DOI : 10.5958/2277-8934.2022.00008.X Vol 29 No 1, p 61-66

MICROSCOPIC, SEROLOGICAL AND MOLECULAR SCREENING OF *Theileria annulata* IN CAMELS (*Camelus dromedarius*) OF SAUDI ARABIA

Jamila S. Al Malki and Nahed Ahmed Hussien
Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

ABSTRACT

The present study was aimed to evaluate the prevalence rate of *Theileria* spp., especially *T. annulata*, in camels of Taif Governorate, Saudi Arabia. A total of 102 blood samples were collected from camels. Parasitological examination of buffy coat smear, ELISA-IgG, and PCR (targeting 16S rRNA and cytochrome b gene sequences) assays were done for *T. annulata* infection detection. The results report a high prevalence rate of *T. annulata* in the examined dromedary's population using ELISA-IgG (32.4%), followed by targeted PCR (25.5%). Then lower detection was reported by buffy coat Giemsa stained (10.8%). Statistical analysis using one-way ANOVA followed by Tukey's at $P < 0.001$ showed significant variation in values within different diagnostic tools and age categories. Both females and males were at risk of parasitic infections, but females have a higher incidence (17.6%, 15.7%, 6.8%). Young age (X≤ 3 years) camels were the most infected category (50.0%, 44.4%, 22.2%) according to age from elder ones. These results could be a baseline for easing a later large-scale epidemiological screening on tropical theileriosis in the camel population in Saudi Arabia.

Key words: *Camelus dromedarius*, KSA, prevalence, Taif, *Theileria annulata*

Theileriosis is one of the most critical tick-borne diseases (TBD) of animals caused by different species of *Theileria*. *Theileria annulata* (*T. annulata*) and *T. parva* are the dominant *Theileria* species that are linked to economic loss and mortality worldwide (Roy *et al,* 2021). In Saudi Arabia, *T. annulata* is the primary causative agent for theileriosis that is transmitted by *Hyalomma* spp. (Hussein *et al,* 1991; De Kok *et al,* 1993). Several studies have reported *T. annulata* from cattle in the eastern province, and Qassim region in Saudi Arabia based on microscopic examination of blood films collected from infected animals (Al-Atiya *et al,* 1991; Hussein *et al,* 1991; Omer *et al,* 2003). However, few studies were done related to dromedary theileriosis in last two decades (Abou-El-Naga *et al,* 2005; El-Fayoumy *et al,* 2005; Hamed *et al,* 2011; Ismael *et al,* 2014; Youssef *et al,* 2015).

The present study aims to assess the prevalence rate of *Theileria* spp., especially *Theileria annulata*, in Taif Governorate, Makkah province, KSA using different diagnostic assays: parasitological, serological, and molecular evaluations in dromedary camels.

Materials and Methods

Three different areas in Taif region were selected from December 2020 to February 2021 to collect blood samples from 102 apparently healthy camels (36 males, aged 1-7 years and 66 females, aged 3-12 years). Blood samples were collected from ear veins (2ml in EDTA tubes) by veterinarians as per their routine examination.

Parasitological diagnosis

Blood and buffy coat smears were done for all samples. All slides were stained by 10% Giemsa, and then examined under a light microscope for *Theileria* spp. diagnosis (Schalm, 1971).

Serological test

According to the instruction manual of Sunlong Biotech® Company (China) blood samples were centrifuged (20 min, 2500 rpm), and then sera were collected for *Theileria* antibodies detection using sandwich-ELISA. Briefly, 50µl of sera in the dilution buffer (with a ratio of 1:4) were added to micro-ELISA strip plate wells (pre-coated with an antigen-specific to *Theileria* spp.). ELISA plate was gently shaken, incubated (30 min, 37°C), and then washed five times with the washing buffer. Next, the reagent of Horseradish Peroxidase (HRP)-conjugate was added to each well, incubated, and then washed. Finally, colour was developed by the addition of chromogen solutions and incubation (15 min, 37°C). Next, 50µl

SEND REPRINT REQUEST TO NAHED A. HUSSIEN email: n.nahed@tu.edu.sa; nahed199@gmail.com

of the stop solution was added to terminate the reaction, and then ELISA plate was read at 450nm. Negative and positive controls supplied within the kit were added in separate wells for data calculations. The presence of *Theileria* spp. was determined by comparing data with their cut-off values. Cut-off (critical value) was calculated as the average value of negative control + 0.15, i.e., a negative OD value < cut-off while a positive OD value ≥ cut-off.

DNA extraction by salting out

Low and high salt buffers, TKM1 and TKM2, were used for DNA extraction. Briefly, the same volumes of blood sample and TKM1 (100mM Tris-HC1, pH 7.4, 250mM sucrose, 10mM EDTA) were mixed and incubated at room temperature for 30 min. Then, the mixture was centrifuged (10 min at 4000 rpm). The supernatant was discarded, and then TKM2 (Tris HCl 10 mM pH 7.6, 10 mM KCl, 10 mM MgCl2, 0.4 M NaCl, and 2mM EDTA), SDS (10%), and proteinase k enzyme (10 mg/ml) were added to the pellet and incubated (30 min, 55°C). Protein was removed with the aid of 6M NaCl, and centrifugation. DNA was precipitated in the supernatant by cold ethanol addition. Finally, the DNA pellet was collected by centrifugation (10,000 rpm, 10 mins), dried, and dissolved in 100 µl autoclaved Milli-Q water (Ahmad *et al,* 2007).

PCR amplification

Firstly, primers were used to target the small subunit ribosomal 16S rRNA sequence of *Theileria* spp. (TH989: 5'-AGTTTCTGACCTATCAG-3' and TH990: 5'-TTGCCTTAAACTTCCTTG-3'). Then positive *Theileria* spp. PCR samples were subjected to another PCR that targeted cytochrome b gene sequence specifically for *T. annulata* (using forward TCyt1F: 5'- ACTTTGGCCGTAATGTTAAAC-3' and reverse TCyt1R: 5'- CTCTGGACCAACTGTTTGG-3' primers) (Masiga *et al,* 1992; Figueroa *et al,* 1993). For both reactions: PCR amplification was done in a total reaction volume of 20 µl: 7 µl H2O, 1 µl (20 pmole) of each forward and reverse primers, 1 µl extracted DNA, and finally 10µl of 2× master mix. The PCR reaction was set up with initial denaturation at 95°C (5 mins), 40 cycles of denaturation at 94°C (30 s), primer annealing at 55°C (60 s), and then primer extension at 72°C (60 s). The final extension at 72°C (10 mins) was necessary done for complete amplification. PCR products of all samples were separated on 1.5 % agarose gel for about 60 min (at 100V), visualised, and then photographed by a gel documentation system. PCR products' size was visually determined by comparing them with a known low molecular weight marker (50-1500 bp).

Statistical analysis

All data collected from parasitological, serological, molecular were analysed statistically. Student t-test was used to differentiate between two related groups. One-way ANOVA was conducted to differentiate between different groups, followed by Tukey's multiple comparisons test using GraphPad software (GraphPad® 2017, San Diego, CA, USA). *** indicates $P \leq 0.001$, ** indicates $P \leq 0.01$, * indicates $P \leq 0.05$ and ns (non-significant) means $P > 0.05$.

Results

Buffy coat examination

Parasitological diagnosis in the whole blood smear showed negative results of all the studied samples (n=102). However, examination of buffy coat smear using Giemsa stain showed n=11 (10.8%) positive *Theileria* spp. (Table 1). *Theileria*'s schizont nuclei appeared stained with Giemsa in the cytoplasm of the infected cells, as shown in Fig 1. According to gender: female positive samples 7/102 (6.8%) showed a statistically high significance in comparison to male samples 4/102 (3.9%) according to the total number of samples at $P < 0.01$ by using student t-test. According to age, young camels from (X≤ 3 years) show the highest prevalence (22.2%), followed by 3>X≤ 6 years (13.9%), then 6>X≤9 years (6.5%), but old camels with age ranges from 9>X≤12 years doesn't show any infection according to parasitological evaluation (0%) ($F (3, 8) = 8183$; $P < 0.001$; R square = 0.9997) by using one-way ANOVA. Tukey's test reports a highly significant difference between all diagnostic groups at $P < 0.001$.

Seroprevalence assessment

Of the 102 sera, samples were diagnosed serologically for the presence of *Theileria* spp. IgG, n=33 (32.4%) positive samples were reported. Table 1 shows a highly significant difference between total positive samples by using different diagnostic assays. In which, IgG seroprevalence using ELISA shows the highest significance (32.4%), followed by targeted PCR (25.5%), and then lower detection was reported by buffy coat Giemsa stained (10.8%) at $F (2, 6) = 8937$; $P < 0.001$; R square = 0.9997 by using one-way ANOVA followed by Tukey's test reports at $P < 0.001$.

Table 1 shows a high significance increase of *Theileria*'s seroprevalence detection in female camel samples 18/102 (17.6%) than males 15/102 (14.7%)

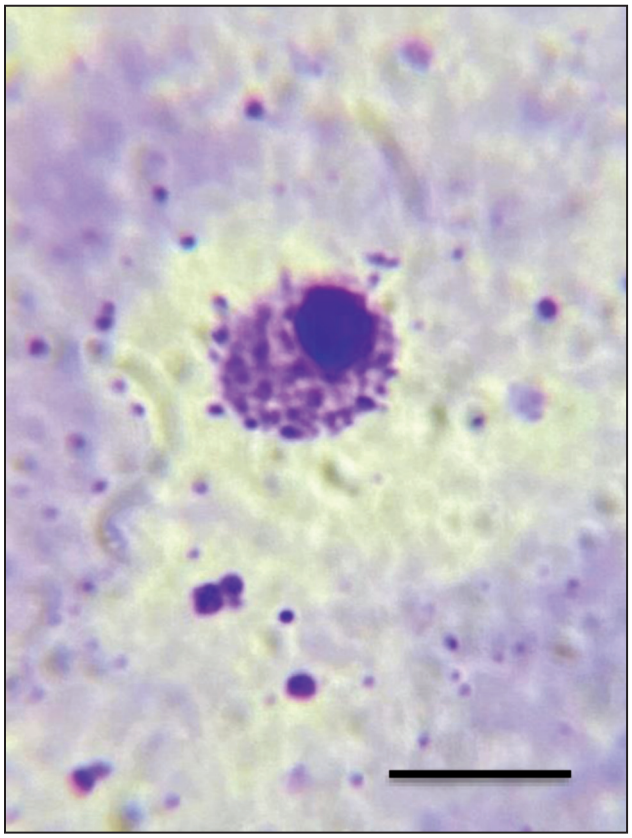

Fig 1. Light micrograph of camels' blood buffy coat smear showing Giemsa-stained schizont nuclei of *Theileria* spp. in the cytoplasm of an infected erythrocyte. Scale-bars: 10 µm.

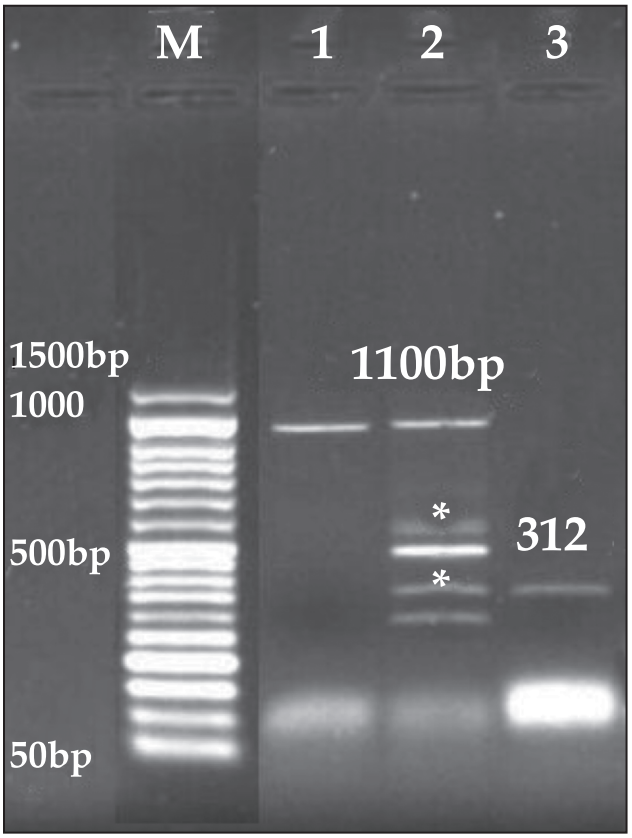

Fig 2. Representative photograph of agarose gel stained with ethidium bromide (1.5%) showing PCR product of small subunit ribosomal 16S rRNA sequence of *Theileria* spp. at 1100 bp (lanes 1 and 2) and cytochrome b gene sequence of *Theileria annulata* at 312 bp (lane 3). Lower bands in lane 2 are non-specific bands. Lane M: Low molecular weight marker (50-1500 bp).

at P ≤ 0.001 using the student t-test. According to age: young camel samples (X≤3 years) show high prevalence (50.0%), followed by 9>X≤12 years (37.5%), then 3>X≤ 6 years (30.6%), and finally 6>X≤9 years (22.6%) were the least detected infected samples using ELISA IgG by using one-way ANOVA (F (3, 8) = 1461; P < 0.001; R square = 0.9982) followed by Tukey's test reports at P < 0.001.

Molecular evaluation

Fig 2 shows a successful amplification of the small subunit ribosomal 16S rRNA sequence of *Theileria* spp. at molecular size 1100bp, and cytochrome b gene sequence specific for *T. annulata*

Table 1. Combined results showing gender, number and age of camels, in addition to parasitological, serological and PCR positive results for *Theileria* spp. diagnosis in all studied samples.

	Number (*n*)	Parasitological evaluation (%)	ELISA- IgG (%)	PCR (%)
Gender				
Female	67	7/67 (10.4%), 7/102 (6.8%)**	18/67 (26.9%), 18/102 (17.6%)***	16/67 (23.9%), 16/102 (15.7%)***
Male	35	4/35 (11.4%), 4/102 (3.9%)	15/35 (42.9%), 15/102 (14.7%)	10/35 (28.6%), 10/102 (9.8%)
Age (X, year)				
X≤ 3	18	4/18 (22.2%)^{a}	9/18 (50.0%)^{a}	8/18 (44.4%)^{a}
3>X≤ 6	36	5/36 (13.9%)^{b}	11/36 (30.6%)^{b}	11/36 (30.6%)^{b}
6>X≤9	31	2/31 (6.5%)^{c}	7/31 (22.6%)^{c}	4/31 (12.9%)^{c}
9>X≤12	16	0 (0.0%)^{d}	6/16 (37.5%)^{d}	3/16 (18.8%)^{d}
Total	**102**	**11/102 (10.8%)**	**33/102 (32.4%)**	**26/102 (25.5%)**

Different letters within the same column refer to statistically significant difference between those groups at P ≤ 0.001 using one-way ANOVA followed by Tukey's test. *** indicates P ≤ 0.001, ** indicates P ≤ 0.01, * indicates P ≤ 0.05 and ns (non-significant) means P > 0.05.

at 312 bp in comparison to the low molecular weight marker ranges from 50 to1500 bp. Table 1 indicates a high significance prevalence rate of female camel samples 16/102 (15.7 %) in comparison to male samples 10/102 (9.8 %) at $P \leq 0.001$ using the student t-test. According to age, also samples from young camels ($X \leq 3$ years) show the highest incidence by using PCR technique (44.4%), followed by $3 > X \leq 6$ years (30.6%), then $9 > X \leq 12$ years (18.75%), and the least was referred to $6 > X \leq 9$ years (12.9%) by using one-way ANOVA ($F(3, 8) = 19069$; $P < 0.001$; R square = 0.9999). Tukey's test revealed a highly significant difference between all diagnostic groups at $P < 0.001$.

Discussion

Theileria spp. belongs to the order Piroplasmida is a common pathogen that is transmitted by ticks. Theileriosis is the second most serious hemoprotozoan disease that affects dromedary camels in tropical and subtropical countries, including Saudi Arabia (Al-Khalifa *et al,* 2009; Durrani *et al,* 2012; El Imam *et al,* 2016). The present study reports a high prevalence rate of *Theileria* spp., especially *T. annulata*, in dromedaries by using different diagnostic examinations; ELISA-IgG (32.4%), PCR (25.5%), and microscopic (10.8%) assessment. There are a lot of research studies that report the prevalence of piroplasm genus, including *Theileria* spp., in cattle in Saudi Arabia, but there is a scarcity regarding camels (Ghafar and Amer, 2019). The present results were consistent with Ismael *et al* (2014) who reported theileriosis (38.73%) in dromedaries in Riyadh region, Saudi Arabia according to parasitological examinations of blood samples using Giemsa-stained blood smears.

Microscopically, *Theileria* spp. was detected in Giemsa-stained buffy coat blood smears with a prevalence rate of 10.8% in camels of the present study. Microscopic examination of buffy coat smear is more specific and sensitive than the whole blood for parasitic diagnosis (Chagas *et al,* 2020). However, it is difficult to identify its species due to parasite developmental changes in size and the form in the infected erythrocyte that differs from host to other (Homer *et al,* 2000). Therefore, we have used a PCR to target the 16S rRNA sequence of *Theileria* spp. and cytochrome b gene sequence, specifically of *T. annulata*. Detection of positive samples was increased by using a molecular assay (25.5%) that is more specific, sensitive, and accurate than microscopic examination (10.8%) (Ullah *et al,* 2021). There is a doubt for the exact speciation of *Theileria* protozoa, therefore, recent studies targeting 18S rRNA gene by traditional PCR was recommended for the confirmation of the camel's *Theileria* species (A'aiz *et al,* 2021).

It is not surprising that the prevalence rate was higher by using ELISA-IgG of *Theileria* spp. (32.4%) than targeted PCR (25.5%). ELISA was able to detect past and recent infections, while PCR could screen only the present parasite infection (Reithinger *et al,* 2003). The present study shows a statistically increase of *T. annulata* incidence in females than in male camels using different diagnostic techniques. This could be attributed to stress on females during gestation and milk production, making them more susceptible to Piroplasmids' infection (Barghash, 2010; Zayed *et al,* 2010).

Moreover, the number of females in the present study is more than males, giving a chance to spread infection within females. Furthermore, we have detected *Theileria* infection in all ages, especially young, with different prevalence rates using various diagnostic tools. According to gender, our findings were consistent with previous study El-Naga and Barghash (2016) they have investigated camels in the Northern West Coast of Egypt infected with *Theileria* spp. with the incidence of infection in females (73.3%) higher than males (71.4%), and the maximum rate of infection was found in age range 6-12. The feeding method could have a role in the incidence of disease throughout different ages; all camels were freely grazed in the studied area (Ullah *et al,* 2021). The higher prevalence in young camels could be due to their not fully developed immune system to combat *T. annulata* infection (Ahmed *et al,* 2008) or more caring of the owner with the adult ones. In addition, a lower *T. annulata* prevalence rate in older camels may be due to the development of concomitant immunity due to their recurrent infections throughout their lifetime (Ilhan *et al,* 1998; Gharbi and Darghouth, 2014).

The high prevalence rate of theileriosis in dromedaries in the studied areas could be corroborated to the high prevalence rate of their vectors. ALanazi *et al* (2018) have detected five different tick species on camels from Saudi Arabia with various prevalence: *Hyalomma dromedarii* (70.6%), along with *Hyalomma impeltatum* (25.3%) *Hyalomma anatolicum* and *Rhipicephalus turanicus* were less prevalent (<2%), and *Haemaphysalis* spp. ticks were found on camels in few limited provinces. They have reported that *H. dromedarii* tick was the only tick from those five species that are positive for *Theileria* spp. DNA.

Therefore, *H. dromedarii* tick acts as a vector of *Theileria* spp. in camels of Saudi Arabia. Their findings agreed with other studies, in which they have identified *H. dromedarii* and *H. anatolicum* as main ticks for *Theileria annulata* transmission in cattle and camel in Hofuf, the Eastern Saudi Arabia region (Omer *et al,* 2021) and *H. dromedarii* in the United Arab Emirates (Al-Deeb *et al,* 2015).

Acknowledgements

This work was supported by Taif University Researchers Supporting Project number (TURSP-2020/299), Taif University, Taif, Saudi Arabia. The authors would like to thank the Ministry of Agriculture and Water in Taif Governorate for facilitating data and sample collections.

Declarations

Ethics approval and consent to participate

Data and samples were collected from veterinarians as per their periodic examination according to the ministerial recommendation. Ministry of Environment, Water and Agriculture (KSA) permitted to collect cattle samples from different locations in Taif for our research study with the number 106082/1074/1442, date 21-02-1442H. Sample's collection and all experimental procedures were performed following a national ethical requirement (National Committee of Bioethics (NCBE) at King Abdulaziz City for Science and Technology (KACST) with number: 10023117, valid till 01 October 2023).

Funding

Grant Number (TURSP-2020/299), Taif University, Taif, Saudi Arabia.

Authors' Contributions

Both authors participated in the design of the study, carried out molecular evaluation and approved the final manuscript. Jamila S. Al Malki collected samples and performed parasitological assay. Nahed Ahmed Hussien performed data analysis and wrote the article draft.

References

A'aiz, NN, Ayyez HN and Neamah AJ. Molecular assay proves the presence of *Theileria annulata* infection in camels in Al-Diwaniyah Province, Iraq. Iranian Journal of Parasitology. (2021); 16(2):289-294.

Abou-El-Naga TR, Abdou TA and Mahmoud MA. Clinicopathological studies on *Theileria annulata* Infection in Siwa Oasis, Egypt. Beni-Suef Veterinary Medical Journal. (2005); 15(2):40-46.

Ahmad S, Ghosh A, Nair DL and Seshadri M. Simultaneous extraction of nuclear and mitochondrial DNA from human blood. Genes and Genetic Systems. (2007); 82:429-432.

Ahmed JS, Glass EJ, Salih DA and Seitzer U. Innate immunity to tropical theileriosis. Innate Immunity. (2008); 14(1):5-12.

ALanazi AD, Abdullah S, Helps C, Wall R, Puschendorf R, ALHarbi SA, Abdel-Shafy S and Shaapan RM. Tick-borne pathogens in ticks and blood samples collected from camels in Riyadh Province, Saudi Arabia. International Journal of Zoological Research. (2018); 14:30-6.

Al-Atiya SA, Elbihari S and Gameel AA. Presence of bovine theileriosis in Saudi Arabia. Veterinary Parasitology. (1991); 38:339-342.

Al-Deeb MA, Muzaffar SB, Abu-Zeid YA, Enan MR and Karim S. First record of a spotted fever group Rickettsia Sp. and *Theileria annulata* in *Hyalomma dromedarii* (Acari: Ixodidae) ticks in the United Arab Emirates. The Florida Entomologist. (2015); 98(1):135-39.

Al-Khalifa M, Hussein H, Diab F and Khalil G. Blood parasites of livestock in certain regions in Saudi Arabia. Saudi Journal of Biological Sciences. (2009); 16:63-67.

Barghash SM. Study of genetic variability and prevalence of *Trypanosoma evansi* in domestic animals in Egypt. Ph.D. thesis, Ain Shams University, Egypt. (2010).

Chagas CRF, Binkienė R, Ilgūnas M, Iezhova T and Valkiūnas G. The buffy coat method: a tool for detection of blood parasites without staining procedures. Parasites and Vectors. (2020); 13(1):104.

De Kok JB, d'Oliveira C and Jongejan F. Detection of the protozoan parasite *Theileria annulata* in Hyalomma ticks by the polymerase chain reaction. Experimental and Applied Acarology. (1993); 17:839-846.

Durrani S, Khan Z, Khattak RM, Andleeb M, Ali M, Hameed H, Taqddas A, Faryal M, Kiran S and Anwar H. A comparison of the presence of *Theileria ovis* by PCR amplification of their ssu rRNA gene in small ruminants from two provinces of Pakistan. Asian Pacific Journal of Tropical Disease. (2012); 2:43-47.

El Imam A, Hassan SM, Gameel AA, El Hussein AM, Taha KM and Oosthuizen MC. Molecular identification of different *Theileria* and *Babesia* species infecting sheep in Sudan. Annals of Parasitology. (2016); pp 62.

El-Fayoumy MM, Abou Elnga TR, Abd El-Baky SMM and Abdou TA. Prevalence of camel theileriosis and its vector tick in North Coast of Egypt. Journal of the Egyptian Veterinary Medical Association. (2005); 65:291-302.

El-Naga TRA and Barghash SM. Blood Parasites in Camels (*Camelus dromedarius*) in Northern West Coast of Egypt. Journal of Bacteriology and Parasitology. (2016); 7:258.

Figueroa JV, Chieves LP, Johnson GS and Buening GM. Multiplex polymerase chain reaction based assay for detection of *Babesia bigemina, Babesia bovis* and *Anaplasma marginale* DNA in bovine blood. Veterinary Parasitology. (1993); 50:69-81.

Ghafar MW and Amer SAM. A preliminary molecular survey of Babesia divergens and first evidence of *Theileria annulata* in cattle from Saudi Arabia. Veterinary World. (2019); 12(2):266-270.

Gharbi M and Darghouth MA. A review of *Hyalomma scupense* (Acari, Ixodidae) in the Maghreb region: from biology to control. Parasite. (2014); 21:12-18.

Hamed MI, Zaitoun AMA, El-Allawy TAA and Mourad MI. Investigation of *Theileria camelensis* in camels infested by Hyalomma dromedarii ticks in Upper Egypt. Journal of Advanced Veterinary Research. (2011); 1:4-7.

Homer MJ, Aguilar-Delfin I, Telford SR, Krause PJ and Persing DH. Babesiosis. Clinical Microbiology Reviews. (2000); 13:451-469.

Hussein HS, Al-Asgah NA, Al-Khalifa MS and Diab FM. The blood parasites of indigenous livestock in Saudi Arabia. Arab Gulf Journal of Scientific Research. (1991); 9:143-160.

Ilhan T, Williamson S, Kirvar E, Shiels B and Brown C. *Theileria annulata*: Carrier State and Immunity a. Annals of the New York Academy of Sciences. (1998); 849(1):109-25.

Ismael AB, Swelum AA, Khalaf AF and Abouheif MA. Clinical, haematological and biochemical alterations associated with an outbreak of theileriosis in dromedaries (*Camelus dromedarius*) in Saudi Arabia. Pakistan Veterinary Journal (2014). 34(2):209-213.

Masiga DK, Smyth AJ, Hayes P, Bromidge TJ and Gibson WC. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. International Journal for Parasitology. (1992); 22:909-918.

Omer OH, El-Malik KH, Magzoub M, Mahmoud OM, Haroun EM, Hawas A and Omar HM. Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. Veterinary Research Communications. (2003); 27:15-25.

Omer SA, Alsuwaid DF and Mohammed OB. Molecular characterisation of ticks and tick-borne piroplasms from cattle and camel in Hofuf, eastern Saudi Arabia. Saudi Journal of Biological Sciences. (2021); 28(3):2023-2028.

Reithinger R, Espinoza JC, Courtenay O and Davies CR. Evaluation of PCR as a diagnostic mass-screening tool to detect Leishmania (Viannia) spp. in domestic dogs (*Canis familiaris*). Journal of Clinical Microbiology. (2003); 41(4):1486-1493.

Roy S, Bhandari V, Barman M, Kumar P, Bhanot V, Arora JS, Singh S and Sharma P. Population genetic analysis of the *Theileria annulata* parasites identified limited diversity and multiplicity of infection in the vaccine from India. Frontiers in Microbiology. (2021); 11:3477.

Schalm OW. Veterinary Hematology. 2nd Ed. Philadelphia: Lea & Febiger. (1971).

Ullah R, Shams S, Khan MA, Ayaz S, Akbar NU, Din QU, Khan A, Leon R and Zeb J. Epidemiology and molecular characterisation of *Theileria annulata* in cattle from central Khyber Pakhtunkhwa, Pakistan. PLoS ONE 16 (9):e0249417. (2021).

Youssef SY, Yasien S, Mousa WM, Nasr SM, El-Kelesh EA, Mahran KM and 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(4):649-56.

Zayed AA, Habeeb SM, Allam NAT, Ashry HMZ, Mohamed AHM, Ashour AA and Taha HA. A critical comparative study of parasitological and serological differential diagnostic methods of *Trypanosoma evansi* infections in some farm animals in Egypt. American-Eurasian Journal of Agricultural and Environmental Sciences. (2010); 8:633-642.