



RESEARCH ARTICLE

Identification and Molecular Characterization of *Theileria annulata* with Associated Risk Factors in Naturally Infected Camels from Selected Districts in Punjab, Pakistan

Faiza Aslam¹, Muti ur Rehman^{1*}, Gulbeena Saleem¹, Kamran Ashraf², Mian Abdul Hafeez^{2*} and Muhammad Saqib³

¹Department of Pathology, University of Veterinary and Animal Sciences Lahore, Pakistan

²Department of Parasitology, University of Veterinary and Animal Sciences Lahore, Pakistan

³Department of Clinical Medicine and Surgery, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad

*Corresponding author: drniazi@uvas.edu.pk; abdul.hafeez@uvas.edu.pk

ARTICLE HISTORY (22-315)

Received: September 15, 2022
Revised: October 29, 2022
Accepted: November 5, 2022
Published online: November 27, 2022

Key words:

Camel
Piroplasmosis
Theileria annulata
PCR
Phylogenetic analysis
Risk factors

ABSTRACT

Camel production in Pakistan is adversely affected by several pathogenic infections and insufficient veterinary facilities. Haemoparasitic diseases significantly affect health and productivity of camels causing a substantial financial burden to camel breeders and owners. The present study was designed for the identification and molecular detection of haemoparasites particularly piroplasms (*Theileria* spp. / *Babesia* spp.) infection in naturally infected local one-humped camels (*Camelus dromedaries*) in Punjab by using parasitological as well as molecular tools like polymerase chain reaction (PCR) followed by phylogenetic analysis. Blood samples (n=400) were collected from camels suspected for piroplasms infections in ten districts of Punjab and processed for blood smears and PCR targeting 18S rRNA gene. The findings revealed that *Theileria* is the most common parasite in camels of all study areas with overall prevalence of 12% and 13.5% by microscopic examination of GSBS and PCR, respectively. The phylogenetic analyses of the isolates on sequencing revealed that all analyzed isolates were closely related to *Theileria annulata* present in NCBI from several parts of the world. However, all samples tested for presence of *Babesia* spp. were found negative by microscopy and PCR. Chi square based risk factors analyses exhibited significant ($P < 0.05$) association between gender, age, tick infestation, previous tick history and prevalence of *Theileria*. In conclusion, current study on haemoparasites is evident for first ever molecular identification of *Theileria annulata* infection in camels of Pakistan along with assessment of potential risk factors associated with disease. Recent outcomes are ascertaining it as a silent killer with damaging effects on immune system.

To Cite This Article: Aslam F, Rehman MU, Saleem G, Ashraf K, Hafeez MA, Saqib M, 2022. Identification and molecular characterization of *Theileria annulata* with associated risk factors in naturally infected camels from selected districts in punjab, pakistan. Pak Vet J. <http://dx.doi.org/10.29261/pakvetj/2022.084>

INTRODUCTION

Camel contributes a key role in pastoral communities (called Rohillas in Cholistan desert) by fulfilling basic necessities of their living such as milk, meat, racing, riding and packing (Khan *et al.*, 2016). It is a widely distributed domestic animal in deserts and semi desert areas of different continents. The global camel population is about 35 million (FAOSTAT 2019). Pakistan is ranked at 8th position globally among camel raising countries with 1.1 million heads (GOP, 2019-2020). In Pakistan, camels are mainly kept by desert people and nomadic pastoralists, in order to cater their socio-economic needs (Ahmad *et al.*, 2010).

Ticks and tick-borne diseases (TBDs) have negative effects on production and reproduction of camels, leading to anemia, fever, wasting and death in heavy infections (El-Naga and Barghash, 2016).

Among piroplasm infections, Theileriosis and Babesiosis are most significant tick-transmitted haemoparasitic diseases of camels caused by the intracellular blood protozoans (Karim *et al.*, 2017). *Theileria* in camels was firstly reported in Russia, named as *Theileria camelensis* (Rutter, 1967) while Babesiosis caused by *Babesia caballi* was diagnosed first time in camels of Sudan (Abd-Elmaleck *et al.*, 2014). *Babesia* species parasitize the erythrocytes of domestic animals and humans, causing anemia in the affected host (Swelum

et al., 2014). Ticks of the genus *Hyalomma* (H) especially, *H. dromedarii* and *H. annatolicum* are accountable for the transmission of piroplasms. In Pakistan *H. dromedary* is the most prevalent (83%) tick species affecting camels (Gadahi *et al.*, 2013).

Diagnosis of haemoparasitic infection in camel through conventional microscopy is a challenging task (Alsaad, 2009). However, PCR being specific and sensitive molecular technique is extensively used for the diagnosis of latent infections (Hussain *et al.*, 2016). The DNA sequencing has appeared as impeccable diagnostic tool established on PCR amplification consuming generic primers that amplify exceptionally conserved ribosomal gene sequences (Ullah *et al.*, 2022). Haemoparasitic diseases in camels are usually neglected in Pakistan. Most documented studies are on camel trypanosomiasis but there is scarce information on theileriosis in camels of Punjab. Therefore, the current study was planned to determine the prevalence of piroplasm infection along with the sequence analysis to study the phylogenetic relationships of detected isolates of local camels in study areas.

MATERIALS AND METHODS

Study area and sample design: The study was carried out from April 2020 to December 2021. A total of 400 blood samples were collected from suspected camels of different age groups (both sexes) in ten selected districts of province Punjab, Pakistan. The representation includes, three distinct geographical regions i.e., Central Punjab (Faisalabad, Jhang), North Punjab (Mianwali, Khushab, Bhakkar) and South Punjab (Rajanpur, Muzaffar Garh, Bahawalpur, Bahawalnagr and Layyah) (Fig. 1). Areas were selected on the basis of population density of camel (Livestock Census, 2006). Sample size was calculated by using the following equation (Thrusfield 2018).

$$N = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$$

Where

N = Number of Samples to be required

P_{exp} = expected prevalence

d = desired precision

Twenty samples (two from each) from apparently healthy animals from all districts were collected as control.

Sample collection: Approximately 5 ml blood was drawn from jugular vein by using sterile syringes and transferred into a sterile K3 EDTA tubes (Atlas Medo-O-Vac Francisco^R) for microscopy and DNA extraction for purpose of amplification by PCR (Modry *et al.*, 2017). Blood samples were brought in ice boxes to Molecular Parasitology Laboratory, UVAS, Lahore.

Parasitological examination: Thin blood smears of all 400 samples were prepared, air-dried and fixed in absolute methanol following the standard protocol. Initial screening of blood smears was carried out to detect the presence of blood protozoans using light microscopy at 40x and 100x (oil immersion objective) (Coles, 1986).

DNA extraction and PCR amplification: After microscopic examination, all blood samples were processed for DNA extraction using WizPrepTM gDNA Mini kit following the manufacturer's instructions.

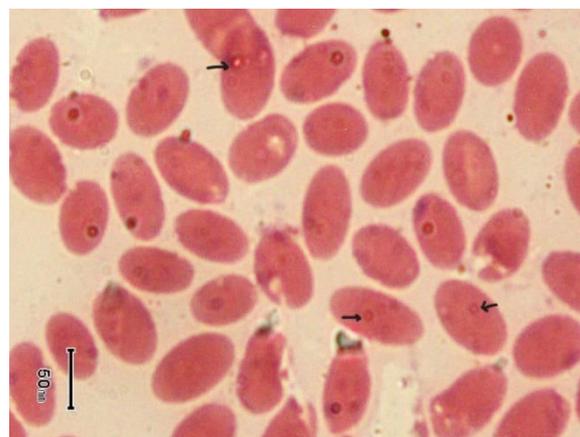


Fig. 1: Intra-Erythrocytic Piroplasm *Theileria* Spp. Indicated By Arrow Heads. at 100x (oil immersion lens).

Concentrations of DNAs were calculated using Nano Drop (A'aiz *et al.*, 2021). For DNA amplification conventional PCR was performed targeting 300 bp region of 18S ribosomal RNA gene with the primers 18SApiF/18SApiR (F: 5'-CGAACGAGACCTTAACC TGCTA-3', R: 5'-GGATCACTCGATCGGTAGGAG-3') described previously (Greay *et al.*, 2018). PCR was performed with a total volume of 25 μ L comprising of 13 μ L of commercial ready to use Master Mix (Green Taq Mix Catalog No. P 131, Vazyme Biotech Co. Ltd), 2 μ L of primers (1 μ L each forward and reverse); 2 μ L (50 ng/ μ L) of DNA; and 8 μ L of double distilled water (Ullah *et al.*, 2022). The cycling conditions were followed as initial denaturation at 95°C for 5 minutes proceeded through 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 45 seconds and last extension at 72°C for 5 min Greay *et al.*, (2018). PCR generated amplicons were fractionated by 1.5% agarose gel electrophoresis at 113 volts, 230MA for 35 minutes to visualize the bands in gel documentation system (Bio Rad Laboratories, USA) with 50 bp standard DNA marker (Thermo ScientificTM) (Ullah *et al.*, 2022). Positive and negative controls were used for every PCR reaction.

DNA Sequencing and phylogenetic analysis: Ten positive PCR products were selected, purified by GeneJET Gel Extraction Kit (Catalog. No; 00520774) and sent to Lab Genetix (Pakistan) for sequence analysis. DNA sequences retrieved were blasted to the databases of the GenBank to find the homologous sequences in accession nos. ON045834, ON045835, ON045836, ON045837, ON045838, ON045839 and phylogenetic tree was constructed. The GenBank databases BLAST (Basic Local Alignment Search Tool) and databanks of the NCBI (Bethesda, MD, USA) (www.blast.ncbi.nlm.nih.gov/Blast.cgi) were employed for analysis (Alanazi *et al.*, 2020). Phylogenetic analysis was performed using MEGA 7 software through neighbor joining method with 1000 bootstrap. The phylogenetic tree was assembled using the Maximum Likelihood method applying Neighbor-Join and BioNJ algorithms (Lan *et al.*, 2021).

Risk factors assessment: Detailed information about each herd (age, sex, location, body condition, tick infestation, previous ailment, vaccination, deworming

status and herd size) was collected on a predesigned questionnaire during sample collection for analysis as well as evaluation of risk factors related with *Theileria annulata* infection in camels.

Statistical analyses: Statistical analyses were executed in R statistical language (R version 4.1.3). The association between various risk factors and *T. annulata* infection was determined by univariable analysis i.e., chi-square test. All the risk factors with p-value less than 0.2 were analyzed at multivariable level using binary logistic regression. Odds ratio (OR) and CIs for each significant variable were identified using a multivariable logistic regression model. All statistics were considered significant at $p < 0.05$. The map was constructed on GIS.

RESULTS

Parasitological identification: Examination of Giemsa's stained blood smears demonstrated the presence of piroplasms (*Theileria*/ *Babesia*) within the erythrocytes in two forms i.e., coma shape and ring shape (Fig. 1). Out of the 400 camels examined, 48 were harboring piroplasms with an overall prevalence of 12%. Highest prevalence was recorded in camels of district Bahawalpur (35.7%) (Table 1).

PCR detection and molecular characterization: PCR results revealed that 13.5% (54/400) blood samples were found infected with *Theileria* piroplasms. Approximately 300 bp of 18S rRNA was amplified (Fig. 2). District wise the highest prevalence (35.7%) of piroplasms was recorded in district Bahawalpur by both microscopy and PCR with no significant variation ($P=0.087$, 0.106) between the districts (Table 1). Among different regions, the highest prevalence was recorded in districts of south Punjab (17.14 %) followed by north (12.86%) and central (3.70%) region with significant ($p < 0.05$) difference (Table 2). None of the samples tested positive by microscopy was found negative by PCR. However, two unidentified samples by microscopic examination got amplified by PCR. None of the samples were found to be co-infected. The phylogenetic analyses of present study isolates revealed the existence of *Theileria annulata* in camels of Punjab. The sequences deposited revealed homology on BLAST with reported isolates and grouped within a clade of *Theileria annulata* isolated from Pakistan and south Asian countries like China, West Bengal, Uterpardesh, Maharashter etc. (Fig. 3). None of the sequences revealed DNA related to *Babesia* spp.

Risk factors associated with *T. annulata* in camels: The univariate analysis revealed that the prevalence of theileriosis was higher in females (17.13%) than males (7.38%) with a significant difference ($p=0.012$). Age wise the highest prevalence (17.45%) was observed in middle age animals (2-5 years), followed by (14.63%) in older animals (>5 y) and (4.60%) in young ones (<2 y) with a significant difference ($P= 0.018$) among different age groups. Tick-infested camels showed the highest prevalence (20.67%) compared with non-infested animals (5.73%) and exhibited significant ($P < .001$) association with *T. annulata* infection in camels. Previous tick history

was found significantly ($P=0.023$) associated with theileriosis in camels. For significant variables values of odds ratio (OR) and 95 % confidence interval were determined by using Multivariate logistic regression Model (Fig. 4).

Other species in surroundings, body conditions, feeding and watering style, deworming status, vaccination status, housing system, flies prevalence, flies control status, purpose of rearing and herd size exhibited statistically non-significant association with *Theileria annulata* infection in camel (Table 3).

Table 1: Comparison of region wise prevalence of *Theileria annulata* through microscopic vs PCR in camels

District	Animals Tested (N)	Microscopy +ve (%)	P-value	PCR+ve (%)	P-value
Jhang	39	02 (05.10)	0.087*	02 (05.10)	0.106*
Faisalabad	15	00 (00.00)		00 (00.00)	
Mianwali	41	05 (12.20)		04 (09.80)	
Khushab	40	04 (10.00)		05 (12.50)	
Bhakkar	90	10 (11.10)		13(14.40)	
Rajan Pur	86	11(12.80)		11 (12.80)	
Muzaffar Garh	38	04(10.50)		06 (15.80)	
Bahawalpur	14	05 (35.70)		05 (35.70)	
Bahawalnagar	22	06 (27.30)		06 (27.30)	
Layyah	15	01 (06.70)		02 (13.30)	
Total	400	48 (12.00)		54 (13.50)	

*The results are not significant at $P > 0.05$

Table 2: Region wise prevalence of *Theileria* in camels

Region	Positive	Negative	Prevalence %	P value
Central	2	52	3.70	0.04
North	22	149	12.86	
South	30	142	17.14	

Central: Faisalabad, Jhang; Northern: Mianwali, Bhakkar, Khushab; Southern: Bahawalnagar, Layyah, Muzaffar Garh, Bahawalpur, Rajan Pur

Table 3: Univariate analysis of risk factors associated with theileriosis in camels of Punjab

Variable	Variable levels	Positi ve	Negati ve	Prevalence (%)	P-value
Gender	Female	43	208	17.13	0.012
	Male	11	138	07.38	*
Age	<2Y	04	083	04.60	0.018
	>5Y	24	140	14.63	*
	2-5Y	26	123	17.45	
Tick infestation	Yes	43	165	20.67	0.000
	No	11	181	05.73	*
Previous tick history	Yes	37	173	17.62	0.023
	No	17	173	08.95	*
Other species in surrounding	Yes	41	225	15.41	0.101
	No	13	121	09.70	
Body condition	Normal	09	091	09.00	0.446
	Emaciated	45	255	15.00	
Deworming status	Yes	02	014	12.50	1.000
	No	52	332	13.54	
Vaccination status	Yes	01	004	20.00	0.511
	No	53	342	13.42	
Housing system	Concrete	01	012	07.69	0.424
	Desert	34	180	15.89	
	Soil	19	154	10.98	
Flies prevalence	Intensive	28	189	12.90	0.900
	S. intensive	26	157	14.21	
Flies control status	Yes	15	089	14.42	0.809
	No	39	257	13.18	
Type of feeding/ watering	Indoor	13	112	10.40	0.330
	Outdoor	41	234	14.91	
Purpose of rearing	Drought	30	179	14.35	0.660
	Exhibition	00	002	00.00	
	Milk/Meat	24	165	12.70	
Herd size	>10	06	055	09.84	0.687
	1-3	23	133	14.74	
	4-6	14	85	14.14	
	7-10	11	73	13.10	

* ($P < 0.05$) significant difference

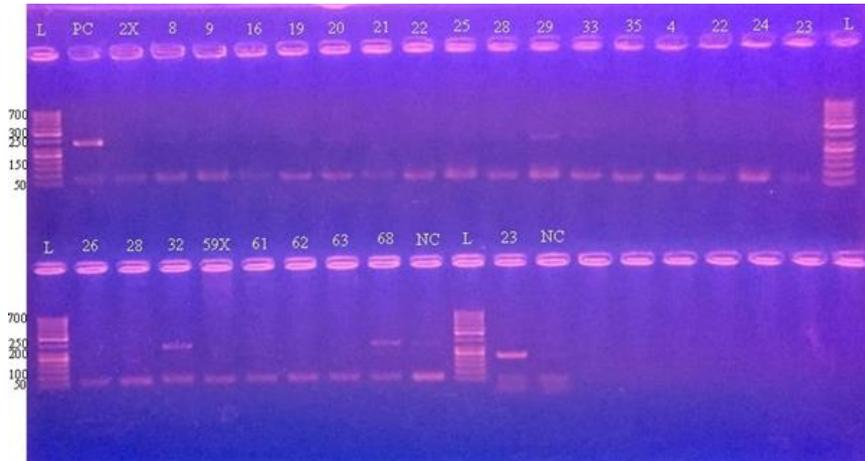


Fig. 2: Agarose gel (1.5%) electrophoretogram stained with ethidium bromide. PCR analysis of the 18s RNA gene revealed a 300 bp band derived from *Theileria annulata* isolates from camel blood samples (29,32, 68). L DNA, 50 base pair ladder, PC (Positive control), Sample lds (2X.. 68), NC (Negative control)



Fig. 3: A phylogenetic tree based on 18S rRNA, constructed with maximum-likelihood (ML) & neighbor joining (NJ) methods, representing phylogenetic relationships between samples in the current study and other reference sequences retrieved from GenBank

DISCUSSION

Theileriosis is declared to be the second most significant hemoprotozoal disease after Trypanosomiasis affecting camels. No data pertaining to epidemiology

and molecular detection of camel theileriosis is yet available in Pakistan. The recent investigation delivers a comprehensive discernment into the epidemiology, etiology, genetic diversity as well as risk factors related to camel theileriosis.

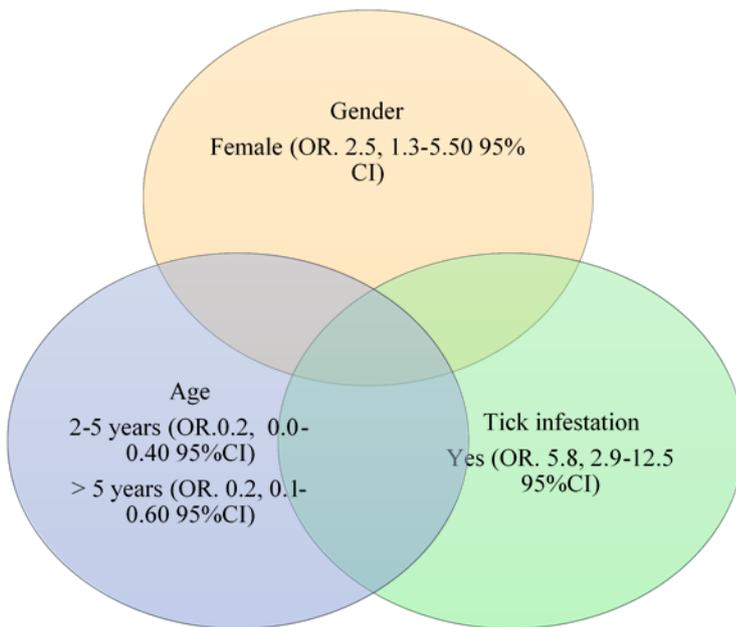


Fig. 4: OR and CIs for significant variables ($p < 0.05$) using a multivariate logistic regression Model. (95% CI=95% confidence interval, OR=Odds ratio).

The preliminary screening of camel blood samples was carried out by microscopic examination with Giemsa-stained blood smears. Out of 400 examined samples, 48 (12%) were positive for erythrocytic forms of piroplasms appeared within the RBCs in rod and coma shape.

These findings are nearly similar with studies in Jordan (10%) by Oncel *et al.*, (2007), lower than 28.88% and 24.5% recorded by Alsaadi and Faraj, (2020) and Ullah *et al.*, (2022) respectively while higher than 6.75% reported in Upper Egypt (Hamed *et al.*, 2011). These variations in prevalence may be attributed to different geographical and climatic conditions of the areas, variable sample size, vector prevalence, animal health status and technical skills of diagnostician (Alsaadi and Faraj, 2020). Piroplasms intermittently identified in blood of carrier animals and can't be detected by direct examination in many cases (Faraj *et al.*, 2019). Molecular detection by PCR assays permitted detection of parasites with far better precision than conventional microscopy (El-Naga and Barghash, 2016) by identifying coinfections, species differentiation, detection at low parasitic levels and chronic infections (Mirahmadi *et al.*, 2022). PCR based findings of this study endorsed that the rate of infection of piroplasms was higher (13.5%) than microscopy which necessitates the substantial role of PCR for piroplasms detection. These findings are in accordance with Ullah *et al.* (2022) and El-Naga and Barghash (2016). Higher and lower prevalence of *Theileria* infection in camels than present study ranged from 4.97% to 75% (Ullah *et al.*, 2022; A'aiz *et al.*, 2021). DNA sequencing and BLAST analysis of the 18S rRNA sequences demonstrated that all positive samples with piroplasmosis were infected with *T. annulata*. These findings are in accordance with Omer *et al.*, (2021) who identified *T. annulata* species in camels with no evidence of *T. camelensis*, *T. dromedarii* and *T. equi* as reported earlier. The majority of reports on occurrence of *T. camelensis* (Ismael *et al.*, 2014) and *T. dromedarii* (Mishra *et al.*, 1987) in camels are based on microscopic detection except Moezi *et al.*, 2016) who amplified sequence of 18S ribosomal DNA fragment to detect *T. camelensis* and *T. dromedarii* in tick infested camels. The results revealed that none of the samples

found positive for *Theileria camelensis* and *Theileria dromedarii*. Since this is the first molecular report regarding the occurrence of *Theileria* infection in camels in Punjab, that's why unable to compare results with any other study from Pakistan.

In phylogenetic tree, the 18S rRNA sequence of *T. annulata* grouped with sequences from south Asian Countries. Owing to existence of hypervariable regions, the 18S rRNA sequences of *T. annulata* have impact in unfolding genetic diversity and crucial for determining evolutionary patterns (Sivakumar *et al.*, 2014).

T. annulata identified in present study is the principal cause of tropical theileriosis in cattle (Mohsin *et al.*, 2022). The occurrence of this pathogen in camels may be owing to the co-inhabitation of these animals in desert areas (Alanazi *et al.*, 2020). This study has provided evidence that piroplasms possess low host specificity and camels can play role in the epidemiology of theileriosis by presenting another host specific piroplasms (Youssef *et al.*, 2015). The current work directed no pathognomic clinical sign in the positive cases. This may be ascribed toward the subclinical and chronic nature of *Theileria* infection in camels.

The nonexistence of Babesia DNA is in resemblance with the Alanazi *et al.*, (2020). The inability to identify Babesia DNA in the existing study may be explicated by the non-availability of the suitable vector or the host specificity of the vector. However, based on findings of the study seems impossible to predict that camels being not infected with Babesia spp. with available information indicating that DNA of *B. caballi* (Mirahmadi *et al.*, 2022), *B. bigemina*, *B. bovis* (El-Naga and Barghash, 2016) exist in camel blood from different parts of the globe.

Current research revealed the *Theileria* infection in male and female camels with a significant difference of prevalence between the genders. The infection in females was 2.5 times higher than males. These outcomes are in agreement with Selim *et al.*, (2021) while in contrast with Ullah *et al.* (2018). The higher rate of infection in female may be due to immunosuppression related to pregnancy, parturition and lactation rendering them more vulnerable to the disease (Amira *et al.*, 2018).

All age groups were found infected with *T. annulata* with variable rates. Middle aged group (2-5y) displayed the higher rate of infection compare to older (>5y) and young ones (< 2y) are in agreement with the previous findings (Alsaadi and Faraj, 2020) while in disagreement to (Mohsin *et al.*, 2022) recorded high infection rate in young animals. Maternal antibodies boost immunity in young calves and protect them from various pathogens (Ullah *et al.*, 2022). The study indicated tick infestation had a significant association with *Theileria* infection in camels. The rate of infection in ticks infested camels was 5.8 times higher (as compare to non-infested). Similar findings were reported by (Ullah *et al.*, 2018). Ticks being the sole vector of *Theileria* are predominantly associated with theileriosis in camels. Higher rate of tick infestation increases the likelihood of infection with *T. annulata* in camels (Selim *et al.*, 2021). Likewise, previous tick history was also found significantly associated (with 2.7 times higher rate of *Theileria* infection in camels. Despite the non-significant effect of other species surrounding on *Theileria* infection in camels, the high rate of infection (in camels surrounded by other species supports the findings of the present study.

Conclusion: Based on the findings we may conclude that *Theileria annulata* is the only prevailing specie identified in the camels of targeted areas. Further investigations on vector role of tick and tick control programs is suggested to decrease the infection rate in camels

Authors contribution: Conceived and designed the experiments: FA, MUR, M.A. Hafeez. Performed the experiments: FA, MUR, GS & KA: Analyzed the data: KA, MUR & MS: Contributed reagents/materials/analysis tools: Wrote the paper: FA, MAH & MS.

REFERENCES

- A'az NN, Ayyez HN and Neamah AJ, 2021. Molecular Assay Proves the Presence of *Theileria annulata* Infection in Camels in Al-Diwaniyah Province, Iraq. *Iran J Parasitol* 16:289-29.
- Abd-Elmaleck BS, Abed GH and Mandourt A, 2014. Some Protozoan Parasites Infecting Blood of Camels (*Camelus dromedarius*) at Assiut locality, Upper Egypt. *J Bacteriol Parasitol* 5: 184.
- Alsaad KM, 2009. Clinical, hematological and biochemical studies of anaplasmosis in arabian one humped camels (*Camelus dromedarius*). *J Anim Vet Advanc* 8:2106-9.
- Alsaadi HH and Faraj AA, 2020. Traditional Study and Seroprevalence of Theileria Spp. In Camels in Middle of Iraq (Wasit, Al-Qadisiyah and Al-Najaf Alashraf). *Plant Arch* 20: 4158-61.
- Ahmad S, Yaqoob M, Hashmi N, *et al.*, 2010. Economic importance of camel: a unique alternative under crisis. *Pak Vet J* 30: 191-7.
- Alanazi AD, Al-Mohammed HI, Alyousif MS, *et al.*, 2020. Species diversity and seasonal distribution of hard ticks (*Acar: Ixodidae*) infesting mammalian hosts in various districts of Riyadh Province, Saudi Arabia. *J Med Entomol* 56:1027-32.
- Amira AH, Ahmed L, Ahmed J, *et al.*, 2018. Epidemiological study on tropical theileriosis (*Theileria annulata* infection) in the Egyptian Oases with special reference to the molecular characterization of *Theileria* spp. *Ticks Tick-borne Dis* 9: 1489-93.
- Coles EH, 1986. *Veterinary clinical pathology*. WB Saunders company. Philadelphia and London.
- El-Naga TRA and Barghash S, 2016. Blood parasites in camels (*Camelus dromedarius*) in Northern West Coast of Egypt. *J Bacteriol Parasitol* 7:258-9.
- FAOSTAT, 2019. *FAO Statistics Division*. Rome, Italy, 2019.
- Faraj AA, Hade BF and Al-Amery AM, 2019. Conventional and molecular study of *Babesia* spp. of natural infection in dragging horses at some areas of Baghdad city, Iraq. *Iraqi J Agri Sci* 50: 909-15.
- Gadahi JA, Bhutto B, Kashif J, *et al.*, 2013. Tick infestation in camels in Thar Desert of Sindh-Pakistan. *Int J Liv Res* 3: 114-8.
- Greay TL, Zahedi A, Krige AS, *et al.*, 2018. Endemic, exotic and novel apicomplexan parasites detected during a national study of ticks from companion animals in Australia. *Parasit Vect* 11: 197-205.
- GOP, Economic Advisor's Wing. Ministry of Finance, Government of Pakistan Islamabad, Pakistan, 2019-20.
- Hamed MI, Zaitoun AM, El-Allawy TA, Mourad MI. 2011. Investigation of *Theileria camelensis* in camels infested by *Hyalomma dromedarii* ticks in Upper Egypt. *J Adv Vet Res* 1:4-7.
- Hussain M, Saeed Z, Gulsher M, *et al.*, 2016. A report on the molecular detection and seasonal prevalence of *Trypanosoma brucei* in Dromedary Camels from Dera Ghazi Khan District in Southern Punjab (Pakistan). *Trop Biomed* 33: 268-75.
- Ismael AB, Swelum AA, Khalaf AF, *et al.*, 2014. Clinical, haematological and biochemical alterations associated with an outbreak of theileriosis in dromedaries (*Camelus dromedarius*) in Saudi Arabia. *Pak Vet J* 34: 209-13.
- Karim S, Budachetri K, Mukherjee N, *et al.*, 2017. A study of ticks and tick-borne livestock pathogens in Pakistan. *PLoS Negl Trop Dis* 11: 5681.
- Khan R, Shahzad MI and Iqbal MN, 2016. Role of Camel in Pastoral Mode of Life and Future Use of rCGH as a Therapeutic Agent in Milk and Meat Production. *PSM Vet Res* 01:32-9.
- Lan Y, Li K, Mehmood K, *et al.*, 2021. Molecular investigation of important protozoal infections in yaks. *Pak Vet J* 41: 557-561
- Mirahmadi H, Ghaderi A, Barani S, *et al.*, 2022. Prevalence of camel babesiosis in southeast of Iran. *Vet Med and Sci*, 8:343-8. <https://doi.org/10.1002/vms3.666>.
- Mishra AK, Sharma NN and Raghavendra RJ, 1987. *Theileria dromedarii* n. sp. from Indian camels (*Camelus dromedarius*). *Riv Parasitol* 4: 99-102
- Modrý D, Hofmannová L, Mihalca AD, *et al.*, 2017. Field and Laboratory diagnostics of parasitic diseases of domestic animals: from Field and laboratory diagnosis of Parasitic Diseases of Domestic Animals; sampling to diagnosis. 2: 20-1.
- Moezi V, Sarani A, Hashemi H, *et al.*, 2016. Molecular study of *Theileria camelensis* and *Theileria dromedarii* strains based on sequence of 18s ribosomal DNA fragment in camels. *J Fundam Appl Sci* 8: 399-406.
- Mohsin M, Hameed K, Kamal M, *et al.*, 2022. Prevalence and risk factors assessment of theileriosis in livestock of Malakand Division, Pakistan. *J Saudi Soc Agri Sci* 21: 242-7
- Omer SA, F Duha, FA Alsuwaid A, *et al.*, 2021. Molecular characterization of ticks and tick-borne piroplasms from cattle and camel in Hofuf, eastern Saudi Arabia. *Saudi J Biol Sci* 28: 2023-8.
- Oncel T, Vural G, Gicik Y, *et al.*, 2007. Detection of *Babesia (Theileria) equi* (Laveran, 1901) Traditional study and seroprevalence of *Theileria* spp. in camels in middle of Iraq (wasit, Al-qadisiyah and Al-najaf alashraf) 4161 in horses in the Kars province of Turkey. *Turkiye Parazitolo Derg* 31:170-2.
- Pakistan Livestock census, 2006. Pakistan Bureau of Statistics, Govt of Pakistan.
- Rutter TG, 1967. Diseases of camels. 2 Protozoan diseases. *Vet Bull* 37:611-8.
- Selim A, Alanazi AD, Sazmand *et al.*, 2021. Seroprevalence and associated risk factors for vector-borne pathogens in dogs from Egypt. *Parasit Vectors* 14:1-11.
- Sivakumar T, Hayashida K, Sugimoto C, *et al.*, 2014. Evolution and genetic diversity of *Theileria*. *Infect Genet Evol* 27:250-63.
- Swelum AA, Ismael AB, Khalaf AF, *et al.*, 2014. Clinical and laboratory findings associated with naturally occurring babesiosis in dromedary camels. *Bull Vet Inst Pulawy* 58:229-33.
- Thrusfield M, 2018. *Veterinary epidemiology*. John Wiley & Sons; UK.
- Ullah N, Durrani AZ, Avais M, *et al.*, 2018. A first report on prevalence of caprine theileriosis and its association with host biomarkers in Southern Khyber Pakhtunkhwa. Pakistan. *Small Rumin Res* 159:56-61.
- Ullah K, Numan M, Alouffi A, *et al.*, 2022. Molecular Characterization and Assessment of Risk Factors Associated with *Theileria annulata* Infection. *Microorganisms* 10:1614. <https://doi.org/10.3390/microorganisms10081614>
- Youssef SY, Yasien S, Mousa WMA, *et al.*, 2015. Vector identification and clinical, hematological, biochemical, and parasitological characteristics of camel (*Camelus dromedarius*) theileriosis in Egypt. *Trop Anim Health Prod* 47:649-56. <https://doi.org/10.1007/s11250-015-0771-1>