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RESEARCH ARTICLE

First report of *Anaplasma marginale* and *Anaplasma ovis* in goats in Kelantan, Malaysia

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ABSTRACT

Anaplasma species are obligate rickettsial intraerythrocytic pathogens that cause an important tick-borne disease of economic importance in livestock production in many countries. *Anaplasma* species have been detected from farm animals worldwide, there is a paucity of information on *Anaplasma* infections in goats from Malaysia. Thus, this study aimed to assess the infection rate and identify *Anaplasma* species and some selected risk factors in goats across selected districts in Kelantan, Malaysia. A total of 411 blood samples were collected from goats and analysed for *Anaplasma* species targeting the *msp4* gene using conventional PCR and sequencing. The infection risk was determined by breed, age, management system and location. Our results indicate an overall infection rate of 30.9% for *Anaplasma* species detected. Interestingly, sequencing of selected amplicons revealed the presence of *Anaplasma ovis* and *A. marginale*. Data analysis revealed a marked statistically significant association between *Anaplasma* infection and some variables such as location (district), farm management system, breed, and age ($P < 0.05$). Specifically, goats raised on intensive management had the highest prevalence of 46.25% (37/80) compared to other management types. Also, with regards to district, goats raised in the coastal region had a higher prevalence of 39.23% (71/181) compared to those raised in inland region 24.35% (56/230). Regarding breed, goats that were of the pure breed had a higher prevalence of *Anaplasma* species infection 38.19% (97/254) compared to crossbreeds with a prevalence of 19.11% (30/157). Lastly, goats <1 year of age had the highest prevalence 42.71% (41/96) followed by those within 1-2 years 38.24% (52/136) while goats > 3 years had the least prevalence 18.99% (34/179). To the best of our knowledge, this is the first report of *A. marginale* and *A. ovis* in goats from north-eastern Peninsular Malaysia. The infected goats were clinically healthy; this revealed the role of goats as a potential reservoir for *A. marginale* and the presence of *A. ovis* in goats in Malaysia. Continuous efforts towards tick control must be sustained to ensure high productive yield and reduced disease burden associated with TBPs of goats in the study area.

Keywords: Small ruminants; tick-borne; haemoparasites; molecular; epidemiology.

INTRODUCTION

Anaplasma, the causative agent of Anaplasmosis is an obligate intracellular, intra-erythrocytic Gram-negative bacterium that infects blood cells of a wide range of domestic and wild mammals including humans (Rymaszewska & Grenda, 2008; Igwenagu *et al*., 2018; Almahallawi *et al*., 2022). Of the nine species within the genus *Anaplasma* that have been documented, *A. ovis, A. capra*, and *A. phagoytophilum* primarily infects sheep and goats. Additionally, *A. marginale* primarily infects cattle. However, it has been found to infect sheep and goats (Yousefi *et al*., 2017; Barbosa *et al*., 2021).

Caprine anaplasmosis caused by *A. ovis* is transmitted by ticks and causes various clinical signs including anaemia, fever, lethargy, jaundice, and abortion (Tibbitts *et al*., 1992; Igwenagu *et al*., 2018; Onyiche & MacLeod, 2023). Infection with *A. ovis* is frequently subclinical and might not induce any changes in health indices (Cabezas-Cruz *et al*., 2019). However, in stressed or debilitated animals, it causes acute illness which is more critical among goats

than sheep (Friedhoff, 1997). On the other hand, *A. marginale*, the causative agent for bovine anaplasmosis, causes a variety of clinical signs, including fever, weight loss, abortion, lethargy, icterus and often death of the animals older than 2 years (Kocan *et al*., 2010).

Ticks are biological vectors and can transmit *Anaplasma* species and the efficiency of the transmission depends on the strains and the availability of the vector species (Scoles *et al*., 2005a, 2005b). At least 45 and 27 ixodid tick species belonging to the major genera of Ixodidae have been attributed to the transmission of *A. marginale* and *A. ovis* respectively (CABI, 2019; Onyiche & MacLeod, 2023). *Rhipicephalus microplus* is one of the vectors for *A. marginale* and *A. ovis*, and it is endemic in Malaysia and Southeast Asia. Although this tick parasitizes cattle primarily, it is also found parasitizing other species (Tan *et al*., 2021).

Microscopic inspection of Giemsa-stained blood smears has traditionally been used for the detection of haemoparasites including *Anaplasma* species. This diagnostic approach is cheap and fast but is also least sensitive and requires the operation of an experienced examiner (Ndung'u *et al*., 1995; Silaghi *et al*., 2017). In recent times, the application of alternative method such as polymerase chain reaction (PCR) have been successful in the detection of tick-borne haemoparasites including infection with *A. ovis* and *A. marginale*, as this diagnostic approach is highly sensitive and specific for the characterization of *Anaplasma* species.

Anaplasma's major surface proteins (MSPs) play a vital part in interactions with both invertebrate and vertebrate hosts (Brayton *et al*., 2006). The MSP2 protein super-family comprises the *msp4* gene, whose function is currently unknown (de la Fuente *et al*., 2002). The *msp4* gene is useful in the genetic characterization and phylogenetic investigation involving several species of *Anaplasma* including *A. marginale, A. phagocytophilum* and *A. ovis* (de la Fuente *et al*., 2005; Belkahia *et al*., 2015; Selmi *et al*., 2021; Onyiche *et al*., 2024).

Globally, the population of domestic goats (*Capra hircus*) is currently estimated at one billion heads (Dhanda *et al*., 2003; Amills *et al*., 2017). Small ruminants account for 5 to 10% of Malaysian National Gross Domestic Product (GDP). This industry is essential for its contribution to food production, national meat and milk self-sufficiency, and supporting rural income (Mohamed, 2007). Kelantan, located in north-eastern Peninsular Malaysia bordering Thailand, is a chiefly agrarian state with abundant small-holder ruminant farms. This state has the lowest GDP per capita in Malaysia, significantly lower than any other state (Malaysia Department of Statistics, 2017). Family subsistence practice such as non-intensive management and multispecies grazing is a common practice that might facilitate interspecies pathogens transmission (da Silva *et al*., 2018).

In Malaysia, infection with *A. marginale* has been investigated in Cattle (Koh *et al*., 2018; Ola-Fadunsin *et al*., 2018). Previous attempts to characterize *Anaplasma* species in goats showed the absence of infection (Tay *et al*., 2014). In this current study, the objective was to genetically characterize *Anaplasma* species in goats in Kelantan, north-eastern Peninsular Malaysia by providing data on the prevalence of infection with *Anaplasma* species in goats and the analysis of some risk factors for the *Anaplasma* infections in order to fill up gaps in our understanding on the epidemiology of *Anaplasma* species infecting small ruminants precisely goats and the knowledge derived thereof will be useful in the development of management methods.

MATERIALS AND METHODS

Ethical statement

All procedures were reviewed and approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Malaysia (UMK/FPV/ACUE/RS/2/2019). Oral consent was sort from the animal owners and approval was granted before sample collection was undertaken.

Study region

The study was conducted from June 2019 to December 2019 covering all ten districts (coastal region: Bachok, Kota Bharu, Machang, Pasir Mas, Pasir Putih, Tumpat and inland region: Gua Musang, Jeli, Kuala Krai, Tanah Merah) in Kelantan (Figure 1).

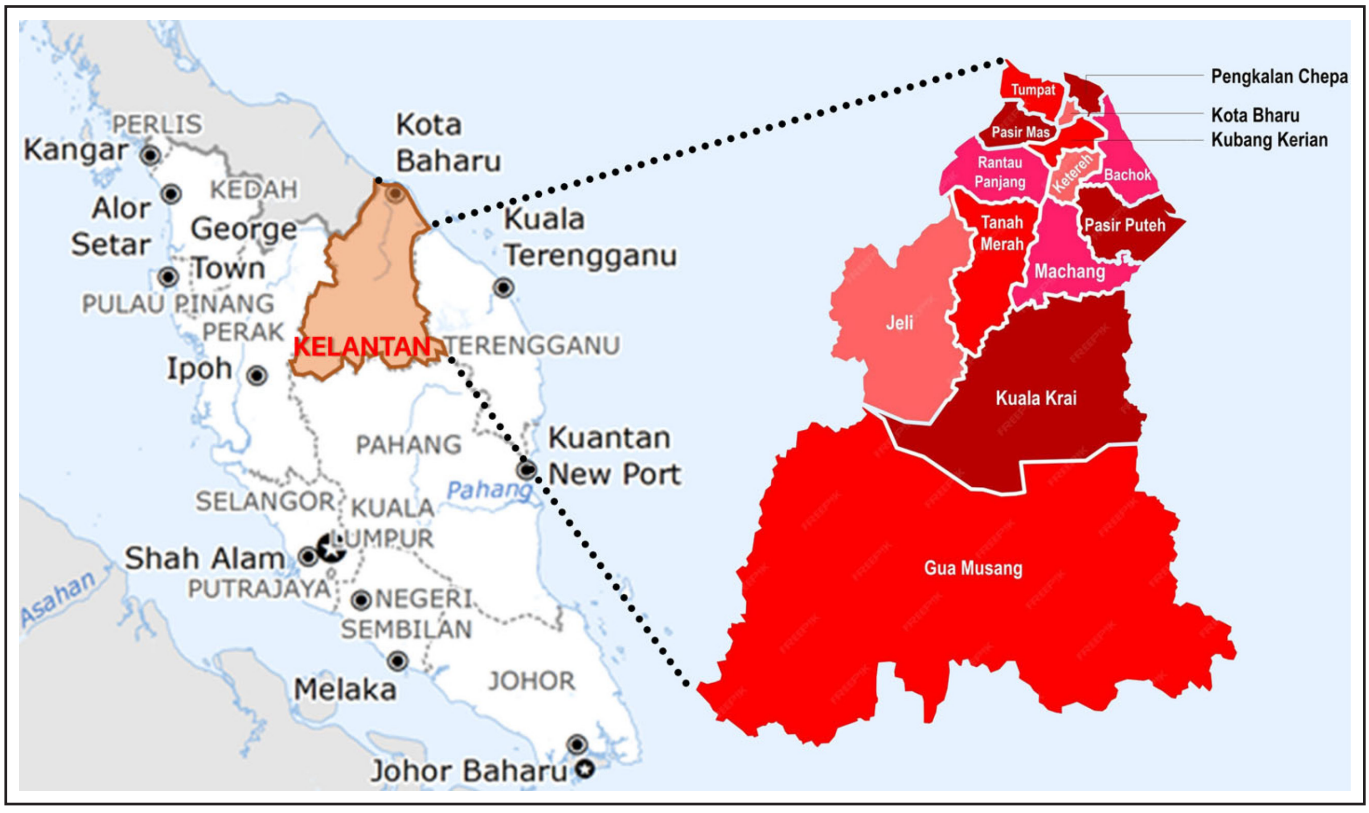

Figure 1. A geographical map of Peninsular Malaysia showing Kelantan and the ten districts in Kelantan. Source: (Wikimedia Commons, n.d.; Freepik, n.d.).

Sampling and data collection

A total of 411 blood samples from goats (327 females and 84 males) were collected in a study for haemoparasitism screening across several farms. These farms were selected based on convenience and agreement from farm owners. Animals from the farms were randomly selected for blood sampling. Physical examinations were first conducted to observe any abnormalities and the body condition score (BCS) and FAMACHA© scores were estimated. As described by Jefferies (1961) and Aumont *et al*. (2004), BCS was measured on a scale of 1 – 5, which was assessed by estimating the amount of muscling and fat cover on the lumber spinous processes and floating ribs. Animals with body scores of 1 to 5 were categorized as emaciated, thin, average, fat and obese, respectively. Also, FAMACHA© score card, the conjunctival colors of the animals were scored on a scale of 1–5 by the same person. The ocular mucous membrane color of each animal was: 1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, slightly anemic; 4 = pink-white, anemic; and 5 = white, severely anemic (Kaplan *et al*., 2004).

The blood samples were taken from each animal through the jugular vein using a VENOJECT™ blood collection needle and tube holder, two ml of each sample was collected in a sterilised ethylene diamine-tetra-acetic acid (EDTA) tube. All collected samples were transported to the laboratory under chill conditions using a cooler box and stored under -80°C immediately after reaching the laboratory, pending further analysis. Data such as breeds (pure breeds includes Anglo-Nubian, Alpine, Boer, Beatle, Jamnapari, Kalahari Red, Saanen, Shami and Toggenburg while cross breeds includes (Anglo-Nubian X, Alpine X, Boer X, Beatle X, Jamnapari X, Katjang X, Kalahari Red X, Saanen X, and Shami X); management system (Intensive, semi-intensive and extensive) and age groups (<1, 1-2, and >3 years) were also recorded during sampling activities. Aging of the animals was carried out using their dentition.

DNA extraction and amplification

DNA was extracted from frozen (-80°C) whole blood using the Macherel-Nagel™ NucleoSpin Genomic DNA Purification kit according to the manufacturer's protocol. PCR amplification was carried out using species-specific primer sets for *A. marginale* F: 5'- CAT CTC CCA TGA GTC ACG AAG TGG C-3' and R: 5'- GCT GAA CAG GAA TCT TGC TCC AAG-3' based on *msp4* gene with an expected amplicon size of 761 base pairs (Shkap *et al*., 2008). PCR was carried out using the 2X GoTaq PCR kit (Promega, USA) and proceeded with 40 PCR cycles of 95°C for 1 min, 65°C 2 mins and 72°C for 1 min and one cycle of 72°C for 10 mins at a MyCyclerTM (Bio-Rad Laboratories, USA) thermal cycler. Nuclease-free water and previously sequenced *A. marginale* PCR products were included as negative and positive controls, respectively, in each PCR run to ensure assay integrity. Amplification was done using the T10 thermocycler (Biorad, USA). Amplicons were electrophoresed on a 2% agarose gel (Life Technologies, USA) at 100 V with Tris-borate acid-EDTA (TBE) buffer, stained with Midori Green Direct (Nippon Genetic, Germany) and viewed under a UV transilluminator (GeneDocTM, Bio-Rad Laboratories). Six amplicons were selected and purified using a Gel/PCR DNA Fragment Extraction Kit (Geneaid, Taiwan). The purified PCR products were sent for sequencing. The obtained sequences were compared with the reference sequences from the National Center for Biotechnology Information (NCBI) GenBank using the Basic Local Alignment Search Tool (BLAST).

Phylogenetic Analysis

The phylogenetic tree was constructed using the aligned *msp4* gene sequences obtained from the preceding analysis via the MAFFT algorithm. Two of the six amplified samples, each representing a different *Anaplasma* species (*A. ovis* and *A. marginale*), were submitted to GenBank following verification with BLAST: *A. ovis* (isolate AO2, accession no.: ON458035) and *A. marginale* (isolate AM1, accession no.: ON458034). Additional *A. ovis* (AO) sequences included accession numbers: MW535731, OP169077, MN198191, MH790274, KU525118, and KU525119. For *A. marginale* (AM), the accession numbers included: MG676458, MG676457, MG676453, MH939155, MF771059, and MF771060. *A. phagocytophilum* (AP) sequences were represented by accession numbers: KM205444, EU008082, KF745727, and KF745728. The outgroup used in this analysis was *Ehrlichia ruminantium* (ER) with accession number CP001102. All the reference sequences were obtained from GenBank and were selected from various localities to ensure diversity in the phylogenetic analysis. This alignment was automatically curated using trimAl with automatic configuration (Capella-Gutiɪrrez *et al*., 2009). For model selection in maximum-likelihood (ML) and Bayesian inference (BI) analyses, W-IQ-TREE was employed (Trifinopoulos *et al*., 2016). The model selection was conducted using the command (–st DNA –m TESTONLY). The selected model for both ML and BI analyses was the General Time Reversible model with empirical base frequencies and four gamma rate categories (GTR+G4+F), obtained from prior model selection in IQ-TREE. The ML analysis was performed using the command (–st DNA –m GTR+G4+F –bb 1000 –alrt 1000 –abayes).Phylogenetic tree visualization was performed using FigTree v1.4.4 (Rambaut, 2014) to represent the results.

Data analyses

The Chi-square test was used to assess the association of the individual factors such as breed, sex, age, FAMACHA© scores, location (district) and farm management system of goats with *Anaplasma* infection. *P*-values were calculated and considered significantly different when $p < 0.05$. Data were compiled and analysed using SPSS V21.0 statistical software.

RESULTS

Clinical examination and findings

Physical examinations on the sampled animals revealed no obvious clinical signs from all the goats examined and no tick infestations found in all the sampled goats. BCS and FAMACHA© scores of the goats mainly fell between 2-3 (98.5%) and 1-2 (92.9%), respectively.

Molecular detection of *Anaplasma* species from blood

PCR amplification of the *msp4* gene from Anaplasma yielded bands of approximately 700 kb after gel electrophoresis (Figure 2). Phylogenetic analysis validated the classification of the study samples, aligning them with their corresponding reference sequences (Figure 3). The *A. marginale* sample (isolate AM1) grouped into the *A. marginale* clade, exhibiting strong maximum-likelihood (ML) and Bayesian inference (BI) support values (ML bootstrap = 98%, BI posterior probability = 1.00), indicating substantial genetic similarity and corroborating species identification. The *A. ovis* sample (isolate AO2), grouped with other A. ovis sequences, exhibiting strong ML and BI support values (ML bootstrap = 97%, BI posterior probability = 1.00), so affirming their classification. By validating the PCR and sequencing results, the tree construction provided further confirmation of the species identification.

Prevalence of *Anaplasma* species and associated risk factors

Out of 411 goats screened for *Anaplasma* species, a total of 127 were positive with an overall prevalence of 30.90%. Statistically significant association was observed between *Anaplasma* species infection and some variables such as location (district), farm management system, breed, and age ($P < 0.05$). Specifically, goats raised on intensive management had the highest prevalence of 46.25% (37/80) compared to other management types (Table 1). Also, with regards to district, goats raised in coastal region had a higher prevalence of 39.23% (71/181) compared to those raised of the inland region 24.35% (56/230) (Table 1). Specific details regarding the prevalence and distribution of *Anaplasma* infection in goats across the various

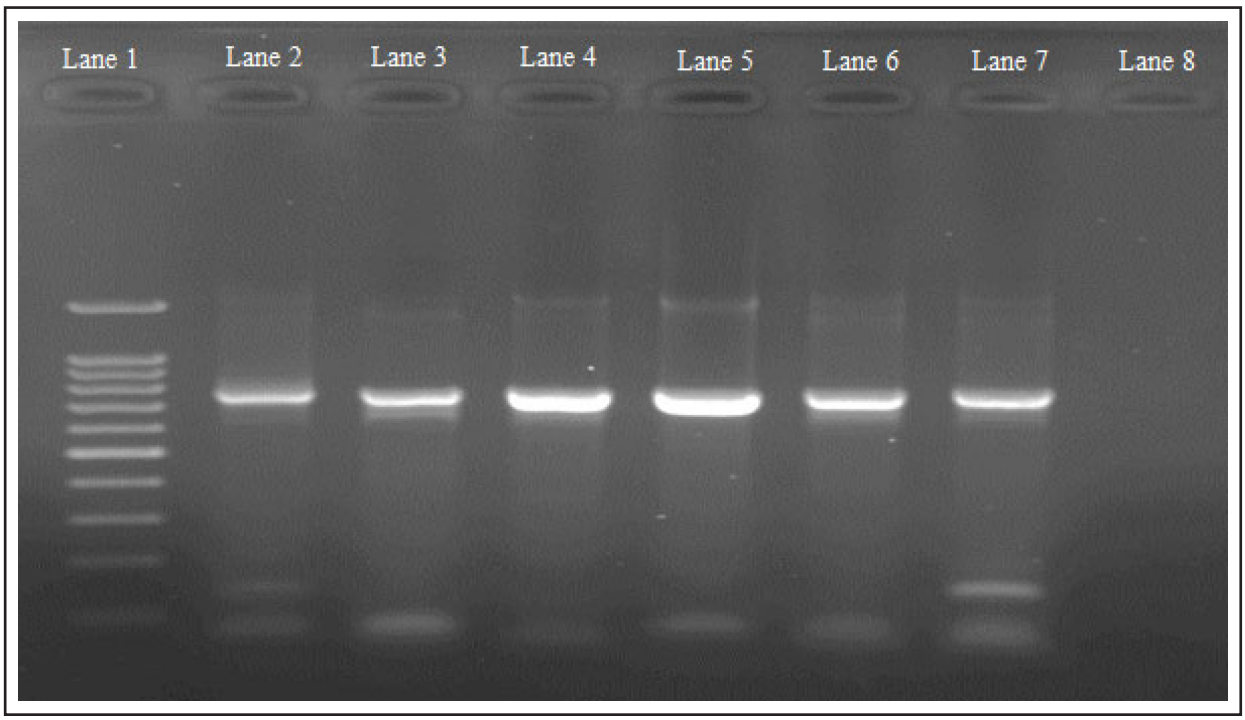

Figure 2. Gel electrophoresis of PCR products targeting the *msp4* gene of *Anaplasma* species. Lane 1: 100bp DNA ladder indicating band size markers. Lane 2: Positive control (*A. marginale*). Lane 3-7: PCR product of *Anaplasma* spp. showing an approximate band size of 761 bp. Lane 8: Negative control (Nuclease-free water).

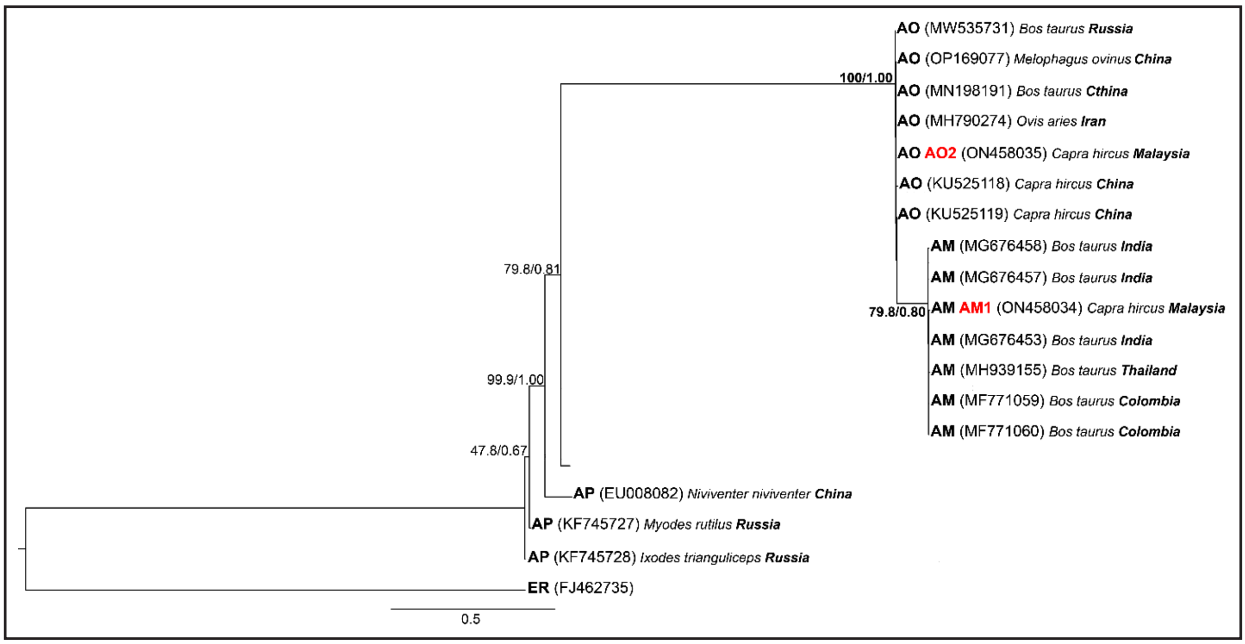

Figure 3. Phylogenetic tree constructed using the *msp4* gene sequences of *Anaplasma* species. The tree includes sequences from this study (*Anaplasma marginale* isolate AM1, accession no.: ON458034; *Anaplasma ovis* isolate AO2, accession no.: ON458035) and reference sequences obtained from GenBank. The tree was generated using maximum-likelihood (ML) and Bayesian inference (BI) methods, with ML bootstrap values and BI posterior probabilities indicated at each node. The *Anaplasma marginale* and *Anaplasma ovis* clades are supported by strong ML/BI values, confirming the classification of the study isolates. *Ehrlichia ruminantium* (accession no.: CP001102) was used as the outgroup.

districts indicate that the infection was present in Bachok (75.6%; 34/45), Kota Bahru (21.1; 8/38), Pasir Mas (26.7%; 12/45), Pasir Putih (43.3%; 13/30) and Tumpat (17.4%; 4/23), all in the coastal region while for the inland region, the districts includes Jeli (7.5%; 3/40), Tanah Merah (75.4%; 43/57) and Kuala Krai (22.2%; 10/45). In Machang (0/40) and Gua Musang (0/48) districts, *Anaplasma* infection was not detected. Concerning breed, goats that were of pure breed had a higher prevalence of *Anaplasma* species infection 38.19% (97/254) compared to cross breeds with a prevalence of 19.11% (30/157). Specifically, pure breeds with *Anaplasma* species prevalence > 50% were Katjang 65.1% (28/43), Jamnapari 55.6% (10/18) and Anglo-Nubian 54.2% (13/24). Other pure breeds include Boer 35.9% (19/53), Kalahari Red 28.6% (10/35) and Saanen 25.4% (17/67). Regarding cross breeds, the specific prevalence of *Anaplasma* species infection include Anglo-Nubian X 22.7% (5/22), Boer X 20.0% (7/35), Beatle X 22.2% (2/9), Jamnapari X 15.4% (2/13), Katjang X 22.7% (10/44), Kalahari Red X 16.7% (2/12) and Saanen X 16.7% (2/12). Lastly, goats <1 year of age had the highest prevalence 42.71% (41/96) followed by those within 1-2 years 38.24% (52/136) while goats > 3 years had the least prevalence 18.99% (34/179) (Table 1).

Table 1. Prevalence and risk factors associated with infection of goats with *Anaplasma* infection in Kelantan, Malaysia

Variables	No examined	No positive (%)	χ^2	P-value
Management system				
Intensive	80	37 (46.25)	16.40	0.0003
Semi-intensive	316	90 (28.48)		
extensive	15	0 (0.0)		
Total	411	127 (30.90)		
District				
Coastal region	181	71 (39.23)	10.50	0.0012
Inland region	230	56 (24.35)		
Total	411	127 (30.90)		
Breed				
Pure breed	254	97 (38.19)	16.54	P<0.0001
Cross breed	157	30 (19.11)		
Total	411	127 (30.90)		
Age (years)				
<1	96	41 (42.71)	21.58	P<0.0001
1-2	136	52 (38.24)		
>3	179	34 (18.99)		
Total	411	127 (30.90)		

DISCUSSIONS

All the goats that were positive with *Anaplasma* species infection appeared to be healthy and no prominent clinical signs associated with anaplasmosis were observed. This finding is in agreement with the studies from Corsica, France and Botswana (Cabezas-Cruz *et al*., 2019; Berthelsson *et al*., 2020). The possible reason for the low or no association link between *A. ovis* infection and the health performance of the goat may be due to natural resistance acquired from *A. ovis* which is widely distributed in Eurasia including Malaysia (Cabezas-Cruz *et al*., 2019; Berthelsson *et al*., 2020). Besides, *A. marginale* also does not seem to inflict any severe clinical signs in goats according to a previous report in Brazil (da Silva *et al*., 2018). The negligible health impact caused by *A. marginale* might also be due to the endemic status of this *Anaplasma* species in Malaysia infesting mainly cattle (Ola-Fadunsin *et al*., 2018). Also, the FAMACHA® score of 1-2 among 92.9% of goats sampled implies that they were not anaemic. Moreover, the majority of the animals have body score of 2 – 3 out of 5, which are relatively healthy without high percentage of fat in the body. Therefore, it is presumed that even the infected goats did not exhibit clinical signs and were relatively healthy.

To the best of our knowledge, this study is the first report of the molecular prevalence of Anaplasmataceae organism (including *A. marginale* and *A. ovis*) infections in goats in Malaysia. We registered an overall prevalence of 30.9%. The prevalence rate of *Anaplasma* spp. in goats varies across different countries with 13.5% in Thailand (Aung *et al*., 2022), 47.4% in Sudan (Lee *et al*., 2018), 71.3% in Mongolia (Enkhtaivan *et al*., 2019), 8.2% in northeastern China (Wang *et al*., 2019), 75.0% in Malawi (Chatanga *et al*., 2021) and 13.6% in Nigeria (Onyiche *et al*., 2022). A molecular prevalence of 45.6% of *Anaplasma* species infection was reported in a meta-analysis from ruminants including sheep, goats and cattle in Southern Africa Development Community Region (Tawana *et al*., 2022). Low or high prevalence in *Anaplasma* species prevalence has been hypothesized to be associated with low or high genetic diversity respectively using the *msp4* gene (Han *et al*., 2017; Tumwebaze *et al*., 2020). Furthermore, differences in prevalence reported across several countries and region may be attributed to differences in the distribution of competent tick vectors, climatic conditions and farm management (Belkahia *et al*., 2014; Tumwebaze *et al*., 2020).

Sheep and goats play pivotal role, serving as reservoirs for various tick species, amplifying the impact of these vectors and perpetuating the life cycles of tick-borne pathogens (Pereira *et al*., 2016). In this study, both *A. ovis* and *A. marginale* DNA was characterized in blood DNA from goats in Kelantan, Malaysia. *Anaplasma marginale* is known to be associated with cattle and causes bovine anaplasmosis. However, increasing molecular evidence shows that this species of *Anaplasma* is also found in other animals, such as goats in Brazil (da Silva *et al*., 2018) and dogs (Hornok *et al*., 2018). Previously, *A. marginale* infection has been molecularly described in cattle from Malaysia (Ola-Fadunsin *et al*., 2018). The finding of *A. marginale* DNA in blood from goats in Malaysia adds to the growing body of literature on *A. marginale* in small ruminants across different countries of the world (Barbosa *et al*., 2021; Aung *et al*., 2022; Silva *et al*., 2024). *Anaplasma ovis*, on the other hand, is a major cause of anaplasmosis in sheep and goats (Wang *et al*., 2017; Cabezas-Cruz *et al*., 2019; Berthelsson *et al*., 2020). The latest work from Tay *et al*. (2014), however, could not detect *Anaplasma* DNA from the goats sampled in Malaysia; thus, the status of *A. ovis* infecting goats in Malaysia still needs to be discovered.

Although across districts, the prevalence varies from 0% to 75.6% among the ten districts sampled that cut across both inland and coastal regions. According to our findings, *Anaplasma* species are currently circulating in at least eight districts from both regions in Kelantan. In both regions, Bachok and Tanah Merah had the highest *Anaplasma* species detection rates of 75%, trailed distantly by other districts at between 7.5% and 43%. Because there is no observed difference in the management, herd health program, deticking or rickettsia prophylaxis, we theorise that the only available explanation for such is the source of goat stocks for the sampled farms. The sourcing for at least the Bachok district with a 75% detection rate was the same, thus suggesting the potential of *Anaplasma* existing in latency over a long period in the goats and ruling out other farm-specific variables such as management and herd health practices. Furthermore, Anaplasmataceae organism infections were detected in goats from both inland and coastal regions. This is because the difference in ambience between the coastal and inland regions is similar. However, the coastal region tends to be warmer and humid all year round, and this may increase tick survival, shorten life cycles, and lengthen tick activity as opined previously in a similar study undertaken in Thailand (Aung *et al*., 2022).

The goat management system in Malaysia appears to significantly influence the occurrence of anaplasmosis in the context of the influence of the management system on the exposure of goats to the rickettsia species and their vectors. This study observed a higher prevalence of anaplasmosis among intensively managed goats compared to extensive and semi-intensive systems, as observed for cattle in India (Zafar *et al*., 2022). The occurrence among intensively managed goats suggests immunological naivety of such goats from infection with *Anaplasma* spp. The goats in extensive management are presumably exposed to tick vector and *Anaplasma* infection early, thereby providing active immunity from subsequent exposures. Furthermore, intensively managed goats are more prone to manual handling than non-intensively managed ones, exposing the goats to the sustained presence of the vectors in the enclosed environment. These findings have been reported for cattle where intensive management was a major risk factor for anaplasmosis in Malaysia, with cattle raised in intensive management having the lowest infection rate compared to other management types (Ola-Fadunsin *et al*., 2018).

In consonance with the management system in Malaysia, the most abundant breed intensively managed is the Katjang, a local Malaysian breed. The high prevalence observed in the Katjang breeds in this study follows the pattern reported for local breeds in other countries where anaplasmosis is endemic (Rajasokkappan & Selvaraju, 2016; Zafar *et al*., 2022). The analogy is similar to Jamnapari goats widely farmed in Malaysia in intensive systems. Even though the within flock prevalence was not assessed in this study, faulty tick (vector) control mechanisms are responsible for the high prevalence in both intensively managed and local goat breeds in Malaysia. A bivariate model study needs to be conducted to compare anaplasmosis occurrence among intensive farms with different vector control systems.

In this study, the infection rate appeared to decrease steadily with increasing age. A study by Rahman *et al*. (2022) is in agreement with our results, where the trend of infection decreasing from young, adult to old animals in Bangladesh. While studies in Europe (Cabezas-Cruz *et al*., 2019; Rubel *et al*., 2021) indicated that older small ruminants tested positive more often than the younger groups; or age did not correlate with detection of *Anaplasma* infection in sheep and horses (Hornok *et al*., 2007; Praskova *et al*., 2011). The exact reasons for these conflicting results require further investigation, as it may due to variations in animal husbandry practices, environmental conditions, vector and *Anaplasma* species.

The *A. marginale msp4* gene has been proposed as a molecular marker for studying the phylogeography for this agent and it has been shown to provide useful phylogenetic and phylogeographic information for New World strains of *A. marginale* (de la Fuente *et al*., 2001, 2003, 2004). However, in this study, distinct strains were not observed when the *msp4* gene sequences of *A. marginale* and *A. ovis* from Malaysia were analysed and compared among the isolates and other strains in the NCBI GenBank. Similar results were obtained with the analysis of *A. marginale msp4* DNA sequences of the Brazil (Vidotto *et al*., 2006, Ramos *et al*., 2019). This gene was then claimed to be highly conserved and stable among heterologous strains (Battilani *et al*., 2017). Additionally, the phylogenetic tree provided in Figure 3 illustrates the relationships between the Malaysian isolates and other globally reported strains, with high maximum-likelihood (ML) and Bayesian inference (BI) support values confirming the classification and demonstrating genetic relatedness. *Anaplasma marginale* of Thailand isolates were shown to have higher sequences diversities among their isolates using *msp2* gene compared to the *msp4* gene (Junsiri *et al*., 2020). Therefore, *msp2* gene is recommended for future use in *A. marginale* strain identification.

CONCLUSION

We detected *A. ovis* and *A. marginale* from farm goats in for the first time Malaysia. This finding provides evidence of the molecular presence of *Anaplasma* species in goats from both coastal and inland region of Kelantan. These findings suggest that goats can serve as potential reservoirs for certain *Anaplasma* species and a potential source of infection for other farm animals. This finding highlights the need for comprehensive surveillance and management efforts to mitigate the risk pose by tick-borne pathogens to livestock's. Further investigations are required to elucidate the underlying epidemiological factors influencing variations in infection rates,

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