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RESEARCH ARTICLE

Molecular Detection of Tick-Borne Pathogens in Captive African Lions (*Panthera Leo*) from Pakistan

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ABSTRACT

African lions (*Panthera leo*) are currently listed as "Vulnerable" on the International Union for the Conservation of Nature (IUCN) red list of threatened species. After habitat destruction and illegal hunting, vector-borne pathogens are the major source of morbidity and mortality in African lions. The aim of the present study was to assess the presence of selected pathogens in African lions from Pakistan. Blood samples were collected from six lions visiting a pet clinic in Lahore, Pakistan. Data on the epidemiological characters (gender, age, mucous membrane, deworming, hematuria, vomiting, and tick infestation) was collected through a questionnaire at the sampling site with the help of lion owner. The blood samples were screened for the amplification of 16S rRNA genes of *Anaplasma* spp., *Ehrlichia canis,* and *Mycoplasma haemofelis*, and 18S rRNA gene of *Babesia* spp. and *Babesia canis* DNA. The results revealed that 2 out of 6 (33.3%) lion blood samples were infected with *Anaplasma* spp. The presence of *B. canis* was also detected in 2 out of 6 (33.3%) lions. Analysis of epidemiological data revealed that all of the studied parameters varied non-significantly ($P > 0.05$) when compared between *B. canis* and *Anaplasma* spp. positive and negative lion blood samples. In conclusion, this is the first report on molecular detection of tick-borne parasites in lions from Pakistan. The data generated in this study will help to understand host parasite interaction of these wild species that would lead towards effective diagnosis and infection control.

Key words: Vector-borne pathogens; PCR; African lions; Pakistan.

INTRODUCTION

Lions (*Panthera leo*), members of the family *Felidae*, are recognized as apex predators that play a key role in regulating ecosystems (André et al., 2014). Genomic studies over the past few years highlight their evolutionary complexity and interactions with other big cat lineages (Antunes et al., 2008; Santos et al., 2025). Lion fossils first appear in Africa during the Late Pliocene, and by the Pleistocene, their range extended across Eurasia and the Americas. Modern genomic analyses of both extinct cave lions and historical lion populations now reveal a clear divergence between cave and modern lion lineages, with no evidence of later interbreeding (de Manuel et al., 2020). Lions eventually disappeared from much of their historic range during the Late Quaternary megafaunal extinctions, largely driven by human hunting pressure, climatic variability, and habitat alterations (Barnett et al., 2009; Makundi, 2025). Infectious diseases particularly those introduced through domestic animals, such as canine distemper virus, bovine tuberculosis, and tick-borne parasites have significantly contributed to the decline of local lion populations, especially in isolated or fragmented groups (Williams et al., 2014; McDermid et al., 2017). Vector-borne diseases arise when blood-feeding arthropods such as mosquitoes, ticks, and fleas transmit infectious pathogens to humans and other animals, posing a major threat to both wildlife and public health (André et al., 2012; Rocklöv & Dubrow, 2020). It is well established that free-ranging wild carnivores face higher exposure to vector-borne infections compared to captive or managed populations (McDermid et al., 2017; Santos et

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al., 2025). Tick- and flea-borne pathogens are capable of causing severe, and sometimes fatal, diseases in lions, negatively impacting their health and contributing to population declines (Kelly et al., 2014; Makundi, 2025). The global rise in these diseases is closely linked to increased human and animal mobility, which facilitates the spread, circulation, and exchange of vectors and infectious agents across regions (Beugnet & Marie, 2009; Rocklöv & Dubrow, 2020). Several tick-borne pathogens, including those causing granulocytic anaplasmosis, babesiosis, and ehrlichiosis, have been identified in lions, highlighting their role in disease transmission and population health risks (Makundi, 2025). Safari parks, zoos, and other captive facilities contribute significantly to the conservation of endangered species (Davis et al., 2010). Nevertheless, animals in these environments remain vulnerable to infections from endemic pathogens, much like their wild counterparts (Abbas et al., 2023; Milošević et al., 2024). Identifying and monitoring the pathogens circulating in parks, farms, and zoos is therefore essential to enhance the effectiveness of conservation and species recovery programs (Gourley et al., 2007; Terio et al., 2021). There is currently limited information on the occurrence of vector-borne diseases in lions (*Panthera leo*) in Pakistan. Therefore, the present study was undertaken to investigate the presence of haemoparasites and associated risk factors in lions from Pakistan using PCR assay.

MATERIALS AND METHODS

Subjects, blood and data collection

Blood samples were collected from 6 apparently healthy client-owned lions, and presented to the pet clinic of the University of Veterinary and Animal Sciences (UVAS) in Lahore, Pakistan for routine checkup during September 2017. Lions were imported by the owner from Africa and were maintained as pet under conducive conditions maintained in a farm house following legal documentation. Blood samples (approximately 3 ml) were collected from the jugular vein into tubes containing 0.5M EDTA solution as an anticoagulant. Clinical and epidemiological data (including gender, age, mucous membrane color, vomiting, deworming, presence/absence of hematuria, and tick infestation) was collected in all animals included in the study. All the experimental protocols and animal handling procedures were approved by the ethical review board of the Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan (IPAB/Eth/2017- 63).

DNA extraction and PCR based blood borne pathogen detection

Genomic DNA was extracted from the collected blood samples using an inorganic salting-out method (sodium chloride precipitation), as described by Ijaz et al. (2025). PCR targeting the 16S (*Ehrlichia canis*, *Anaplasma* spp., *Mycoplasma haemofelis*) and 18S (*Babesia* spp., *Babesia canis*) rRNA genes was conducted. Details of oligonucleotide primers and PCR assays used in the screening of the samples are listed in Table 1.

The PCR reaction mixtures and thermocycling conditions were performed for each pathogen with minor modifications as described by Földvári et al. (2005) for *B. canis*, Braga et al. (2012) for *M. haemofelis*, Chaudhry (2012) for *Babesia* spp., Gal et al. (2008) for *E. canis* and Noaman et al. (2009) for *Anaplasma* spp. PCR products were visualized under a UV Trans illuminator (Dongsheng Biotech China) after electrophoresis on a 2% agarose gel stained with Ethidium Bromide. In all amplification series, positive (genomic DNA of *Ehrlichia canis*, *Mycoplasma haemofelis,* and *Babesia canis*) and negative (RNase-free water) controls were included. Amplicons of positive samples (*B. canis* and *Anaplasma* spp.) were excised from the gel and purified using NucleoSpin Gel and PCR Clean-up (Macherey Nagel, Germany) for sequencing.

Statistical analysis

All the data is represented as Mean ± Standard Error of mean. The significance level was set at $P < 0.05$. Results were statistically analyzed by the Statistical package Minitab (version 17). Epidemiological and clinical parameters were compared between parasite-positive and negative animals by using Fischer' s exact test.

Table 1. Oligonucleotide primer sequences, their targeted genes, annealing temperatures, and amplicon generated for the amplification of blood-borne parasites in lion samples during the present study.

Pathogens	Targeted gene	Primer sequence	PCR amplicon (bp)	Annealing temperature (°C)	Reference
Anaplasma spp.	16S rRNA	F5'AGAGTTTGATCCTGGCTCAG 3' R5'GTTAAGCCCTGGTATTTCAC 3'	577	56	Noaman et al. (2009)
Ehrlichia canis	16S rRNA	F5'AACACATGAAG TCG AAC GGA-3' R5'TATAGGTACCGT CAT TATCTTCCCTAT-3'	400	62-52 Touch down PCR	Gal et al. (2008)
Mycoplasma haemofelis	16S rRNA	F5´GACTTTGGTTTCGGCCAAGG-3´ R5´CGAAGTACTATCATAATTATCCCTC-3´	393	54	Braga et al. (2012)
Babesia spp.	18S rRNA	F5'CCG TGC TAA TTG TAG GGC TAA TAC-3' R5'GGAC TACG A CG GTATCTGATCG-3'	800	58	Chaudhry, (2012)
Babesia canis	18S rRNA	F5'AGGGAGCCTGAGAGACGGCTACC-3' R5'TTAAATACGAATGCCCCCAAC-3'	450	60	Rene-Martellet et al. (2013)

RESULTS AND DISCUSSION

To our knowledge, there is no information available in the literature regarding the presence of tick and flea-borne parasites in lion populations in Pakistan. The present study was designed to report the presence of various haemoparasites in six lions' blood samples collected from the country. Analysis of the results revealed that PCR had amplified *Anaplasma* spp. and *Babesia canis* each in 2 out of 6 (33.3%) blood samples of the animals (Fig. 1A and B).

However the DNA of *Babesia* spp., *Ehrlichia canis* and *Mycoplasma haemofelis* was not detected in the screened African lion blood samples (Table 2). Also, the sequences of *B. canis* and *Anaplasma* spp. were found futile in the present study because the sequence result did not show a promising peak despite the fact that the positive purified PCR products of both species were sent two times for sequencing.

Torina et al. (2007) reported 10% prevalence of *A. phagocytophilum* in *P. leo* from Fasano Safari Park in Italy by PCR. Kelly et al. (2014) analyzed blood samples of 86 captive lions from Zimbabwe for the molecular detection of *A. phagocytophilum* and found 7% of lions were infected with this pathogen. McDermid et al. (2017) reported that blood samples collected from lions (N = 13) were analyzed for *Anaplasma* spp. using reverse line blot hybridization assay from Botswana and found all lions were negative. Githaka et al. (2012) reported the presence of *Babesia* spp. in two captive anaemic lions (*P. leo*) using a touch-down PCR targetting 18S rRNA gene of parasite from Nairobi Kenya, and the BLAST search for these amplified sequences had a close match with *B. canis* (98%). McDermid et al. (2017) screened blood samples of 13 lions from Botswana for molecular detection of the 18S rRNA gene of different species of *Babesia* and found that all of these were generally infected with the *Babesia* species while only two lions were specifically positive for *B. canis*. Munson et al. (2008) collected the blood samples of 6 lions from Africa and analysed them using molecular methods for the presence of 18S rRNA gene *B. canis* and found that all samples were negative. PCR-based analysis had also been carried out on 86 captive lions collected from Zimbabwe and 73% of lions were found infected with *Babesia* species (Kelly et al., 2014). All these studies documented above show a wide range of prevalence for both groups of parasites under investigation. The differences in prevalence rates reported in these studies are probably due to different climatic and geographical distributions and vector abundance (Razzaq et al., 2015).

Analysis of data revealed that none of the studied parameters was found associated with the presence of *B. canis* and *Anaplasma* spp. (Table 3 and 4). These results are in agreement with those reported by McDermid et al. (2017) as they had conducted a similar study in lions for molecular detection of *Babesia* spp. and reported that the age and gender of analyzed lions were not associated with *Babesia* spp. infection.

Despite this is the first ever report from Pakistan regarding the presence of blood borne infectious agents in captive pet lines, small sample number and lack of pathogen confirmation are the limiting factors for this study. Keeping in view that lions are among the endangered species and the sample collection is not an easy task, the small sample number can be justified. Regarding DNA sequencing, it is rare that the parasite DNA is amplified (as we enrolled apparently healthy animals). So, once we fail to get a presentable sequence due to many factors including sequence delay or contamination, it is always difficult to reamplify pathogenic sequences. For the future studies, we recommend a nested PCR based approach to enhance the chances of successful DNA sequencing.

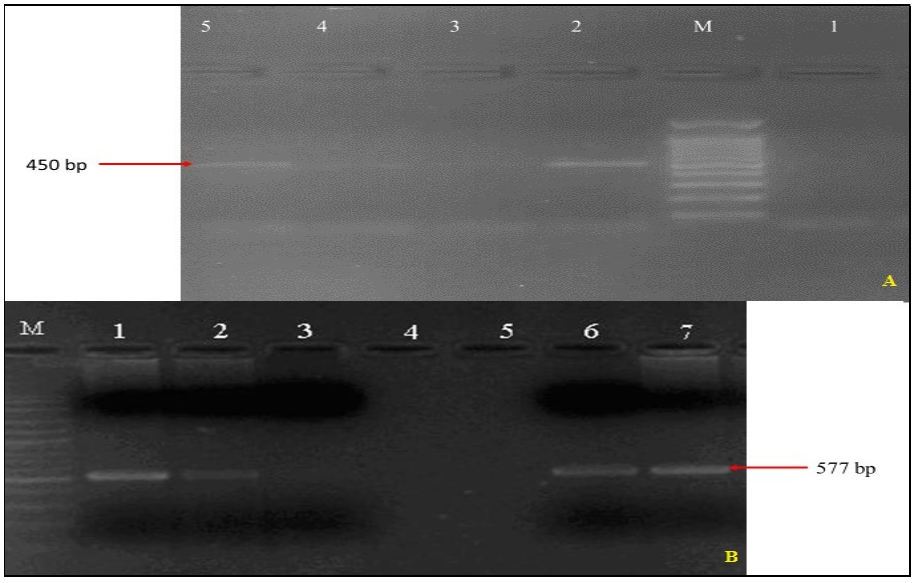

Fig. 1: A. PCR amplification of 16 S rRNA gene of *Babesia canis* in blood samples from present study. Lanes: M, 100 bp DNA ladder; lanes 1 and 3 presenting parasite negative, lane 2 presenting positive control while lanes 4 and 5 presenting *Babesia canis* positive *Panthera leo*. **B.** PCR amplification of 16S rRNA gene in *Anaplasma* spp. in *Panthera leo* blood samples. Lanes: M presenting the 100 bp DNA ladder; lane 1 is showing the positive control; lanes 6 and 7 presenting *Anaplasma* spp. poitve while lanes 2-5 presenting bacteria negative *Panthera leo* blood samples.

Table 2: Prevalence of studied blood borne parasites in the blood samples of African lion *(Panthera leo)* collected from Lahore. Percentage prevalence for each pathogen is given in parenthesis.

Serial No.	Pathogen	Prevalence
1	*Anaplasma spp.*	2/6 (33.3 %)
2	*Babesia* spp.	0/6 (0 %)
3	*Ehrlichia canis*	0/6 (0 %)
4	*Babesia canis*	2/6 (33.3 %)
5	*Mycoplasma haemofelis*	0/6 (0 %)

Table 3: Association of *Babesia canis* prevalence with the studied epidemiological and clinical parameters describing African lions enrolled during the present study. N represents the total number of collected samples. % prevalence of *Babesia canis* is given in parenthesis. P-value indicates the results of Fischer exact test calculated for each parameter.

Parameters	N		*B. canis.* +ve	P - value
Animal sex	Male	4	02 (50%)	0.5
	Female	2	00 (0%)	
Age	>1 year	4	01 (25%)	1
	<1 year	2	01 (50%)	
Mucous Membrane	Normal	6	02 (33.3%)	
	Pale	0	00 (0%)	#
Hematuria	Present	0	00 (0%)	#
	Absent	6	02 (33.3%)	
Vomiting	Present	0	00 (0%)	
	Absent	6	02 (33.3%)	#
Dewormed	Yes	6	02 (33.3%)	
	No	0	00 (0%)	#
Tick Infestation	Present	0	00 (0%)	
	Absent	6	02 (33.3%)	#
Body Condition	Good	6	02 (33.3%)	#
	Poor	0	00 (0)	
Hydration status	Normal	6	02 (33.3%)	
	Dehydrated	0	00 (0%)	#

P>0.05 = Non-significant; # Statistical analysis was not possible.

Table 4: Association of *Anaplasma* spp. prevalence with epidemiological and clinical parameters of African lions enrolled during the present study. N represents the total number of collected samples. % prevalence of *Anaplasma* spp., is given in parenthesis. P-value indicates the results of Fischer exact test for each parameter.

Parameters	N		*Anaplasma spp.* +ve	P - value
Gender	Male	4	01 (25%)	
	Female	2	01 (50%)	1
Age	>1 year	4	01 (25%)	
	<1 year	2	01 (50%)	1
Mucous Membrane	Normal	6	02 (33.3%)	#
	Pale	0	00 (0%)	
Hematuria	Present	0	00 (0%)	#
	Absent	6	02 (33.3%)	
Vomiting	Present	0	00 (0%)	#
	Absent	6	02 (33.3%)	
Dewormed	Yes	6	02 (33.3%)	3
	No	0	00 (0%)	
Tick Infestation	Present	0	00 (0%)	#
	Absent	6	02 (33.3%)	
Body Condition	Good	6	02 (33.3%)	#
	Poor	0	00 (0)	
Hydration status	Normal	6	02 (33.3%)	
	Dehydrated	0	00 (0%)	

P>0.05 = Non-significant; For these parameters statistical analysis was not possible.

Conclusion

In conclusion, *B. canis* and *Anaplasma* spp. were detected in captive pet lions from Lahore, Pakistan. This study provided the first molecular evidence of tick-borne pathogens (*B. canis* and *Anaplasma* spp.) in lions in Pakistan. As we have little information available in literature regarding the parasites infecting the wild lions, the data generated in this study will help to understand host parasite interaction of these wild species that would lead towards effective diagnosis and infection control that will improve the health and quality of life of these wild animals.

DECLARATIONS

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Competing interests: The authors declare that they have no competing interests.

Data Availability Statement: Data will be available upon request from the corresponding author.

Ethics approval and consent to participate: All the experimental protocols and animal handling procedures were approved by the ethical review board of the Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan (IPAB/Eth/2017- 63).

Author's Contribution: Formal Analysis, investigations, original draft writing, reviewing, and editing: Asma Hassan, Omeira Ibrahim, and Maria Shahzeen. Resources: Asim Khalid Mehmood and Waseem Ijaz, Data validation, data curation, and supervision: Maryam Ijaz.

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