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Prevalence of Anaplasmosis and Its Associated Risk Factors in Camels at Lakki Marwat, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Background: Anaplasmosis is a tick-borne rickettsial disease affecting a wide range of domestic animals, including camels. In camels, infections are often subclinical or occur as co-infections, but clinical cases can present with fever, anemia, weight loss, ataxia, anorexia, jaundice, and lymph node enlargement. This study provides the first investigation of camel anaplasmosis in Lakki Marwat, aiming to determine its prevalence and associated risk factors in this region. **Methods:** A cross-sectional survey of 384 one-humped camels (Camelus dromedarius) was conducted from July 2024 to June 2025. Blood samples were collected and examined by Giemsa-stained thin blood smear microscopy for Anaplasma parasites. Packed cell volume (PCV) was measured to assess anemia, and a competitive ELISA was used on sera to detect Anaplasma antibodies. Data on host factors (age, sex, breed, body condition), husbandry, and tick infestation were recorded. Associations between potential risk factors and infection status were analyzed using chi-square tests for categorical variables and t-tests for continuous variables. **Results:** Microscopic examination revealed an overall Anaplasma infection prevalence of 13.5% (52/384). Anaplasma marginale was the most common species detected, followed by A. centrale, including a few co-infections. Tick-infested camels had a significantly higher infection rate (25.0%) than tick-free camels (5.4%) ($p < 0.001$). Camels in poor body condition showed higher infection prevalence (30.0%) than those in good condition (8.3%) ($p < 0.001$). No significant differences in infection were observed by age, sex, breed, or village origin ($p > 0.05$). Infected camels had lower mean PCV (≈22%) compared to uninfected (≈29%) ($p < 0.001$), indicating anemia. Serologically, 19.0% of camels had anti-Anaplasma antibodies, confirming additional subclinical exposures. **Conclusion:** Camel anaplasmosis is present in the study region with an overall infection prevalence of about 13%, and tick infestation and poor body condition are key risk factors. The disease largely occurs subclinically, associated with anemia in infected animals. These findings underscore the need for improved tick control and preventive strategies to mitigate anaplasmosis in camels. Future studies should incorporate molecular diagnostics to identify Anaplasma species and further explore epidemiological dynamics in Pakistan's camel populations.

INTRODUCTION

Anaplasmosis is an infectious disease of blood cells caused by obligate intracellular bacteria of the genus Anaplasma (family Anaplasmataceae) (Aldujaily et al. 2023). It is primarily transmitted by hard ticks (e.g. Hyalomma, Rhipicephalus, Dermacentor, Ixodes spp.) and can also be spread mechanically by biting flies or contaminated instruments (Alanazi et al. 2020). Anaplasma species such as *A. marginale, A. centrale, A. ovis, A. platys*, and *A. phagocytophilum* infect a wide range of hosts including ruminants, canines, and camelids (Alsubki et al. 2022).

Camels (*Camelus dromedarius*) are susceptible to anaplasmosis, though infections in camels are often subclinical or part of mixed parasitic infections (Azmat et al. 2018). Clinical camel anaplasmosis, when it occurs, may manifest as fever, anemia, emaciation, incoordination, anorexia, jaundice, or lymphadenopathy. Such cases have been documented as subclinical anaplasmosis that only

became apparent under stress or concurrent disease, as reported in Indian camels by (Sudan et al. 2014). In general, chronic carrier states are common – infected animals can remain asymptomatic carriers, with disease exacerbations precipitated by stressors like drought or poor (Elhaig et al. 2024).

Ticks are considered the principal vectors for camel anaplasmosis, and camel-rearing regions with high tick burdens are at risk for the disease. Studies from Saudi Arabia have identified various tick-borne pathogens (including Anaplasma spp.) in dromedary camels, and Anaplasma DNA has been detected in approximately 6.5% of camels in Riyadh province (Morgan et al. 2006). In Egypt and North Africa, camel anaplasmosis appears to be more prevalent: for example, Anaplasma antibodies were found in up to 47.4% of camels in some Egyptian regions, and PCR-based tests showed infection rates as high as 66.6% (Mahmoud et al. 2023). Other reports worldwide have ranged from as low as ~6% to over 60% prevalence depending on the area and diagnostic method (Sudan et al. 2014). Such variation is attributed to differences in tick abundance, diagnostic sensitivity, and herd management practices. Overall, these studies indicate that anaplasmosis is an emerging health concern for camels in tropical and subtropical regions. In Pakistan, information on camel anaplasmosis has until recently been very limited or neglected (Si et al. 2022).

Camels play an important socio-economic role in arid parts of the country, including Khyber Pakhtunkhwa province, yet hemoparasitic diseases in camels have not been well studied. A recent serological survey in Punjab province provided the first evidence of Anaplasma exposure in Pakistani camels, reporting an 8.5% seroprevalence and identifying risk factors such as region, tick infestation, and poor body condition (Khan et al. 2019). However, there are no published data from Khyber Pakhtunkhwa province.

Lakki Marwat is its district with a significant camel population managed under traditional pastoral systems. Ticks are commonly observed on local camels, suggesting a potential risk of tick-borne infections. This study was therefore designed to investigate the prevalence of camel anaplasmosis in Lakki Marwat district and to identify associated risk factors under local conditions. The study aimed to determine the prevalence of Anaplasma infection in camels of Lakki Marwat by microscopic and serological methods, and to evaluate host and management factors associated with infection risk in this population.

MATERIALS AND METHODS

Study Area and Population

The study was conducted in Lakki Marwat district of Khyber Pakhtunkhwa, Pakistan. The district lies in a semi-arid region with hot summers and mild winters, an environment conducive to ticks. The study population consisted of one-humped camels (*C. dromedarius*) kept by smallholder pastoralists in four villages. Camels in this area are managed under extensive free-grazing conditions and are used for transport, milk, and meat. Both sexes and all age groups of camels were included. For analysis, camels were categorized by age group as: <3 years (young), 3–7 years (adult), and >7 years (old). Body condition score (BCS) was assessed for each camel using a standard camel body condition scoring method (scores 1–5) as described by (Kamili et al. 2006). For analysis, BCS was further grouped as poor (emaciated, score 1–2), moderate (score 3), or good (well-conditioned, score 4–5). The breed of each camel was recorded (most were of the local "Gaddi" breed common to the region, versus a few animals originating from other breeds). The presence or absence of tick infestation on each camel was noted by thorough physical examination, focusing on tick predilection sites.

Study Design and Sample Size

A cross-sectional study design was employed from July 2024 to June 2025, covering all seasons in order to account for any temporal variation in infection. Camels were selected by simple random sampling from each village's available herds. Assuming no prior data on camel anaplasmosis in the area, the expected prevalence was conservatively set at 50% (maximal uncertainty) for sample size calculation. Using Thrusfield's formula for sample size in prevalence studies (with 95% confidence level and 5% precision), the minimum required samples were 384 camels (Thrusfield 2018).

$N=Z^2\times P_{exp}(1-P_{exp})\ /d^2$

$N=(0.05)^2(1.96)^2/0.50\times0.50\approx384.$

A total of 384 camels were thus sampled (approximately 96 from each of the four villages). Each animal was assigned a unique ID and its sex, age, breed, body condition, origin (village), and tick infestation status were recorded on a data sheet.

Sample Collection

Blood samples were collected from the jugular vein of each camel using sterile vacutainer needles. Approximately 3–4 mL of blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes for hematology and smear preparation. To ensure aseptic collection, the venipuncture site was disinfected and a new needle was used for each animal. Each sample tube was labeled with the camel's ID and details (age, sex, etc.). Samples were kept in an icebox with cold packs and transported the same day to the Veterinary Research Center, Lakki Marwat for analysis.

Laboratory Analysis

In the laboratory, thin blood smears were prepared immediately from each EDTA blood sample. Smears were air-dried, then fixed in absolute methanol for 2 minutes. Fixed smears were stained with Giemsa stain (10% v/v in phosphate buffer) for 30 minutes. After staining, slides were gently rinsed with tap water, air-dried, and a drop of immersion oil was added. Each smear was examined under a microscope at 100× magnification (oil immersion) for the presence of Anaplasma parasites. At least 50 fields were systematically observed per slide before declaring a sample negative. Anaplasma organisms were identified by their characteristic morphology and intraerythrocytic position. *Anaplasma marginale* appears as small (0.3–1.0 µm), dense, round purple inclusion bodies located at the margins of red blood cells, whereas *A. centrale* typically appears as similar round bodies more centrally located in the erythrocyte. Smear-positive samples were classified as *A. marginale* or *A. centrale* based on these criteria, and if inclusions of both types were seen, the sample was noted as a co-infection. In addition to microscopy, a competitive

ELISA (cELISA) was performed on the serum of each sample to detect antibodies against Anaplasma spp. A commercial Anaplasma antibody test kit (cELISA, VMRD Inc., USA) was used following the manufacturer's protocol. This assay targets the conserved major surface protein 5 (MSP5) of *A. marginale*/*A. centrale*/*A. ovis* and has high sensitivity and specificity in camelids. Briefly, serum samples and controls were added to the ELISA plate wells pre-coated with Anaplasma antigen; after incubation and washing, an anti-MSP5 monoclonal antibody conjugate was applied. The presence of Anaplasma-specific antibodies in the sample competes with the conjugate, resulting in reduced color development. Optical densities were measured on an ELISA reader and percent inhibition calculated. Samples with ≥30% inhibition (per kit instructions, equivalent to a cutoff P value of 0.42) were considered seropositive for Anaplasma exposure (Bellezze et al. 2023). This serological test cannot distinguish Anaplasma species, but it provides an overall seroprevalence indicating past or current infection.

Hematological Analysis

To assess the impact of infection on the camels' health, packed cell volume (PCV) was measured for each blood sample as an indicator of anemia. Microhematocrit capillary tubes were filled with well-mixed EDTA blood and centrifuged in a hematocrit centrifuge at 12,000 rpm for 5 minutes. The PCV was read using a hematocrit reader as the percentage of red blood cell column height to total blood column height. Camels with PCV < 24% were classified as anemic. This threshold (24%) is a standard anemia cutoff for adult cattle and camelids in tropical areas (Ibrahim et al. 2010). Mean PCV values were compared between Anaplasma-infected and uninfected camels.

Data and Statistical Analysis

All field and laboratory data were entered into Microsoft Excel and then analyzed using SPSS v25.0 (IBM Corp.). Prevalence of anaplasmosis was calculated as the number of microscopy-positive camels divided by the total number examined, expressed as a percentage. For serology, seroprevalence was calculated similarly using the cELISA results. Descriptive statistics were used to summarize the sample characteristics and infection rates. The association between Anaplasma infection status (positive/negative by microscopy) and categorical risk factors (sex, age group, breed, body condition, tick infestation, and origin village) was evaluated using chi-square (χ^2) tests. A separate chi-square test was also used to assess the relationship between anemia (PCV < 24%) and infection. For each factor, odds ratios (OR) were considered in interpretation, but a formal multivariable logistic regression was not performed due to the moderate number of positives. For continuous variables like PCV, Student's t-test was applied to compare the mean values between infected and uninfected groups. In all analyses, a two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

Out of the 384 camels examined, 52 were positive for Anaplasma on Giemsa-stained blood smears, yielding an overall infection prevalence of 13.5% (Table 1). Microscopic examination identified Anaplasma inclusion bodies consistent with *A. marginale* in most positive cases. A smaller number of cases showed centrally located inclusions suggestive of *A. centrale*. Specifically, 35 camels (9.1% of the total sample) were positive for *A. marginale*, 12 camels (3.1%) for *A. centrale*, and 5 camels (1.3%) appeared to have mixed infections with both types (Table 2).

These results indicate *A. marginale* as the predominant species infecting camels in the area, with occasional *A. centrale* infections and co-infections. In addition to the active infections detected by microscopy, serological testing by cELISA revealed a higher exposure rate. A total of 73 camels (19.0%) tested positive for anti-Anaplasma antibodies, suggesting that a number of camels had been exposed to Anaplasma without detectable parasites on blood smears. Notably, all 52 smear-positive camels were also positive on the cELISA, and an additional 21 camels were seropositive despite having no observable parasites on microscopy. This difference between microscopy prevalence (13.5%) and seroprevalence (19.0%) reflects the presence of subclinical or cleared infections that are below the microscopic detection threshold. No clinical signs were overt in most infected camels at the time of sampling; only a few of the heavily infected animals showed mild signs of pallor and lethargy, and these corresponded to those with the lowest PCV values.

Camel anaplasmosis was associated with a significant reduction in red blood cell parameters. The mean packed cell volume of Anaplasma-positive camels was 22.5% (±4.1 SD), compared to 29.3% (±3.2) in negative camels. This difference was statistically significant ($t = -11.69$, $df \approx 382$, $p < 0.001$), indicating that infected camels were, on average, moderately anemic. Consistent with this, 67% of the infected camels (35 out of 52) had PCV < 24% (the anemia threshold) compared to only 18% of uninfected camels (60 out of 332). A chi-square test confirmed that anemia (PCV < 24) was significantly associated with infection ($p < 0.001$). These results support that Anaplasma infection contributes to anemia in camels, likely due to destruction of erythrocytes by the parasite. Other hematological parameters were not comprehensively measured in this field study; however, the marked drop in PCV among infected animals highlights the pathogenic impact of even subclinical anaplasmosis. Infected camels in this study did not show severe leukocyte abnormalities outwardly, though previous research indicates that chronic anaplasmosis can alter white blood cell counts as well.

Camels carrying ticks had a significantly higher likelihood of Anaplasma infection. Among 160 camels that were tick-infested at examination, 40 (25.0%) were Anaplasma-positive, whereas only 12 out of 224 tick-free camels (5.4%) were positive. This difference was highly significant ($\chi^2 = 30.76$, $df = 1$, $p < 0.001$), indicating a strong association between tick infestation and infection (Table 1). In practical terms, camels with ticks were about 5–6 times more likely to be infected than those without ticks (estimated OR ≈ 5.9). This underscores ticks as the principal vector for Anaplasma transmission in camels. There was a significant inverse relationship between body condition and infection prevalence. Camels in poor body condition (emaciated) showed the highest infection rate:

30.0% (15/50) were infected. In contrast, only 12.0% (30/250) of camels with moderate condition and 8.3% (7/84) of those in good condition were infected. The trend of higher infection in poorer-conditioned animals was significant ($\chi^2 = 14.02$, df = 2, $p < 0.001$). This suggests that malnourished or thin camels are more susceptible to Anaplasma infection, or conversely that infection may contribute to loss of condition. Notably, many of the infected camels were in suboptimal condition, which could be a consequence of chronic parasitism or other concurrent diseases. Age – Camel age was not found to be a significant risk factor in this study. Young camels under 3 years of age had an infection prevalence of 11.3% (9/80), adults 3–7 years old had 12.5% (25/200), and older camels above 7 years had 17.3% (18/104) positive (Table 1). The results suggest that camels are equally likely to be infected at any age, or that age-related differences are minor in this endemic setting. There was no clear evidence of either young animals being more susceptible or older animals accumulating more infection in this cross-section of the population.

The prevalence of infection was slightly higher in male camels (14.9%, 20/134) than in female camels (12.8%, 32/250), but this difference was not statistically significant ($\chi^2 = 0.34$, df = 1, $p = 0.56$). Therefore, sex did not appear to be a determining factor for anaplasmosis in the sampled camels. Both males and females, which typically share similar grazing environments in these communities, had comparable risk in our study.

Almost all camels sampled were of the local "Gaddi" breed, with a small number of non-local breed animals that had been brought into the area. Infection prevalence in local breed camels was 12.9% (40/310) versus 16.2% (12/74) in the non-local group. This difference was not significant ($\chi^2 = 0.56$, df = 1, $p = 0.45$). Given the small subset of non-local breed camels, no clear breed predisposition can be concluded. It appears that susceptibility to Anaplasma infection is not strongly breed-dependent among dromedary camels, although almost all are one-humped camels with presumably similar genetic background.

We investigated whether camels from different villages or management units had different infection rates. The prevalence ranged from 10.0% in village B to 21.6% in village D (Table 1). Village D had the highest positivity (16/74), while villages A, B, and C were around 11–13% (14/120, 10/100, 12/90 respectively). These variations did not reach statistical significance ($\chi^2 = 5.56$, df = 3, $p = 0.13$). It is possible that village D's higher rate is due to chance or minor differences in husbandry or tick control, but overall the infection is fairly uniformly present across the region. All villages practice similar extensive grazing and had comparable tick burdens on animals, which may explain the lack of a strong "cluster" effect by location.

The seroprevalence of Anaplasma infection in camels as assessed by cELISA was found to be almost similar across sex and age groups, indicating no statistically significant association ($p > 0.05$). Both male and female camels showed nearly identical seropositivity (18.7% and 19.2%, respectively), suggesting that gender does not play a major role in susceptibility. Similarly, camels aged <3 years, 3–7 years, and >7 years exhibited very close seroprevalence rates (18.8%, 19.0%, and 19.2%, respectively), reflecting a uniform exposure risk across age brackets. However, a highly significant association ($p < 0.001$) was observed between body condition score (BCS) and seroprevalence. Camels with poor BCS (score 1–2) had a much higher seroprevalence (40.0%) compared to those with moderate (16.0%) or good body condition (15.5%), indicating that animals in a debilitated state are significantly more prone to infection, likely due to compromised immunity. Tick infestation was another strong predictor of infection: seroprevalence in tick-infested camels was 31.3%, compared to just 10.3% in non-infested animals ($p < 0.001$). This reinforces the role of ticks as the principal vector in disease transmission. Regarding village origin, the seroprevalence ranged from 17.8% to 20.3% among the four localities, but this variation was not statistically significant ($p = 0.7$), indicating relatively homogenous environmental exposure risks across the study area (Table 3).

A comparison of hematological profiles between infected and uninfected camels revealed statistically significant differences ($p < 0.001$) across all parameters. Infected camels had significantly lower packed cell volume (PCV) values (21.60 ± 3.76%) compared to uninfected ones (29.28 ± 3.21%), consistent with anemia resulting from parasitemia and red blood cell destruction. Hemoglobin concentrations also reflected this trend, being markedly reduced in infected animals (8.35 ± 1.06 g/dL vs. 11.14 ± 1.28 g/dL), reinforcing the diagnosis of normocytic, normochromic anemia frequently associated with Anaplasma infection. RBC counts were significantly lower in the infected group (7.12 ± 0.98 × 10^6/µL) compared to uninfected camels (8.62 ± 0.90 × 10^6/µL), confirming the erythrocyte-damaging nature of Anaplasma organisms. Interestingly, white blood cell (WBC) counts were elevated in infected camels (12.36 ± 2.77 × 10^3/µL), a finding that may reflect a reactive leukocytosis or underlying inflammation. These hematological changes align with those typically seen in rickettsial blood-borne infections and highlight the utility of routine hemograms in clinical suspicion and diagnosis (Table 4).

Multivariate logistic regression revealed that tick infestation and poor body condition were the most significant independent risk factors associated with Anaplasma infection. Tick-infested camels were nearly six times more likely to be infected (AOR = 5.93, 95% CI: 3.02–11.63, $p < 0.001$), strongly affirming the vector-borne nature of the disease. Similarly, camels in poor physical condition (score 1–2) were 3.12 times more likely to test positive than those in good condition ($p = 0.010$), indicating a susceptibility bias in nutritionally or physiologically compromised animals. Moderate body condition did not show a significant association ($p = 0.31$), suggesting a threshold effect where only severely emaciated animals are predisposed. Other factors, including age, sex, and breed, did not have a statistically significant impact on infection status ($p > 0.05$), although slight trends toward higher odds in older and male animals were observed. These findings underscore the importance of both vector control and animal management practices in mitigating disease burden (Table 5).

Analysis of seasonal trends in Anaplasma prevalence revealed that summer posed the highest risk for both

active infection (microscopy-positive: 30.0%) and exposure history (cELISA-positive: 34.0%). This peak may be attributed to optimal environmental conditions for tick proliferation and activity during hot and humid months, facilitating pathogen transmission. Spring also showed moderately high prevalence levels (microscopy: 18.8%; seroprevalence: 20.8%), likely due to the onset of vector activity. By contrast, winter recorded the lowest infection rates (microscopy: 11.1%; seroprevalence: 13.3%), consistent with reduced tick activity in colder months. Autumn data were intermediate (microscopy: 14.3%; seroprevalence: 16.3%), likely reflecting the tapering vector activity. These findings emphasize the importance of seasonal vector control measures and surveillance, particularly during the high-risk summer months, to prevent disease outbreaks (Table 6).

Among microscopy-positive camels, clinical assessment revealed a consistent pattern of disease manifestation. The most frequently observed clinical signs were anemia (78.8%) and tick infestation (76.9%), both classical features of anaplasmosis. Emaciation (65.4%) and lethargy (50.0%) were also common, reflecting chronic disease progression and systemic debilitation. Fever (>39.5°C) was recorded in nearly half the infected animals (48.1%), which aligns with the febrile nature of acute rickettsial infections. Interestingly, icterus (jaundice) was noted in 21.2% of the cases, likely due to hemolysis and resultant hyperbilirubinemia, although this is a less frequently reported finding in camelids compared to bovines. The clinical spectrum recorded in this study highlights the nonspecific but consistent signs of anaplasmosis in camels and supports the need for integrating clinical surveillance with laboratory diagnostics to ensure early and accurate case identification (Figure 1).

Table 1

Prevalence of Anaplasmosis in Camels by Demographic and Management Factors in Lakki Marwat

Factor	Category	No. Examined	No. Positive	Prevalence (%)	χ^2 (p-value)
Sex	Male	134	20	14.9	0.34 (p = 0.56)
	Female	250	32	12.8	
Age group	< 3 years	80	9	11.3	1.80 (p = 0.41)
	3–7 years	200	25	12.5	
	> 7 years	104	18	17.3	
Body condition	Poor (score 1–2)	50	15	30.0	**14.02 (p < 0.001)**
	Moderate (score 3)	250	30	12.0	
	Good (score 4–5)	84	7	8.3	
Breed	Local (Gaddi)	310	40	12.9	0.56 (p = 0.45)
	Non-local (other)	74	12	16.2	
Tick infestation	Yes	160	40	25.0	**30.76 (p < 0.001)**
	No	224	12	5.4	
Origin (village)	A	120	14	11.7	5.56 (p = 0.13)
	B	100	10	10.0	
	C	90	12	13.3	
	D	74	16	21.6	

Table 2

Distribution of Anaplasma species identified in camels by microscopy

Infection category	No. of camels (% of total)
Uninfected (no Anaplasma)	332 (86.5)
Infected (any Anaplasma)	52 (13.5)
– *Anaplasma marginale*	35 (9.1)
– *Anaplasma centrale*	12 (3.1)
– Co-infection (A. m + A. c)	5 (1.3)
Seropositive (Anaplasma antibodies)	73 (19.0)

Table 3

Seroprevalence of Anaplasma Infection in Camels by Risk Factors

Factor	Category	No. Examined	No. Seropositive	Seroprevalence (%)	χ^2	p-value
Sex	Male	134	25	18.7	0.02	0.88
	Female	250	48	19.2		
Age Group	< 3 years	80	15	18.8	0.14	0.93
	3–7 years	200	38	19.0		
	> 7 years	104	20	19.2		
Body Condition	Poor (score 1–2)	50	20	40.0	**16.82**	**< 0.001**
	Moderate (score 3)	250	40	16.0		
	Good (score 4–5)	84	13	15.5		
Tick Infestation	Yes	160	50	31.3	**22.36**	**< 0.001**
	No	224	23	10.3		
Origin (Village)	Abdul Khel	120	24	20.0	1.35	0.7
	Aba Khel	100	18	18.0		
	Pezu	90	16	17.8		
	Darkha	74	15	20.3		

Based on cELISA detection of anti-Anaplasma antibodies. A camel was considered seropositive if % inhibition ≥ 30%.

Table 4

Hematological Parameters in Anaplasma-Infected and Uninfected Camels

Parameter	Infected Mean ± SD	Uninfected Mean ± SD	t-value	p-value
PCV (%)	21.60 ± 3.76	29.28 ± 3.21	−11.7	<0.001
Hb (g/dL)	8.35 ± 1.06	11.14 ± 1.28	−13.5	<0.001
RBC (10^6/µL)	7.12 ± 0.98	8.62 ± 0.90	−10.9	<0.001
WBC (10^3/µL)	12.36 ± 2.77	9.41 ± 2.51	+8.45	<0.001

Table 5

Multivariate Logistic Regression of Risk Factors Associated with Camel Anaplasmosis (Microscopy Positive Cases)

Factor	Category	Adjusted Odds Ratio (AOR)	95% CI	p-value
Tick Infestation	Present	5.93	3.02–11.63	<0.001 **
Body Condition	Poor vs Good	3.12	1.31–7.41	0.010 *
Body Condition	Moderate vs Good	1.56	0.65–3.78	0.31

Age Group	>7 yrs vs <3 yrs	1.61	0.68–3.85	0.28
Sex	Male vs Female	1.21	0.62–2.36	0.56
Breed	Non-local vs Local	1.31	0.61–2.82	0.47

Outcome variable: Anaplasma infection status (positive vs. negative); method: binary logistic regression.

Table 6

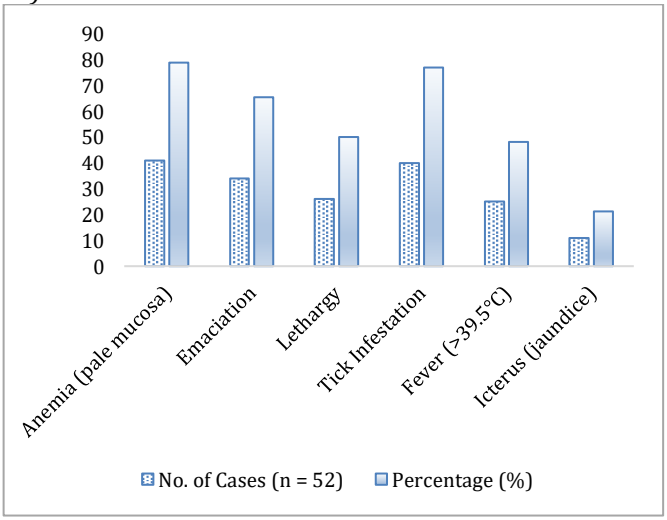
Seasonal Prevalence of Anaplasmosis in Camels

Season	No. Examined	Microscopy Positive	Microscopy Prevalence (%)	cELISA Seropositive	Seroprevalence (%)
Winter (Dec–Feb)	90	10	11.1	12	13.3
Spring (Mar–May)	96	18	18.8	20	20.8
Summer (Jun–Aug)	100	30	30.0	34	34.0
Autumn (Sep–Nov)	98	14	14.3	16	16.3

Distribution of microscopy-positive and cELISA-positive cases across seasons.

Figure 1

Clinical Signs Observed in Microscopy-Positive Camels (n = 52)



DISCUSSION

This study presents the first detailed investigation of camel anaplasmosis in Khyber Pakhtunkhwa, Pakistan, revealing that Anaplasma infection is present in the camel population of Lakki Marwat district with an overall microscopy prevalence of 13.5%. This finding is in line with the range of prevalences reported for camel anaplasmosis in other regions using various diagnostic methods. For example, a molecular survey in Punjab, Pakistan found a 13.33% prevalence of Anaplasma in camels (Shoulah et al. 2023), and several studies worldwide have reported infection rates in the single to low double digits (approximately 6–20%) under endemic conditions (Shoulah et al. 2023). Our observed prevalence falls into this lower range of what has been termed "low endemicity" for camel anaplasmosis (Abou El-Naga et al. 2012). In contrast, much higher values have been recorded in parts of North Africa. Abou El-Naga (2012) in Egypt reported a 47.4% infection rate by microscopy and up to 67.4% by PCR in camels from the Nile Delta and coastal areas (Gul et al. 2021). Such high prevalences likely reflect areas with intense tick challenge and possibly differences in camel management or breed susceptibility. These comparisons suggest that the epidemiology of camel anaplasmosis is geographically diverse.

Our results, around 13% infection, indicate that southern Khyber Pakhtunkhwa is an endemic area of modest infection prevalence, more comparable to the situation in Punjab (where 8.5% seroprevalence was recently reported) (Abbas et al. 2024b), than to the hyper-endemic foci in parts of Africa. It is important to note that the diagnostic approach influences prevalence estimates. We used Giemsa-stained blood smears for primary detection, which is a practical field method but has lower sensitivity than molecular or serological tests (Parvizi et al. 2020). The complementary cELISA in our study detected nearly 5.5% more camels with Anaplasma-specific antibodies than were found by microscopy. This indicates that a subset of camels had been exposed and mounted an immune response despite having no detectable parasitemia at the sampling time. Such animals could be latent carriers with parasite levels below microscopical detection or those that recently cleared the infection. The difference between infection prevalence and seroprevalence highlights the subclinical nature of camel anaplasmosis: many camels may harbor Anaplasma at low levels or as past infections without showing parasites on a blood smear (Badshah et al. 2023).

In camels, infections are often chronic and usually appear subclinical or as co-infections without distinctive (Tabor 2022). This is consistent with our field observations – none of the infected camels showed severe disease, and only a few showed mild anemia or lethargy. The impact on hematological health, however, was evident. Infected camels had significantly lower PCV (mean ~22%) compared to uninfected camels (~29%). This mirrors findings by Aldujaily et al. (2023) who reported that camels naturally infected with subclinical anaplasmosis had notably reduced hemoglobin levels and hematocrit compared to healthy camels (Aldujaily et al. 2023). The anemia in anaplasmosis is caused by the destruction of red blood cells as the rickettsial organisms multiply within erythrocytes and eventually cause their rupture. Up to 15% or more of RBCs can be parasitized in acute infections, leading to hemolytic anemia (Heidari et al. 2023).

Our results indicate that even subclinical infections impose a physiological cost, potentially reducing the work capacity and productivity of camels over time due to lower oxygen-carrying capacity of the blood. Field observers have noted that chronically infected camels may have reduced stamina and slight weight loss (though these signs are subtle). Moreover, anemia could make camels more vulnerable to other stressors. Interestingly, Heidari et al. (2023) found an increased leukocyte count in subclinically infected camels, suggesting a sustained immune response or stress leukogram (Getange et al. 2021). In contrast, other reports Azmat et al. (2018) noted neutropenia and lymphopenia in camels with anaplasmosis (Azmat et al. 2018), likely reflecting an acute phase of infection. These discrepancies imply that hematological profiles might

differ between acute and chronic stages of camel anaplasmosis.

In our study, we likely captured mostly chronic or subacute cases, given the mild nature of infections and the management system. A major finding of this study is the identification of risk factors that significantly correlate with infection status. Chief among them is tick infestation. We documented a six-fold higher prevalence of anaplasmosis in camels that had ticks on their body versus those without ticks (25% vs 5%). This strong association is expected, as numerous studies confirm that Anaplasma spp. are primarily tick-borne pathogens (Getange et al. 2021). Ticks of the genus Hyalomma, which are abundant on camels in our region, are known vectors of camel anaplasmosis and other hemoparasites (Abbas et al. 2024a). Our findings align with the recent serosurvey in Punjab which also found tick-infested camels at much higher odds of being seropositive (OR ~38). Similarly, a study reported that camels with tick infestation were at significantly increased risk of Anaplasma infection (El-Alfy et al. 2024), echoing the consensus that tick control is crucial in preventing anaplasmosis. It is worth noting that mechanical transmission (via biting flies or reuse of needles) is also possible, but in our context, the overwhelming contribution of ticks is clear. Nearly all infected camels had ticks, indicating that tick exposure is likely a necessary condition for infection in this environment. This underscores a practical point: controlling tick infestations on camels (through acaricides, pasture management, and grooming) should drastically reduce the transmission of anaplasmosis (Zafar et al. 2022). The camels' nutritional and body condition status also emerged as a significant risk factor.

We found a marked gradient: camels in poor condition had the highest infection rates (30%), moderate condition had intermediate infection (12%), and well-conditioned camels had the lowest (8%). This trend may have two, not mutually exclusive, interpretations. First, camels in poor body condition might have weaker immunity or more exposure to vectors (for instance, weaker animals may have more ticks, or malnutrition could impair their ability to clear infections) (Guzman et al. 2018). Second, chronic Anaplasma infection itself can contribute to deterioration of body condition over time due to the sustained anemia and potential anorexia during acute phases. Cross-sectional data cannot confirm causality, but the association is consistent with reports that emaciated (poor-BCS) camels are more susceptible to anaplasmosis (Chaibi et al. 2024). Azmat et al. (2018) also observed higher Anaplasma infection in camels with poor body condition, likely linked with heavy tick loads and concurrent diseases in those animals (Azmat et al. 2018). Our results reinforce that body condition score is a useful risk indicator: a thin camel in this region is significantly more likely to be carrying Anaplasma than a well-fed one. From a management perspective, improving nutrition and overall health of camels could enhance their resistance to infections or their tolerance of parasitism. Interestingly, age and sex did not show significant effects on infection prevalence in our study. All age groups were almost equally affected, suggesting that camels are exposed to ticks and infection early in life and continue to be at risk throughout adulthood. Some other studies have reported age-related differences: for instance, a study in Pakistan noted that middle-aged camels (3–5 years) had higher Anaplasma seroprevalence than older camels (Ghafar et al. 2025), which was hypothesized to be because older camels might develop partial immunity after repeated low-level exposures. Chaibi et al. (2024) similarly observed that cattle over five years often have immunity that reduces clinical anaplasmosis (Ghafar et al. 2025).

In our data, the oldest camels (>7 years) had a slightly higher raw prevalence (17.3%) than younger ones (~11–12%), but not significantly so. This could be due to limited sample size in subgroups or the fact that even older camels remain exposed to ticks enough to still contract infections. Regarding sex, previous findings have been inconsistent: some researchers found female camels more likely to be seropositive, possibly due to stresses of pregnancy and lactation causing some immune suppression. For example, a study reported females had significantly higher infection rates than males, whereas a Pakistani study by Azmat et al. noted males had higher infection (perhaps because males often roam more or are less tended) (Azmat et al. 2018). Our results showed no meaningful difference between sexes (M: 14.9% vs F: 12.8% $p > 0.5$), agreeing with the large Punjab survey which also found no sex effect (8.5% in both genders) (Parvizi et al. 2020).

It is possible that management in our area does not differ drastically by sex – both male and female camels graze in the same areas and receive minimal differential care, so their exposure risk is similar. Thus, we conclude that sex and age are minor factors in this endemic setting, with both male/female and young/old camels equally needing protection from ticks and infection. No significant difference was observed by breed or herd origin. Almost all camels in the study were the indigenous one-humped dromedary, with only minor breed variations (which in camels are not as genetically pronounced as in cattle). It is expected that species (dromedary vs Bactrian) might have some difference in susceptibility, but all our camels were dromedaries. Within Pakistan's dromedaries, no literature exists suggesting one breed line is more resistant to anaplasmosis. Our limited data showed a slightly higher infection percent in "non-local" camels (16.2%) than local (12.9%), but this could be due to chance given the small number of non-local animals. All in all, breed is likely not a crucial determinant for infection – management and environment play bigger roles. Similarly, the four villages we sampled all presented anaplasmosis, with prevalence ranging 10–22%. Although one village (D) had a higher rate, the difference was not statistically significant. All villages are in the same district and share similar climate and grazing ecology. Camels may also move between villages, as herders sometimes travel, which can homogenize infection risk across nearby areas (Selim et al. 2022). Therefore, anaplasmosis appears to be widespread at a low-moderate level throughout the study region, rather than confined to any particular hotspot in our sampling frame.

Our finding that A. marginale was more common than *A. centrale* in camels is noteworthy. A. marginale is classically a bovine parasite, but accumulating evidence shows it can also infect camels and other ruminants

(Ashraf et al. 2021). *A. centrale* is a less pathogenic bovine Anaplasma, often used as a live vaccine strain for cattle in some countries. The presence of *A. centrale* in 3% of our camels suggests spillover or circulation of multiple Anaplasma species in the area. It is possible that the camels acquire A. marginale from ticks that previously fed on infected cattle, as cattle are common in Khyber Pakhtunkhwa and often graze in proximity. Indeed, multi-host ticks like *Hyalomma anatolicum* can transmit *A. marginale* between cattle and camels (Ashraf et al. 2021).

Our study did not employ PCR, so we could not confirm if Candidatus *A. camelii* or other novel strains were present. However, the occurrence of co-infections on smears hints that camels may harbor more than one Anaplasma. This has implications for diagnosis and control: mixed infections might modulate disease severity and influence the sensitivity of tests. Future molecular characterization in this region would be valuable to identify exactly which Anaplasma species (or strains) are circulating in camels and ticks. Despite being mostly subclinical, camel anaplasmosis has tangible impacts. In our study, infected camels showed reduced hematological values, which can translate to diminished work output (for draft or transport camels) and possibly lower milk yields due to poorer health. While none of the camels died or showed acute fatal anaplasmosis, the disease could potentially exacerbate under harsh conditions. For instance, if a camel already infected with Anaplasma faces a drought or heavy work, the borderline anemia could worsen and precipitate clinical illness. There are reports of clinical anaplasmosis outbreaks in camels causing fever, jaundice, and even death in severely infested animals, especially when co-infections like trypanosomes are present. This indicates that even if ordinarily silent, anaplasmosis in camels is a disease worth monitoring and managing.

Our study underscores the importance of tick control and general health management in camels. Regular acaricide treatment, manual removal of ticks (grooming), and improving housing (e.g., keeping camels in tick-safer enclosures at night) could significantly reduce infection rates. Although our study did not specifically measure grooming practices or tick control usage, anecdotal information from owners indicated minimal tick control measures are in place (which likely explains the high tick infestation rate observed). Educating camel owners about the economic losses from subclinical diseases and encouraging integrated tick management could help. Moreover, maintaining good nutrition for camels not only improves their condition but might enhance their immune response to infections, potentially lowering the burden of Anaplasma and other parasites. Another practical implication is the need for surveillance and veterinary attention to camel health. Historically, camels have been considered hardy animals with few diseases, leading to relative neglect in veterinary services. Our findings contribute to the evidence that camels in Pakistan do harbor infections like anaplasmosis which can affect their productivity. This highlights that veterinary authorities should include camel populations in tick-borne disease control programs. Currently, vaccines for bovine anaplasmosis exist in some regions (e.g., using *A. centrale* as an attenuated vaccine in cattle), but no vaccines are available for camels. The control must therefore rely on prevention (tick control) and treatment. Tetracycline antibiotics are effective against Anaplasma and could be used to treat valuable animals showing signs of anemia or illness due to anaplasmosis. However, mass treatment is not practical for subclinical cases; thus, preventing infection in the first place by managing ticks is far more sustainable. In terms of the broader epidemiological picture, our study provides baseline data for Khyber Pakhtunkhwa province. It confirms that like other parts of Pakistan, such camels are exposed to Anaplasma spp. (our seroprevalence ~19%).

Camels could be serving as additional reservoirs or sentinels for these pathogens. There is also a public health aspect: certain Anaplasma species (e.g., *A. phagocytophilum*) can infect humans, though those have not been reported in camels here. Nonetheless, tick control on camels can have collateral benefits in reducing tick-borne disease risk for people (since Hyalomma ticks from camels can bite humans and transmit diseases like Crimean-Congo hemorrhagic fever virus). Therefore, improving camel health through parasite control aligns with the One Health concept as well.

Limitations

This research has some limitations that should be acknowledged. First, the diagnostic method of microscopy, while specific, has limited sensitivity – especially in chronic carriers with low parasitemia. We likely underestimated the true prevalence of Anaplasma infection by relying on smear detection. The inclusion of cELISA helped reveal additional exposures, but we did not perform PCR confirmation. Molecular testing would have allowed precise identification of the Anaplasma species involved (e.g., to detect if Candidatus Anaplasma camelii was present). The lack of PCR means our species identification (marginale vs centrale) was based solely on morphology, which, while generally reliable, is not foolproof. Secondly, the study was geographically limited to one district and a total of four villages. Thus, the results may not be fully generalizable to all of Khyber Pakhtunkhwa or Pakistan – there could be areas with different tick ecologies or husbandry practices that experience higher or lower anaplasmosis prevalence. Third, as a cross-sectional study, we could only assess associations, not causal relationships. For example, while we found poor body condition associated with infection, we cannot definitively say infection caused poor condition or vice versa in all cases. Longitudinal studies would be useful to see if camels lose condition after becoming infected or if poor condition predisposes to infection. Another limitation is that we did not systematically evaluate seasonal effects. Our sampling covered a one-year span, but we did not analyze data by season in detail. Season can influence tick populations – for instance, summer might increase tick activity and infection risk. In the Punjab study, season (summer) was a significant factor for higher seroprevalence. In our data, we did not record each sample's month, so we might have missed seasonal peaks. Finally, resource constraints meant we only measured one hematological parameter (PCV) and did not

evaluate others like white blood cell differentials or biochemistry, which could provide more insight into subclinical disease effects. Despite these limitations, the study provides a foundational dataset for camel anaplasmosis in the region. Future research incorporating more advanced diagnostics (PCR sequencing) and broader sampling (multiple districts and seasons) would build upon these findings.

Practical Implications

The demonstration of Anaplasma infection in camels of Lakki Marwat has several practical implications for veterinary health management in the region. First, it underlines the importance of implementing effective tick control programs for camels. Given the strong link between tick infestation and infection, regular use of acaricides (such as pour-on or spray formulations) on camels, along with environmental tick management in animal enclosures, should significantly reduce transmission. Training camel owners in simple tick control practices and routine grooming could help lower the burden of ticks and thereby Anaplasma infections. Second, the findings call for greater awareness among livestock owners and field veterinarians regarding camel anaplasmosis. Camels with vague signs like poor condition or mild anemia might be overlooked, but our study indicates such signs could be due to subclinical anaplasmosis. Educating owners to recognize when a camel is underperforming or looking pale, and encouraging them to seek veterinary advice, can lead to timely treatment (e.g. with oxytetracycline) and prevention of productivity losses. Third, this research provides baseline data that can be used by animal health authorities to include camels in broader tick-borne disease surveillance and control initiatives. Camels are valuable assets for local livelihoods (providing transport, milk, meat, and income); protecting them from diseases like anaplasmosis will have economic benefits. By highlighting risk factors such as poor nutrition, the study also implies that improving general husbandry (adequate feeding, mineral supplementation) may enhance camels' resilience to infections.

CONCLUSION

This study establishes that Anaplasma infections are present in the camels of Lakki Marwat, Khyber Pakhtunkhwa, Pakistan, at an appreciable prevalence (~13.5% by microscopy; ~19% by serology). The infections are mostly subclinical yet associated with significant anemia, indicating a hidden impact on animal health. *Anaplasma marginale* appears to be the dominant infecting species in camels here, with occasional *A. centrale* and mixed infections, suggesting multi-host transmission cycles involving camels and other livestock. Crucially, tick infestation and poor body condition were identified as key risk factors that heighten the likelihood of infection. In contrast, age, sex, and breed showed no significant influence in this population. These findings point to ticks as the main driver of camel anaplasmosis and underscore the importance of tick control and good husbandry in prevention. To our knowledge, this investigation is the first of its kind in Khyber Pakhtunkhwa and adds to the emerging recognition of camel anaplasmosis in South Asia. We recommend implementing integrated tick management strategies for camel herds and improving nutritional and health care for these animals. Further research – employing molecular diagnostics, broader geographic sampling, and longitudinal monitoring – is warranted to fully elucidate the epidemiology of Anaplasma in camels and to inform effective control measures.

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