Pertanika J. Trop. Agric. Sci. 49 (1): 25 - 42 (2026)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Serological Survey and Risk Factors of Bovine Anaplasmosis in a Breeding Population of Mafriwal Cattle in Johor, Malaysia

Nur-Amalina Nasruddin[1], Nur-Sabrina Ahmad Mustaza[1], Muhammad-Ali Hanapiah Ab Manap[1,3], Erkihun Aklilu[1], Farina Mustaffa-Kamal[4], and Basripuzi Nurul Hayyan[1,2]*

[1]Department of Veterinary Paraclinical Studies, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan, Malaysia
[2]UMK Veterinary Laboratory Diagnostics, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan, Malaysia
[3]Pusat Ternakan Haiwan Air Hitam, Peti Surat 526, Jalan Batu Pahat, 86009 Kluang, Johor, Malaysia
[4]Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

ABSTRACT

Bovine anaplasmosis is a significant cattle disease with economic implications caused by intra-erythrocytic bacteria, *Anaplasma marginale* and *Anaplasma centrale*. In Malaysia, where the dairy industry is growing to meet increasing demands, understanding disease epidemiology is crucial. This cross-sectional study investigated the seroprevalence of bovine anaplasmosis in 242 Mafriwal cattle population in a government commercial dairy farm using commercial cELISA kit and its associations with various risk factors using Chi-square test. The study revealed a high seroprevalence of 79.75%, with lactating cattle having the highest seropositivity (95.08%) among the other management groups. However, no significant association ($p < 0.05$) was found between packed cell volume (PCV) and seropositivity, although a higher proportion of seropositive cattle (82.73%) have a low PCV. Cattle that were kept in semi-intensive housing had a higher seropositivity (81.87%) than those managed under intensive system (73.33%) but the housing type did not significantly affect the seropositivity. There is no significant correlation between the molecular findings of bovine anaplasmosis and seropositivity, yet polymerase chain reaction (PCR) confirmed 82.14% of seropositive cases and 76.47% of PCR negative samples is seropositive. Seropositivity increase from 68.85% in 2021 to 90.83% in 2022, indicating a potential rise in the prevalence of bovine anaplasmosis over time. This study

ARTICLE INFO

Article history:
Received: 11 April 2025
Accepted: 08 December 2025
Published: 30 January 2025

DOI: https://doi.org/10.47836/pjtas.49.1.02

E-mail addresses:
malinanasruddin.nan@gmail.com (Nur-Amalina Nasruddin)
sabrinamustaza@gmail.com (Nur-Sabrina Ahmad Mustaza)
alihanapiah@dvs.gov.my (Muhammad-Ali Hanapiah Ab Manap)
erkihun@umk.edu.my (Erkihun Aklilu)
farina@upm.edu.my (Farina Mustaffa-Kamal)
basripuzi@umk.edu.my (Basripuzi Nurul Hayyan*)
* Corresponding author

revealed that bovine anaplasmosis is prevalent among Mafriwal cattle population in studied farm, and it is significantly associated with management groups and the year of sampling. Increased monitoring and control measures are needed to curb the spread of bovine anaplasmosis. Further research is warranted to explore additional risk factors and epidemiological aspects, benefiting cattle health and production in Malaysia.

Keywords: Anaplasma, bovine anaplasmosis, cELISA, Mafriwal, serological, seropositivity

INTRODUCTION

Anaplasma marginale is the primary cause of bovine anaplasmosis, a common tick-borne disease among cattle. This intracellular haemoparasite poses serious health risks leading to decreased productivity and weight loss. While in severe cases, it causes mortality in the affected cattle population (Abba et al., 2016; Smith, 2015). In general, haemoparasites infection causes notable economic losses to the dairy industry worldwide due to reduced milk production, increased costs associated with treatment of diseases and the consequent effect on the efficiency and profitability of dairy production systems (Bitrus et al., 2018; Hanzlicek et al., 2016; Machado et al., 2015).

Bovine anaplasmosis, also known as gall sickness is a disease caused by the intraerythrocytic bacteria, *Anaplasma marginale*. Meanwhile, *Anaplasma centrale*, is a closely related *Anaplasma* sp. but typically causes milder form of bovine anaplasmosis. It had been used as a live vaccine to protect against *A. marginale* infections in some regions worldwide (Ierardi, 2025). The disease is transmitted through the bites of infected ticks and leads to the development of haemoparasites within red blood cells of the hosts. The infections result in a variety of clinical symptoms, including fever, weakness, inappetence, dehydration, constipation, jaundice, depression, laboured breathing, and abortion, with haemolytic anaemia as a primary symptom of bovine anaplasmosis (Abba et al., 2016; Das et al., 2021; Jaswal et al., 2013). The anaplasmosis infection may persists in the host, making the infected animals as asymptomatic carriers for the disease (Seo et al., 2018). The complexity of bovine anaplasmosis epidemiology is attributed to the multifaceted connections between haemoparasite, host, and vector (Paramanandham et al., 2019).

A gold standard method for the detection of bovine anaplasmosis are the microscopic examination of Giemsa-stained blood smears. However, polymerase chain reaction (PCR), which is a molecular-based diagnostic method, yields high sensitivity and specificity for *Anaplasma* spp. detection. This diagnostic tool provides a rapid and inexpensive diagnosis, and it is particularly useful in animals with low parasitemia (El-Ashker et al., 2015; Jalali et al., 2013). Serological methods, including enzyme-linked immunosorbent assay (ELISA) and immunofluorescence test (IFAT) aids in the identification of subclinical or chronic infection of bovine anaplasmosis, where the pathogen may not be detectable by

microscopic examination or PCR (Ashuma et al., 2014). These methods have become the essential tools to determine the seroprevalence of *A. marginale* in the dairy herds (Curtis & Coetzee, 2021). These assays will provide valuable information for disease surveillance through a rapid and relatively inexpensive screening of large cattle herd. While these tests require some laboratory equipment, such as, incubator and plate reader, and are not directly accessible to farmer, it can be conducted through regional veterinary laboratories. This makes the assay a feasible option for routine herd-level monitoring in many farms setting. Additionally, it had been proven that serology methods yield a higher prevalence for the diagnosis of bovine anaplasmosis (Nur-Amalina et al., 2023). The diagnosis offers valuable epidemiology information to assess the disease prevalence for the development of targeted control measures (Parvizi et al., 2020; Spare et al., 2020). This will reduce the morbidity and mortality, and improve the productivity of the herd, ultimately minimising the economic losses caused by the infections (Scariot et al., 2022).

The connection between seropositivity and parameters like management group, housing system and health status enable researchers to understand the factors effecting the disease development among the Mafriwal population. The susceptibility toward bovine anaplasmosis varies with the age of cattle since older animals are more susceptible than younger cattle (Eleftheriou et al., 2022; Zim et al., 2024). Some studies reported that female cattle are significantly vulnerable to anaplasmosis infection compared to bulls (Atsuwe et al., 2024; Badshah et al., 2023). Additionally, higher seropositivity were reported in cattle managed in larger herd size, with inadequate acaricide treatments and practicing pasture grazing (Selim et al., 2021; Zim et al., 2024). Clinical conditions, such as anaemia, fever, and jaundice, are strongly correlated with bovine anaplasmosis infection (Değirmençay et al., 2022). According to Scariot et al. (2022), mastitis and retained placenta are significantly associated with anaplasmosis infection among dairy cattle. Furthermore, the prevalence of bovine anaplasmosis was reportedly higher in summer since tick population reach their peak activity level at warmer months. Therefore, environmental factor including seasonal changes serve as one of the potential risk factors for bovine anaplasmosis infection (Abdela et al, 2018; Oliveira et al., 2021). Meanwhile, the diagnoses of bovine anaplasmosis in Malaysia yield a high prevalence through microscopic, molecular and serological diagnostic methods (Agina et al., 2021; Bitrus et al., 2018; Nur-Sabrina et al., 2024; Ola-Fadunsin et al*.,* 2018). Therefore, the knowledge of epidemiological factor affecting the susceptibility of Malaysian cattle, particularly Mafriwal, is crucial as this disease are prevalent in Malaysia.

Mafriwal breed was developed to supply the needs for local dairy products by Malaysian consumers. The Department of Veterinary Service (DVS) Malaysia developed a tropicalised dairy breed, Mafriwal, in the 1970s by selectively breeding Sahiwal and Friesian cattle. The Mafriwal cattle population has been maintained in a Malaysian government commercial farm located in the southern region. This breed prevails within

Malaysia's dairy industry because of their ability to tolerate various environmental stressors including their endurance in a tropical climate (Mastura et al., 2019; Panandam & Raymond, 2005). Notwithstanding their tolerance to various environmental stressors, a recent study confirmed the presence of *A. marginale* in the Mafriwal population. Based on their study, parasitism significantly impacts on the milk yield and body weight of Mafriwal cattle (Nur-Sabrina et al., 2024). Despite the widespread prevalence of bovine anaplasmosis, the studies on bovine anaplasmosis in Malaysia remained limited (Hayyan & Nasruddin, 2025). The gap in scientific literature highlighted the need to have a region-specific epidemiological study. Therefore, this study was carried out to determine the seroprevalence rate of *Anaplasma* spp., while exploring the risk factors associated with bovine anaplasmosis among Mafriwal cattle population.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional observational study was conducted in a breeding population of Mafriwal cattle at a government-operated commercial dairy farm in Johor, a state in the Southern region of Peninsular Malaysia, on December 2021 and June 2022.

Cattle Management

The Mafriwal cattle population in this study was classified into four management groups namely calves below one year old, yearlings, lactating cows, and dry cows. Newly born calves were managed intensively where they were fed with colostrum twice daily at 10% of their body weight, followed by fresh raw milk, fresh fodder, and calf starter pellets at two months old. Weaning occurred at 100 days or until they reached 90 kg. Calves remained under intensive housing until they became yearlings. Yearlings were transitioned semi-intensive housing, where they were released into the paddock with signal (*Brachiaria decumbens*) and guinea (*Panicum maximum*) grasses, practicing a rotational grazing system. Yearlings were supplemented with concentrates including palm kernel cake and molasses once daily. Later, as the calves reached two years old, they were mixed with selected bulls on pasture and subsequently went through gestation, lactation and dry cow groups. Nonetheless, gestating cows were excluded from this study to prevent stress during sampling.

Sample Collection and Storage

Blood samples were obtained from the coccygeal vein of 242 Mafriwal cattle of different management groups namely calves below one year old (n = 60), yearlings (n = 61), lactating cows (n = 61), and dry cows (n = 60). All Mafriwal cattle in the farm except the pregnant

cows were included in the sampling to increase statistical power and to estimate the true seroprevalence of bovine anaplasmosis in the population. The blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-coated blood collection tubes for the purpose of determining the packed cell volume (PCV) of the cattle and for molecular identification of *A. marginale.* The PCV was performed using the fresh samples and the remaining blood samples were aliquoted and placed in 1.5 mL microcentrifuge tube at -20 ℃ until DNA extraction. The serum samples were collected from serum-separator tubes (SST) for antibody detection using ELISA. Serum samples were kept in 1.5 mL microcentrifuge tube at -20 ℃ until use.

Blood Sample Analysis

Packed Cell Volume

An aliquot of blood samples collected in the EDTA tubes were drawn into hematocrit capillary tubes by capillary action and sealed at one end with Cristaseal (Hawksley and Son Ltd, United Kingdom). The sealed capillary tubes were centrifuged at 220 Relative Centrifugal Force (RCF) for five minutes and read with a rotoreader (Basripuzi et al., 2018). In this study, PCV values below 0.24 L/L were assigned as "low," while values of 0.24 L/L and above were categorized as "normal" according to Bull et al. (2003).

Polymerase Chain Reaction

The DNA of *A. marginale* and *A. centrale* was extracted from each blood sample using the Geneaid Gsync™ DNA extraction kit according to the manufacturer's instructions (Geneaid Biotech Ltd. New Taipei City, Taiwan). The extracted DNA were stored in 1.5 mL microcentrifuge tube at -20 °C until further use (Nur-Sabrina et al., 2024). The MSP4 gene of *A. marginale* and 16s rRNA gene of *A. centrale* were amplified on a MyCycler™ thermocycler (Bio-Rad, USA) using published primers and thermocyclic profile presented in Table 1 (Shkap et al., 2008).

The DNA products were electrophoresed at 400W/100V for 40 minutes on 2 % agarose gel (Promega Madison, USA) with Tris-acetic acid-EDTA (TAE) buffer, and stained with Midori Green dye (Nippon Genetics, Europe). Visualisation of DNA fragments was performed using the GelDoc™ EZ Imager.

Enzyme-linked Immunosorbent Assay (ELISA)

Samples and reagents preparations for ELISA were performed following the manufacturer's recommended protocol (VMRD, USA). Each undiluted serum sample of 50 µL was added in duplicate to individual wells of a recombinant MSP5-coated plate from Anaplasma Antibody Test Kit, cELISA V2 (VMRD, USA). The undiluted positive and negative

Table 1
Primers used for the amplification of Anaplasma spp.

Anaplasma spp.	Targeted gene	Oligonucleotide sequence	Thermocycler profile
A. marginale	MSP4	Reverse: CATCTCCCATGAGTCACGAAGTGGC Forward: GCTGAACAGGAATCTTGCTCCAAG	ID: 95°C/5mins D: 95°C/1mins A: 65°C/2mins E: 72°C/1min No of cycles: 40 FE: 72°C/10mins
A. centrale	16s rRNA	Reverse: CATCTCCCATGAGTCACGAAGTGGC Forward: GCTGAACAGGAATCTTGCTCCAAG	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

Note. min = minutes; ID = initial denaturation; D = denaturation; A = annealing; E = extension; FE = final extension; bp = base pair

control sera of 50 µL were run in duplicate and triplicate, respectively. The assay plate was then incubated at 23±2 ℃ for 60 minutes before the serum samples and controls were discarded. The plate was washed twice with 1:10 diluted washing solution. Then, 50 µL of monoclonal antibody-peroxidase conjugate in 1:100 of dilution buffer was added to each well and incubated at 23±2 ℃ for 20 minutes. The plate was washed four times. A total of 50 µL of substrate solution was added to each well and incubated for 20 minutes at 23±2 ℃. The reaction in each well was stopped by adding 50 µL of stop solution. The optical density (OD) of each well was read at 630 nm on a microplate absorbance spectrophotometer (Bio-Rad, USA).

Test Validation and Results Interpretation

Test validation involved the utilisation of both negative and positive controls. The ELISA assay was considered valid if the mean OD of the triplicate negative controls fell within the range of >0.40 and ≤2.10, and the mean OD of duplicate positive controls exhibited ≥ 30% inhibition.

Results were interpreted based on the percentage inhibition (I%) which was calculated as: $I\% = 100\ [1- (OD_{Sample} / OD_{Negative\ control}]$, where OD_{Sample} was the OD of sample and $OD_{Negative\ control}$ was the mean OD of negative control. Serum samples with < 30% of inhibition is considered negative for antibody against bovine anaplasmosis while serum samples with ≥ 30% of inhibition were positive.

Statistical Analysis

Descriptive statistics for categorical variables were performed by calculating frequencies and percentages for each category. Chi-square test was used to analyse the data using R software (version 4.3.1) to determine the associations between different management groups (calves below one year old, yearlings, lactating cows, dry cows), PCV level (low, normal), housing system (intensive, semi-intensive), molecular identification of *A. marginale* (positive, negative) and year of sampling (2021, 2022) with seropositivity at 95% confidence interval.

RESULTS

The findings of this study revealed that 79.75% sampled (193/242) cattle in this population were tested seropositive for bovine anaplasmosis. A significant association between the different management groups and seroprevalence was observed ($\chi^2(3) = 9.98$, $p = 0.019$) in which the lactating group had the highest seroprevalence (95.08%). A total of 115 (82.73%) seropositive cattle were detected with low PCV levels (n = 139) while only 78 (75.73%) seropositive cattle were detected with normal PCV levels (n = 103). However, there was no statistically significant association between the PCV levels and seropositivity of anaplasmosis ($\chi^2(1) = 2.55$, $p = 0.110$).

Similarly, no significant association was observed between the type of housing systems and the seroprevalence ($\chi^2(1) = 2.04$, $p = 0.153$) although cattle reared in semi-intensive housing (n = 182, 81.87%) have a higher seroprevalence compared to intensive housing (n = 60, 73.33%). The results showed that 82.14% (115/140) of the PCR-positive samples were found to be seropositive, while 76.47% (78/102) of the PCR-negative samples were found to be seropositive. Nonetheless, there is no significant association between PCR results and seropositivity of the samples towards bovine anaplasmosis ($\chi^2(1) = 1.18$, $p = 0.277$).

In 2021, a total of 84 animals were found to be seropositive, accounting for 68.85% of the sampled population (n = 122). In 2022, there were 109 seropositive animals, representing 90.83% of the sampled cattle in the same population (n = 120). A highly significant association between the seroprevalence of bovine anaplasmosis and the year of sampling ($\chi^2(1) = 18.10$, $p = 0.000$) is shown in Table 2.

DISCUSSION

This study demonstrates a high seroprevalence (79.75%) of bovine anaplasmosis among the tested Mafriwal cattle population which are kept for commercial dairy production and breeding purposes. In contrast, a lower seroprevalence of anaplasmosis using the same cELISA kit as used in the current study (51.11%) was reported among randomly sampled dairy cattle of various breeds from different farms in Selangor, Malaysia (Bitrus et al., 2018).

Table 2
The association between seroprevalence with age group, PCV, housing type, molecular identification, and year of sampling

Variable		Number of animal's tested	Number of seropositive animals	Percentage (%)	X^2	df	p
Management group	Calves	60	44	73.33	9.98	3	0.019*
	Yearlings	61	47	77.05			
	Lactating	61	58	95.08			
	Dry	60	44	73.33			
PCV	Low (<0.24 L/L)	139	115	82.73	2.55	1	0.110
	Normal (≥0.24 L/L)	103	78	75.73			
Housing type	Intensive	60	44	73.33	2.04	1	0.153
	Semi-intensive	182	149	81.87			
Molecular identification of *Anaplasma* spp.	Positive	140	115	82.14	1.18	1	0.277
	Negative	102	78	78.47			
Year	2021	122	84	68.85	18.10	1	0.000***
	2022	120	109	90.83			

Note. X^2 = chi square; df = degree of freedom; p = p-value; * = p-value < 0.05; ** = p-value < 0.01; *** = p-value < 0.001

The seroprevalence of bovine anaplasmosis vary as the management system of each farm affect the seroprevalence of bovine anaplasmosis differently (Ola-Fadunsin et al*.,* 2018).

In the present study, lactating cattle, classified under management groups variable, exhibited the highest seropositivity rate (95.08%). In contrast, other management groups, including calves, yearlings, and dry cows, had relatively lower seropositivity rates. The statistically significant relationship between management groups and seropositivity ($\chi^2(3) = 9.98$, $p = 0.019$) in the present study, suggested that production stage and physiological status influenced the susceptibility of Mafriwal cattle to anaplasmosis. While management groups are not strictly age categories, lactating and dry cows are generally older animals, whereas calves represent younger cattle. Therefore, the overlap may partially explain the agreement with Bitrus et al. (2018) and Debbarma et al. (2020), which reported high seropositivity observed in cattle greater than 3 years old. However, these studies revealed that the age of animal and the seropositivity are not statistically significant ($p > 0.05$) whereas our study suggested otherwise. These findings are attributed by the differences in study design, classification criteria or epidemiological factors. The use of management

group in the current study likely captured physiological and exposure risks, which may not be reflected when considering age alone. Furthermore, Manap et al. (2024) found that Mafriwal cattle were more susceptible to haemoparasite infections with advancing production stage, in which the yearlings, lactating cows, and dry cows were estimated with higher odds ratio to be infected in comparison to the calves, likely due to cumulative exposure to anaplasmosis or physiological stress. While Atif et al. (2013) suggested that seroprevalence of bovine anaplasmosis increased with age, Ola-Fadunsin et al. (2018) revealed that a higher prevalence was observed in younger cattle. However, the present study suggested that age and production stage play a significant role, in agreement with Vetrivel et al. (2017) in which bovine anaplasmosis was found to be significantly associated with age of dairy cattle. Young calves may receive passive humoral antibodies from their dam through colostrum, whereas cattle over five months old typically gain immunity through exposure to pathogen and clinical infection, building their own active immune response (Vlasova & Saif, 2021). The exposure to infections and immunological progression may explain the lower seropositivity observed in calves and the higher seroprevalence among lactating and dry cows in the present study. Previous findings revealed that the seropositivity in lactating group are higher (60 %) than the peripartum, dry, and pregnant groups (Da Silva & Da Fonseca, 2013). Although transient immunosuppression is well-recognised during pregnancy, lactating cows also experience the transient reduction in immune function during early lactation due to physiological stress of milk production, which makes them more susceptible to the bovine anaplasmosis (Aktas & Özübek, 2017). Moreover, the lactating cows experience various physiological changes and stressors due to the demands of milk production, including nutrient partitioning towards lactation and the production of colostrum. Therefore, it compromises the immune function and increase the susceptibility to anaplasmosis infection. Furthermore, high levels of prolactin and progesterone in lactating cattle can lead to a temporary suppression of the immune system, making them more susceptible to infections like bovine anaplasmosis (Aleri et al., 2016; Debbarma et al., 2020; Vlasova & Saif, 2021).

In addition, no association was observed between the seroprevalence of bovine anaplasmosis and the PCV level in the Mafriwal cattle population ($\chi^2(1) = 2.55$, $p = 0.110$). The PCV were observed to be significantly lower ($p < 0.05$) in cattle with anaplasmosis as it often induces changes in the animal's blood parameters, making hematology and biochemical tests valuable tools for diagnosis (El-Ashker et al., 2015; Bezabih et al., 2017, Abdela et al., 2018; World Organisation for Animal Health [OIE], 2018). However, the non-significant association observed between PCV and seropositivity are because PCV reflect the ongoing parasitaemia. Animal infected with anaplasmosis often become a carrier, maintaining seropositivity without exhibiting haematological changes (Hairgrove et al., 2015). Bovine anaplasmosis causes haemolytic anaemia, where the red blood cell count

in the bloodstream drops, resulting in reduced oxygen-carrying capacity. As a result, the PCV levels in an infected animal's blood decrease. However, mild parasite infections may not result in significant anaemia as shown in animals with stronger infections (Hayyan et al*.,* 2020). Complete blood count (CBC) may reveal characteristics such as anaemia and altered red blood cell indices. The ability of *Anaplasma* spp. to penetrate red blood cells may explain the difference in average PCV values between calves with anaplasmosis and those who are unaffected. The reticuloendothelial system, specifically the spleen, contains macrophages that are principally responsible for removing this pathogen from the organism after it has gone through cycles of replication within erythrocytes (Abdela et al., 2018). Additionally, PCV and serum biochemical analysis help in assessing the severity of the disease.

Although this study revealed that cattle reared in the semi-intensive housing system exhibited a higher seropositivity rate (81.87%) compared to those reared in the intensive housing system (73.33%), the differences was not statistically significant ($p = 0.153$). These findings are in contrast with a study by Sajid et al. (2014), which showed that cattle raised under intensive management system had a significantly lower prevalence of *A. marginale* infection in comparison to the cattle raised in extensive and semi-intensive systems. Bovine anaplasmosis can be transmitted biologically via tick's bite and biting flies; or mechanically via contaminated fomites including needle and surgical equipment; or through transplacental transmission (Aubry & Geale, 2011). Therefore, the farm management and housing system can potentially influence the exposure of the animals to bovine anaplasmosis. Cattle reared in extensive and semi-intensive system are more likely to be in contact with the tick vectors of *Anaplasma* spp. including *Rhipicephalus (Boophilus) microplus* and reservoir animals, particularly the wildlife. Ticks play a significant role as the primary vector for *A. marginale* by sustaining the pathogen in the natural environment while reservoir animals naturally maintain the pathogen and serve as a source of infection (Tucker et al*.,* 2016; Salinas-Estrella et al., 2022). However, cattle reared in the intensive system are kept at a higher stocking density than extensive and semi-intensive cattle, which increases the likelihood of *Anaplasma* spp. infections through the potential mechanical vectors and stress.

Polymerase chain reaction (PCR) is a molecular technique that can be used to detect the presence of *Anaplasma* spp. DNA in the blood samples of cattle. This approach is highly sensitive and specific, making it possible to identify the pathogen even before the onset of clinical signs (Chi et al., 2013). The PCR can directly confirm active infection, and often used as a diagnostic assay (Chi et al., 2013; Singh et al., 2012). In this study, molecular and serological diagnosis showed that 82.14% (115/242) of the Mafriwal cattle population were infected with bovine anaplasmosis. However, the molecular analysis revealed no evidence of association ($\chi^2(1) = 1.18$, $p = 0.277$) between PCR and serological

results, suggesting possible differences in the detection of current infection versus past exposure or latent carrier states. The presence of antibodies can be detected through serological tests, such as ELISA, which are commonly used for epidemiological studies. However, serological test does not distinguish between past and active infection, leading to discrepancies when compared to PCR tests that detect the pathogen's DNA (Aubry & Geale, 2011; Spare et al., 2020). Previous molecular work on bovine anaplasmosis among Mafriwal cattle population revealed high prevalence of anaplasmosis caused by *A. marginale* and *A. centrale* (Nur-Amalina et al., 2024). Nevertheless, PCR alone does not provide a definitive diagnostic because it only identifies active anaplasmosis and unable to detect past exposure. Therefore, while PCR is a valuable diagnostic tool for identifying active infections, serological results provide a better cumulative exposure history of the disease.

Serological tests, including ELISA, have higher sensitivity for detecting antibodies against *Anaplasma* spp. However, PCR is the preferred method for diagnosing anaplasmosis because it detects active infection and enables better differentiation between *Anaplasma* subspecies (Jalali et al., 2013; Sharma et al., 2015; Shabana et al., 2018). Furthermore, the complex relationship between PCR and seropositivity in bovine anaplasmosis may depend on the stage of infection and the immune status of the animal. During the acute phase of infection, both PCR and seropositivity are expected to be positive. However, it may take a period of time for the immune system produces sufficient levels of antibodies before it can be detected. Therefore, PCR often yield earlier positive results than serology (Hamou et al., 2012). In chronic infections or carrier animals, PCR positivity can persist even when antibody level dropped. This is primarily due to long-term pathogen persistence in host tissues while antibody levels can decrease over time (Aubry & Geale, 2011). In comparison, seropositive animals with negative PCR results may reflect prior exposure to *A. marginale* but the body had already cleared the infection. This aligns with our findings that 76.47% of PCR-negative animals were seropositive, highlighting that the two diagnostic methods detect infection at different stages, which explained the discrepancies of the results. Additionally, although the United State Department of Agriculture's (USDA) cleared and approved the commercial cELISA kit, cross-reactivity has been observed in both *Ehrlichia* genotypes and *Anaplasma phagocytophilum* (*A. phagocytophilum*) (Al-Adhami et al., 2011; Dreher et al., 2005). Overall, both methods have established their role in the diagnosis and monitoring of bovine anaplasmosis with complementing information on cattle infection status (Hansmann et al., 2019; Jalali et al., 2013; Moniuszko-Malinowska et al., 2021).

Temporal trend means the changes and patterns of specific events. In the case of bovine anaplasmosis, it describes the evolution, spread, and fluctuation of the disease over time. This analysis provides additional insights into factors driving the dynamics of bovine anaplasmosis, such as environmental changes, management practices and the effectiveness

of control measures (Leal Filho et al., 2022; Sunder et al., 2019;). Temporal trend analysis of the seroprevalence have revealed some notable patterns. One notable pattern includes the potential increase of disease prevalence over time through the observation of the ascending seroprevalence in the sampled population from 68.85% in 2021 to 90.83% in 2022 with significant associations between seroprevalence and sampling year (($\chi^2(1) = 18.10$, $p = 0.000$)). Factors that can be considered affecting this temporal variation include climate change, vector dynamics, varying management practice or other epidemiological factors (Hanzlicek et al., 2016). The study of temporal trends enables researchers to understand the epidemiology of bovine anaplasmosis by detecting significant patterns in bovine anaplasmosis occurrence and predicting future trends. The knowledge assists researchers and relevant individuals or organisations in directing management strategies, implement control measures, and understanding and preparing for potential risks that be posed to cattle populations in the near future.

The findings of this study revealed a high seroprevalence of *Anaplasma* spp. in the studied Mafriwal cattle population. The findings aligned with previous studies reporting that crossbred cattle are highly susceptible to anaplasmosis infection compared to the local breed (Atif et al., 2013; Ola-Fadunsin et al., 2018). These findings concludes that the prolonged exposure of *Anaplasma* spp. for many generations has given the local cattle the ability to develop resistance against the pathogen or vector causing bovine anaplasmosis. Furthermore, the resiliency and hardiness of the local breeds are through the acclimatisation to the specific conditions. Their susceptibility to the infection decreased as they adapt to the local environment through the development of physiological and behavioural traits (Ola-Fadunsin et al., 2018). Contradicting to other local cattle breed, Mafriwal, a Friesian-Sahiwal cross, may have a relatively higher bovine anaplasmosis seroprevalence as a result of different genotypic composition and management conditions. Sahiwal cattle are well-adapted to tropical climates, but Friesian genetics may introduce traits that make them highly susceptible to diseases such as bovine anaplasmosis. A previous study revealed that Friesian-Holstein cattle are genetically more susceptible to tick-borne diseases due to specific BoLA alleles such as DRB32 and DRB316, while Sahiwal cattle carry resistance-associated alleles like DRB314 and 41, contributing to their resilience against infections such as anaplasmosis (Duangjinda et al., 2013).

Cattle seroprevalence of bovine anaplasmosis was high in Mafriwal cattle population in a commercial government farm in Kluang, Johor. Housing systems, PCR result and PCV level were not significantly associated with seropositivity against anaplasmosis; however, seropositivity has shown a significant association with age group and year of samplings. The observed increase in seroprevalence over time highlights the need for improved monitoring and control strategies, such as regular screening and effective vector control. These measures are crucial to reduce economic losses and to enhance the overall health and productivity of cattle herds. In the current study, gestating cows were excluded from

sampling to minimise stress and potential risks to both dam and foetus during pregnancy. Therefore, the effect of pregnancy status of Mafriwal cattle on anaplasmosis infection and the immunity remains an important area for future research. The findings from this study could provide knowledge for the development of management strategies to improve productivity of the cattle.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Ministry of Higher Education Malaysia for the Fundamental Research Grant Scheme (FRGS/1/2020/WAB04/UMK/02/5) and the Department of Veterinary Services for the contribution in this study.

REFERENCES

Abba, Y., Jesse, F. F. A., Sadiq, M. A., Ibrahim, H. H., Chung, E. L. T., Bitrus, A. A., Lila, M. A. M. & Hambali, I. U. (2016). Clinical management and gross pathological findings of a severe anaplasmosis in a dairy cow. *Journal of Advanced Veterinary and Animal Research, 3*(2), 195199. https://doi.org/10.5455/javar.2016.c150

Abdela, N., Ibrahim, N., & Begna, F. (2018). Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia. *Acta Tropica, 177*, 9-18. https://doi.org/10.1016/j.actatropica.2017.09.010

Aktas, M., & Özübek, S. (2017). Outbreak of anaplasmosis associated with novel genetic variants of *Anaplasma marginale* in a dairy cattle. *Comparative Immunology, Microbiology and Infectious Diseases, 54*, 20-26. https://doi.org/10.1016/j.cimid.2017.07.008.

Al-Adhami, B., Scandrett, W. B., Lobanov, V. A., & Gajadhar, A. A. (2011). Serological cross-reactivity between *Anaplasma marginale* and an *Ehrlichia* species in naturally and experimentally infected cattle. *Journal of Veterinary Diagnostic Investigation, 23*(6), 1181-1188. https://doi.org/10.1177%2F1040638711425593

Aleri, J. W., Hine, B. C., Pyman, M. F., Mansell, P. D., Wales, W. J., Mallard, B., & Fisher, A. D. (2016). Periparturient immunosuppression and strategies to improve dairy cow health during the periparturient period. *Research in Veterinary Science, 108*, 8-17. https://doi.org/10.1016/j.rvsc.2016.07.007

Atif, F. A., Khan, M. S., Muhammad, F., & Ahmad, B. (2013). Sero-epidemiological study of *Anaplasma marginale* among cattle. *Journal of Animal and Plant Sciences, 23*(3), 740-744.

Atsuwe, T., Adulugba, O., Udo, E., & Chikwendu, J. (2024). Occurrence of anaplasmosis among cattle in Makurdi Metropolis, Benue State, Nigeria. *Sahel Journal of Veterinary Sciences*, *21*(3), 10-15. https://doi.org/10.54058/dxhp0n49.

Aubry, P., & Geale, D. W. (2011). A review of bovine anaplasmosis. *Transboundary and Emerging Diseases, 58*(1), 1-30. https://doi.org/10.1111/j.1865-1682.2010.01173.x

Ashuma, S. A., Kaur, P., Bal, M. S., & Singla, L. D. (2014). Application of multiplex PCR for the simultaneous detection of natural infection of theileriosis, babesiosis, and trypanosomosis in cattle. *Journal of Veterinary Parasitology*, *28*(2), 112-116.

Agina, O. A., Shaari, M. R., Isa, N. M. M., Ajat, M., Zamri-Saad, M., Mazlan, M., Muhamad, A. S., Kassim, A. A., Ha, L. C., Rusli, F. H. and Masaud, D. (2021). Molecular detection of *Theileria* species, *Anaplasma* species, *Candidatus Mycoplasma haemobos, Trypanosoma evansi,* and first evidence of *Theileria sinensis*-associated bovine anaemia in crossbred Kedah-Kelantan x Brahman cattle. *BMC Veterinary Research*, *17*, 1-20. https://doi.org/10.1186/s12917-021-02902-0

Badshah, F., Ullah, K., Kamal, M., Rafiq, N., Usman, T., Ríos-Escalante, P., & Said, M. (2023). Epidemiological analysis of anaplasmosis in cattle from Khyber Pakhtunkhwa, Pakistan. *Veterinary World*, *16*(11)*,* 2287-2292. https://doi.org/10.14202/vetworld.2023.2287-2292.

Basripuzi, N. H., Salisi, M. S., Isa, N. M. M., Busin, V., Cairns, C., Jenvey, C., & Stear, M. J. (2018). Boer goats appear to lack a functional IgA and eosinophil response against natural nematode infection. *Veterinary Parasitology, 264,* 18-25. https://doi.org/10.1016/j.vetpar.2018.10.014

Bezabih, M., Shabula, Z., & Beyene, N. (2017). Prevalence of bovine trypanosomiasis in Dara District Sidama Zone, Southern Ethiopia. *Journal of Parasitology and Vector Biology, 9*(9)*,* 132-136. https://doi.org/10.5897/JPVB2015.0226.

Bitrus, A. A., Jesse, F. F. A., Abba, Y., Pei, J. L. X., Peter, I. D., Hambali, I. U., Haron, A.W., Lila, M.A.M. & Saharee, A. A. (2018). Seroprevalence of anaplasmosis in dairy cattle from Peninsular Malaysia. *Advances in Animal and Veterinary Sciences, 6*(2), 70-74. http://dx.doi.org/10.17582/journal.aavs/2018/6.2.70.74

Bull, B. S., Fujimoto, K., Houwen, B., Klee, G., Van Hove, L., Van Assendelft, O. W., Bunyaratvej, A., Buttarello, M., Davis, B., Koepke, J.A. & Lewis, S.M. (2003). International Council for Standardisation in Haematology (ICSH) recommendations for "surrogate reference" method for the packed cell volume. *Laboratory Hematology, 9*, 1-9.

Chi, Q., Liu, Z., Li, Y., Yang, J., Chen, Z., Yue, C., Luo, J., & Yin, H. (2013). Development of a real-time PCR assay for detection and quantification of *Anaplasma ovis* infection. *Transboundary and Emerging Diseases, 60*(2), 119-24. https://doi.org/10.1111/tbed.12139.

Curtis, A. K., & Coetzee, J. F. (2021). Assessment of within-herd seroprevalence of *Anaplasma marginale* antibodies and associated decreased milk production in an Iowa dairy herd. *Applied Animal Science, 37*(2), 126-131. https://doi.org/10.15232/aas.2020-02110

Da Silva, J. B., & Da Fonseca, A. H. (2013). Analysis of the risk factors related to the immune humoral anti-*Anaplasma marginale* in dairy cattle. *Semina-Ciencias Agrarias, 34*(2), 777-784. https://doi.org/10.5433/1679-0359.2013v34n2p777

Das, D., Sarma, K., Roychoudhury, P., Chethan, G., Ravindran, R., Islam, S., Prasad, H., Rajesh, J., Behera, B., & Choudhury, F. (2021). Gross and histopathological findings of naturally occurring *Anaplasma marginale* infection in cattle. *Indian Journal of Animal Research, 56*(12), 1552-1556. https://doi.org/10.18805/IJAR.B-4283.

Debbarma, A., Pandit, S., Jas, R., Baidya, S., Chandra Mandal, S., Sarathi Jana, P., & Das, M. (2020). Prevalence of tick-borne haemoparasitic diseases in cattle of West Bengal, India. *Biological Rhythm Research, 51*(2), 310-317. https://doi.org/10.1080/09291016.2018.1528693

Değirmençay, Ş., Küçükler, S., Özdemir, S., & Kaman, R. (2022). Evaluation of erythrocyte arginase activity, plasma nitric oxide concentration and oxidative stress status in cattle with anaplasmosis. *Veterinary Parasitology, 314*, Article 109855. https://doi.org/10.1016/j.vetpar.2022.109855.

Dreher, U. M., De La Fuente, J., Hofmann-Lehmann, R., Meli, M. L., Pusterla, N., Kocan, K. M., Woldehiwet, Z., Braun, U., Regula, G., Staerk, K.D.C & Lutz, H. (2005). Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. *Clinical and Vaccine Immunology, 12*(10), 1177-1183. http://doi.org/10.1128/CDLI.12.10.1177-1183.2005

Duangjinda, M., Jindatajak, Y., Tipvong, W., Sriwarothai, J., Pattarajinda, V., Katawatin, S., & Boonkum, W. (2013). Association of BoLA-DRB3 alleles with tick-borne disease tolerance in dairy cattle in a tropical environment. *Veterinary Parasitology, 196*(3-4), 314-20. https://doi.org/10.1016/j.vetpar.2013.03.005.

El-Ashker, M., Hotzel, H., Gwida, M., El-Beskawy, M., Silaghi, C., & Tomaso, H. (2015). Molecular biological identification of *Babesia, Theileria*, and *Anaplasma* species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. *Veterinary Parasitology, 207*(3-4), 329-334. https://doi.org/10.1016/j.vetpar.2014.12.025

Eleftheriou, A., Cole, D., Kieffer, J., & Pesapane, R. (2022). Molecular prevalence of *Anaplasma marginale* and associated risk factors in beef cattle herds from Ohio: A cross-sectional study. *Journal of the American Veterinary Medical Association*, *1*(5), 1-5. https://doi.org/10.2460/javma.22.05.0204.

Fereig, R., Mohamed, S., Mahmoud, H., AbouLaila, M., Guswanto, A., Nguyen, T., Mohamed, A., Inoue, N., Igarashi, I., & Nishikawa, Y. (2017). Seroprevalence of *Babesia bovis, B. bigemina, Trypanosoma evansi,* and *Anaplasma marginale* antibodies in cattle in southern Egypt. *Ticks and Tick-borne Diseases, 8*(1), 125-131. https://doi.org/10.1016/j.ttbdis.2016.10.008.

Guarnizo, T., Alvarez, D., Díaz-Sánchez, A., Cabezas-Cruz, A., Gutiérrez, L., Marrero, S., & Corona-González, B. (2020). Epidemiology and genetic diversity of *Anaplasma marginale* in Zamora-Chinchipe, Ecuador. *Ticks and Tick-borne Diseases, 11*(3), Article *101380*. https://doi.org/10.1016/j.ttbdis.2020.101380.

Hairgrove, T., Schroeder, M. E., Budke, C. M., Rodgers, S., Chung, C., Ueti, M. W., & Bounpheng, M. A. (2015). Molecular and serological in-herd prevalence of Anaplasma marginale infection in Texas cattle. *Preventive veterinary medicine*, *119*(1-2), 1-9. https://doi.org/10.1016/j.prevetmed.2015.02.006

Hamou, S. A., Rahali, T., Sahibi, H., Belghyti, D., Losson, B., Goff, W., & Rhalem, A. (2012). Molecular and serological prevalence of *Anaplasma marginale* in cattle of North Central Morocco. *Research in Veterinary Science, 9*(3), 1318-1323. https://doi.org/10.1016/j.rvsc.2012.02.016

Hansmann, Y., Jaulhac, B., Kieffer, P., Martinot, M., Wurtz, É., Dukic, R., Boess, G., Michel, A., Strady, C., Sagez, J., Lefèbvre, N., Talagrand-Reboul, E., Argemi, X., & Martino, S. (2019). Value of PCR, serology, and blood smears for human granulocytic anaplasmosis diagnosis, France. *Emerging Infectious Diseases, 25*, 996-998. https://doi.org/10.3201/eid2505.171751.

Hanzlicek, G. A., Raghavan, R. K., Ganta, R. R., & Anderson, G. A. (2016). Bayesian space-time patterns and climatic determinants of bovine anaplasmosis. *PLoS One, 11*(3), Article e0151924. https://doi.org/10.1371/journal.pone.0151924

Hayyan, B. N. & Nasruddin, N. A., (2025). Exploring global research trends in bovine anaplasmosis: A bibliometric approach. In *AIP Conference Proceedings*, *3275*, 1. https://doi.org/10.1063/5.0248774

Hayyan, B. N., Sharma, R. S., Raimy, N., Nisha, M., Hussain, K., Busin, V. M., Jenvey, C.J., Cairns, C & Stear, M. J. (2020). Kinetics of IgA and eosinophils following a low-dose, predominantly *Haemonchus contortus* infection of Boer goats. *Parasite Immunology, 42*(6), Article e12707. https://doi.org/10.1111/pim.12707

Ierardi, R. A. (2025). A review of bovine anaplasmosis (*Anaplasma marginale*) with emphasis on epidemiology and diagnostic testing. *Journal of Veterinary Diagnostic Investigation*, 10406387251324180. https://doi.org/10.1177/10406387251324180

Jalali, S., Khaki, Z., Kazemi, B., Bandehpour, M., Rahbari, S., Razijalali, M., & Yasini, S. (2013). Molecular detection and identification of *Anaplasma* species in sheep from Ahvaz, Iran. *Iran Journal of Veterinary Research, 14*(1), 50-56. https://doi.org/10.22099/IJVR.2013.1389.

Jaswal, H., Bal, M. S., Singla, L. D., Gupta, K., & Brar, A. P. S. (2015). Pathological observations on clinical *Anaplasma marginale* infection in cattle. *Journal of Parasitic Diseases, 39*(3), 495-498. https://doi.org/10.1007/s12639-013-0384-4

Leal Filho, W., Ternova, L., Parasnis, S. A., Kovaleva, M., & Nagy, G. J. (2022). Climate change and zoonoses: a review of concepts, definitions, and bibliometrics. *International Journal of Environmental Research and Public Health, 19*(2), 893. https://doi.org/10.3390/ijerph19020893

Machado, R. Z., Silva, J. B. D., André, M. R., Gonçalves, L. R., Matos, C. A., & Obregón, D. (2015). Outbreak of anaplasmosis associated with the presence of different *Anaplasma marginale* strains in dairy cattle in the states of São Paulo and Goiás, Brazil. *Revista Brasileira de Parasitologia Veterinária, 24*(4), 438-446. http://dx.doi.org/10.1590/S1984-29612015078

Manap, M. A. H. A., Mustaza, N. S. A., Nasruddin, N. A., Abdullah, W. L. W., Yaakub, H., & Hayyan, B. N. (2024). Molecular prevalence of *Anaplasma marginale, Babesia bigemina,* and *Theileria orientalis* and their associations with Mafriwal cattle's age groups. *Journal of Advanced Veterinary and Animal Research*, *11*(2), Article 384. https://doi.org/10.5455/javar.2024.k787

Mastura, Y., Shariffah, N., Nor Aini, W., Muhamad, A. H. A., Mohd, H. A., & Chandrawathani, P., (2019). Growth performance of Mafriwal dairy cattle in Malaysia. *Malaysian Journal of Veterinary Research, 10*(1), 43-50.

Moniuszko-Malinowska, A., Dunaj, J., Andersson, M., Chmielewski, T., Czupryna, P., Groth, M., Grygorczuk, S., Zajkowska, J., Kondrusik, M., Kruszewska, E., & Pancewicz, S. (2021). Anaplasmosis in Poland - Analysis of 120 patients. *Ticks and Tick-borne Diseases, 12*(5), Article 101763. https://doi.org/10.1016/j.ttbdis.2021.101763.

Nur-Amalina, N., Nur-Sabrina, A. M., Muhamad-Ali, H. A. M., & Basripuzi, N. H. (2023). Bovine anaplasmosis: A systematic review with meta-analysis on the global prevalence. *Tropical Biomedicine*, *40*(4), 375-82. https://doi.org/10.47665/tb.40.4.001

Nur-Amalina, N., Nur-Sabrina, A. M., Muhamad-Ali, H. A. M., Mohammad-Sabri, A. R., Nor-Azlina, A. A., & Basripuzi, N. H. (2024). Molecular identification and phylogenetic analysis of *Anaplasma marginale* and *Anaplasma centrale* isolated from commercial Mafriwal cattle in Johor, Malaysia. *Tropical biomedicine*, *41*(4), 385-391. https://doi.org/10.47665/tb.41.S.001.

Nur-Sabrina, A. M., Muhamad-Ali, H. A. M., Nur-Amalina, N., Wan-Ladiana, W. A., Hasimah, H., Salma, C. W., Wan, K.L., & Hayyan, B. N. (2024). Relationship between parasitism with milk yield and body weight of Mafriwal cattle. *Veterinary Integrative Sciences, 22*(1), 141-159. https://doi. org/10.12982/VIS. 2024.012.

Ola-Fadunsin, S. D., Gimba, F. I., Abdullah, D. A., Sharma, R. S. K., Abdullah, F. J. F., & Sani, R. A. (2018). Epidemiology and risk factors associated with *Anaplasma marginale* infection of cattle in Peninsular Malaysia. *Parasitology International, 67*(6), 659-665. https://doi.org/10.1016/j.parint.2018.06.013

Oliveira, M. C. S., Alencar, M. M., Giglioti, R., Beraldo, M. C. D., Aníbal, F. F., Correia, R. O., Boschini, L., Chagas, A. C. S., Bilhassi, T. B., & Oliveira, H. N. (2013). Resistance of beef cattle of two genetic groups to ectoparasites and gastrointestinal nematodes in the state of São Paulo, Brazil. *Veterinary Parasitology, 197*(1-2), 168-175. https://doi.org/10.1016/j.vetpar.2013.06.021

Panandam, J. M., & Raymond, A. K. (2005). Development of the Mafriwal dairy cattle of Malaysia. *AGTR Case Study*. International Livestock Research Institute, Nairobi, Kenya. https://hdl.handle. net/10568/3599.

Paramanandham, K., Mohankumar, A., Suresh, K., Jacob, S., & Roy, P. (2019). Prevalence of *Anaplasma* species in India and the World in dairy animals: A systematic review and meta-analysis. *Research in Veterinary Science, 123*, 159-170. https://doi.org/10.1016/j.rvsc.2019.01.013.

Parvizi, O., El-Adawy, H., Melzer, F., Roesler, U., Neubauer, H., & Mertens-Scholz, K. (2020). Seroprevalence and molecular detection of bovine anaplasmosis in Egypt. *Pathogens, 9*(1), 64. https://doi.org/10.3390/pathogens9010064

Rodríguez-Vivas, R. I., Grisi, L., Pérez de León, A. A., Villela, H. S., Torres-Acosta, J. F. D. J., Fragoso Sánchez, H., Romero Salas, D., Rosario Cruz, R., Saldierna, F., & García Carrasco, D. (2017). Potential economic impact assessment for cattle parasites in Mexico. Review. *Revista Mexicana de Ciencias Pecuarias, 88*(1), 61-74. https://doi.org/10.22319/rmcp.v8i1.4305

Sajid, M. S., Siddique, R. M., Khan, S. A., Iqbal, Z., & Khan, M. N. (2014). Prevalence and risk factors of anaplasmosis in cattle and buffalo populations of district Khanewal, Punjab, Pakistan. *Global Veterinaria, 12*(1), 146-153. https://doi.org/10.5829/idosi.gv.2014.12.01.8252

Salinas-Estrella, Elizabeth, Itzel Amaro-Estrada, Mayra E. Cobaxin-Cárdenas, Jesús F. Preciado de la Torre, and Sergio D. Rodríguez. (2022). Bovine Anaplasmosis: Will there ever be an almighty effective vaccine? *Frontiers in Veterinary Science, 9*, 946545. https://doi.org/10.3389/fvets.2022.946545

Scariot, C., Scariot, J., De Souza Ramos, I., Gonçalves, L., Calchi, A., André, M., Machado, R., Costa, M., Kreutz, L., Zanella, R., & Vieira, M. (2022). Bovine anaplasmosis as a risk factor for retained placenta, mastitis, and abomasal displacement in dairy cattle. *Research in Veterinary Science*, *154*, 145-150. https://doi.org/10.1016/j.rvsc.2022.12.011.

Selim, A., Manaa, E., Abdelhady, A., Said, M. B., & Sazmand, A. (2021). Serological and molecular surveys of *Anaplasma* spp. in Egyptian cattle reveal high *A. marginale* infection prevalence. *Iranian Journal of Veterinary Research, 22*(4), Article 288. https://doi.org/10.22099/ijvr.2021.40587.5879

Seo, M., Ouh, I., Lee, H., Geraldino, P., Rhee, M., Kwon, O., & Kwak, D. (2018). Differential identification of *Anaplasma* in cattle and potential of cattle to serve as reservoirs of *Anaplasma capra*, an emerging tick-borne zoonotic pathogen. *Veterinary Microbiology, 226*, 15-22. https://doi.org/10.1016/j.vetmic.2018.10.008.

Shabana, I., Alhadlag, N., & Zaraket, H. (2018). Diagnostic tools of caprine and ovine anaplasmosis: A direct comparative study. *BMC Veterinary Research, 14(1)*, Article 165. https://doi.org/10.1186/s12917-018-1489-x.

Sharma, A., Singla, L., Kaur, P., & Bal, M. (2015). PCR and ELISA *vis-à-vis* microscopy for detection of bovine anaplasmosis: A study on associated risk of an upcoming problem in North India. *Scientific World Journal, 2015*(1). Article 352519. https://doi.org/10.1155/2015/352519.

Shkap V., Leibovitz B., Krigel Y., Molad T., Fish L., Mazuz M., Fleiderovitz L., & Savitsky I. (2008). Concomitant infection of cattle with the vaccine strain *Anaplasma marginale* ss *centrale* and field strains of *A. marginale. Veterinary Microbiology, 130*, 277-284. https://doi.org/10.1016/j.vetmic.2008.02.013

Singh, H., J., Haque, M., Singh, N., & Rath, S. (2012). Molecular detection of *Anaplasma marginale* infection in carrier cattle. *Ticks and Tick-borne Diseases, 3*(1), 55-58. https://doi.org/10.1016/j.ttbdis.2011.10.002.

Smith, B.P. (Ed.). (2015). *Large animal internal medicine-E-book*. Elsevier Health Sciences (pp. 1054-1056).

Spare, M., Hanzlicek, G., Wootten, K., Anderson, G., Thomson, D., Sanderson, M., Ganta, R., Reif, K., & Raghavan, R. (2020). Bovine anaplasmosis herd prevalence and management practices as risk-factors associated with herd disease status. *Veterinary Parasitology: Regional Studies and Reports*, *277S*, Article 100021. https://doi.org/10.1016/j.vpoa.2019.100021

Sunder, J., Sujatha, T., De, A. K., Bhattacharya, D., Perumal, P., Kumar, L., & Kundu, A. (2019). Temporal and spatial epidemiological trend of emerging livestock diseases of Andaman and Nicobar Islands, India. *International Journal of Applied Research in Veterinary Medicine, 17*(2).

Tucker, T., Aly, S., Maas, J., Davy, J. & Foley, J. (2016). Investigation of *Anaplasma marginale* seroprevalence in a traditionally managed large California beef herd. *Veterinary Medicine International, 2016*, Article 6186078. https://doi.org/10.1155/2016/6186078

Vetrivel, D., Pandian, A., & Shree, J. (2017). A study on predisposing factors for the prevalence of anaplasmosis in dairy cattle. *Journal of Entomology and Zoology Studies, 5*, 1228-1230.

Vlasova, A. N., & Saif, L. J. (2021). Bovine immunology: Implications for dairy cattle. *Frontiers in Immunology, 12*, Article 643206. https://doi.org/10.3389/fimmu.2021.643206

World Organization for Animal Health. (2018). Bovine anaplasmosis. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 6th edition. pp.99-1003. OIE.

Zim, M., Ahmed, N., Ahmed, M., Miah, M., Sajib, M., Rabbi, M., Rahman, M., Roy, B., & Talukder, M. (2024). First seroprevalence survey of bovine anaplasmosis: An emerging tick-borne disease in commercial livestock and dairy farms in Bangladesh. *Parasitology*, *151*, 992-1000. https://doi.org/10.1017/s0031182024001495.