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Original Article

Seroprevalence and associated risk factors of caprine arthritis encephalitis (CAE) and peste des petits ruminants (PPR) among deer in an institutional farm in Malaysia

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Abstract

Caprine arthritis encephalitis (CAE) and peste des petits ruminants (PPR) are significant viral diseases impacting small ruminants, leading to substantial economic losses and posing a potential threat to livestock health. Although seroprevalence evidence of these viruses has been documented in ruminants, there is a lack of data regarding their presence among farmed deer in Malaysia. This preliminary study aimed to determine the seroprevalence and associated risk factors for CAEV and PPRV infections among deer in Malaysia. A total of ninety-two blood sera from an institutional deer farm were screened for CAEV and PPRV antibodies using commercially available indirect ELISA test kits. The results revealed 8.7% (95% CI = 4.47-16.23) apparent and 8.38% (95% CI = 3.72-16.7) true prevalence for CAEV and 4.35% (95% CI = 1.70-10.65) apparent and 3.99% (95% CI = 1.18-10.70) true seroprevalence for PPRV among deer. Univariable analysis indicated that age ($\chi^2 = 4.434$, $p = 0.707$) and sex ($\chi^2 = 1.071$, $p = 0.377$) were not significantly associated with CAEV seropositivity, while the herd group ($\chi^2 = 4.733$, $p = 0.053$) showed a significant association. Conversely, PPR seroprevalence was higher in young deer (8.0%) compared to adults (2.69%), with female deer demonstrating a higher prevalence (5.36%) than males. Additionally, the older herd exhibited a higher true prevalence of PPR (5.07%) compared to the newer herd (2.32%). This study provides the first serological evidence of CAEV and PPRV among deer in Malaysia and justifies further epidemiological investigations in order to reduce the potential threat to animals.

Keywords: caprine arthritis encephalitis, Malaysia, peste des petits ruminants, seroprevalence

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Introduction

CAE is an infectious multisystemic viral disease in goats caused by the caprine arthritis encephalitis virus (CAEV). The virus is classified as a Small Ruminant Lentivirus (SRLV). It is characterized as a small, enveloped and single-stranded RNA virus that belongs to the *Retroviridae* family (Al-Ani and Vestweber, 1984). It is believed that CAEV spreads primarily through two main transmission routes: vertical and horizontal. Vertical transmission (from mother to offspring) occurs by ingesting contaminated milk and colostrum, while horizontal transmission (animal-to-animal) could occur by respiratory infection (Blacklaws, 2012), bodily fluids, or even through direct contact with infected animals (Brajon *et al*., 2012; Rowe and East, 1997). However, recent studies have indicated that the virus can also be transmitted through non-maternal transmission routes (Leymaster *et al*., 2015). Factors such as animal density, the duration of exposure of uninfected animals to infected groups, and housing conditions play a significant role in virus transmission. In goats, the disease is mainly manifested by encephalitis and severe arthritis, which affect all sexes, ages, and breeds (Alamerew *et al*., 2022; Mosa *et al*., 2022). In addition, other clinical signs include swelling of the joint capsules, which may lead to lameness and reduced growth rate (Waseem *et al*., 2015; Jesse *et al*., 2017). CAE virus infection has been associated with medical conditions such as mastitis, pneumonia, and synovitis. High CAEV prevalence within a herd often leads to reduced milk production, necessitating the culling of infected animals, which can cause substantial economic losses for the ruminant industry (Norouzi *et al*., 2015; Peterson *et al*., 2022). This virus infection was first documented in 1974, and since then, numerous countries have reported cases in their livestock (Al-Ani & Vestweber 1984). In Malaysia, the first CAEV case was recorded by Noordin *et al*. (2010), who observed clinical signs and lesions consistent with CAEV in an affected goat herd. In addition, through post-mortem examination and histopathological analysis, Ling *et al*. (2013) identified a case of CAEV infection in a Boer cross kid. The first serological survey of CAEV in small ruminants reported a prevalence rate of 8.8% in Selangor (Jesse *et al*., 2018) and 21.4% in other states (Paul *et al*., 2021).

On the other hand, another RNA virus gaining attention in small ruminant livestock worldwide is peste des petits ruminants (PPR) disease, widely known as sheep and goat plague, which is caused by the peste des petits ruminants virus (PPRV) (Taylor and Barrett, 2007). This virus is classified within the genus *Morbillivirus* within the family *Paramyxoviridae* (Wohlsein and Singh, 2014). It primarily replicates in the cranial lymph nodes, including those in the oropharynx and mandibular regions, as well as in the tonsils and other lymph nodes of the animals. Symptoms such as fever, conjunctivitis, rhinotracheitis, ulcerative stomatitis, and gastroenteritis result from PPRV infection in small ruminants (Taylor and Barrett, 2007). In severe cases, it can also cause pneumonia. In naive populations, morbidity and mortality rates can reach as high as 90-100%, while in endemic areas, these rates tend to drop to around 20% (Banyard *et al*., 2010). Most of the transmission occurs through direct contact between infected and healthy animals, either via aerosols released from discharges of infected animals such as discharges from the eyes, nose, and mouth, as well as loose feces that contain high titers of virus. Transmission also occurs through fomites, contaminated water, food, and bedding of the infected animals (Munir, 2014). Similarly, communal grazing may spread the disease between flocks belonging to the same village (Taylor and Barrett 2007).

Caprine arthritis encephalitis virus (CAEV) and peste des petits ruminant virus (PPRV) are two significant viral diseases that primarily affect small ruminants, such as goats, sheep, and, as recent studies indicate, deer populations. These viral infections pose a serious threat to the health and productivity of affected animals, resulting in substantial economic losses in the livestock and wildlife sectors (Dhar *et al*., 2002; Singh and Prasad, 2008). Although both diseases are well-documented in domestic livestock, their impact on wildlife, especially deer, remains largely unexplored, particularly in Southeast Asia. In Malaysia, the presence of CAEV and PPRV infections in deer could have notable economic and ecological impacts. From an economic perspective, infected captive deer may suffer from reproductive inefficiencies, higher mortality rates, as well as slowed growth rates, leading to financial losses for farmers and conservation programs (Singh and Prasad, 2008). Moreover, both the infections in wild deer populations could hinder disease control efforts, as infected wildlife could act as reservoirs, facilitating transmission to domestic animals. From a conservation standpoint, the spread of these viruses could have a significant impact on Malaysia's biodiversity and wildlife management strategies, as it threatens the sustainability of both native and farmed deer populations (Meng, 2012). Therefore, understanding the epidemiology of CAEV and PPRV in deer populations is crucial for formulating optimal control and prevention measures. For instance, enhancing surveillance programs, improving diagnostic techniques, and implementing stronger biosecurity measures are essential for controlling the risks associated with these viral infections. This study aims to provide insights into the current status of CAEV and PPRV infections in Malaysia and the contributing risk factors for these infections among deer livestock.

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. In Malaysia, there is currently no vaccination program for deer against PPRV and CAEV. 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. Individuals were manually restrained whenever possible before withdrawing 5 mL of whole blood by jugular venipuncture for serum extraction. Epidemiological variables of the deer, such as age, sex, and herd, were also recorded. The deer were further categorized according to their age group as young (≤ 2 years), young adult (2 to 3 years) and adults (> 3 years).

Detection of serum antibody by ELISA: The ID Screen® PPR Competition (ID.VET, France) and ID Screen® MVV/CAEV VISNAS (ID.VET, France) are indirect enzyme-linked immunosorbent assay (ELISA) screening tests and were used to detect serum antibodies against PPRV (PPRC ver 0821) and CAEV (VISNAS ver 0922) respectively, following manufacturer's 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% ≥ 60%) were interpreted as positive for CAEV, while samples with S/N % ≤ 50 % were interpreted as positive for PPR. The indirect enzyme-linked immunosorbent assay (ELISA) screening tests were used to detect serum antibodies against CAEV (VISNAS ver 0922)

Statistical analysis: 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 analyze the data. Risk factors such as age, sex, and a herd of the sampled deer are identified as the independent variable, and seroprevalence to CAEV and PPRV infections were identified as the dependent variable. The Chi-square test using the Fisher's Exact method was computed using the Statistical Package for Social Sciences (SPSS) Software version 25.0 to determine the relation between the seroprevalence of CAEV and PPRV infections (dependent variable) and the age, sex, 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 utilized to calculate a multiple binary logistic regression analysis forward selection to evaluate the contributing risk factors for seroprevalence of CAEV and PPRV infections in deer.

Results

The results indicated an apparent seroprevalence of 8.7% (95% CI = 4.47-16.23) and a true seroprevalence of 8.38% (95% CI = 3.72-16.7) for CAEV. In addition, anti-PPRV antibodies were detected in four animals, suggesting an apparent prevalence of 4.35% (95% CI = 1.70-10.65) and a true prevalence of 3.99% (95% CI = 1.18-10.70) among the deer livestock (Table 1).

Table 1 The apparent and true Seroprevalence of CAEV and PPRV in deer livestock in Selangor, Malaysia.

Variables	Tested	CAEV				PPRV			
		Apparent	95% CI	True	95% CI	Apparent	95% CI	True	95% CI
Age									
Young	23	1(4.35)	0.77-0.99	3.58	-0.97-21.95	0(0.0)	0.00-14.31	-0.64	-0.64-14.60
Young adult	37	6 (16.22)	7.65-31.14	16.68	7.23-33.15	3(8.11)	2.80-21.30	8.00	2.34-22.05
Adult	32	1(3.12)	0.55-15.74	2.24	-1.04-16.16	1(3.12)	0.55-15.74	2.69	-0.47-16.13
Sex									
Male	21	3(14.29)	4.98-34.64	14.55	4.28-37.02	0(0.00)	0.00-15.46	-0.64	-0.64-15.38
Female	71	5(7.04)	3.05-15.45	6.56	2.15-15.84	4(5.63)	2.21-13.61	5.36	1.72-13.85
Herd									
New	36	6(16.67)	7.87-31.89	17.18	7.47-33.99	1(2.78)	0.49-14.17	2.32	-0.49-14.45
Old	56	2(3.57)	0.98-12.12	2.73	0.13 -12.16	3(5.36)	1.84-14.61	5.07	1.32-14.92
Total	92	8(8.7)	4.47-16.23	8.38	3.72-16.7	4(4.35)	1.70-10.65	3.99	1.18-10.70

CI: confidence interval.

Seroprevalence of CAEV varied by age, with young adult deer showing the highest rate at 16.22% (95% CI = 7.65-31.14). In comparison, the young age group had a lower rate of 4.35% (95% CI = 0.77-0.99), while adult deer exhibited a prevalence of 3.12% (95% CI = 0.55-15.74) (Table 2). In terms of sex, female deer had a prevalence rate of 7.04% (95% CI = 3.05-15.45), while male deer exhibited a higher rate of 14.29% (95% CI = 4.98-34.64). When examining seroprevalence according to herd, the new herd presented a higher rate of 16.67% (95% CI = 7.87-31.89) compared to the old herd, which had a rate of 3.57% (95% CI = 0.98-12.12). The results demonstrate a significant association between the seroprevalence of CAEV and the herd type ($\chi^2 = 4.733$, $p = 0.053$) among the deer examined (Table 2). However, no association was found between the seroprevalence of CAEV and either sex ($\chi^2 = 1.071$, $p = 0.377$) or age ($\chi^2 = 4.434$, $p = 0.707$) in the deer population investigated.

In the case of PPRV, the distribution of infections by age, sex, and herd indicated that young deer (< 2 years) had the highest seroprevalence at 8.0% (95% CI = 2.34-22.05), compared to adult deer, which recorded a seroprevalence of 2.69% (95% CI = -0.47-16.13) (Table 2). Among the different sexes, only the female deer tested positive, with a true prevalence of 5.36% (95% CI = 1.72-13.85), while no infections were detected in the males. Additionally, the older herd exhibited a higher true prevalence rate of 5.07% (95% CI = 1.32-14.92), in contrast to the new herd, which had a true prevalence of 2.32% (95% CI = -0.49-14.45). The chi-square univariable analysis results indicated no significant associations between PPRV seropositivity and age ($\chi^2 = 2.418$, $p = 0.448$), sex ($\chi^2 = 1.237$, $p = 0.570$), or herd ($\chi^2 = 0.351$, $p = 1.000$) among the deer examined in this study (Table 2).

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

Variables	Tested	CAEV				PPRV			
		Positive	Negative	χ^2	*P*-value	Positive	Negative	χ^2	*P*-value
Age									
Young	23	1 (4.3)	2 2 (95.7)			0 (0.0)	23 (100.0)		
Young adult	37	6 (16.2)	31 (83.8)	4.434	0.707	3 (8.1)	34 (91.9)	2.418	0.448
Adult	32	1 (3.1)	31 (96.9)			1 (3.1)	31 (96.9)		
Sex									
Male	21	3 (14.3)	18 (85.7)	1.071	0.377	0 (0.0)	21 (100.0)	1.237	0.570
Female	71	5 (7.0)	66 (93.0)			4 (5.6)	67 (94.4)		
Herd									
New	36	6 (16.7)	30 (83.3)	4.733	0.053*	1 (2.8)	35 (97.2)	0.351	1.000
Old	56	2 (3.6)	54 (96.4)			3 (5.4)	53 (94.6)		

χ2: Chi-square, *P*-values (Fisher's Exact test) with an asterisk (*) are statistically significant

Discussion

This study is the first to report the prevalence of caprine arthritis-encephalitis virus (CAEV) and peste des petits ruminant virus (PPRV) infections among deer in Malaysia. CAEV infection is a known economically important viral disease causing persistent fatigue and painful arthritis in ruminants. As a transboundary disease, CAE disease needs to be studied in more detail in other ruminants such as deer, so that the emphasis on control and prevention strategies on farms can be strengthened. This study showed that the CAEV seroprevalence in the herd of deer in an institutional farm in Selangor, Malaysia, was 8.38%. This CAEV seroprevalence aligns closely with the findings of Olech *et al*. (2020), who reported a 9% seroprevalence among European Red Deer (*Cervus elaphus*) in Poland. A higher seroprevalence of 11.1% was recorded among European mouflons during a study conducted by Kuhar *et al*. (2022) in Slovenia. In contrast, Olech *et al*. (2024) documented markedly lower CAEV seroprevalence rates in deer livestock, noting a rate of 0.64% in Red Deer (*Cervus elaphus*) and 0% in both Roe Deer (*Capreolus capreolus*) and Fallow Deer (*Cervus Dama*). The observed discrepancies may stem from the distinct epidemiology of CAEV in small ruminant livestock versus wildlife populations, along with variations in the diagnostic tests employed in these studies (Minardi *et al*., 2013; Kalogianni *et al*., 2021). This might be attributed to the specific environment management and ecological factors associated with semi-intensive farming systems. For instance, animals are maintained in closer proximity, which can facilitate transmission through respiratory secretions, contaminated equipment, or shared feed and water sources (Blacklaws, 2012). Besides that, the potential for interspecies transmission could happen where deer cohabit or share pastures with domestic small ruminants, which could increase the risk of CAEV exposure, as lentiviruses such as CAEV are known to cross species barriers under certain conditions (Shah *et al*., 2004). On a cautionary note, it should not be interpreted as evidence of broader regional endemicity of CAEV in Southeast Asia. Continuous surveillance can ascertain the true distribution and host range of CAEV in this region. This study observed 14.29% seropositivity in males and 7.04% in females, and the findings in this study are not in agreement with the study conducted in Italy by Gufler *et al*. (2007) and this may be due to the limited sample size enrolled for this study. However, the result of this study differs slightly with regard to age, as the young age group was most affected by CAEV. The higher seroprevalence of caprine arthritis encephalitis virus (CAEV) observed in the semi-farmed deer herd may be attributed to specific management and ecological factors associated with semi-intensive farming systems. In such environments, animals are maintained in closer proximity, which can facilitate horizontal transmission through respiratory secretions, contaminated equipment, or shared feed and water sources. Moreover, the potential for interspecies transmission, particularly in regions where deer cohabit or share pastures with domestic small ruminants, could increase the risk of CAEV exposure, as lentiviruses such as CAEV are known to cross species barriers under certain conditions (Shah *et al*., 2004).

PPRV is a devastating infectious disease of small ruminants due to its high mortality, nearing 80-90% (Kumar *et al*., 2014; Parida *et al*., 2015) and high morbidity that can reach as high as 100% during the outbreaks (Kumar *et al*., 2014; Munir, 2014), and its potential to spread across local and international boundaries. This characteristic of PPRV has given it a reputation as an emerging transboundary disease that is targeted for eradication by the World Organization for Animal Health (WOAH). The current true prevalence of PPRV recorded in deer is comparable to a previous report of 4.58% among cattle and buffaloes in Southern India (Balamurugan *et al*., 2011) and 6.4% in sheep and goats in Ethiopia (Waret-Szkuta *et al*., 2008). Previous studies have reported 43.33% seroprevalence of PPRV among small ruminants and 59.09% in large ruminants in Pakistan (Khan *et al*., 2008), and 52.9% among sheep and goats in the Republic of Chad (Mahamat *et al*., 2018). Another study by El-Yuguda *et al*. (2013) has reported 71.6% in sheep, 51.6% in goats, 27.8% in camels, and 16.7% in cattle from North-eastern Nigeria. Other researchers have also demonstrated that wildlife species are susceptible to PPRV infection. For instance, Aguilar *et al*. (2020) in South Sudan have reported a true seroprevalence of 71.4% among the Tiang (*Damaliscus lunatus tiang*). However, the current study's result is higher than 2.6% seroprevalence of PPRV reported by a previous study involving sheep and goats in Malaysia (Jimale *et al*., 2024). The difference in detection rates may be due to the species differences in susceptibility, differences in management practices, and differences in climatic conditions during sampling since PPRV often had outbreaks during the rainy season or the dry cold

season (Taylor and Barrett, 2007; WOAH, 2024), and finally the existing control measures such as vaccination and other herd health programs in place. The spreading of PPRV is most likely due to sharing the same grazing area, especially if the area is grazed by small ruminants such as goats and sheep, introducing the virus from the infected and susceptible animals (Balamurugan *et al*., 2011) through aerosol or clinical excretions (saliva, lacrimal, feces) to the naïve deer herd (Aguilar *et al*., 2020). Infected animals can recover through good nursing and good animal husbandry, and they developed lifelong immunity, so they cannot be re-infected with PPRV again and do not carry or shed the virus, underscores the possibility of lower PPRV prevalence rate in this study (Taylor and Barrett 2007; Parida *et al*., 2015).

The higher seropositivity rate observed among young animals compared to adults in the context of PPRV and CAEV can be attributed to multiple immunological factors. For PPRV, young goats and sheep often display elevated seropositivity due to maternally derived antibodies acquired via colostrum, which can persist for up to 3-4 months and remain detectable by serological assays (Diallo *et al*., 2007). This passive immunity may not indicate active infection but can still produce a positive result in enzyme-linked immunosorbent assays (ELISAs), thus inflating seroprevalence estimates in this age group. Similarly, CAEV has been identified to transmit vertically to kids from infected female goats and maternal antibodies are also transferred through colostrum, contributing to transient seropositivity in neonates without active viral replication (Adams *et al*., 1983; Hasegawa *et al*., 2017). On a cautionary note, this is a limitation in this study where a higher number of young animals positive for PPRV and CAEV could not distinguish whether the detected antibody occurred in natural infection or from maternal transmission. Adults, on the other hand, may show reduced seropositivity due to waning antibody levels in the absence of natural re-exposure or experienced seroreversion or maintain low antibody titers below assay detection thresholds, particularly in chronic CAEV infections (Rowe & East 1997)). These combined factors explain the paradoxical pattern of higher seropositivity in younger animals compared to adults in populations exposed to PPRV and CAEV.

CAEV-infected animals are known to become permanent carriers, retaining the virus and transmitting it either maternally, via fomites, or through close contact in grazing areas. This increases the likelihood of new infections. Consequently, the deer population plays a crucial role as a potential source of infection for other animals. To control CAE disease among small ruminant livestock, it is important to consider CAEV carrier animals within the deer population in Malaysia in a holistic manner. Although there is currently no vaccination program for PPRV in Malaysia, caution is required when interpreting serological test results to differentiate between active and passive infections within a population. Therefore, more sensitive and specific molecular-based antigen detection tests, such as polymerase chain reaction (PCR) and high-throughput sequencing technology, should be employed in future investigations. These methods could provide greater diagnostic information regarding the genetic diversity and virulence determinants of the PPRV. This study thus offers essential epidemiological information about the serological status and risk factors associated with CAEV and PPRV infections in deer in Malaysia. However, it is important to note that serological surveys and tests are screening tools and do not confirm whether an infection is active or passive, highlighting the need for continuous surveillance of these infections in animals.

In conclusion, to the best of our knowledge, this study is the first documented evidence of serum antibodies towards CAEV and PPRV among deer (*Cervus timorensis*) in Malaysia. Further studies are needed to know the overall seroprevalence of this disease among deer livestock in Malaysia and studies to isolate and characterize the strain of CAEV and PPRV circulating among deer in Malaysia.

Conflicts of interest: There were no conflicts of interest that may have biased the work reported in this study.

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