Tropical Biomedicine 41(S): 385-391 (2024)
https://doi.org/10.47665/tb.41.S.001

RESEARCH ARTICLE

Molecular identification and phylogenetic analysis of *Anaplasma marginale* and *Anaplasma centrale* isolated from commercial Mafriwal cattle in Johor, Malaysia

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ARTICLE HISTORY

Received: 19 July 2024
Revised: 16 October 2024
Accepted: 16 October 2024
Published: 31 December 2024

ABSTRACT

Bovine anaplasmosis is a tick-borne disease in cattle which is mainly caused by *Anaplasma marginale* and *Anaplasma centrale*. It poses significant economic burdens and threat on livestock industries worldwide. This study aimed to identify *Anaplasma* spp. infecting the commercial Mafriwal cattle in Johor, Malaysia and investigate their phylogenetic relationship in the population. In this study, polymerase chain reaction (PCR) targeting the MSP4 gene for *A. marginale* and the 16s rRNA gene for *A. centrale* were performed. These assays were conducted on blood samples collected from 242 Mafriwal cattle. BLAST analysis and phylogenetic trees were constructed to analyze the genetic relationships between the *Anaplasma* spp. The results revealed 57.85% of the sampled population were infected with *Anaplasma* spp., 21.90% with *A. marginale*, 9.50% with *A. centrale* and 26.45% with both *A. marginale* and *A. centrale*. BLAST analysis showed 100% similarities between *A. marginale* sequences from this study and the sequence from a goat in Brazil. Similarly, *A. centrale* sequences were closely related to strains from tick vector, *Rhipicephalus (Boophilus) microplus* in Panama with 100% similarity. Phylogenetic analysis confirmed distinct clades for *A. marginale* and *A. centrale*, indicating genetic diversity and specific species differentiation. The findings highlight the endemicity of bovine anaplasmosis in Malaysian cattle populations and potential cross-border transmission routes. Moreover, this study provides the first report of *A. centrale* prevalence in Malaysia, emphasizing the importance of ongoing surveillance and management efforts. Understanding the genetic diversity and species differentiation of these pathogens is crucial for designing effective control strategies and vaccine development. In conclusion, this study enhances our understanding of the prevalence and genetic dynamics of bovine anaplasmosis among Mafriwal cattle in its largest population in Malaysia for better diagnosis and effective control measures.

Keywords: Tick-borne disease; anaplasmosis; *Anaplasma* spp.; phylogenetic tree; Mafriwal cattle.

INTRODUCTION

Bovine anaplasmosis is a tick-borne disease in cattle caused by *Anaplasma* spp. namely *Anaplasma marginale, Anaplasma centrale, Anaplasma phagocytophilum* and *Anaplasma bovis* (Dahmani *et al*., 2015). Bovine anaplasmosis causes significant economic losses in livestock industries worldwide due to decreased milk yield, weight loss, abortions, and eventually death of the animal with control measures varying by geographical location (Namratha & Ramesh, 2020; Waruri *et al*., 2021; Salinas-Estrella *et al*., 2022b).

Globally, tick species from the genera *Rhipicephalus, Dermacentor* and *Ixodes* have been identified as vectors for bovine anaplasmosis (Battilani *et al*., 2017). The most prevalent genus of ticks in Malaysia is *Haemaphysalis* spp. (20 species), followed by *Amblyomma* spp. (eight species) and *Dermacentor* spp. (seven species) (Koh *et al*., 2017; Kazim *et al*., 2022). The *Anaplasma* spp. are also reportedly transmitted through mechanical vectors including *Tabanus* spp. flies, *Chrysops* spp., *Stomoxys* spp., *Haematobia* spp., *Hippelates* spp. and *Psorophora* spp. In addition, the transplacental and iatrogenic transmission of pathogen through fomites and blood transfusion has also been reported (Dantas-Torres & Otranto, 2017).

Among *Anaplasma* spp. reported to cause bovine anaplasmosis, *A. marginale* and *A. centrale* are known to be the primary causative agents of bovine anaplasmosis (Sisson *et al*., 2017; Hove *et al*., 2018). *A. marginale*, a small, coccoid to ellipsoidal, non-motile haemoparasite that resides and replicates in membrane-bound vacuoles within the cytoplasm is considered highly virulent and the most prevalent in cattle (Kocan *et al*., 2010; Aktas & Özübek, 2017; Nitture *et al*., 2020; Falghoush *et al*., 2023). It had been reported to cause progressive anaemia in the infected host (Battilani *et al*.,

2017) that lead to progressive hemolytic anemia, abortions, high fever, dyspnea, icterus, loss of condition, milk production, and death to the infected animal (Waruri *et al*., 2021; Das *et al*., 2022). Morphologically, *A. marginale* appeared as a dense, rounded intraerythrocytic body situated on or near the margin of the erythrocyte, whereas *A. centrale* concentrated in the central part of the infected erythrocyte (OIE, 2023).

While *A. marginale* is considered as a highly pathogenic intra-erythrocytic haemoparasite in cattle, *A. centrale* causes a milder clinical sign in cattle and it is considered as a naturally attenuated *Anaplasma* subspecies (Rar & Golovljova, 2011). The *A. centrale* has been extensively used as a live vaccine against *A. marginale* in many countries to reduce severe symptoms of bovine anaplasmosis (Bell-sakyi *et al*., 2015; Falghoush *et al*., 2023). Furthermore, it had been reported that cattle infected with *A. centrale* may developed immunity against *A. marginale* (Belkahia *et al*., 2015; Brown & Barbet, 2016; Bellezze *et al*., 2023). Nonetheless, live vaccine for bovine anaplasmosis is not available in Malaysia. Currently, there is no published report on the prevalence of *A. centrale* among the local cattle population. Therefore, detection of *A. centrale* in bovine anaplasmosis cases is important due to its potential as a live vaccine for the cattle populations in this country.

Mafriwal cattle is the Malaysian Friesian-Sahiwal crossbreed that was selectively developed to supply the local needs of dairy products. The breeding program aimed to develop a dual-purpose breed that could thrive in Malaysian tropical climate while exhibiting high milk production and good beef qualities (Mastura *et al*., 2019). The adaptability of Mafriwal cattle to local conditions and resistance to local diseases makes them feasible for smallholder farms and large commercial operation. Nonetheless, a recent report revealed that 100% of the screened Mafriwal cattle (n=129) in a nucleus farm were microscopically positive with *Anaplasma* spp., indicating active infections of *Anaplasma* spp. in the population (Manap *et al.*, 2024). The finding was also consistent with Ola-Fadunsin *et al*. (2017), which suggests that bovine anaplasmosis is a prevalent disease among cattle populations in Malaysia.

The diagnosis of bovine anaplasmosis is commonly made through the presence of *A. marginale* on stained blood smears from clinically infected animals during the acute phase of infections (Aubry & Geale, 2011; Atif, 2015; Abba *et al*., 2016). However, the combination of molecular diagnostic assays such as PCR, DNA microarray system and serological assays like enzyme-linked immunosorbent assay (ELISA), may reveal the true prevalence of bovine anaplasmosis in the population as the assay considers asymptomatic animals as well as animals with low parasitic infections (Amorim *et al*., 2014; El-Ashker *et al*., 2015; Ahmed *et al*., 2022).

Based on a systematic review on the global prevalence of bovine anaplasmosis (Nur-Amalina *et al*., 2023) only four studies reported the prevalence of bovine anaplasmosis in Malaysia as of 2023 (Rahman *et al*., 2012; Tay *et al*., 2014; Koh *et al*., 2018; Bitrus *et al*., 2018). Furthermore, the lack of study on Mafriwal cattle population warrants a need to investigate the prevalence of *A. marginale* and *A. centrale* to further explain the epidemiology of the disease (Nur-Amalina *et al*., 2023). Thus, this study was conducted to identify monospecies infection and co-infection of *A. marginale* and *A. centrale* isolated from the commercial Mafriwal cattle in Johor, Malaysia as well as to investigate their phylogenetic relationships based on gene sequence analysis.

MATERIALS AND METHOD

Study site, duration and ethical approval

The study was conducted in a commercial government farm located in Johor between December 2021 and May 2022. The study was performed under the approval of the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (UMK/FPV/ACUE/RES/003/2021).

Sample collection and storage

Blood samples were collected from the coccygeal vein of 242 Mafriwal cattle belonging to different age groups, namely calves of less than 1-year-old (n = 60), yearlings (n = 61), lactating cows (n = 61) and dry cows (n = 60). Samples were collected in ethylenediaminetetraacetic acid (EDTA) coated blood collection tube and kept at -6°C until use.

DNA extraction, amplification, sequencing and BLAST analysis

The DNA extraction of *A. marginale* and *A. centrale* was conducted from blood samples using Geneaid Gsync™ DNA extraction kits (Geneaid Biotech Ltd. New Taipei City, Taiwan) based on manufacturer's protocol. The extracted DNA were stored at -20°C until used for DNA amplification. DNA amplification was performed using primer previously described by Nur-Sabrina *et al*. (2024) for *A. marginale* and Kawahara *et al*. (2006) for *A. centrale* with slight modification on thermocycler profile (Table 1).

Table 1. Primers used for *Anaplasma* spp. DNA amplification

Targeted gene	Oligonucleotide sequence	Thermocycler profile	Size (bp)
A. marginale (MSP4)	Reverse: CATCTCCCAT GAGTCACGAAGTGGC Forward: GCTGAACA GGAATCTTGCTCCAAG	ID: 95°C/5mins D: 95°C/1mins A: 65°C/2mins E: 72°C/1min No of cycles: 40 FE: 72°C/10mins	761 bp
A. centrale (16s rRNA)	Reverse: CATCTCCCA TGAGTCACGAAGTGGC Forward: GCTGAACAGG AATCTTGCTCCAAG	ID: 94°C/5mins D: 94°C/30s A: 58.5°C/30s E: 72°C/1min No of cycles: 39 FE: 72°C/5mins	426 bp

bp = base pair.

Polymerase chain reaction (PCR) for each DNA marker was performed in a total volume of 25.0 µl which consisted of 12.5 µl of GoTaq® Green MasterMix (Promega, USA), 5.5 µl of nuclease-free water (Promega, USA), 1.0 µl of forward primer, 1.0 µl of reverse primer, and 5.0 µl of extracted DNA template. The DNA amplifications were performed in a MyCycler™ thermocycler (Bio-Rad, USA). The PCR products were electrophoresed on 400W/100V on 1.5% agarose gel (Promega, USA) with Tris-acetic acid-EDTA (TAE) buffer (Vivantis Technologies Sdn Bhd, Malaysia) and stained with Midori Green Dye (Nippon Genetics, Europe) for 40 minutes. The DNA fragments were visualized under UV transilluminator (GelDoc™ EZ Imager, Bio-RAD, USA).

After the amplification, 13 positive PCR products of *A. marginale* and eight positive products from *A. centrale* were chosen as representatives. The unpurified positive amplicons, forward and reverse primers were sent for purification and Sanger sequencing (Apical Scientific Sdn. Bhd, Malaysia). The sequences obtained were compared to known haemoparasite gene fragment sequences available in the National Centre for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST).

Multiple sequence alignment and phylogenetic analysis

The sequences were submitted to GenBank and further aligned using the Clustal Omega multiple alignment tool available in MEGA 11. The phylogenetic trees were constructed using RaXML and branch support were given using 1000 bootstrap replicates (Stamatakis, 2015). The phylogenetic trees were visualized using FigTree. The

sequences for Anaplasmataceae including *A. marginale, A. centrale, A. phagocytophilum* and *A. bovis* from different countries and host species were used to construct the phylogenetic tree. *Eimeria bovis* from the family Eimeriidae was used as the outgroup for the analysis (Table 2).

Table 2. Accession numbers and species included in the phylogenetic analysis

Family	Genus	Species	Accession no.
Anaplasmataceae	*Anaplasma*	*Anaplasma marginale*	OP437580* – OP437584* OP965393* – OP965400* JN572928 ON458034.1 MG967665 KU497714 MT268094 MT173814 MK140740 MF771079 OM256483 KX989517 HM640938
		Anaplasma centrale	OR888548* – OR888555* KM401900 MN653235 ON333780 MH338229 MH341119 AB588978 KP062966 KU686784 MH503922
		Anaplasma phagocytophilum	OQ869778 FJ538291 OL884353 AY176588 MW238338 EU436154 AB196721 NR044762 GQ428333
		Anaplasma bovis	KF055356 MZ146328 MH244925 KJ183084 EU682764 OR623817 KU870666 MF197898 KX115423 KF055364
Eimeriidae	*Eimeria*	*Eimeria bovis*	OR026526

* = Accession number of collected sample.

RESULTS

Molecular identification of *A. marginale* and *A. centrale*

The amplification of *Anaplasma* spp. revealed a total of 57.95% (140/242) of the total Mafriwal population were infected with bovine anaplasmosis. Single infection of *A. marginale* was found in 21.90% (53/242) followed by *A. centrale* (23/242, 9.50%) whereas, mixed infection of *A. marginale* and *A. centrale* was observed in 26.45% (64/242) cattle (Figure 1).

BLAST and phylogenetic analyses

The BLAST analysis of MSP4 sequences of *A. marginale* (OP437580 – OP437584; OP965393 – OP965400) shows that all samples derived from the present study were 100% similar to *A. marginale* isolated from the blood sample of a goat in Brazil (MT268094.1). The 16s rRNA sequence of *A. centrale* (OR888548 – OR888555) obtained from this study were 100% similar to *A. marginale* isolated from the *Rhipicephalus (Boophilus) microplus* in Panama (OR724728.1) (Table 3).

The phylogenetic analysis of *Anaplasma* spp. namely MSP4 gene of *A. marginale* and 16s rRNA gene of *A. centrale, A. phagocytophilum* and *A. bovis* formed three main clusters, implying different *Anaplasma* species for each clade (Figure 2). *Anaplasma marginale* sequences obtained from the present study (OP437580 – OP437584; OP965393 – OP965400) formed a monophyletic clade (100%) and display a close relationship (98%) to *A. marginale* sequences obtained from the blood samples of cattle from Malaysia (MT173814.1; OM256483.1), Sudan (KU497714.1), Thailand (MK140740.1), India (KX989517.1) and Iran (MG967665.1). The phylogenetic analysis also revealed that the *A. marginale* sequences obtained from this study were closely related to *A. marginale* sequences obtained from several hosts of ruminant

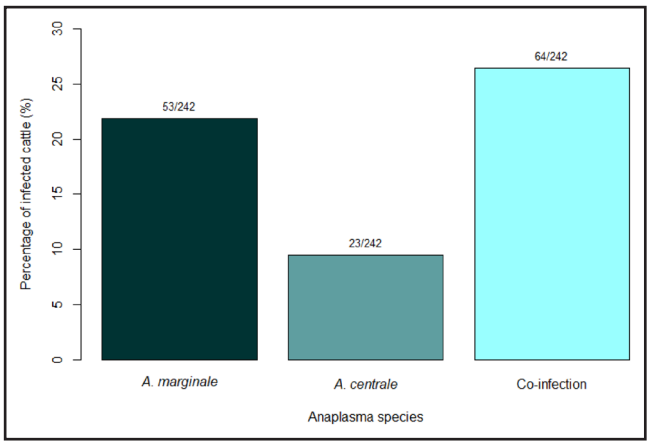

Figure 1. Percentage of Mafriwal cattle infected with single and co-infection of *Anaplasma marginale* and *Anaplasma centrale* in the sampled population.

Table 3. Basic Local Alignment Search Tool (BLAST) analysis of sequences in the present study

Targeted gene	Species	Accession number	Percent identity (%)	Host	Accession number
MSP4	*Anaplasma marginale*	OP437580 – OP437584 OP965393 – OP965400	100	Goat (Unknown breed)	MT268094.1
16s rRNA	*Anaplasma centrale*	OR888548 – OR888555	100	*Rhipicephalus* (*Boophilus*) *microplus*	OR724728.1

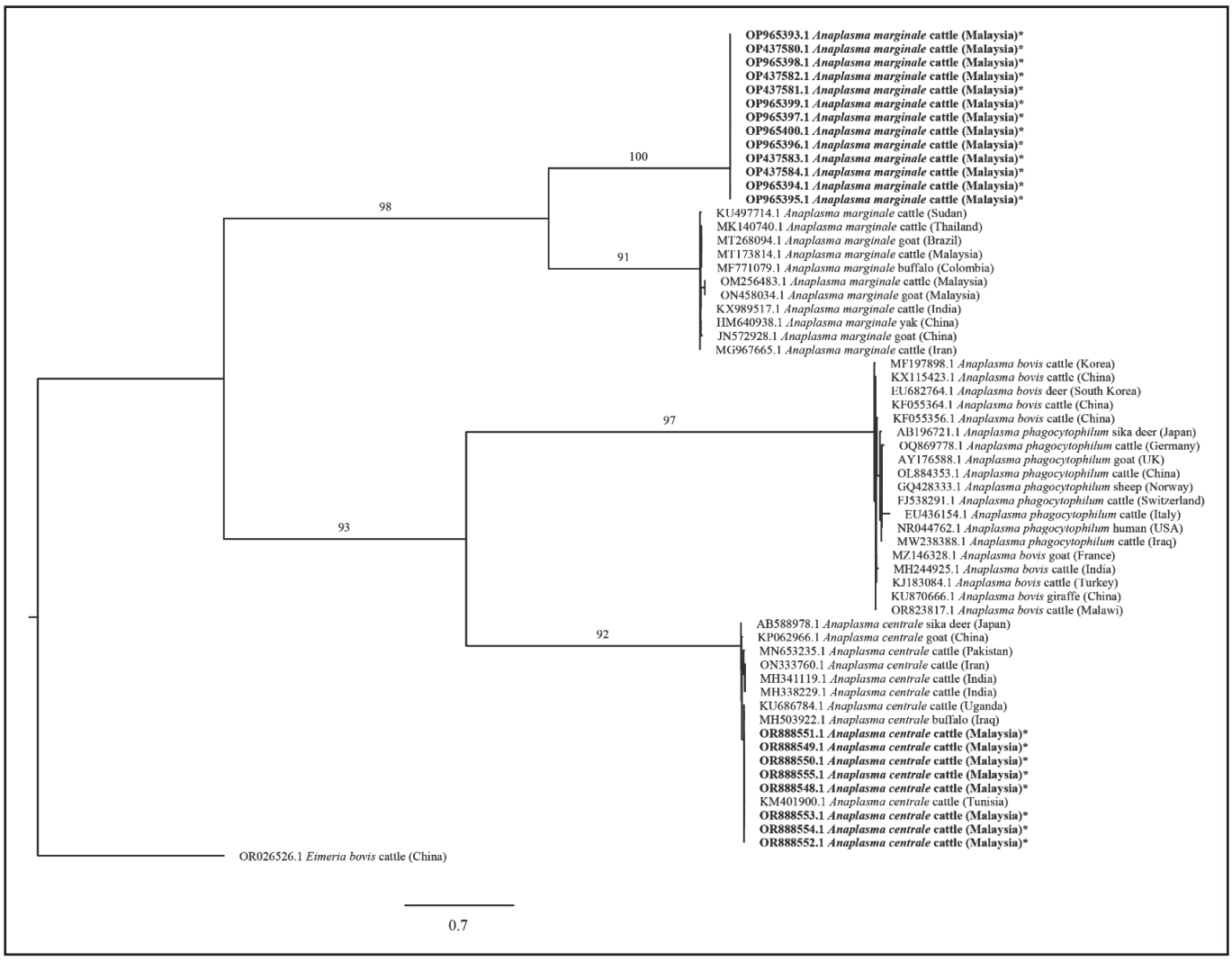

Figure 2. Phylogenetic tree of Anaplasma marginale MSP4 sequences and Anaplasma centrale 16s rRNA sequences, identified in the blood samples of Mafriwal cattle in the sampled population with other reference of Anaplasma spp. sequences isolated from different host species in different geographical areas. The numbers above branches represent bootstrap percentages of 1000 replicates. Scale bar represents the number of nucleotide substitutions per site. Sequence OR026526.1 Eimeria bovis from China was chosen as the outgroup. The sequences obtained from Mafriwal cattle in this study were bold and indicated with asterisk (*).

species including goats (MT268094.1; ON458034.1; JN572928.1), buffalo (MF771079.1), and yak (HM640938.1).

Based on the phylogenetic analysis, *A. phagocytophilum*, *A. bovis* and *A. centrale* were related (93%), although *A. centrale* sequences obtained from this study (OR888549.1 – OR888555.1) formed a monophyletic clade (92%) with the sequence references of *A. centrale* derived from the blood samples of cattle from Pakistan (MN653235.1), Iran (ON333760.1), India (MH341119.1; MH338229.1), Uganda (KU686784.1) and Tunisia (KM401900.1). Additionally, the *A. centrale* sequences isolated from this study were 92% related to the sequences obtained from the blood samples of sika deer (AB588978.1), goat (KP062966.1) and buffalo (MH503922.1) (Figure 2).

DISCUSSION

Bovine anaplasmosis which causes by *Anaplasma* spp. is a widespread and economically significant disease affecting cattle globally (Nur-Amalina *et al*., 2023). The tick-borne disease is caused by several *Anaplasma* spp. including *A. marginale, A. centrale, A. bovis* and *A. phagocytophilum* (Dahmani *et al*., 2015). However, the main causative agent of anaplasmosis among cattle population is *A. marginale* and *A. centrale* (Sisson *et al*., 2017; Hove *et al*., 2018). Some literatures suggested that *A. marginale* is the most prevalent among the pathogenic *Anaplasma* spp. globally (Aktas & Özübek, 2017; Nitture *et al*., 2020; Ferreira *et al*., 2022; Falghoush *et al*., 2023; Nur-Amalina *et al*., 2023). Recent studies revealed that *A. marginale* is the most predominant haemoparasite species infecting Mafriwal cattle population with prevalence rate of up to 100% (Nur-Sabrina *et al*., 2024; Manap *et al*., 2024). Clinical signs observed in cattle infected with *Anaplasma* spp. includes fever, anemia, jaundice, weakness, and respiratory distress (Hairgrove *et al*., 2015; Bal *et al*., 2017; Jurković *et al*., 2020). Furthermore, it causes a rapid decline of milk production in dairy cattle and abortion in pregnant animal, thus lead to a significant economic loss to the dairy cattle industry worldwide (Howden *et al*., 2010; Szabára *et al*., 2016; Henker *et al*., 2020; Waruri *et al*., 2021; Das *et al*., 2022). *Anaplasma centrale* is known to cause mild clinical sign in the infected cattle, but it rarely causes clinical outbreak in the field (Rar & Golovljova, 2011; OIE, 2023). Therefore, *A. centrale* was widely used in Australia, South America, South Africa, and Israel as a live vaccine against *A. marginale* for cattle (Aubry & Geale, 2011; Dantas-Torres & Otranto, 2017).

Interestingly, although previous studies have reported various clinical signs in infected cattle, the sampled population in the present study did not exhibit any of these clinical manifestations.

This could be due to several factors, including genetic resistance, host immunological differences, and the overall health of the cattle. Asymptomatic carriers are frequently suggestive of a well-adapted host-pathogen interaction, in which the pathogen persists without producing significant disease. This dynamic has important implications for disease control since it may result in underreporting of illness prevalence and delayed responses to outbreaks. Asymptomatic cattle with bovine anaplasmosis have significant implications for disease control, preventative measures and the overall health and productivity of the population. They can act as reservoir for *Anaplasma* spp., facilitating the undetected disease transmission within and between herds. Therefore, the transmission of bovine anaplasmosis prolonged as the pathogen might keep spreading across the vector population. The presence of asymptomatic cattle in the population makes it difficult to detect the infection through routine clinical observations, leading to an underestimation of disease prevalence. Economically, previous studies have shown that parasitic infections significantly impact milk yield and body weight in dairy cattle (Nur-Sabrina *et al*., 2024). Additionally, anaplasmosis has been found to reduce rumination and activity by 34% and 11%, respectively, in dairy heifer (Teixeira *et al*., 2022). Moreover, haemoparasitism are most prevalent in calves of 1 to 2-year-old (Manap *et al*., 2024), leading to significant financial losses for farms due to reduced growth rates, increased veterinary costs, and higher mortality rates among younger cattle group. These findings highlight the indirect impact of asymptomatic carriers in exacerbating financial losses due to undetected disease progression.

The present study revealed a substantial molecular prevalence of both *A. marginale* and *A. centrale* in the commercial Mafriwal cattle population in Johor, Malaysia. Interestingly, this is the first discovery of *A. centrale* in ruminants in Malaysia. The prevalence rates of *A. marginale* (21.90%), *A. centrale* (9.50%) and co-infection of both species (26.45%) indicate a significant burden of anaplasmosis in the studied cattle population. According to Belkahia *et al*. (2015), co-infection of *A. marginale* and *A. centrale* is common among cattle. The prevalence rates of *Anaplasma* spp. in Mafriwal cattle aligned with Agina *et al*. (2021) in which 26.23% of Kedah-Kelantan × Brahman cattle population in Pahang, Malaysia were detected with this haemoparasite. On the other hand, the prevalence rates of *A. marginale* were higher in studies involving cattle farms of various breeds in all states of Peninsular Malaysia indicating high endemicity with variations across different regions of the country (Ola-Fadunsin *et al*., 2018; Koh *et al*., 2018). The prevalence rates of *Anaplasma* spp. reported in this study were also consistent with findings in the other countries such as Turkey, Ecuador, Korea, and the Philippines; hence highlighting the global impact of anaplasmosis on cattle populations (Aktas *et al*., 2011; Ybañez *et al*., 2013; Tana-Hernández *et al*., 2017; Seo *et al*., 2018). Nur-Sabrina *et al*. (2024) reported the molecular prevalence of *A. marginale* among Mafriwal cattle in its largest population in Malaysia increased from 39% to 62% in 2021 and 2022, respectively. The same study also reported significant effects of parasitism on the body weight and milk yield of the cattle. Hence, presence of *A. marginale* highlighted the importance of early disease management in the Mafriwal cattle population. The increasing prevalence of *A. marginale* and the documented economic loss on growth performance and milk yield emphasize the importance of immediate intervention to mitigate the financial impact of anaplasmosis on dairy and beef industries. The current findings on the co-infection in this study highlight the complexity of managing anaplasmosis since concurrent infections might worsen the clinical and subclinical impacts on the productivity of cattle. Therefore, addressing this challenge is crucial to the health and productivity of the cattle population, thereby ensuring the sustainability of dairy and beef cattle production in Malaysia.

In this study, BLAST analysis revealed all sequence representatives of *A. marginale* were 100% similar to the MSP4 gene sequence of *Anaplasma* spp. obtained from the blood sample of a goat in Northeastern Brazil. This finding emphasizes the genetic homogeneity, global distribution and the widespread prevalence of *A. marginale* among variety of host species. Moreover, the similarities of *A. marginale* in the current study to the *A. marginale* obtained from the Brazilian goat highlight the potential role of domesticated livestock to the transmission of bovine anaplasmosis within agricultural settings. Analysis of eight sequence representatives of *A. centrale* showed 100% similarities to 16s rRNA uncultured *Anaplasma* sp. obtained from the tick-vector, *Rhipicephalus* (*Boophilus*) *microplus* in Panama (OR724728.1). Previous study suggested that *R.* (*Boophilus*) *microplus* is one of the most prevalent tick species in Peninsular Malaysia, beside *Haemaphysalis bispinosa* (Tay *et al*., 2014; Ola-Fadunsin *et al*., 2021). Therefore, the analysis proved that *R.* (*Boophilus*) *microplus* is an important biological vector for the transmission of *Anaplasma* spp. in Malaysian cattle population. While cattle are known as the primary hosts, the other host species including deer, goats and yaks serve as the potential reservoirs for *Anaplasma* spp. (Silveira *et al*., 2012; Koh *et al*., 2018). The zoonotic potential of anaplasmosis, as indicated by infections in humans underlines the need for effective control strategies (Chochlakis *et al*., 2010). The co-existence of *Anaplasma* spp. in various host species and the detection of *A. marginale* in wildlife populations also emphasize the intricate ecological dynamics of these species (Henrichs, 2014). The identification of similar strain in geographically distant regions suggests the role of vectors and animal movement in spreading the pathogen across borders. Thus, stricter biosecurity and vector control measures must be implemented at a regional and global level to mitigate the risk of cross-border transmission.

The global distribution of *A. marginale* and *A. centrale* shown in the phylogenetic tree (Figure 2) emphasizes the importance of understanding the geographical spread of these haemoparasites. The phylogenetic analysis demonstrated the formation of distinct clades, highlighting the genetic diversity of *Anaplasma* spp. In this study, the isolated *A. marginale* gene sequences formed a monophyletic clade closely related to the strains from various ruminant species in different geographical locations. Furthermore, the close relationships observed between sequences from Malaysia and other countries imply potential cross-border transmission routes thus highlighting the importance of international collaboration in surveillance and control efforts to mitigate the spread of bovine anaplasmosis. Such findings emphasized the need for comprehensive monitoring programs and coordinated responses to prevent the introduction and establishment of *Anaplasma* spp. in the other regions. Moreover, this study supports existing evidence that *A. marginale* and *A. centrale* are distinct species with morphological differences, genetic variation, and specific gene structures that affirm their separation (Hove *et al*., 2018; Khumalo *et al*., 2018). Understanding the genetic diversity and distinct species status of *A. marginale* and *A. centrale* is crucial in designing targeted control strategies and developing effective vaccines as *Anaplasma* spp. has high genetic variability, which can lead to new species development with significant veterinary and economic relevance (Battilani *et al*., 2017; Rar *et al*., 2021). The use of live blood vaccine is not entirely safe, although the strain of *A. centrale* used in vaccine is of reduced virulence (OIE, 2023). Therefore, there is a need for a commercial worldwide effective vaccine against bovine anaplasmosis, as current vaccine candidates have not been successful indicating the need for further evaluation and development of effective vaccines (Sarli *et al*., 2020; Salinas-Estrella *et al*., 2022a).

CONCLUSION

This study provides valuable insights into the prevalence, genetic diversity and species differentiation through the phylogenetic analysis between *A. marginale* and *A. centrale* isolated from the largest population of Mafriwal cattle in Malaysia. The most intriguing finding was the first detection of *A. centrale* in the Malaysian breed cattle population and its phylogenetic relationship with the related references around the world. Thus, the potential of *A. centrale* strain obtained from this study as a live vaccine to control bovine anaplasmosis among Malaysian cattle populations should be explored. The economic impact of bovine anaplasmosis accentuates the need for effective diagnosis, monitoring, and treatment. Hence, understanding the dynamics of *Anaplasma* spp. infections is crucial for the implementation of targeted control measures and minimizing the economic impact on livestock. Furthermore, studying the vector of bovine anaplasmosis could be beneficial for gaining deeper insights into its epidemiology and developing a more comprehensive control approach.

ACKNOWLEDGEMENT

We would like to acknowledge the Ministry of Higher Education Malaysia for the financial support in this study through the Fundamental Research Grant Scheme (FRGS/1/2020/WAB04/UMK/02/5).

Conflict of Interest

The author declares that they have no conflict of interest.

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