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MOLECULAR INVESTIGATION AND ASSOCIATED RISK FACTORS OF THEILERIOSIS IN ONE-HUMPED CAMELS IN JHANG, PAKISTAN

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Abstract

The camel is a valuable source of food, wool and hides in many countries, including Pakistan. The animal is understudied concerning the molecular diagnosis of vector-borne protozoa, particularly theileriosis, thus, the present study reports the characterization of the blood of dromedary camels through molecular tools. A total of 126 blood samples were collected from one-humped camels, and the blood smears were prepared and examined microscopically using Giemsa staining. The PCR assay was carried out for the detection of Theileria (T.) annulata, followed by sequence analysis of the positive samples. Thirty-six (34.61%) samples were found to be positive for T. annulata through microscopy and PCR. The disease was non-significantly associated with sex and age, but significantly associated with parasitic infestation. It was concluded that T. annulata was highly prevalent in the camel population of Jhang, Punjab, Pakistan, and higher authorities should take measures to control this disease. Further studies are suggested to investigate their impact on the general health and productivity of animals.

Keywords: *Camel, Molecular detection, Pakistan, PCR, Theileria*

INTRODUCTION

The camel is a multipurpose, resilient animal and withstands harsh circumstances of arid and semi-arid areas due to its unique physiological adaptations. Pakistan ranks at 8th globally among camel-raising countries, with 21 breeds and a camel population of 1.1 million (1) (). One humped camels (*Camelus dromedarius*) is utilized for transportation, as well as milk and meat production (2). Although camels are usually resistant to various infections but theileriosis is the most important and highly prevalent tick-borne disease of the species (3, 4). Protozoa like theileria, babesia and anaplasma are usually transmitted by ticks in the livestock including camelids (5, 6). The ticks involved in the transmission of these diseases are Hyalomma and Ixodid species, distributed worldwide and thus cause considerable economic losses in health and production (7, 8). About 235 species of tick genera are considered as the vectors for different pathogens including protozoa, viruses, rickettsia and bacteria (9, 10). However, ticks are obligatory blood-sucking ecto-parasites that usually infest various animals including humans and become the carrier for various pathogens (11, 12). Theileriosis caused by various parasitic species including *T. annulata*, *T. ovis*, *T. mutans* and *T. equi* are considered as mild to moderate pathogens to camels. *Theileria annulata* is intracellular erythrocytic protozoan that predominantly appears small oval to ring-shaped in the blood smears (13). It is considered endemic in the tropical and sub-tropical territories including Asia, Middle East, Southern Europe and North Africa (14). The clinical findings in camels infected with theileriosis are fever, emaciation, rough

body coat, ocular discharge, diarrhea and enlargement of lymph nodes (15). Theileriosis had been described and frequently studied in other livestock species (16 , 17) but only few studies are found in the literature in camels (18).

The detection of *T. annulata* through classical microscopic examination by using Giemsa stain is helpful in early diagnosis of the disease (19). Additionally, the molecular detection of parasites by using polymerase chain reaction (PCR) is suggested to be a valuable tool to further confirm the diagnosis (1, 5, 20, 21). As, PCR-based diagnosis of theileriosis in camels has not yet been conducted in Pakistan. Therefore, the current study was planned to detect the theileria on molecular basis along with associated risk factors in camels in Pakistan.

MATERIALS AND METHODS

STUDY AREA

The blood samples (n=126) were collected from the suspected camels showing the clinical signs of Theileriosis in the district Jhang, Pakistan. This area is located in Punjab, Pakistan at an Altitude of approximately 518 feet above sea level, with coordinates 31.2715° N Latitude and 72.3281° E Longitude.

A written permission was obtained from each camel owner for the use of samples for scientific purpose before collecting the blood samples. The data relating to potential causative risk factors i.e. age, sex and presence of ticks were collected on a predesigned questionnaire.

BLOOD SAMPLING AND SMEAR EXAMINATION

Approximately, 5ml of blood was collected aseptically via jugular venipuncture using a disposable plastic syringe. The samples were promptly transferred into purple-capped vacutainers (BD® spray-coated with K_2 EDTA) and immediately transported to the Post-Graduate diagnostic laboratory, University of Veterinary and Animal Sciences, Sub-campus, Jhang for preliminary analysis. Thin blood smears were prepared, stained with Giemsa and Wright stains and examined under the light microscope (Olumpus) at 100X to identify the *T. annulata* parasite on the basis of intracellular characteristic features, as described earlier (20). The camels were initially declared as positive for the disease, based on the presence of protozoa in the stained smears.

DNA EXTRACTION

The blood specimens were subjected to DNA purification by using QIAamp DNA Mini Kit (51304, USA) by using 200 µl of EDTA added whole blood (22, 23). The final elution volume was 50 µl and the DNA concentration and purity was determined via NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific Inc, USA), as described (24).

MOLECULAR DETECTION OF *T. ANNULATA*

For molecular identification of *T. annulata*, partial amplification of 18S rRNA gene with a product size of 500 bp was employed by using primer set PIRO-A1 (5'-AGGGAGCCTGAGAGACGGCTACC-3') and PIROB (5' -TTAAATACGAATGCCCCCAAC-3') as described elsewhere (25).

PCR reaction mixture (25µl), was prepared by mixing 12.5 µl master mixture (2x, VizPure, W1402, Republic of Korea), 2µl (10 pmol/µl) each forward and reverse primers, 4µl of template DNA and 4.5 µl nuclease-free water (26, 27). Thermal cycler (4375305 Veriti™, Applied biosystems, USA) was used with thermal cycling parameters as follows; 95°C for 10 min (initial denaturation), 40x cycles (denaturation, 94°C for 30s, annealing, 59°C for 30s, extension, 72°C for 30 s) and a final extension at 72°C for 7 minutes. Subsequently, the PCR product was run in 1.3% agarose gel at 90 volts for 40 minutes, as described earlier (27, 28).

STATISTICAL ANALYSIS

The data on the prevalence of *T. annulata* in the camels were analyzed using the Pearson Chi Square test with a 95% confidence interval, utilizing the OpenEpi software.

RESULTS

A total of 36 samples were observed positive both microscopically and through PCR for *T. annulata*. It is pertinent to mention that all the sampled animals were infested with ticks. The overall prevalence observed at 95% C.I. was 28.57% (36/126). The amplified samples showed 500-bp band specific for 18S rRNA gene of *T. annulata* (Fig. 1). The sequence analysis revealed three distinct 18S rRNA sequences ranging from 443–646bp. Nucleotide BLAST analysis (was done at UVAS, Lahore) indicated that all sequences shared over 90% identity with *T. annulata* sequences available at public databases. The newly identified partial 18S rRNA gene sequences of *T. annulata* from this study were submitted to GenBank under the Accession numbers MW300321, MW392284 and MW392285 (https://www.ncbi.nlm.nih.gov/nuccore/).

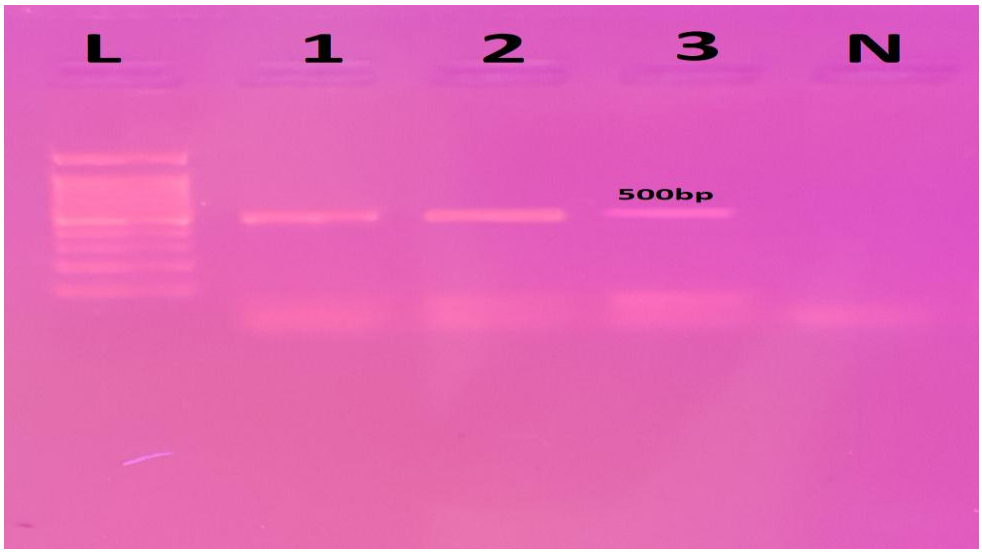

Fig. 1. *Theileria annulata* band of ~500bp on 1.3 % agarose gel electrophoresis. DNA ladder (100 bp) (L); lanes 1, 2, 3 are positive samples; negative control (N)

The potential risk factors associated with the prevalence of *T. annulata* were analyzed (Table I). It was revealed from the analysis that the prevalence of *T. annulata* was significantly ($p<0.05$) associated with the age ($p=0.045$) and presence of ectoparasites infestation ($p=0.008$), whereas, there was no significant difference between male and female camels ($p=0.214$). The prevalence was found to be higher in the camels of age below than six years and infested with ticks.

Table I. Prevalence of *T. annulata* associated with different risk factors

Risk factors	Category	Tested samples	Positive (n)	% Prevalence at 95% CI	p-value
Age	<5 Year	78	27	34.61	0.055
	>5 Year	48	9	18.75	
Sex	Male	67	16	23.88	0.214
	Female	59	20	33.89	
Ectoparasitic infestation	Yes	75	28	37.33	0.008
	No	51	8	15.68	

Table I shows the prevalence of *T. annulata* in camels based on age, sex and ectoparasitic infestation. The prevalence is presented as the percentage of positive cases within each category, along with the corresponding 95% confidence interval (CI). P-values represent the degree of statistical significance, with values below 0.05 deemed statistically significant.

DISCUSSION

This study offers comprehensive insights into the genetic characterization of *T. annulata* and the associated risk factors for camel Theileriosis in Punjab, Pakistan. Theileriosis is a tick-borne disease of a great concern in camels at worldwide. In this study, a 28.57% prevalence of *T. annulata* was observed in the camels reared in the region of Jhang district. This prevalence (28.57%) is quite higher than 13.5% reported in a previous study conducted at 10 different districts of Punjab in Pakistan (1). The variation in the prevalence may be attributed to multiple factors including the health status of the animal (29), an increase in the vectors

populations (30), as well as the feeding and management characteristics of camels population under the study (31).

In tropical and subtropical countries, theileriosis is considered as the 2nd most significant hemoprotozoal disease affecting dromedary camels after trypnosomiasis (20, 32, 33) and various theileria species are implicated as the causative agents of the disease. However, in Egypt, *Theileria camelensis* is recognized as the primary cause of camel theileriosis. The current work showed that 28.57% (36/126, with a 95% C.I.) of the camels examined were infected with the erythrocytic form of *T. annulata*. Few of the authors mentioned that such infection is asymptomatic most of the time in the camelids (5, 6). Therefore, we performed microscopic examinations of other apparently healthy animals through Giemsa-staining of blood smears. Moreover, the PCR assay was subsequently performed to identify *T. annulata*, followed by sequence analysis of the positive samples (Fig. 1). Hence, it has been observed that 36/126 (28.57%) samples were found to be positive for *T. annulata* by light microscopy and PCR.

It is worth mentioned that three randomly selected amplicons of 18S rRNA gene were sequenced via Sanger sequencing method through a commercial service provider (Macrogen, Korea). Raw reads were visualized and trimmed with Chromas v.1.45 (7). The sequence data were submitted to the GenBank database at the National Center for Biotechnology Information (NCBI) and the submitted sequences were analyzed via blastn suite of NCBI. Based on query coverage and percentage identity, similar sequences submitted from other countries of the region were selected and aligned to our sequences by using ClustalW. The multiple sequence alignment was further processed for construction of a phylogenetic tree by using MEGA X 10.0.5 software (34, 35). The neighbor joining tree was constructed based on Tamura 3-parameter (Gamma) model with 1000 bootstrap replicates.

CONCLUSIONS AND RECOMMENDATIONS

This study represents an increased prevalence (28.57%) of the disease by molecular diagnosis and phylogenetic analysis of *Theileria annulata* in one humped camels (*Camelsus dromedarius*) at the district Jhang. Our finding reveals that this high prevalence of the infection in this region underscores the need of effective control measures to mitigate the spread of this pathogen. Regular screening of animals using molecular diagnostic tools should be established to develop an effective monitoring strategy for the early detection and timely intervention of *Theileria annulata* infections. In light of the high prevalence observed, it is recommended that comprehensive tick control and disease prevention programs be implemented to reduce the burden of infection. Awareness and training programs for farmers are essential to promote timely reporting of theileriosis cases and adoption of targeted treatment protocols. A collaborative approach involving government authorities, veterinary institutions, and local farmers is crucial for developing and maintaining sustainable control strategies for this disease. Furthermore, additional studies focusing on the epidemiology and economic impact of camel theileriosis in Pakistan should be encouraged to enhance understanding and inform evidence-based policy decisions.

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Authors' contribution:

MA, MK, Conceptualization; MA, MK Data curation; AS, AR, HU Formal analysis; HU, IA Investigation; MAS, AR Methodology; MK, AN Supervision; HMA, AS Writing original draft; IA, MK, HMA, AS, TR, MAS, AR, HU, IA Writing review & editing. All authors critically reviewed the manuscript and gave their consent for publication of the final version of the manuscript.

Conflict of interest:

The authors declare no known financial or personal conflicts of interest related with this paper.

Data availability statement:

All the data of this study are mentioned in the results and tables of the manuscript file.

References:

1. Aslam F, Saleem G, Ashraf K, Hafeez MA, Saqib M. Identification and molecular characterization of Theileria annulata with associated risk factors in naturally infected camels from selected districts in Punjab, Pakistan. Pakistan Veterinary Journal. 2023;43(1):233-242.
2. Zahoor J, Kashif M, Nasir A, Bakhsh M, Rehman AU, Sikandar A, Nazar AW, Rizwan M. Sero-epidemiology, therapeutic study and the risk factors associated with Trypanosomiasis (Surra) in camels in district Jhang, Punjab, Pakistan. Continental Vet J 2023; 3(1):42-48.
3. El-Alfy ES, Abbas I, Saleh S, Elseadawy R, Fereig RM, Rizk MA, Xuan X. Tick-borne pathogens in camels: a systematic review and meta-analysis of the prevalence in dromedaries. Ticks and Tick-borne Diseases. 2024;15(1):102268.
4. Onyiche TE, Răileanu C, Tauchmann O, Fischer S, Vasić A, Schäfer M, Biu AA, Ogo NI, Thekisoe O, Silaghi C. Prevalence and molecular characterization of ticks and tick-borne pathogens of one-humped camels (Camelus dromedarius) in Nigeria. Parasites & Vectors. 2020;13:1-6.
5. Amer MM, Galon EM, Soliman AM, Do T, Zafar I, Ma Y, Li H, Ji S, Mohanta UK, Xuan X. Molecular detection of tick-borne piroplasmids in camel blood samples collected from Cairo and Giza governorates, Egypt. Acta Tropica. 2024;256:107252.
6. Pardinilla LM, Aljaberi S, Procter M, Hamdan L, Pasha SK, Al Aiyan A, Qablan MA. The prevalence of selected vector-borne diseases in dromedary camels (Camelus dromedarius) in the United Arab Emirates. Veterinary Parasitology: Regional Studies and Reports. 2024;50:101006.
7. Alanazi AD, Al-Mohammed HI, Alyousif MS, Said AE, Salim B, Abdel-Shafy S, Shaapan RM. Species diversity and seasonal distribution of hard ticks (Acari: Ixodidae) infesting mammalian hosts in various districts of Riyadh Province, Saudi Arabia. Journal of medical entomology. 2019;56(4):1027-32.
8. Hussain A, Hussain S, Yu A, Varga C, De Leo GA, Smith RL. Geographical epidemiology of Hyalomma anatolicum and Rhipicephalus microplus in Pakistan: A systematic review. Plos one. 2024;19(8):e0309442.
9. Djiman TA, Biguezoton AS, Saegerman C. Tick-Borne Diseases in Sub-Saharan Africa: A Systematic Review of Pathogens, Research Focus, and Implications for Public Health. Pathogens. 2024;13(8):697.
10. Ossa-López PA, Ramírez-Chaves HE, Rivera-Páez FA. Pathogens associated with ticks (Acari: Ixodidae) and mammals in the Orinoquia region of Colombia: An approach to understanding vector-pathogen-host interactions. Acta Tropica. 2024;256:107282.
11. Alvi MH, Rehman A, Jamil T, Iqbal MZ, Durrani AZ, Khan AU, Usman M, Sauter-Louis C, Conraths FJ. Impact of Farm Management Practices on Tick Infestation in Punjab's Livestock: A Comprehensive Epidemiological Study. Animals. 2024;14(16):2437.
12. Zheng X, Zhang X, Huang X, Yue X, Wang Y. Biodiversity of Ectoparasites and Molecular Detection of Bartonella in Ectoparasites Infesting Rhinolophus Affinis in Yunnan Province, China. Pakistan Veterinary Journal. 2024;44(3).
13. Marwaha S, Brar B, Jain VK, Poonia R, Prasad M. Multiplex PCR for rapid differential diagnosis of co-prevalent species of Theileria (Theileria annulata and Theileria orientalis) in cattle. Parasitology Research. 2023;2(5):1189-97.
14. Mahmoud HY, Tanaka T, Ali AO, Emeish WF. Molecular detection and characterization of Anaplasma ovis, Theileria ovis, and Theileria lestoquardi in sheep and goats in Luxor, Egypt. BMC Veterinary Research. 2024;20(1):260.
15. Verma R, Das G, Kumar S, Nath S, Rai A, Soni A, Mandal S. Molecular investigation of bovine tropical theileriosis outbreak in an organized dairy cattle farm in Madhya Pradesh, India. Parasitology Research. 2023;122(9):2079-89.
16. Atif FA, Nazir MU, Roheen T, Mehnaz S, Hussain I. Antitheilerial efficacy of juglone, buparvaquone and oxytetracycline against tropical theileriosis in naturally infected crossbred cattle. Pakistan Veterinary Journal. 2023;44(1):129-34.

17. Tumer KC, Dincer PF, Babacan S, Yerlikaya Z. Urinary Neutrophil Gelatinase-Associated Lipocalin, Cystatin C and Clusterin as Biomarkers for Acute Kidney Injury in Cattle with Tropical Theileriosis. Pakistan Veterinary Journal. 2023;43(2).
18. Youssef SY, Yasien S, Mousa WM, Nasr SM, El-Kelesh EA, Mahran KM, Abd-El-Rahman AH. Vector identification and clinical, hematological, biochemical, and parasitological characteristics of camel (Camelus dromedarius) theileriosis in Egypt. Tropical Animal Health and Production. 2015;47:649-56.
19. Madkour BS, Karmi M, Youssef MA, Abdelraouf A, Abdel-Rady A. Epidemiological and diagnostic investigation on bovine theileriosis in Aswan Governorate, Egypt. Journal of Parasitic Diseases. 2023;47(1):124-30.
20. Zahoor J, Kashif M, Nasir A, Bakhsh M, Qamar MF, Sikandar A, Rehman A. Molecular detection and therapeutic study of Trypanosoma brucei evansi from naturally infected horses in Punjab, Pakistan. Polish Journal of Veterinary Sciences. 2022;25(3):429-35.
21. Bibi S, Abbas S, Zaman MA, Gul R, Batool AI, Khalil I, Sikandar A, Atif FA. Comparative efficacy of imidocarb dipropionate with additive in naturally infected cattle against bovine babesiosis. Veterinary Parasitology. 2025:110495.
22. Shakir MZ, Rizvi F, Masoud F, Rafique A, Usmani MW, Naseem MN, Usman M. Seroprevalence, Molecular Confirmation and Application of Bacteriophage as A Potential Prophylactic Therapy Against Fowl Typhoid Disease in Commercial Poultry Birds. Pakistan Veterinary Journal. 2024;44(4):1185-92.
23. Zhong W, Liu R, Lan R, Chen J, Tian J, Wang R, Wang D, Yin C. Potential Negative Impact of Actinobacteria Phylum on Middle-Aged Equines Based on 16s rDNA Analysis. Pakistan Veterinary Journal. 2024;44(4).
24. Dupain C, Ali HM, Mouhoub TA, Urbinati G, Massaad-Massade L. Induction of TTF-1 or PAX-8 expression on proliferation and tumorigenicity in thyroid carcinomas. International Journal of Oncology. 2016;49(3):1248-58.
25. Földvári G, Hell E, Farkas R. Babesia canis canis in dogs from Hungary: detection by PCR and sequencing. Veterinary parasitology. 2005;127(3-4):221-6.
26. Alberfkani MI, Swar SO, Almutairi LA, Hasan HK, Ahmed AE, Khalid HM, Mero W. Molecular Characterization and Phylogenetic Analysis of 18S rRNA, gp60 and HSP70 Genes of Cryptosporidium parvum Isolated from Cattle Owners and Cattle using Nested PCR. Pakistan Veterinary Journal. 2024;44(4).
27. Ali HM, Urbinati G, Chapuis H, DesmaEle D, Bertrand JR, Couvreur P, Massaad-Massade L. Effects of siRNA on RET/PTC3 junction oncogene in papillary thyroid carcinoma: from molecular and cellular studies to preclinical investigations. PLoS One. 2014;9(4):e95964.
28. Abed DA, Hamzah KJ, Abdul-kareem S, Mehrzad J. Bacteriological and Molecular Study of Some Urinary Tract Infections Bacteria in Human and Cows in Babylon Province. Pakistan Veterinary Journal. 2024;44(4).
29. Spolidorio MG, Labruna MB, Zago AM, Donatele DM, Caliari KM, Yoshinari NH. Hepatozoon canis infecting dogs in the State of Espírito Santo, southeastern Brazil. Veterinary Parasitology. 2009;163(4):357-61.
30. Otranto D, Dantas-Torres F, Weigl S, Latrofa MS, Stanneck D, Decaprariis D, Capelli G, Baneth G. Diagnosis of Hepatozoon canis in young dogs by cytology and PCR. Parasites & vectors. 2011;4:1-6.
31. de Azevedo Gomes L, Moraes PH, do Nascimento LD, O'Dwyer LH, Nunes MR, Rossi AD, Aguiar DC, Goncalves EC. Molecular analysis reveals the diversity of Hepatozoon species naturally infecting domestic dogs in a northern region of Brazil. Ticks and tick-borne diseases. 2016;7(6):1061-6.
32. Selim A, Alshammari A, Marzok M, Salem M, Al-Jabr OA, Gattan HS. Molecular prevalence and associated risk factors of Theileria annulata infections in dromedary camels in Egypt. Tropical Animal Health and Production. 2023;55(5):335.
33. El-Naga TR, Barghash SM. Blood parasites in camels (Camelus dromedarius) in Northern West Coast of Egypt. J. Bacteriol. Parasitol. 2016;7(1):258.
34. Ayan A, Celik BA, Celik OY, Akyildiz G, Kilinc OO, Ayan OO, Oguz FE, Goz Y, Yuksek N, Yilmaz AB, Myrzhiyeva A. First Report of Zoonotic Cryptosporidium parvum Subtype IIaA15G2R1 in Dogs in Türkiye. Pakistan Veterinary Journal. 2024;44(4).
35. Khan MK, Mahmood MS, Ali S, Khatoon A, Umar S, Sabir Mughal MA, Azeem A, Chatha AK, Abbas Z, Rafique A. Prevalence and Characterization of Buxtonella sulcata in Bovines in Pakistan Using Morphological and PCR-Based Approaches. Pakistan Veterinary Journal. 2024;44(4).