Full Length

REAL-TIME PCR DETECTION OF *ANAPLASMA SPP*. IN DROMEDARY CAMELS IN QATAR

M. Kavitha[1]*, S. Uma[2], M. Samar[3], D. Sudha[4], P. Vanathi[5]

Department of Genetics
Tharb Camel Hospital
Qatar

ABSTRACT

In recent years, Qatar's population has grown quickly, which has led to a rise in camels (Camelus dromedarius, one-humped). This increased the risk of infection. Six species of bacteria of the genus Anaplasma are responsible for the tick-borne disease anaplasmosis, which affects people and livestock. The most significant species for human and veterinary health are Anaplasma marginale and Anaplasma phagocytophilum. The study aimed to detect Anaplasma spp in camel blood samples, utilizing RT-PCR analysis with genetic PCR solution kits [GPS]. A total of 800 camel blood samples collected in an aseptic condition from the jugular vein in 5ml EDTA tubes were used for bacterial DNA isolation. The thermal profile was set up with initial denaturation at 95°C for 1 min, denaturation at 95°C for 15 s of 40 cycles, and extension at 60°C for 60 s. The amplification curve shows the absolute quantification. A mean CT value of 28.5 was considered positive, indicating the presence of Anaplasma spp. This demonstrates the widespread occurrence of anaplasmosis in the region and poses a risk to public health as well as the economy. To maximize future strategic control programs and stop expansion to nearby nations, monitoring, species and subspecies identification, and control measure implementation are crucial tools.

Key words: Anaplasmosis, dromedary camel, Anaplasma spp, RT-PCR, CT value**.**

Received :13.06.2025 Revised : 10.09.2025 Accepted : 12.09.2025

INTRODUCTION

Dromedary camels (*Camelus dromedarius*) are essential as a source of meat, milk, and transportation, for the economy and way of life in arid and semi-arid areas. However, tick-borne illnesses (TBDs), especially those brought on by obligatory intracellular bacteria of the genus *Anaplasma* (Order: Rickettsiales; Family: *Anaplasmataceae*), are progressively compromising their well-being and productivity. *Anaplasma ovis, Anaplasma marginale*, and other genetically different strains of the ruminant-infecting *Anaplasma* species have been found in camels in Asia, the Middle East, and Africa. Anaplasmosis is a tick-borne disease of considerable

[1]Lab In-Charge, A1 Shumookh Integrated Service, Shahaniya - Qatar. *Corresponding Author Email: kavitharaj.m@gmail.com
[2]Research and Development, Sigma Biocore Innovations, Ambur, Tamil Nadu, [3]Researcher
[4]Assistant Professor, Department of Biochemistry, Islamiah Women's Arts and Science College, Vaniyambadi - 635751
[5]Professor and Head, Department of Microbiology, Bharathidasan Arts and Science College, Erode.

veterinary and public health importance, caused by obligate intracellular, Gram-negative bacteria of the genus *Anaplasma*, which belong to the α-proteobacteria group within the order Rickettsiales (Kocan *et al*., 2010). This genus includes several species that affect a wide range of hosts: *A. marginale, A. centrale*, and *A. bovis* primarily infect cattle; *A. ovis* causes ovine and caprine anaplasmosis; *A. platys* is associated with canine infectious cyclic thrombocytopenia; and *A. phagocytophilum*, the only zoonotic species, is responsible for human granulocytic anaplasmosis and tick-borne fever in ruminants (Torina *et al*., 2012; Atif *et al*., 2021). These pathogens, which often infect hematopoietic cells and replicate within parasitophorous vacuoles, can cause anemia, fever, weight loss, and even mortality, particularly in older or immunocompromised animals. Transmission occurs mainly through ticks but may also result from mechanical routes and contaminated instruments. Anaplasmosis is especially prevalent in tropical and subtropical climates and represents a major constraint on livestock productivity, particularly among smallholder farmers, due to economic losses from reduced animal performance and increased veterinary costs (Abdallah *et al*., 2020). Although camels (*Camelus dromedarius*) are generally resilient animals, emerging evidence suggests that they may harbor *Anaplasma spp.*, raising concerns about their epidemiological role as potential reservoirs. *Anaplasma spp.* infections in camels are frequently asymptomatic, which results in underdiagnosis and a hidden infection reservoir. Fever, lethargy, weight loss, and anemia are examples of clinical indications that can be confused with other illnesses when they occur. The sensitivity and specificity of conventional diagnostic techniques, such as serological testing or microscopic analysis of stained blood smears, are insufficient to identify low-level infections or distinguish closely similar species (de la Fuente *et al.,* 2005).

Molecular techniques, especially real-time quantitative PCR (qPCR), have become the gold standard for the sensitive and specific detection of *Anaplasma* DNA in blood samples, enabling identification of subclinical infections and more accurate surveillance of disease prevalence (Torina *et al*., 2012). In this study, the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, USA) was utilized to detect *Anaplasma spp.* in camel blood specimens. This high-throughput platform offers advanced analytical capabilities and enhanced sensitivity, making it ideal for both diagnostic and epidemiological purposes (Thermo Fisher Scientific, 2023). Our objective was to assess the presence of *Anaplasma spp.* in camels using species-specific primers and probes, thereby contributing to improved understanding and control of anaplasmosis in the region.

MATERIALS AND METHODS

Study location

The region investigated in Al Shahaniya, Qatar, has a desert climate with an arid and hot summer characterized by temperatures ranging between 25 and 46°C, and a relatively mild winter, with minimal rainfall.

Sample collection

800 blood samples from both healthy and diseased camels (*Camelus dromedarius*) were taken in total. Between March and September of 2021, all samples were gathered from nearby camel farms in Qatar's Alshahaniya area. Samples were rapidly preserved after collection and brought to the lab in an icebox for further analysis.

Isolation of Bacterial DNA

Extraction of genomic DNA was done using three commercial kits (QIA amp DNA Micro Kit, Qiagen, Hilden, Germany) and by the Magnapure compact nucleic acid isolation kit (Roche). King Fisher Duo prime kit. Following the manufacturer's instructions.

Real-time PCR

In order to identify the presence of bacteria, the extracted DNA was used for the real-time PCR analysis. Each DNA sample was subjected to analysis for the presence of *Anaplasma spp, Coxiella burnetii, Theileria spp, Babesia spp,* and *Brucella spp.* Individual kits were used to detect the presence of bacteria by amplification using the SYBR Green real-time PCR (rt-PCR) assay in Quantsudio 6 Flex (Applied Biosystem). For each of the aforementioned organisms, the PCR mix was prepared using a GPS (Genetic PCR Solutions) kit. The total volume of PCR mix was carried out with Master mix(5x), primer-probe, water, and 3.8 µL of DNA, making up the PCR reaction mix, which has a total volume of 15 µL. Both negative (no DNA) and control positive (pathogen-positive kit control) DNA were used in all PCR experiments. The thermal profile was set up as follows, with initial denaturation at 95 °C for 1 min, denaturation at 95 °C for 15 s of 40 cycles, and extension at 60°C for 60 s (Table 1).

Electrophoresis

PCR products were examined on 1 % agarose mix. Add (1:5µL) of loading buffer and PCR product and 50 base pair Ladder 6µL, adjust the mode to 100V and time to 40min. Transfer the gel onto the UV illuminator and check the separated bands. The stained bands were visualized on iBright gel documentation system.(Thermo Fisher).

RESULTS

A total of 800 camel blood samples were analyzed for the presence of *Anaplasma spp.* using species-specific SYBR Green-based real-time PCR assays on the QuantStudio 6 Flex Real-Time PCR System. Out of these, 445 samples (55.6%) tested positive for *Anaplasma spp.,* indicating a high prevalence of infection within the sampled population. The positive samples exhibited a mean cycle threshold (Ct) value of approximately 27.77, suggesting a moderate to high concentration of bacterial DNA in most of these cases (Fig.1). These findings highlight the significant presence of *Anaplasma spp.* circulating among camels in the Al Shahaniya region. In addition to the positive samples, 67 samples (8.3%) were classified as "suspect." These samples

showed late amplification curves with Ct values typically ranging from 35 to 38, which is near the limit of detection for the assay. The remaining 288 samples (36.0%) showed no amplification signal within the defined Ct cutoff, and were therefore classified as negative. These negative results suggest the absence of detectable Anaplasma DNA, though the possibility of very low pathogen loads below the assay's detection threshold cannot be entirely ruled out.

Electrophoresis Confirmation

Selected PCR products were further validated via electrophoresis on a 1% agarose gel. Clear and distinct DNA bands were observed for samples that showed qPCR amplification, confirming the specificity of the PCR products. No bands were detected in negative controls, ruling out contamination. (Fig.2)

DISCUSSION

The current study demonstrates a high prevalence of Anaplasma spp. infection among camels in the Al Shahaniya region of Qatar, with 55.6% of tested samples yielding positive results via real-time PCR. This finding underscores the significant role camels may play as potential reservoirs for Anaplasma spp., and raises public health and veterinary concerns, particularly in arid environments where camels are of economic and cultural importance. The relatively high proportion of positive samples aligns with findings from similar studies in other arid or semi-arid regions. For example, a study conducted in Saudi Arabia by Al Khodair *et al*., (2020) reported Anaplasma DNA in 48.2% of camel blood samples, while research in Sudan documented even higher prevalence rates, exceeding 60% in certain regions (El Imam *et al*., 2017). These regional similarities suggest a common epidemiological pattern in Middle Eastern and North African countries, possibly due to overlapping ecological factors such as the widespread presence of competent tick vectors, close camel herding practices, and environmental conditions favorable to tick survival and transmission.

The mean Ct value of 27.77 observed in this study indicates a moderate to high bacterial load in positive samples. This is significant because lower Ct values are typically associated with active or acute infections, which may coincide with more prominent clinical signs in the affected animals. Although clinical data were not systematically collected in this study, field observations suggest that some camels with positive PCR results exhibited nonspecific signs such as lethargy, fever, and decreased appetite. These signs are commonly associated with Anaplasma infections, although confirmation would require further hematological and serological evaluation.

Interestingly, 8.3% of the samples were classified as "suspect" due to high Ct values (35-38), which approach the detection limit of the assay. Such results may be due to low DNA concentrations, possibly resulting from early infection stages, sample degradation, or inhibitory substances. While not confirmed as positive, these suspect results warrant further analysis, including re-

extraction, repeat PCR, or use of alternative confirmatory tests. These suspect results may reflect low pathogen load, possibly due to early-stage infection, chronic carrier status, or sample degradation. As suggested in previous studies (Kocan *et al*., 2010), chronic infections with low bacteremia are common in *Anaplasm*a-infected ruminants and may go undetected without sensitive molecular techniques. Re-testing of these samples or use of a confirmatory test such as nested PCR or sequencing could help resolve their infection status. The 36.0% of samples that tested negative may truly be free of *Anaplasma spp.* infection or may carry pathogen levels below the detection threshold of the assay. It is also possible that infections by other *Anaplasma* species not targeted by the assay were present, or that DNA degradation occurred during sample transport and storage despite the cold chain precautions. $\Delta Rn = Rn+ - Rn-$, where: Rn+ is the fluorescence signal from the reaction well after amplification. Rn− is the baseline signal before significant amplification occurs. It reflects the height of the amplification curve. A high ΔRn, with a good signal, indicates strong amplification

Even though the GPS kits are for several species of *Anaplasma*, qPCR yields the expected amplification in real time, we employed gel electrophoresis after PCR to confirm the size of the PCR product. The lanes were approximately the same height as in the ladder, indicating successful amplification of *Anaplasma spp*. Lane 1 contains the DNA ladder, which confirms the approximate size of the bands in the sample lanes. Bright bands in the sample lanes correspond to the expected size of 422 bp, while faint or absent bands indicate negative or low-concentration samples.

These findings have practical implications. The high infection rate underscores the importance of regular screening and tick control programs, particularly in herd management systems where animals are frequently exposed to tick-infested environments. Given that *Anaplasma spp.* are primarily transmitted by ticks, particularly *Hyalomma spp.,* in desert environments, controlling tick populations may significantly reduce infection rates. Moreover, the potential zoonotic risk, although low for some *Anaplasma* species, warrants closer surveillance, especially among workers in close contact with camels, such as herders and veterinarians.

Overall, the findings indicate that more than half of the tested camel population harbored *Anaplasma* DNA, underscoring a potential endemic transmission cycle in the region. The combination of positive, suspect, and negative results reflects the variability in infection status among individual animals and supports the importance of continuous monitoring and control strategies for tick-borne pathogens in camel herds.

According to data from this study, camels from Qatar's Al Shahaniya province have *Anaplasma spp* infections, As Qatar's economy continues to grow, and people and their pets are moving in and out of the country. This factor may increase the likelihood of introducing and spreading infection. Therefore, regular veterinary

checkups and appropriate prophylaxis should be encouraged for Qatari pets and camels. Additionally, when arranging travel for animals to and from the Middle East, thorough diagnostic screening should be conducted.

CONCLUSIONS

This study expands the information on the distribution routine screenings and prophylactic measures are strongly recommended. For safe trading of animals and animal products, comprehensive epidemiological studies will be useful to clarify the course of anaplasmosis, considering current and future dispersion of the vertebrate host and tick vector. This will support the establishment of effective countermeasures for the future.

ACKNOWLEDGEMENT

All authors are thankful to the Management, Dr. Ashraf El-Sharabasy Coo, and Mr. L.S.Rajan, Senior Lab tech of Tharb Camel Hospital, Qatar for providing the space to carry out this research work. I would like to praise and thank God the Almighty, who has granted countless blessings, knowledge, and opportunity to accomplish this work.

DECLARATIONS

FUNDING

This research established no external funding

CONFLICT OF INTEREST

No conflicts of interest.

Table.1. Thermal Profile

Cycle	Step	Temperature	Time	Description
1X	1	95ºC	15 min	Initial denaturation
40X	2	95ºC	15 s	Template denaturation
	3	60ºC	60 s	Annealing
1X	5	72ºC	7 min	Final extension

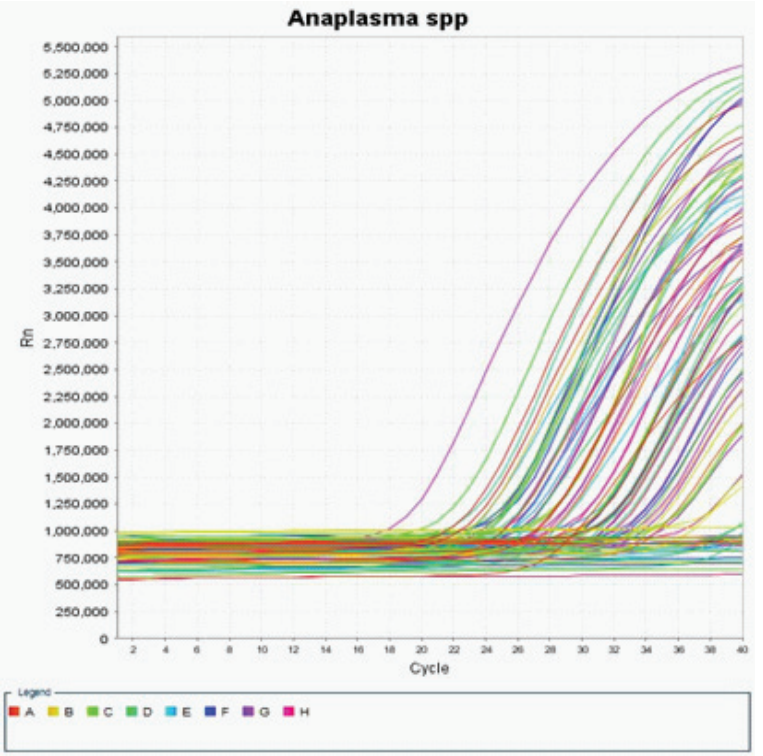

Fig.1. Amplification curve (Rn vs Cycle number) of *Anaplasma* spp

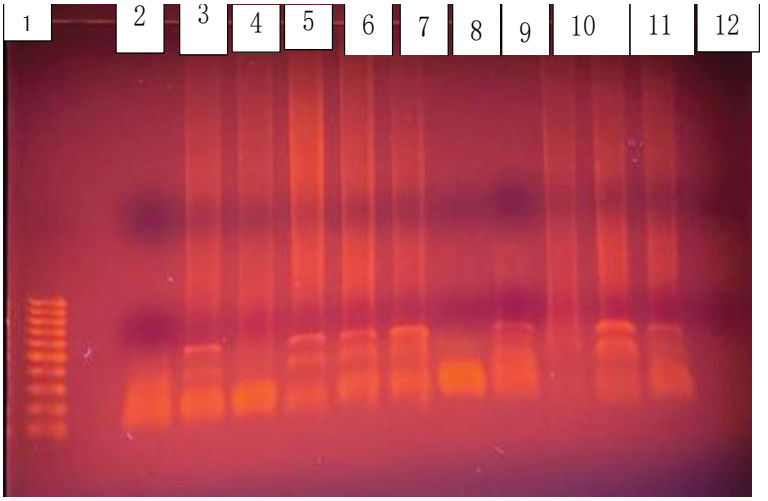

Fig.2. Lane 1- DNA ladder, Lane 2-7 & 9,11-12 Sample DNA [Lane 4-positive control, Lane 10-Negative control]

REFERENCE

Abdallah, M.M., Abdel-Razik, A.R., Ahmed, S.A., El-Dakhly, K.M. and El Namaky, A.H. (2020). Molecular detection and phylogenetic characterization of *Anaplasma spp.* in camels from Egypt. *Ticks and Tick-borne Diseases*, **11**(4): 101402.

Al Khodair, K.M., Alanazi, A.D., Alouffi, A.S., Alyousif, M.S., Alshahrani, M.Y. and Al-Khalifa, M.S. (2020). Molecular detection of *Anaplasma spp.* in dromedary camels from Saudi Arabia. *Veterinary World*, **13**(2): 325–330.

Atif, F.A., Mehnaz, S., Qamar, M.F., Roheen, T., Sajid, M.S., Ehtisham-Ul-Haque, S., Kashif, M. and Ben Said, M. (2021). Epidemiology, diagnosis, and control of canine infectious cyclic thrombocytopenia and granulocytic anaplasmosis: emerging diseases of veterinary and public health significance. *Veterinary Sciences*, **8**(12): 312.

De la Fuente, J., Torina, A., Naranjo, V., Nicosia, S., Alongi, A., La Barbera, G., Caracappa, S. and Kocan, K.M. (2005). Infection with *Anaplasma* phagocytophilum in a dog from Sicily, Italy: case report. *Annals of the New York Academy of Sciences*, **1063**(1): 346–348.

El Imam, A.H., Gameel, A.A., El Hussein, A.M. and Taha, K.M. (2017). Prevalence and molecular characterization of *Anaplasma spp.* in camels in Sudan. *Parasites and Vectors*, **10**(1): 312.

Kocan, K.M., de la Fuente, J., Blouin, E.F. and Garcia-Garcia, J.C. (2010). *Anaplasma* marginale (Rickettsiales: *Anaplasmataceae*): Recent advances in defining host–pathogen adaptations of a tick-borne rickettsia. *Parasitology*, **137**(8): 1241–1250.

Thermo Fisher Scientific, 2023. QuantStudio™ 6 Flex Real-Time PCR System—User Guide. [online] Available at: https://www.thermofisher.com [Accessed 13 Jun. 2025].

Torina, A., Alongi, A., Naranjo, V., Scimeca, S., Nicosia, S., Di Marco, V., Caracappa, S. and de la Fuente, J. (2012). Infection of horses with *Anaplasma* phagocytophilum in Sicily, Italy. *Veterinary Journal*, **191**(1): 121–123.