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Molecular evidence of the high prevalence of *Anaplasma* species in camel populations in Pakistan

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ABSTRACT

Anaplasmosis is a tick-borne disease caused by *Anaplasma* species, impacting livestock and wildlife worldwide. Despite previous studies on the molecular epidemiology of *Anaplasma* in camels across various countries, limited data exist for Pakistan. This study aimed to determine the prevalence and genetic diversity of *Anaplasma* species in camels from Bahawalpur, Pakistan, using molecular techniques. A total of 94 blood samples were collected from camels via jugular venipuncture and subjected to DNA extraction. Polymerase chain reaction (PCR) was performed targeting the 16S rRNA gene, followed by sequencing and phylogenetic analysis. The overall prevalence of *Anaplasma* species was found to be 40.4%, with 38 samples testing positive. BLAST analysis revealed 17 isolates as *A. capra*, 14 as *A. platys*, 3 as *A. ovis*, 2 as *A. marginale*, and 2 as *A. camelii*. Phylogenetic analysis using the Maximum Likelihood method grouped the identified Anaplasma species into four distinct clades, with *A. capra* and *A. marginale* showing a close genetic relationship. Univariable statistical analysis revealed no significant associations between Anaplasma prevalence and host factors such as: sex (P=0.5457), age (P=0.464), body condition (P=0.3269), pregnancy status (P=0.7530), or parity (P=0.8057). The results suggest that camels could act as potential reservoirs for multiple *Anaplasma* species, contributing to the epidemiological complexity of anaplasmosis in Pakistan. This study provides the first molecular characterization of *Anaplasma* species in Pakistani camels, highlighting the need for further research on transmission dynamics and potential zoonotic implications. Enhanced surveillance and preventive strategies are recommended to mitigate the impact of anaplasmosis in camel populations and associated livestock.

Key words: *Anaplasma*; camels; prevalence; phylogenetic analysis; Pakistan

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Introduction

Anaplasma species are obligate intracellular bacteria that cause a variety of tick-borne illnesses that are significant for both humans and animals worldwide. They are members of the family *Anaplasmataceae*, in the order *Rickettsiales* (KOLO, 2023). Anaplasmosis is a disease that causes emaciation in animals, particularly in ruminants, including sheep, goats, and cattle (OIE, 2017). However, *Anaplasma centrale*, *Anaplasma marginale*, *Anaplasma platys*, *Anaplasma bovis*, and *Anaplasma phagocytophilum* may also infect camels (KOCAN et al., 2004; LORUSSO et al., 2016). The disease is transmitted both mechanically by insect vectors (biting flies and ticks) and iatrogenically by contaminated needles. More than 20 species of blood-sucking ticks are found, e.g. hard ticks such as *Rhipicephalus* spp., *Boophilus* spp., *Dermacentor* spp., and *Ixodes* (DANTAS-TORRES and OTRANTO, 2016; CONSTABLE et al., 2017).

In camels, anaplasmosis is often a co-infection or a subclinical illness. Clinical symptoms seen may be anemia, jaundice, fever, anorexia, emaciation, mild ataxia, or enlarged lymph nodes (SELMI et al., 2019; RAR et al., 2021). The lack of pathognostic clinical signs makes a definite diagnosis challenging. Thus, awareness and suitable diagnostic techniques in disease-endemic areas are crucial (ALSUBKI et al., 2022). Confirmatory techniques used are microscopy, serological and molecular procedures. These techniques can be employed independently or in combination. Romanowsky-stained blood smears are typically used in animals suspicious for anaplasmosis. Although considered as the gold standard method it fails to identify subclinical carriers (AUBRY and GEALE, 2011). *Anaplasma* specific antibodies can also be found using enzyme-linked immunosorbent assay (ELISA), a complement fixation test, a card agglutination test, and an indirect fluorescent antibody test (SELIM et al., 2021; AL-MOSOY, 2023). Known drawbacks are low sensitivity and the inability to distinguish between the various types of *Anaplasma* (PARVIZI et al., 2020). For identification of *Anaplasma* at the species level and phylogenetic studies, PCR is used (BATTILANI et al., 2017; BEN-SAID et al., 2018).

Various studies have been conducted on the molecular epidemiology of anaplasmosis in camels from Iran, Nigeria, Egypt, Iraq, Malaysia, China, and various other countries (LI et al., 2015; BAHRAMI et al., 2018; KOH et al., 2018; FARAJ, 2019; ONYICHE et al., 2020; SALMAN et al., 2022). This knowledge on camel anaplasmosis is lacking in Pakistan. In this study, we conducted a molecular survey to determine the prevalence of different *Anaplasma* species in camels, and evaluate the relationship between many proposed variables influencing the prevalence of camel anaplasmosis.

Materials and methods

Study location. This study was conducted in Bahawalpur, which is one of the southern districts of the Punjab province of Pakistan with a camel production history. The district has a desert environment with hot summers and mild winters. This climate promotes tick population growth, especially during the warmer months. Sampling of blood from camels was carried out during May 2021.

Sampling and DNA extraction. The convenience simple random sampling technique was carried out and 94 blood samples (about 3mL) were drawn directly from the jugular vein into EDTA-coated vacutainers and labeled appropriately. Animal demographic information, such as age, sex, body condition score, pregnancy status and parity number, were recorded in a questionnaire. The blood was transported to the Laboratory of Veterinary Preventive Medicine and Public Health, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan, under cool conditions. DNA extraction from whole blood was carried out using a GeneJET Genomic DNA Purification Kit (Thermo Scientific™, K0722) strictly following the manufacturer's instructions.

PCR and sequencing. Amplification of the partial 16S rRNA gene (345 bp) was carried out using a previously designed forward primer (5′-GGTACCYACAGAAGAAGTCC-3′) and reverse primer (5′-TAGCACTCATCGTTTACAGC-3′) (PAROLA et al., 2000). The PCR conditions used are shown in Table 1.

Table 1. Primer sequences and PCR cycling conditions for the amplification of the Anaplasma 16S rRNA gene

Gene	Primer sequence (5'-3')		Conditions					Amplicon length	Reference
			Initial denaturation	Denaturation	Annealing	Extension	Final Extension		
			35 cycles						
16S rRNA	Forward	GGTACCYACAGAAGAAGTCC	95°C for 3 min	95°C for 15 Sec	55°C for 15 Sec	72°C for 30S	72 °C for 5 min	345 bp	[22]
	Reverse	TAGCACTCATCGTTTACAGC							

The PCR reaction had a total volume of 25 µl, containing 5× Taq buffer at 5 µl concentration, together with 10 pmols of each primer, 0.2 mM dNTPs, 0.5 µl Ex Taq DNA polymerase at 5 U/µl (TaKaRa, Kusatsu, Japan) and 2 mM MgCl2, genomic DNA extract at ≥ 20 ng and RNAse-free water. The agarose gel contained 1.5% (w/v) concentration for amplicon visualization. The sequencing process required five microliters of amplicon, while the remaining amplicon material went toward sequencing analysis. The PCR products were positioned then cleaned using the GeneJet Gel extraction kit (Thermo ScientificTM, K0691) before Macrogen (Korea) performed the sequencing process.

Molecular analysis. DNA sequence alignment and manual analysis for misread nucleotide bases were performed using Unipro UGENE software (OKONECHNIKOV et al., 2012). Isolate identity was validated using the NCBI BLAST program. The NJ model and Jukes-Cantor nucleotide distance measure were used to generate a phylogenetic tree using MEGA 11 (TAMURA et al., 2021). The 1000 bootstrap repeats provided statistical support for branch specificity. To assess association strength, the *Ehrlichia* sequence was employed as an outgroup.

Statistical analysis. Data were categorized and a 95% confidence interval (CI) was obtained using the method given by NEWCOMBE (1998). The chi-square test and odds ratios were calculated using Statistix and WinPepi software (ABRAMSON, 2011).

Results

The study revealed a 40.4 percent prevalence of *Anaplasma* species in selected camels from the southern region of Pakistan. A total of 38 samples tested PCR-positive, and sequencing, followed by BLAST analysis identified 17 isolates as *A. capra*, 3 as *A. ovis*, 2 as *A. marginale*, 2 as *A. camelli*, and 14 as *A. platys*. The generated sequences, after alignment with the reference sequences present in GenBank, were deposited in the NCBI GenBank repository under the accession numbers PV249364-PV249377 (*A. platys*), PV249384-PV249386 (*A. ovis*), PV249403-PV249419 (*A. capr*a), PV258749-PV258750 (*A. camelli*) and PV262380-PV262381 (*A. marginale*). Additionally, the study examined the impact of various risk factors on the prevalence of *Anaplasma* species, including sex, age, body score, pregnant status, and parity. The results are presented in detail for each risk factor in Table 2.

The prevalence of *Anaplasma* infection in camels is not significantly influenced by their sex. Although female camels exhibited a slightly higher prevalence of 44.4% (95% CI: 32-57.62) compared to males, 35% (95% CI: 22.13-50.49), the chi-square test showed no significant association (P=0.5457). This implies that sex alone does not play a major role in *Anaplasma* prevalence in camels.

There were no significant associations between age and *Anaplasma* prevalence in camels. Even though camels older than six years had the highest prevalence of 48.3% (95% CI: 31.39-65.57), the chi-square test resulted in a non-significant P-value (P=0.464). Camels aged between 3 and 6 years, and camels up to 3 years did not differ significantly in

prevalence. These findings suggest that while older camels may have a slightly higher prevalence, age alone is not a decisive factor for *Anaplasma* infection.

Body condition scores showed a trend towards significance for influencing *Anaplasma* prevalence. Camels with poor body condition had the highest prevalence of 58.6% (95% CI: 40.74-74.49), followed by those with very weak condition with 50% (95% CI: 9.45-90.55). However, the chi-square test yielded a p-value of 0.3269, indicating that the association was not statistically significant. This suggests that while there may be a trend, other factors could be at play in determining *Anaplasma* prevalence.

The pregnancy status of the animal was also investigated to check whether this factor has a role in the prevalence of *Anaplasma* species. However, there was no significant correlation found between pregnancy status and *Anaplasma* prevalence. The prevalence of *Anaplasma* in non-pregnant camels was 47.1% (95% CI: 31.45-63.26), while pregnant camels had a prevalence of 40% (95% CI: 21.88-61.34). The chi-square test result showed a non-significant p-value of 0.7530, indicating that pregnancy status alone does not impact *Anaplasma* prevalence in camels.

Table 2. Univariable analysis of camel demographic and physiological factors associated with Anaplasmosis prevalence in Pakistan

Variable	Category	Positive/Tested	Prevalence (95% CI)	OR (95% CI)	Chi-Square (p-value)
Sex	Female	24/54	44.4 (32-57.62)	1.27 (0.59-2.74)	0.37 (0.5457)
	Male	14/40	35 (22.13-50.49)	Ref.	
Age	More than 6 Year	14/29	48.3 28 (31.39-65.57)	1.86 (0.66-5.23)	1.53 (0.4644)
	>3 to ≤ 6 Years	17/38	44.7 (30.15-60.30)	1.73 (0.64-4.67)	
	Up to 3 years	7/27	25.9 (13.17-44.68)	Ref.	
Body Condition Score	Poor	17/29	58.62 (40.74-74.49)	3.81 (0.80-18.05)	3.45 (0.3269)
	Very weak	1/2	50 (9.45-90.55)	3.25 (0.29-36.95)	
	Moderate	18/50	36 (24.14-49.86)	2.34 (0.50-10.85)	
	Good	2/13	15.4 (4.32-42.23)	Ref.	
Pregnancy Status	Non-pregnant	16/34	47.06 (31.45-63.26)	1.18 (0.43-3.19)	0.10 (0.7530)
	Pregnant	8/20	40 (21.88-61.34)	Ref.	
Parity	2nd	4/7	57.14 (25.04-84.18)	2.86 (0.29-28.03)	0.98 (0.8057)
	1st	9/17	52.94 (30.96-73.83)	2.65 (0.32-21.92)	
	Zero	10/25	40 (23.40-59.26)	2.00 (0.25-16.15)	
	3rd	1/5	20 (3.62-62.45)	Ref.	

The number of times a camel has given birth also did not show any significant impact on the prevalence of *Anaplasma* species. Camels in the second parity group had the highest prevalence of 57.14% (95% CI: 25.04-84.18), followed by those in the first parity group with 52.9% (95% CI: 30.96-73.83). However, after conducting the chi-square test, the p-value was found to be non-significant at 0.8057, indicating that parity is not a determining factor for *Anaplasma* prevalence.

A total of 17, 14, 3, 2, and 2 isolates showed the highest similarity with Anaplasma capra, A. platys, A. ovis, A. marginale, and A. camelii, respectively. The identity of each sequence was confirmed through BLAST analysis. The percentage identity of each Anaplasma sequence obtained in this study was 100% when compared to the respective sequences already deposited in the GenBank database.

We employed both the Maximum Likelihood technique and the Jukes-Cantor model to investigate evolutionary history. The tree has a best maximum likelihood score of -4482.14. A heuristic search was initiated with multiple trees that were produced through automated execution of Neighbor-Join and BioNJ algorithms on the Jukes-Cantor model-based distance matrix estimation, followed by selection of topologies with optimal log likelihood scores. Each internal node in the tree features the representation of sites containing at least 1 unambiguous base in at least 1 sequence for its descendent clades. The phylogenetic tree revealed 4 distinct clades (Fig. 1). Clade-1 and clade-3 were the most diverse. Clade-1 chiefly consisted of *A. marginale*, *A. centrale*, *A. capra* and *A. ovis* sequences, while in clade-3 *A. marignale*, *A. capra*, *A. ovis*, *A. platys* and *A. bovis* were present. This clade harbored *Anaplasma* species sequences from Australia, China, Israel, Italy, Japan, the Netherlands, the Philippines, South Korea and Uganda. Clade 1 and clade 3 were formed by 14 (36.84%) and 8 (21.05%) sequences isolated in this study. *Anaplasma* species sequences from the USA (MK736870), South Korea (MH794247) and China (MH255939) were also present in this cluster. In clade-2, *A. capra* sequences originated in the current study were present. Clade-4 consisted of a total of 14 (36.84%) sequences of *A. platys* (n=12) and *A. camelli* (n=2) found in this investigation. In this study, no sample was positive for *A. bovis*, *A. centrale* and *A. phagocytophilum*. *Anaplasma platys* (Iran and Egypt) and *A. camelli* (Kenya) were also clustered in the same clade. All sequences showed a distant place from *Ehrlichia chaffensis* which was used as an outgroup.

A Maximum Likelihood method-based phylogenetic tree demonstrates the evolutionary connections between *Anaplasma* species which exist in Pakistani camels. Multiple *Anaplasma* species separated into four genetic lineages based on the analytical results. The arrangement of isolates delivers important genetic information regarding *Anaplasma*'s variations and its relationship with its camel hosts, as well as transmission mechanisms.

The Clade 1 collection brings together *Anaplasma marginale* along with *Anaplasma capra* and their reference sequences together with isolated strains from the study subjects. The Pakistani *A. capra* isolates show phylogenetic grouping with *A. marginale* which indicates a possible genetic link between these microorganisms. Most of the phylogenetic branches maintain values higher than 90%, which gives powerful evidence for genealogical relationships. This clade contains *A. capra* despite the fact that this bacterium primarily infects small ruminants, which suggests camels have adapted the bacterium through evolution or transmission occurring across different species.

Clade-2 contains *Anaplasma centrale* with its fellow members *Anaplasma ovis* and several *A. capra* isolates retrieved from Pakistan. The clade-2 classification contains *A. ovis* infections which exclusively affect sheep and goats, as well as *A. ovis* in a distinct separate cluster. Analysis of camel-derived *A. capra* with these species suggests camels could play a role as intermediate hosts or reservoirs, and thereby increase the epidemiological diversity of *Anaplasma* infections in Pakistan. Bootstrap values in sub-branches decreased because the genetic variations between isolates might stem from regional separations as well as adaptations based on specific hosts.

The third clade mostly comprises *Anaplasma platys*, along with the inclusion of *Anaplasma bo-*

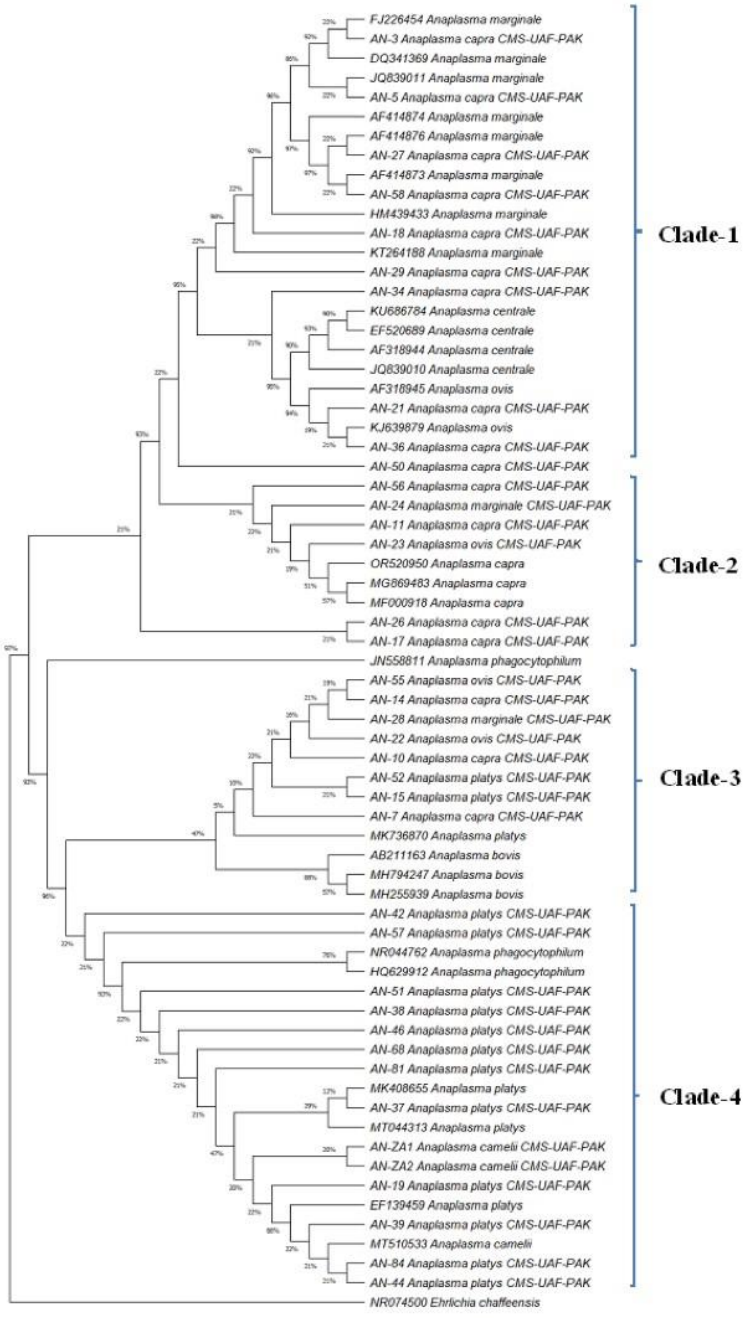

Fig. 1. Maximum likelihood phylogenetic tree of *Anaplasma* species based on 16S RNA gene sequences, showing evolutionary relationships between the isolates from this study and previously reported isolates worldwide

vis and *Anaplasma phagocytophilum*. *A. platys* infects platelets and exists mainly in dogs, while *A. bovis* and *A. phagocytophilum* occur naturally in ruminants along with wild mammals. The *A. platys* isolates obtained from camels contribute to our understanding of the range of hosts involved with this pathogen by showing that camels act as new infection hosts. The moderate bootstrap values detected in the clade branches represent the genetic variations between these isolates because *A. platys* and *A. bovis* have presumably adapted to different hosts. *Anaplasma platys* in camels suggests that

this pathogen can be transmitted by common ticks between dogs and camels.

The fourth clade contains *Anaplasma camelii* and other *A. platys* isolates observed after the scriptonal description of *A. camelii*. The sole detection of *A. camelii* in camels means that this bacteria has a strong specialized relationship with its host species, and indicates its probable distinct evolutionary lineage that has evolved within camels. Experts have found this species in dromedary camels before, yet it is uncertain how pathogenic this bacterium can be. The genetic relationship between *A. camelii* and *A. platys* becomes visible from their cluster analysis, while separate evolutionary paths separate these strains. The placement of *Ehrlichia chaffeensis* as an outgroup proves that *Anaplasma* species remain distinct from other associated genera.

None of the strains *A. bovis*, *A. centrale* and *A. phagocytophilum* were detected in any of the samples tested. The lack of their detection in this research does not rule out the possibility of these organisms being present in camels because additional investigations with larger specimen sizes and using different diagnostic methods could determine their epidemiological status in these species.

Discussion

Camels are versatile animals found worldwide in semi-arid conditions. They usually provide transportation for people in deserts. They also add to the nutritional protein supply with meat and milk (MULLAICHARAM, 2014). Despite their vast benefits, the camel population is in danger due to several health risks. Parasitic ailments are one of the primary limitations in the livestock industry, which impact both the quality and quantity of milk and meat. A significant vector-borne illness that affects camels and other ruminants is anaplasmosis, leading to major economic losses. In this study, we investigated the molecular prevalence of different *Anaplasma* species in camels, and compared our sequences with sequences from all over the world (ISMAEL et al., 2016; BAHRAMI et al., 2018; ALANAZI et al., 2020).

This study revealed the alarmingly high occurrence (40.4%) of anaplasmosis in apparently healthy camels in southern parts of Pakistan. In contrast, recent studies reported 5 and 13.3 percent of anaplasmosis in camels (DURRANI et al., 2017; AZMAT et al., 2018). Global studies on camel anaplasmosis indicate that the prevalence can range from 4.65% up to 95.5% (GHAFAR and SHOBRAK, 2014; BASTOS et al., 2015; LI et al., 2015; GHARBAN and AL-TAEE, 2016; ISMAEL et al., 2016; AIT-LBACHAH et al., 2017). Nevertheless, differences in prevalence estimates can be attributed to varying sample sizes, diagnostic techniques, the demographics of study areas, and endemicity.

Parasitic diseases cause severe problems for animal health. Various factors contribute to disease occurrence. In this study, major factors which can contribute to a disease were analyzed. The results showed that sex is not a significant factor, although female camels exhibited a slightly higher prevalence of 44.4% (95% CI: 32-57.62) compared to 35% in males. Our results are in accordance with the findings of studies from France, Sub-Saharan Africa, and Tunisia (MAURIZI et al., 2009; BELKAHIA et al., 2015). This study contradicts the findings of DURRANI et al. (2017). An explanation for these variations is that some camel owners only maintain females for breeding, which makes them more vulnerable to tick infection. Furthermore, immunosuppression in females can occur during pregnancy and lactation, and last for up to two years. Females are also prone to more stress factors than males, such as pregnancy, parturition, concurrent infections such as parasitism, or malnutrition, which finally result in low immunity. In addition, females are kept untethered and are free in their movements in comparison to male camels, which may be a contributory factor for anaplasmosis in these camels.

Another factor analyzed was age which also showed a non-significant correlation to *Anaplasma* prevalence. In this study, the >6 years age group had the highest prevalence (48.28%), followed the by >3 to ≤6 years group (44.74%) and the group younger than 3 years (25.93%). The same trend was observed in studies from Saudi Arabia, Nigeria, and China (LI et al., 2015; ALANAZI et al., 2020; ONYICHE et al., 2020).

The analysis of the results of risk variables linked to the presence of *Anaplasma* spp. DNA in blood revealed that the body condition score was also not statistically significantly linked to the disease, which is supported by AZMAT et al. (2018). This finding contradicts investigations that found that a poor body condition is a significant factor in *Anaplasma* prevalence (ONYICHE et al., 2020; ALSUBKI et al., 2022). No correlation was found either with the factors parity and pregnancy status in this study.

The sequencing results revealed only two samples positive for *A. camelli*. The possible reason for this could be attributed to the fact that camels in Pakistan are reared together with other ruminants. Sharing of ticks between camels and other livestock species may lead to a heavy burden of *Anaplasma* species in camels.

In this study, our species identification relied not only on PCR amplification but also on sequencing and phylogenetic analysis to differentiate between *Anaplasma* species. We acknowledge that while our approach effectively identified *Anaplasma* species, incorporating species-specific primers in future studies could further enhance the precision of species differentiation, especially for detecting low-prevalence species. It is suggested that subsequent studies should consider multi-gene approaches, such as targeting the *msp4* or *groEL* genes, along with 16S rRNA, to improve species resolution.

An assessment of the risk factors that may be associated with the disease and a description of the epidemiological status of anaplasmosis in camels contribute to a better understanding of the disease's dynamics and can promote the development of better management strategies. Thus, we recommend studies to cover a broader geographical area and increase the sample size in future to provide better insights into the prevalence of the disease. Moreover, inclusion of biting flies and tick vectors in future studies may provide deeper insights into the epidemiology of camel anaplasmosis. Furthermore, future studies should make use of techniques that allow amplification of long gene fragments to identify *Anaplasma* better at species level with high confidence.

Conclusions

In conclusion, this study provides important insights into the prevalence and diversity of *Anaplasma* species in camels in Bahawalpur, Punjab, Pakistan. With an overall prevalence of 40.4%, the findings suggest that *Anaplasma* species are relatively common in the region, but no significant associations were found between infection rates and the risk factors examined. The phylogenetic analysis revealed notable diversity among the strains, though certain species, such as Anaplasma bovis, Anaplasma centrale, and Anaplasma phagocytophilum, were absent. These results highlight the complexity of camel anaplasmosis, and underline the need for more comprehensive diagnostic tools and larger-scale studies. Further research should focus on examining tick vectors and expanding the scope of epidemiological investigations to understand better the transmission dynamics and impact of this disease in camels across Pakistan.

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Authorship contributions statement

Ali Hassan and Muzafar Ghafoor are the co-first authors and contributed equally to this study.

A.H., M.S.S., M.S., and M.A.A. conceptualized the study. The methodology was designed by M.A.A., A.A., M.S., M.G., M.H.H., A.R.S., and K.A. Formal analysis was carried out by T.J., M.A.A., M.Z.A., A.R.S., M.S.S., S.U.R., and M.S. Writing of the original draft was done by M.A.A., A.A., and A.H. while G.M., M.G., M.H.H., and M.S.S. edited the draft. M.S. supervised the project.

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Declarations of competing interest

The authors declare no competing interests.

Data availability statement

All data supporting the conclusions of this article are included in the article.

References

ABRAMSON, J. H. (2011): WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol. Perspect. Innov. 8, 1.
https://doi.org/10.1186/1742-5573-8-1

AIT-LBACHA, H., Z. ZOUAGUI, S. ALALI, A. RHALEM, E. PETIT, M. J. DUCROTOY, H. J. BOULOUIS, R. MAILLARD (2017): "*Candidatus anaplasma camelii*" in one-humped camels (*Camelus dromedarius*) in Morocco: a novel and emerging anaplasma species? Infect. Dis. Poverty 6, 1.
https://doi.org/10.1186/s40249-016-0216-8

ALANAZI, A. D., V. L. NGUYEN, M. S. ALYOUSIF, R. R. S. MANOJ, A. S. ALOUFFI, R. DONATO, A. SAZMAND, J. A. MENDOZA-ROLDAN, F. DANTAS-TORRES, D. OTRANTO (2020): Ticks and associated pathogens in camels (*Camelus dromedarius*) from Riyadh Province, Saudi Arabia. Parasit. Vectors 13, 110.
https://doi.org/10.1186/s13071-020-3973-y

AL-MOSOY, A. S. S. (2023): Serological Prevalence of Anaplasmosis in Buffaloes of Wasit Province, Iraq. Ann. Rom. Soc. Cell. Biol. 27, 138-145.

ALSUBKI, R. A., F. M. ALBOHAIRY, K. A. ATTIA, I. KIMIKO, A. SELIM, M. Z. SAYED-AHMED (2022): Assessment of Seroprevalence and Associated Risk Factors for Anaplasmosis in *Camelus dromedarius*. Vet. Sci. 9, 57.
https://doi.org/10.3390/vetsci9020057

AUBRY, P., D. W. GEALE (2011): A review of bovine anaplasmosis. Transbound. Emerg. Dis. 58, 1-30.
https://doi.org/10.1111/j.1865-1682.2010.01173.x

AZMAT, M., M. IJAZ, S. H. FAROOQI, A. GHAFFAR, A. ALI, A. MASUD, S. SALEEM, A. REHMAN, M. M. ALI, K. MEHMOOD, A. KHAN, H. ZHANG (2018): Molecular epidemiology, associated risk factors, and phylogenetic analysis of anaplasmosis in camel. Microb. Pathog. 123, 377-384.
https://doi.org/10.1016/j.micpath.2018.07.034

BAHRAMI, S., H. HAMIDINEJAT, A. R. G. TAFRESHI (2018): First molecular detection of *Anaplasma phagocytophilum* in dromedaries (*Camelus dromedarius*). J. Zoo Wildl. Med. 49, 844-848.
https://doi.org/10.1638/2017-0165.1

BASTOS, A. D. S., O. B. MOHAMMED, N. C. BENNETT, C. PETEVINOS, A. N. ALAGAILI (2015): Molecular detection of novel Anaplasmataceae closely related to *Anaplasma platys* and *Ehrlichia canis* in the dromedary camel (*Camelus dromedarius*). Vet. Microbiol. 179, 310-314.
https://doi.org/10.1016/j.vetmic.2015.06.001

BATTILANI, M., S. DE ARCANGELI, A. BALBONI, F. DONDI (2017): Genetic diversity and molecular epidemiology of *Anaplasma*. Infect. Genet. Evol. 49, 195-211.
https://doi.org/10.1016/j.meegid.2017.01.021

BELKAHIA, H., M. BEN-SAID, L. SAYAHI, A. ALBERTI, L. MESSADI (2015): Detection of novel strains genetically related to *Anaplasma platys* in Tunisian one-humped camels (*Camelus dromedarius*). J. Infect. Dev. Ctries. 9, 1117-11125.
https://doi.org/10.3855/jidc.6950

BEN-SAID, M., H. BELKAHIA, L. MESSADI (2018): *Anaplasma* spp. in North Africa: A review on molecular epidemiology, associated risk factors and genetic characteristics. Ticks Tick Borne Dis. 9, 543-555.
https://doi.org/10.1016/j.ttbdis.2018.01.003

CONSTABLE, P. D., K. W. HINCHCLIFF, S. H. DONE, W. GRÜNBERG (2017): Veterinary Medicine. A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 11th ed., Saunders Publishers, pp. 965-969.

DANTAS-TORRES, F., D. OTRANTO (2016): Anaplasmosis. In: Arthropod Borne Diseases. (Marcondes, C. B. Ed.), Springer nature, pp. 215-222.
https://doi.org/10.1007/978-3-319-13884-8_15

DURRANI, A. Z., Z. BASHIR, I. RASHEED, N. U. SARWAR (2017): Epidemiological study of common diseases and their risk factors in camels in South Punjab, Pakistan. Microb. Pathog. 108, 6-12.
https://doi.org/10.1016/j.micpath.2017.04.011

FARAJ, A. A. (2019) Parasitological and molecular study of camel anaplasmosis in Al-Najaf Province, Iraq. Biochem. Cell. Arch. 19, 3217-3222.
https://doi.org/10.35124/bca.2019.19.2.3217

GHAFAR, M., M. SHOBRAK (2014): Molecular detection and characterization of *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis, in some animals suspected to be competent reservoirs in Taif district, Kingdom of Saudi Arabia. Life. Sci. J. 11, 63-69.

GHARBAN, H. A. J., H. S. AL-TAEE (2016): Seroclinical diagnosis of Anaplasma marginale bacteria in carrier arabian one-humped camels. Basrah J. Vet. Res. 15, 346-359.

ISMAEL, A. B., A. A. A. SWELUM, A. F. KHALAF, A. N. ALOWAIMER (2016): First evidence of natural anaplasmosis in *Camelus dromedarius* in Saudi Arabia. J. Camel Pract. Res. 23, 95-100.
https://doi.org/10.5958/2277-8934.2016.00014.X

KOCAN, K. M., J. DE LA FUENTE, E. F. BLOUIN, J. C. GARCIA-GARCIA (2004): *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology 129, S285-S300.

https://doi.org/10.1017/s0031182003004700

KOH, F. X., C. PANCHADCHARAM, F. T. SITAM, S. T. TAY (2018): Molecular investigation of *Anaplasma* spp. in domestic and wildlife animals in Peninsular Malaysia. Vet. Parasitol. Reg. Stud. Reports 13, 141-147.

https://doi.org/10.1016/j.vprsr.2018.05.006

KOLO, A. (2023): *Anaplasma* Species in Africa-A Century of Discovery: A Review on Molecular Epidemiology, Genetic Diversity, and Control. Pathogens 12, 702.

https://doi.org/10.3390/pathogens12050702

LI, Y., J. YANG, Z. CHEN, G. QIN, Y. LI, Q. LI, J. LIU, Z. LIU, G. GUAN, H. YIN, J. LUO, L. ZHANG (2015): *Anaplasma* infection of Bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang, China. Parasit. Vectors 8, 313.

https://doi.org/10.1186/s13071-015-0931-1

LORUSSO, V., M. WIJNVELD, M. S. LATROFA, A. FAJINMI, A. O. MAJEKODUNMI, A. G. DOGO, A. C. IGWEH, D. OTRANTO, F. JONGEJAN, S. C. WELBURN, K. PICOZZI (2016): Canine and ovine tick-borne pathogens in camels, Nigeria. Vet. Parasitol. 228, 90-92.

https://doi.org/10.1016/j.vetpar.2016.08.014

MAURIZI, L., J. L. MARIÉ, C. COURTIN, S. GORSANE, D. CHAL, B. DAVOUST (2009): Seroprevalence survey of equine anaplasmosis in France and in sub-Saharan Africa. Clin. Microbiol. Infect. 15, S68-S69.

https://doi.org/10.1111/j.1469-0691.2008.02191.x

MULLAICHARAM, A. R. (2014): A review on medicinal properties of Camel milk. World J. Pharm. Sci. 2, 237-242.

NEWCOMBE, R. G. (1998): Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat. Med. 17:857-872.

OIE (2017): Office International des Epizooties. Terrestrial manual. Section 2.4. Bovine, chapter 2.4.1. Bovine anaplasmosis. WOAH

OKONECHNIKOV, K., O. GOLOSOVA, M. FURSOV (2012): UGENE team. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28, 1166-1167.

https://doi.org/10.1093/bioinformatics/bts091

ONYICHE, T. E., C. RĂILEANU, O. TAUCHMANN, S. FISCHER, A. VASIĆ, M. SCHÄFER, A. A. BIU, N. I. OGO, O. THEKISOE, C. SILAGHI (2020): Prevalence and molecular characterization of ticks and tick-borne pathogens of one-humped camels (*Camelus dromedarius*) in Nigeria. Parasit. Vectors 13, 428.

https://doi.org/10.1186/s13071-020-04272-2

PAROLA, P., V. ROUX, J. L. CAMICAS, I. BARADJI, P. BROUQUI, D. RAOULT (2000): Detection of Ehrlichiae in African ticks by polymerase chain reaction. Trans. R. Soc. Trop. Med. Hyg. 94, 707-708.

https://doi.org/10.1016/s0035-9203(00)90243-8

PARVIZI, O., H. EL-ADAWY, U. ROESLER, H. NEUBAUER, K. MERTENS-SCHOLZ (2020): Performance Analysis of *Anaplasma* Antibody Competitive ELISA Using the ROC Curve for Screening of Anaplasmosis in Camel Populations in Egypt. Pathogens 9, 165.

https://doi.org/10.3390/pathogens9030165

RAR, V., S. TKACHEV, N. TIKUNOVA (2021): Genetic diversity of *Anaplasma* bacteria: Twenty years later. Infect. Genet. Evol. 91, 104833.

https://doi.org/10.1016/j.meegid.2021.104833

SALMAN, D., T. SIVAKUMAR, D. OTGONSUREN, M. E. MAHMOUD, E. K. ELMAHALLAWY, A. KHALPHALLAH, A. M. E. Y. KOUNOUR, S. A. BAYOMI, M. IGARASHI, N. YOKOYAMA (2022): Molecular survey of *Babesia*, *Theileria*, *Trypanosoma*, and *Anaplasma* infections in camels (*Camelus dromedaries*) in Egypt. Parasitol. Int. 90, 102618.

https://doi.org/10.1016/j.parint.2022.102618

SELIM, A., E. MANAA, A. ABDELHADY, M. BEN-SAID, A. SAZMAND (2021): Serological and molecular surveys of *Anaplasma* spp. in Egyptian cattle reveal high *A. marginale* infection prevalence. Iran. J. Vet. Res. 22, 288-297.

https://doi.org/10.22099/ijvr.2021.40587.5879

SELMI, R., M. DHIBI, M. BEN-SAID, H. BEN YAHIA, H. ABDELAALI, H. AMEUR, S. BACCOUCHE, A. GRITLI, M. MHADHBI (2019): Evidence of natural infections with *Trypanosoma*, *Anaplasma* and *Babesia* spp. in military livestock from Tunisia. Trop. Biomed. 36, 742-757.

TAMURA, K., G. STECHER, S. KUMAR (2021): MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38, 3022-3027.

https://doi.org/10.1093/molbev/msab120

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SAŽETAK

Anaplazmoza je krpeljima prenosiva bolest uzrokovana vrstama iz roda *Anaplasma*, koja zahvaća stoku i divlje životinje širom svijeta. Unatoč provedenim istraživanjima molekularne epidemiologije anaplazmi u deva u raznim zemljama, za Pakistan postoje samo ograničeni podaci. Cilj je rada bio ustanoviti prevalenciju i gensku raznolikost anaplazmi u deva iz grada Bahawalpura u Pakistanu, primjenom molekularnih metoda. Ukupno su 94 uzorka krvi prikupljena od deva venepunkcijom jugularne vene i podvrgnuta DNA ekstrakciji. Primijenjena je lančana reakcija polimerazom (PCR) na genu *16S* rRNA, nakon čega je provedeno sekvenciranje i filogenetska analiza. Ukupna je prevalencija anaplazmi bila 40,4%, s 38 pozitivnih uzoraka. BLAST analiza pokazala je da 17 izolata pripada vrsti *A. capra*, 14 vrsti *A. platys*, 3 vrsti *A. ovis*, 2 vrsti *A. marginale*, i 2 vrsti *A. camelii*. Primjenom metode najveće vjerojatnosti, identificirane anaplazme razvrstane su u četiri filogenetske skupine/kladusa, pri čemu su *A. capra* i *A. marginale* pokazale najveću gensku povezanost. Univarijantna statistička analiza nije otkrila znakovitu povezanost prevalencije anaplazmi i pokazatelja kao što su spol (P=0,5457), dob (P=0,464), kondicija (P=0,3269), graviditet (P=0,7530) i paritet (P=0,8057). Rezultati upućuju na to da bi deve mogl biti potencijalni rezrevoari za brojne vrste anaplazmi, pridonoseći epidemiološkoj složenosti anaplazmoze u Pakistanu. Ovo istraživanje donosi prvi molekularni opis anaplazmi u deva u Pakistanu, ističući potrebu za daljnjim istraživanjima dinamike prijenosa i potencijalnih zoonotskih implikacija. Preporučuje se pojačan nadzor i primjena preventivnih mjera kako bi se ublažio učinak anaplazmoze u populaciji deva i stoke koja je s devama u kontaktu.

Ključne riječi: *Anaplasma*; deve; prevalencija; filogenetska analiza; Pakistan