ruminants. Although both *C. burnetii* and MAP infections have been reported in domestic ruminants worldwide, there is no data on their seroprevalence status among deer in Selangor, Malaysia. This study investigated the seroprevalence status of *C. burnetii* and MAP among deer in Malaysia. Ninety-two blood sera collected from an institutional deer farm were screened using commercial Multi-species ID Screen indirect ELISA tests to detect specific anti-*C. burnetii* (FQS-MS/1117) and anti-MAP (PARAS Ver 0516) antibodies. The ELISA results showed a 14.1% (95% CI = 8.5-22.7) apparent and 14.8% (95% CI = 8.4-24.4) true prevalence for *C. burnetii* and a 2.2% (95% CI= 0.6-7.6) apparent and 1.3% (95% CI= -0.45-7.40) true prevalence for MAP among deer. There were statistically significant associations between seropositivity to *C. burnetii* and the age groups ($\chi 2$= 7.325, P = 0.011) or herds of deer ($\chi 2$= 9.732, P = 0.001). To the best of our knowledge, this study provides the first preliminary serological evidence of *C. burnetii* and MAP in *Cervus timorensis*, suggesting potential threats to animal and public health in Malaysia.

Keywords: Deer, *Coxiella burnetiid*, *Mycobacterium avium paratuberculosis*, seroprevalence, Malaysia

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Introduction

Coxiella burnetii is a zoonotic Gram-negative pathogenic bacterium that causes coxiellosis in animals and Q fever in humans (Celina and Cerný, 2022). The organism is present in body secretions or excretions (Agerholm, 2013). Transmission in animals may be airborne by inhalation or vector-borne by ticks, while human infection may be due to direct anthroponotic transmission from animals through body secretions/excretions, tick-borne transmission, or indirect occupational exposure (Celina and Cerný, 2022). Coxiellosis is mostly asymptomatic, but metritis, infertility, abortion, premature delivery, stillbirth, and the birth of weak neonates may be observed (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; Eldin *et al.*, 2017). Q fever causes mild flu-like symptoms or severe fatal complications in humans (Angelakis and Raoult, 2010). It is a disease of economic and public health significance (Keshavamurthy *et al.*, 2019; Jesse *et al.*, 2020), and wildlife species, including deer, serve as reservoir hosts (Celina and Cerný, 2022). Although *C. burnetii* has been detected and reported in humans (Rai *et al.*, 2011; Khor *et al.*, 2018) and livestock (Nurkunasegran *et al.*, 2017; Khor *et al.*, 2018; Jesse *et al.*, 2020) in Malaysia, there is still a lack of published information on its seroprevalence status and risk factors among wild ruminants and farmed deer.

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a zoonotic bacterium that causes paratuberculosis or Johne's disease (JD) in ruminants (Ssekitoleko *et al.*, 2021; Yu *et al.*, 2022) and Crohn's disease in humans (Hermon-Taylor *et al.*, 2000). The MAP organism is shed in the feces, milk, and colostrum of infected animals, and transmission occurs via the fecal-oral route (Khol *et al.*, 2017). Johne's disease has a global distribution and is observed in domestic (Barrero-Domínguez *et al.*, 2019; Pourmahdi Borujeni *et al.*, 2021) and wild (Budhe *et al.*, 2014; Matos *et al.*, 2017) ruminants. Although JD is a notifiable disease, it is grossly underreported due to its subclinical course and lack of routine monitoring mechanisms in many countries (Manning and Collins, 2001). Although there is a recent report on prevalence of MAP among small ruminant livestock in Malaysia (Jimale *et al.*, 2024), there is paucity of information on its seroprevalence among deer in the country.

This study was necessary due to the global economic and zoonotic impacts of Q fever and paratuberculosis and the recognition of wildlife as a reservoir and source of infection for domestic ruminants and man, coupled with the lack of epidemiological data on the seroprevalence status of these important zoonoses in Malaysia. In this study, we investigated the seroprevalence status of Q fever and paratuberculosis in farmed deer livestock to shed light on the epidemiology of *C. burnetii* and MAP and furnish preliminary data for implementing further studies and designing comprehensive control programs in Malaysia.

Materials and Methods

Study design and sampling: The study received ethical clearance from the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM/IACUC/AUP-U042/2023). The deer stock (*Cervus timorensis*) belonged to an institutional farm located within latitude 2.982962 and longitude 101.729190 in Selangor, Malaysia. The deer herds were semi-intensively managed, openly grazed during the day with supplemented pelletized concentrate feeding, and were allowed to breed naturally. A total of 92 blood sera comprising 36 fresh samples collected between January and August 2023 (new herd) and 56 archived serum samples collected in 2017 (old herd) were included in this study. Sample collection was done conveniently based on availability due to the difficulty in restraining the deer. Whenever possible, individuals were manually restrained before withdrawing 5 mL of whole blood by jugular venipuncture for serum extraction. Epidemiological variables of the deer, such as age, gender, and herd, were also recorded. The deer were further categorized according to their age group as young (≤ 2 years) and adults (> 2 years).

Detection of serum antibody by ELISA: Commercial Multi-species ID Screen (ID.VET, France) indirect ELISA screening tests were used to detect serum antibodies directed against *C. burnetii* (FQS-MS/1117) and MAP (PARAS Ver 0516) according to manufacturers instructions. The optical densities of ELISA microplates were measured at 450 nm wavelength using an ELISA microplate absorbance reader (Infinite® 200 PRO). The samples with ($S/P\% > 70\%$) were interpreted as positive for MAP, while samples with $S/P\% > 50\%$ were interpreted as positive for *C. burnetii*.

Statistical analysis: The Epitools statistical calculator was used to calculate the apparent and true prevalence of *C. burnetii* or MAP with their respective 95% confidence intervals (CI) (Rogan and Gladen, 1978; Sergeant, 2018). The Chi-square test using the Fisher's Exact method was computed using the Statistical Package for Social Sciences (SPSS) Software version 29.0 macOS to determine the associations between the seroprevalence of *C. burnetii* or MAP infection as the dependent variables and the age, gender, or herd of deer as the independent variables.

Results and Discussion

The ELISA assays revealed 1.32% (95% CI = -0.5-7.4) and 14.8% (95% CI = 8.4-24.4) true prevalence for MAP and *C. burnetii* (Table 1) with significant associations between *C. burnetii* infection and the age groups ($\chi2 = 7.325$, $P = 0.011$) and herds ($\chi2 = 9.732$, $P = 0.001$) of deer. This study is the first report on the prevalence of *C. burnetii* and MAP infection among deer in Malaysia. The result of this study agrees with previous reports of 12.3% for *C. burnetii* in China (Cong *et al.*, 2015), 15.4% in Spain (Ruiz-Fons *et al.*, 2008), and 14.5% in the United States (Kirchgessner *et al.*, 2013). However, our result is higher than previous reports of 1.4% (Yangho *et al.*, 2011), 9.18% (Shin *et al.*, 2014) in Korea, and 3.4% in eastern Australia (Voss *et al.*, 2023). The high seroprevalence of *C. burnetii* infection observed among deer livestock in this study is due to optimal environmental conditions

(Mori and Roest, 2018). Grazing of deer in semi-intensive management, as seen in this study, increases their risk of exposure to the natural tick vectors of *C. burnetii* (Nusinovici *et al.*, 2015). *Hyalomma marginatum marginatum* and *Hyalomma lusitanicum* increased the risk of coxielliosis in Spain (Ruiz-Fons *et al.*, 2008). The higher seropositivity in adult deer may be due to repeated exposure with increasing age (Rizzo *et al.*, 2016). Moreover, their proximity to cattle and goat livestock may lead to cross-infection (Celina and Cerný, 2022). Although a high seroprevalence of *C. burnetii* infection is accompanied by reproductive issues in animals and humans (Ullah *et al.*, 2022), no clinical cases of abortion, stillbirths, or infertility were previously reported on the farm. Thus, the absence of clinical disease or history of reproductive disorders in the studied deer farm has led us to suggest that the observed high seropositivity could be attributed to past exposure of deer to *C. burnetii* because serological testing used in this study only indicates exposure. Without further molecular diagnostics, it is not likely to suggest that the presence of antibodies indicates an active infection.

The current observation of 1.3% seropositivity to MAP among captive deer livestock agrees with previous reports of 1.9%, 3.8, 3.4% in various types of deer in Portugal (Tryland *et al.*, 2004), 1.7% in Mauritius (Jori *et al.*, 2014), and 0.5% (Sleeman *et al.*, 2009) and 0.3% (Raizman *et al.*, 2005) in the United States. However, our result is lower than 16% in Mexico (Lozano-Cavazos *et al.*, 2021), 17.6% in China (Meng *et al.*, 2015), and 30.2% in Spain (Reyes-García *et al.*, 2008). The MAP organism is known to be very hardy and survives for up to 48 weeks outside the host in water bodies and sediments (Whittington *et al.*, 2019). The result represents a significant risk for domestic animals and other wildlife due to their potential to harbor subclinical disease (Sleeman *et al.*, 2009). MAP infection may potentially spill over from deer to domestic animals and wildlife in areas that practice semi-intensive or extensive management systems (Geraghty *et al.*, 2014).

This study provides preliminary serological data showing a high level of exposure to *C. burnetii* among deer livestock, suggesting potential threats to animal and public health in Malaysia. Furthermore, this study is, to the best of our knowledge, the first documented evidence of serum antibodies towards *C. burnetii* and MAP in *Cervus timorensis* in Malaysia. Although no vaccination was practiced against Q fever and paratuberculosis in the herds to suggest vaccine antibodies, further studies using more sensitive molecular diagnostics are needed to confirm and characterize MAP and *C. burnetii* circulating among deer and elucidate their potential role in the epidemiology of coxielliosis and paratuberculosis in Malaysia. A one-health approach towards controlling paratuberculosis and Q fever is recommended among livestock and people with occupational exposure to MAP and *C. burnetii*, such as veterinarians, animal health workers, abattoir workers, animal traders, environmental workers, and public health care workers.

Table 1 The apparent and true Seroprevalence of *Coxiella. burnetii* and *M. avium paratuberculosis* in deer livestock in Selangor, Malaysia

Variables	Tested	*Coxiella. burnetii*				*M. avium paratuberculosis*			
		Apparent	95 % CI	True	95 % CI	Apparent	95 % CI	True	95 % CI
Age									
Young	59	4 (6.8)	2.7-16.2	6.5	1.9-17.1	0 (0.0)	0.0- 6.1	-1.12	-0.1-5.7
Adult	33	9 (27.3)	15.1-44.2	29.5	15.8-48.6	2 (6.1)	0.2-19.6	5.69	0.8-20.9
Gender									
Male	22	1 (4.5)	0.8-21.8	4.0	-0.9-23.4	0 (0.0)	0.0-14.9	-0.01	-0.0-15.6
Female	70	12 (17.1)	10.1-27.6	18.1	10.2-29.9	2 (2.9)	0.8-9.8	2.09	-0.2-9.9
Herd									
Old	56	13 (23.2)	14.1-39.1	24.9	14.7-39.1	2 (3.6)	0.9-12.1	2.89	0.0-12.5
New	36	0 (0.0)	0.0-9.6	-1.1	-1.1-9.7	0 (0.0)	0.0-9.6	-1.12	-1.1-9.7
Overall	**92**	**13 (14.1)**	**8.5-22.7**	**14.8**	**8.4-24.4**	**2 (2.2)**	**0.6-7.6**	**1.3**	**-0.5-7.4**

CI: confidence interval.

Table 2 Univariable analysis for the associations between seropositivity and epidemiological variables

Variables	Tested	*Coxiella. burnetii*				*M. avium paratuberculosis*			
		positive	negative	$\chi 2$	p-value	positive	negative	$\chi 2$	p-value
Age									
Young	59	4 (6.8)	55 (93.2)	7.325	0.011*	0 (0.00)	59 (100)	3.655	0.126
Adult	33	9 (27.3)	24 (72.7)			2 (6.1)	31 (93.9)		
Gender									
Male	22	1 (4.5)	21 (95.5)	2.189	0.179	0 (0.0)	22 (100)	0.643	1.000
Female	70	12 (17.1)	58 (82.9)			2 (2.9)	68 (97.1)		
Herd									
Old	56	13 (23.2)	43 (76.8)	9.732	0.001*	2 (3.6)	54 (96.4)	1.314	0.518
New	36	0 (0.0)	36 (100)			0 (0.0)	36 (100)		

$\chi 2$: Chi-square, P-values (Fisher's Exact test) with an asterisk (*) are statistically significant.

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