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Phylogenetic Analysis and Detection of Drug Resistance Gene in *Theileria annulata* Isolated from Buffaloes

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ABSTRACT

Bovine theileriosis, caused by *Theileria annulate*, is disease affecting cattle and buffaloes worldwide. The current study aimed to screen the blood samples of 30 naturally suspected local buffaloes infected with *Theileria* species. The blood samples were initially examined by light microscopic and then the positive samples were subjected to PCR reactions. All 30 animals indicated clinical symptoms, such as high fever, loss of appetite, the presence of the hard tick, and enlargement of lymph nodes. The amplified products of *18S rRNA* were analyzed, along with molecular detection of the drug-binding site alterations and interrelated changes in the *cytochrome b* (*cyto b*) gene. Blood smears revealed the presence of infected erythrocytes with *Theileria* spp. The PCR results confirmed infection in samples when DNA amplified with partial *18S rRNA* and *cyto b* genes. The sequencing data were obtained from GeneBank using the accession numbers OM937770.1, ON207523.1, ON207525.1, ON207524.1, ON207526.1, and ON207527.1 Following BLAST analysis (Basic Local Alignment Search Tool), genetic differences were observed between the Iraqi isolate OM937770.1 and strains from India, Iran, and Turkey. The data obtained from the current study may reveal the genetic alteration of the local strain in the drug-target codons, which are found in one isolate and are different from the GenBank isolates. The results suggest that the failure of buparvaquone therapy might be due to the resistance to *cyto b* gene.

Keywords: Buffalo, Buparvaquone, Gene, *Theileria annulata*

INTRODUCTION

Bovine theileriosis is a worldwide prevalent disease in cattle and buffalo, caused by the tick-borne hemoprotozoan parasite known as *Theileria annulate* (Bilgic et al., 2010; Abdullah and Ali, 2021; Ullah et al., 2021). Several genera of hard ticks (Ixodidae) can transmit the disease and the clinical manifestations include fever, swollen lymphoid tissue, jaundice, and high mortality (Ali et al., 2013; Abdel Rahman and Ismaiel, 2018). Theileriosis negatively affect dairy and livestock animals productivity, leading to significant losses in the industry due to decreased milk output and weight loss (Gharbi et al., 2006). Compared to other vector-borne diseases, theileriosis prevalence is higher than *Anaplasma* spp. (11%) but lower than babesiosis (29%) across the world (Paramanandham et al., 2019; Jacob et al., 2020; Abid et al., 2021). In buffaloes, the infection rates of babesiosis, theileriosis, and anaplasmosis were 51.44%, 15.74%, and 13.99 %, respectively (Anwar, 2018). This discrepancy may be attributed to the fact that most studies have focused on diagnosing theileriosis in cattle rather than buffalos, with cattle traditionally considered the primary host for theileriosis (AL-Judi, 2001; Sallemi et al., 2018; Kawan, 2019).

Apart from the difficulty of species identification, the blood smear technique is not suitable for detecting infections with low parasite levels (Nayel et al., 2012; Rafiullah et al.,2019; Arwa and Kawan, 2022). Serological approaches for detecting *Theileria* species are insensitive due to cross-reactions and the loss of antibodies in long-term carriers (Passos et al., 1998). Therefore, the present study aimed to evaluate the prevalence of *Theileria annulata* (*T. annulata*) among buffaloes in Iraq and identify drug-binding site alterations in codons in the resistant isolates.

MATERIALS AND METHODS

Ethical approval

The project received approval and funding from the local committee of animal care at the College of Veterinary Medicine, University of Baghdad, Iraq, under reference number 706, dated 23/3/2022.

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Sample collection

The study included blood samples of 30 Bubalus bubalis buffalos (28 female and 2 male) aged 1-3 years old, presenting symptoms, such as fever and enlargement of lymph nodes. During the examination of the animals' bodies, the presence of the hard tick species *Haylomma anatolicum* was observed. Subsequently, the animals were treated with buparvaquone (Buparvon, ALKE, Istanbul®), an approved anti-parasitic drug, at a single dose of 2.5 mg/(Saruhan and Paşa, 2008). The treatment was administered in a licensed private veterinary clinic located in southeast Iraq during the early summer of 2021. After the treatment, 5 ml of blood was collected from the jugular vein of each animal using an EDTA-coated glass tube. The blood samples were then transferred to the laboratory of the Zoonotic Diseases Research Unit, University of Al-Qadissyia College of Veterinary Medicine, Iraq, with an icebox.

Microscopic examination

Thin blood films were prepared immediately from the collected blood samples, and subsequently dried, and fixed by 100% Ethanol (BDH, England). Samples were transferred to the laboratory in a slide box for Giemsa staining and examination under a light microscope (Olympus CX21, Philippines) based on the technique described by Soulsby (1982). The slides smear were stained with Giemsa solution (England), for at least 20 minutes. Finally, the slides were examined using a microscope with an oil immersion lens at a magnification of X100.

Genomic materials extraction

The DNA extraction process was performed on the 30 collected blood samples. For this purpose, 200 µl of whole blood was utilized, and the extraction was conducted using the G-spin genomic kit (iNt RON Biotech. Seongnam, Si, S. Korea) following the manufacturer's instructions. The DNA was successfully isolated from the samples, resulting in concentrations ranging 150-350 ng/µl. Due to issues related to purity and DNA concentration, only 13 samples were used in the subsequent analyses. The concentration and purity of genomic DNA were assessed using a NanoDrop (Thermo/USA), with acceptable 260/230 ratios used for PCR amplification.

Polymerase chain reaction

The primers used in the study were designed according to a corresponding reference sequence, available in the GenBank database of *T. annulata*, 18S rRNA (570bp) gene (OQ411265 and MN432518 (partial) Unpublished data). The forward primer sequence used was F: CAGGCTTTCG CCTTGAATAG, while the reverse primer sequence was R: ACCACCACCCAAAGAATCAA. For the *cytochrome b* (*cyto b*) gene (891 bp), the forward primer employed was F: CGGTTGGTTTGTTCGTCTTT and reverse primer was R: CGAACTCTTGCAGAGTCCAAT (supplied from Bioneer [Korea] in conjunction with the AddStart Taq Master [2x Conc.] 1.0 ml kit/ ADDBIO, INC). The PCR reaction mixture was prepared with a total volume of 20 µl. Each primer (1.5 µl) was added, along with 10 µl of the master mix. A volume of 2 µl of DNA was included, and the remaining volume was filled with deionized PCR water to reach the total volume. The thermocycler reaction protocol included desaturation (at 95°C for 30 seconds), annealing (at 58°C for 30 seconds), extension (at 72°C 1 minute, and final extension at 72°C 5 minutes) for 33 cycles. The amplified products were electrophoresed with 1.5% agarose gel at 80 volts, stained in the ethidium bromide, and visualized with a UV transilluminator reader.

DNA sequencing method

All PCR products were subjected to Sanger dideoxy sequencing technology, following the method described by Hsiao (2019). The amplification of *18S rRNA* gene, and *cyto b* gene was performed, and sequencing was done using the Sanger sequencing system (forward and reverse reaction for each products, Bioneer Company, Korea). The resulting sequence was aligned together for phylogenetic tree analysis using Unweighted Pair Group Method Arithmetic. (UPGM; Wheeler and Kececioglu, 2007). For the tree-building procedure, NCBI-BLAST alignment and Neighbor Distances in MEGA software were used. All data obtained in the current research were submitted to GenBank ON207523.1, ON207524.1, ON207525.1, ON207526.1ON207527.1, and OM937770.1.

RESULTS

The microscopic characterization of the stained blood film indicated erythrocytes infected with the *Theileria* species in only 6 positive samples out of 13 running reactions (Figure 1). These positive samples were obtained from animals exhibiting clinical symptoms, such as high fever, loss of appetite, enlargement of lymph nodes, and pale mucus membranes.

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PCR, sequencing and phylogenetic tree construction

Due to certain limitations, only six out of thirteen PCR-amplified reactions for both the *rRNA* gene, and *cyto b gen* were selected for Sanger dideoxy sequencing technology (Hsiao, 2019, Figure 2). The obtained partial *18S rDNA* sequences were aligned with the corresponding sequences from the GenBank® database (Figure 3). However, only six readable and clean reaction data were obtained from the sequencing process.

The study involved aligning the obtained sequences with corresponding data available in the GenBank database. The alignment was performed for the deduced *cyto b* amino acid sequences, using the Clustal W default setting of MEGA v6.0 (Figure 4). Evolutiocnary distances were calculated using the Unweighted Pair Group MethodArithmetic (UPGMA) method. The phylogenetic tree constructed based on the *cyto b* gene showed distinct distances between local between *T. annulata* isolates and Indian, Iranian, and Turkish strains.

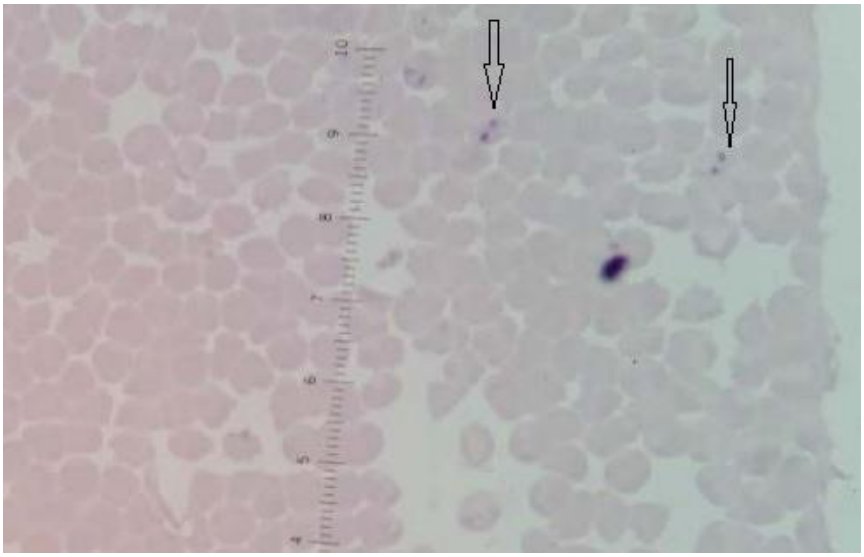

Figure 1. Microscopic examination of stained blood sample by Giemsa staining indicating *Theileria* species (arrows) is in the erythrocytes of a buffalo in Iraq (100 X magnification)

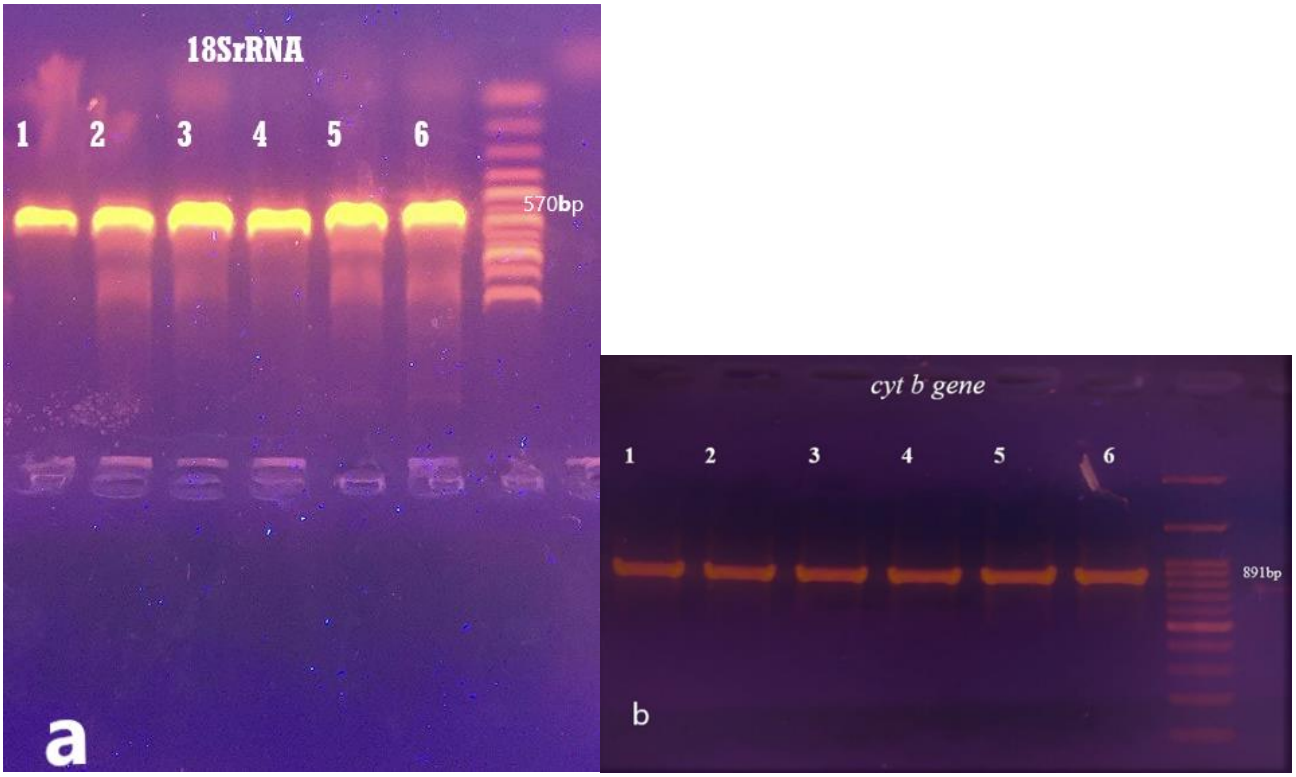

Figure 2. The electrophoresed 1.5% agarose gel using the *Theileria annulata* 18S rRNA and cyto b genes PCR products from buffalo DNA. **a:** Lanes 1-6 represent positive samples at 570bp for 18S (rRNA) gene. **b:** Lanes 1-6 represent positive samples at 891 bp for cyt b gene in buffalo.

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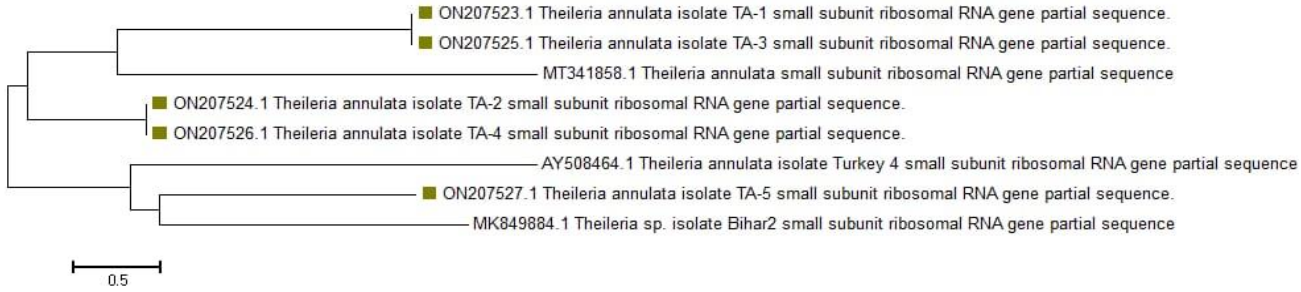

Figure 3. Constricted genetic tree shows Iraqi *Theileria* isolates based on the 18S ribosomal (r.) RNA gene (Green Square). Evolutionary distances were calculated using the (UPGMA) Unweighted Pair Group Method Arithmetic, Wheeler and Kececioglu 2007) method in MEGA v6 in buffalo.

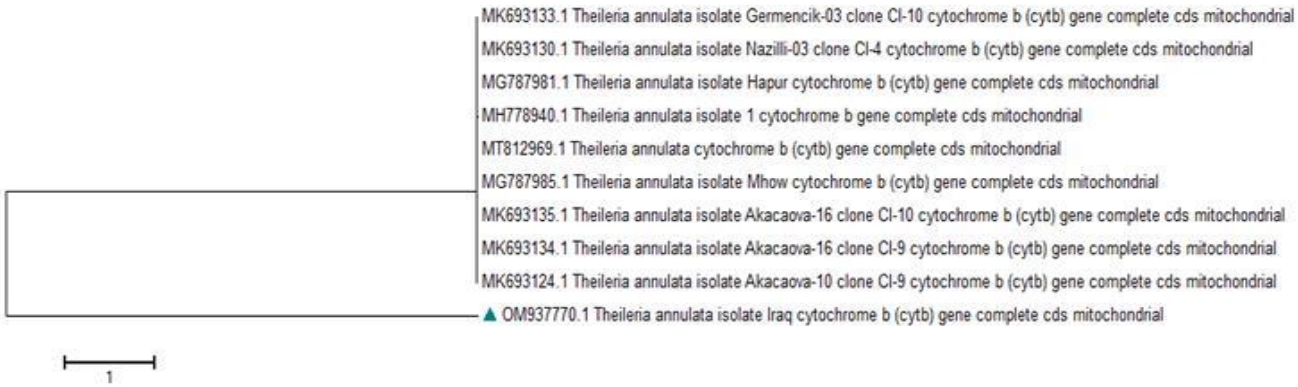

Figure 4. Phylogenetic tree analysis of local *Theileria annulata* isolates based on the (cyto b) gene (green label). The evolutionary distances were calculated using the UPGMA method in MEGA v6.0 (Kumar et al., 2016) in buffalo.

DISCUSSION

Since PCR is a more sensitive method, the results from this investigation were consistent with previous studies (Hasso and Al-Nashy, 2002; Gharbi et al., 2006; Alhaboubi et al., 2017). False-negative diagnoses of theileriosis through blood smear examination often occur due to the various structural configurations of piroplasms (Edith et al., 2018; Farooq et al., 2019; Al-Amery and Al-Amery, 2022). PCR-based molecular diagnosis may be used to circumvent the drawbacks of blood smear testing and investigate the parasite's prevalence throughout a large herd of cattle (Hasso and Al-Nashy, 2002; Faraj 2019; Al-Abedi and Al-Amery, 2021). The tree analysis was conducted using a neighbor-joint algorithm. All the Iraqi obtained sequences of the 18S rDNA aligned together with 100% identity. The isolates ON207523.1 ON207525.1 were close to each other with 100% identity for Indian buffalo isolates with variation with ON207524.1 and ON207526.1 which were close to each other in 100% identity. On the other hand, ON207527.1 was closely attached to Turkish cattle isolates and Indian Buffalo isolates. This result ensures the stability of the *18S rRNA* gene of *T. annulata* in different hosts and the cross-relation of transmission factors and similarity of the buffaloes' origin in Iraq.

Previous studies have indicated that buparvaquone, like other hydroxynaphthoquinones, could well act by binding to the *cyto b* location and preventing the parasite's electron transport pathway (Goodman et al., 2017). The *T. annulata* may become quite drug-resistant, especially in endemic areas, such as Iran, Turkey, and Tunisia, which might make it very difficult for bovine livestock to thrive in these areas (Mhadhbi et al., 2015). The identified drug is a Qo inhibitor that specifically targets the coenzyme Q binding pocket in the c*yto b* gene, leading to effective inhibition of mitochondrial respiration. However, it commonly fails to treat patients with single or double mutations in the Qo binding region of the *cyto b* gene. This finding helps explain the observed genetic distance between the resistant Iraqi isolate (OM937770.1) and strains from India, Iran, and Turkey (Figure 4, Parveen et al., 2021). This is further supported by a study performed in Iraq suggesting several mutations in the *cyto b* gene may have been responsible for the resistance of the parasite against buparvaquone (Alfatlawi et al., 2021).

Theileriosis is of high susceptibility in exotic breeds and crossbred bovines, including buffalo, and its significant effect on animal health leads to economic losses (Al-Taiy et al., 2020). There is no doubt that the treatment with long-standing protocols, may develop drug resistance, especially in the exotic breeds of the infected animals.

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CONCLUSION

Molecular approaches have shown higher specificity in detecting the prevalence of theileriosis compared to blood smear examinations. In this study, it was found that buparvaquone, the primary hydroxynaphthoquinone drug, has become ineffective against tropical theileriosis due to the emergence of drug resistance. The *Cytochrome b* gene plays a crucial role as a target gene and marker in characterizing and understanding the failure of buparvaquone therapy caused by drug resistance.

DECLARATIONS

Availability of data and materials

The author's declared that all data and materials supporting the results of this study are available.

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Ethical consideration

The authors considered all the ethical concerns, including plagiarism, the double submission, and the originality of the presentation.

Authors' contributions

All authors contribute equally to the research plan. Shehala R. Feidhel, and Howaida H. Abed, collected samples and prepared them for laboratory work. Amer R. Alhaboubi contributed to molecular application and DNA analysis. All authors contribute in writing the manuscript and have agreed to publish the last version and revisions. Amer R. Alhaboubi was the correspondent of the submitted article.

Competing interests

All authors declared no conflict of interest

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REFERENCES

Abdel Rahman MM and Ismaiel WM (2018). Comparative study for diagnosis of babesiosis and theileriosis in different age groups of cattle in some localities in Egypt with treatment trials. Egyptian Veterinary Medical Society of Parasitology Journal, 14(1): 1-14. DOI: https://www.doi.org/10.21608/evmspj.2019.33911

Abdullah SH and Ali SA (2021). Molecular investigation and phylogeny of *Theileria* spp. from naturally infected sheep and the first report of *Theileria* sp. OT3 in Sulaymaniyah governorate/Iraq. Polish Journal of Veterinary Sciences, 24(2): 201-209. DOI: https://www.doi.org/10.24425/pjvs.2021.136809

Abid K, Bukhari S, Asif M, Sattar A, Arshad M, Aktas M, Ozubek S, Shaikh RS, and Iqbal F (2021). Molecular detection and prevalence of *Theileria ovis* and *Anaplasma marginale* in sheep blood samples collected from Layyah district in Punjab, Pakistan. Tropical Animal Health and Production, 53: 439. DOI: https://www.doi.org/10.1007/s11250-021-02870-5

Al-Abedi GJK and Al-Amery AMA (2021). Molecular diagnosis and phylogenetic analysis of babesia species isolated from ticks of infested cattle in Wasit Governorate, Iraq. Iraqi Journal of Agricultural Sciences, 52(1): 136-145. DOI: https://www.doi.org/10.36103/ijas.v52i1.1245

Al-Amery AR and Al-Amery AMA (2022). Molecular diagnosis of *Cryptosporidium* ssp. In water buffaloes at Babylon province, Iraq. Iraqi Journal of Agricultural Sciences, 53(1): 147-156. DOI: https://www.doi.org/10.36103/ijas.v53i1.1519

Alfatlawi MA, Jasim AA, Jarad NE, and Khlaif SF (2021). Clinical and molecular identification of ruling *Theileria annulata* strains in cattle calves in Al-Diwaniyah province, Iraq. Iraqi Journal of Veterinary Science, 5(1): 115-119. DOI: http://www.doi.org/10.33899/ijvs.2020.126429.1319

Alhaboubi AR, Pollard DA, and Holman PJ (2017). Molecular and morphological characterization of a haemogregarine in the alligator snapping turtle, *Macrochelys temminckii* (*Testudines: Chelydridae*). Parasitology Research, 116: 207-215. DOI: https://www.doi.org/10.1007/s00436-016-5280-2%20

Ali Z, Maqbool A, Muhammad K, Khan M, and Younis M (2013). Prevalence of *Theileria annulata* infected hard ticks of cattle and buffalo in Punjab, Pakistan. The Journal of Animal & Plant Sciences, 23(1): 20-26. Available at: http://www.thejaps.org.pk/docs/v-23-1/04.pdf

AL-Judi AMH (2001). Treatment of mange in buffaloes with Abamectin. Iraqi Journal of Veterinary Medicine, 25(1): 194-198. DOI: https://www.doi.org/10.30539/ijvm.v25i1.1160

Al-Taiy HK, Al-Salhi ATH, and Al-Mashadani ALJ (2020). Development extension service to meeting the requirements of buffalo breeders in Iraq. Iraqi Journal of Agricultural Sciences, 51(1): 432-442. DOI: https://www.doi.org/10.36103/ijas.v51i1.942

Anwar K (2018). Epidemiology of tick-borne infection in ruminants in Peshawar. The Journal of Advances in Parasitology, 5(1): 6-10. Available at: http://nexusacademicpublishers.com/uploads/files/JAP_5_1_6-10.pdf

Arwa RK and Kawan MH (2022). Microscopic examination of ovine Babesiosis at Baghdad city. Iraqi Journal of Agricultural Sciences, 53(4): 798-801. DOI: https://www.doi.org/10.36103/ijas.v53i4.1591

Bilgic HB, Karagenç T, Shiels B, Tait A, Eren H, and Weir W (2010). Evaluation of cytochrome b as a sensitive target for PCR based detection of *T. annulata* carrier animals. Veterinary Parasitology, 174(3-4): 341-347. DOI: https://www.doi.org/10.1016/j.vetpar.2010.08.025

Edith R, Harikrishnan TJ, Gomathinayagam S, Kumarasamy P, and Senthilkumar TMA (2018). *Cytochrome b* gene based molecular survey of *Theileria annulata* infection in cattle in Tamil Nadu, India. Journal of Entomology and Zoology Studies, 6(5): 2356-2359. Available at: https://www.entomoljournal.com/archives/2018/vol6issue5/PartAB/6-5-305-115.pdf

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 Journal of Agricultural Sciences, 50(3): 909-915. DOI: https://www.doi.org/10.36103/ijas.v50i3.707

Farooq U, Tufani NA, Malik HU, and Mir MS (2019). Clinical and morpho-molecular epidemiology of bovine theileriosis in Kashmir, India. Indian Journal of Animal Research, 53(3): 375-381. Available at: https://arccarticles.s3.amazonaws.com/webArticle/Final-attachment-published-B-3512.pdf

Gharbi M, Sassi L, Dorchies P, and Darghouth MA (2006). Infection of calves with *Theileria annulata* in Tunisia: Economic analysis and evaluation of the potential benefit of vaccination. Veterinary Parasitology, 137(3-4): 231-241. DOI: https://www.doi.org/10.1016/j.vetpar.2006.01.015

Goodman CD, Buchanan HD, and McFadden GI (2017). Is the mitochondrion a good malaria drug target?. Trends Parasitology, 33(3): 185-193. DOI: https://www.doi.org/10.1016/j.pt.2016.10.002

Hasso SA and Al-Nashy NA (2002). Single and mixed blood protozoa infection with *Anaplasma* and Theileria in Buffaloes (*Bubalus bubalis*) in Baghdad-Iraq. Iraqi Journal of Veterinary Medicine, 26(1): 149-152. DOI: https://www.doi.org/10.30539/ijvm.v26i1.1133

Hsiao SJ (2019). Sources of error in molecular diagnostic analyses. Accurate results in the clinical laboratory. Chapter 21, pp. 337-347. DOI: https://www.doi.org/10.1016/B978-0-12-813776-5.00021-2

Jacob SS, Sengupta PP, Paramanandham K, Suresh KP, Chamuah JK, Rudramurthy GR, and Roy P (2020). Bovine babesiosis: An insight into the global perspective on the disease distribution by systematic review and meta-analysis. Veterinary Parasitology, 283: 109136. DOI: https://www.doi.org/10.1016/j.vetpar.2020.109136

Kumar S, Stecher G, and Tamura K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870-1874. DOI: https://www.doi.org/10.1093%2Fmolbev%2Fmst197

Mhadhbi M, Chaouch M, Ajroud K, Darghouth MA, and BenAbderrazak S (2015). Sequence polymorphism of *cytochrome b* gene in *Theileria annulata* Tunisian isolates and its association with buparvaquone treatment failure. PloS one, 10(6): e0129678. DOI: https://www.doi.org/10.1371/journal.pone.0129678

Nayel M, El-Dakhly KM, Aboulaila M, Elsify A, Hassan H, Ibrahim E, and Yanai T (2012). The use of different diagnostic tools for *Babesia* and *Theileria* parasites in cattle in Menofia, Egypt. Parasitology Research, 111(3): 1019-1024. DOI: https://www.doi.org/10.1007/s00436-012-2926-6

Paramanandham K, Mohankumar A, Suresh, KP, Jacob SS, and Roy P (2019). Prevalence of *Anaplasma* species in India and the world in dairy animals: A systematic review and meta-analysis. Research in Veterinary Science, 123: 159-170. DOI: https://www.doi.org/10.1016/j.rvsc.2019.01.013

Parveen A, Alkhaibari AM, Asif M, Almohammed HI, Naqvi Z, Khan A, Aktas M, Ozubek S, Farooq M, and Iqbal F (2021). Molecular epidemiology of *Theileria annulata* in cattle from two districts in Punjab (Pakistan). Animals, 11(12): 3443. DOI: https://www.doi.org/0.3390/ani11123443

Passos LMF, Bell-Sakyi L, and Brown CGD (1998). Immunochemical characterization of *in vitro* culture-derived antigens of *Babesia bovis* and *Babesia bigemina*. Veterinary Parasitology, 76(4): 239-249. DOI: https://www.doi.org/10.1016/S0304-4017(98)00095-8

Rafiullah A, Rahman K, Khan A, Ali A, Khan A, and Sajid NK (2019). Prevalence of *Theileria parva* in large ruminants through conventional and molecular techniques in district Lakki Marwat and Peshawar (Pakistan). Sarhad Journal of Agriculure, 35: 320-329.

Sallemi S, Rjeibi MR, Rouatbi M, Amairia S, Ben Said M, Khamassi Khbou M, and Gharbi M (2018). Molecular prevalence and phylogenetic analysis of *Theileria 323nnulate* and *Trypanosoma evansi* in cattle in Northern Tunisia. Veterinary Medicine Science, 4(1): 17-25. DOI: https://www.doi.org/10.1002/vms3.79

Saruhan B and Paşa S (2008). Therapeutic efficacy of buparvaquone (buparvon) in cattle with theileriosis. Turkiye Parazitolojii Dergisi, 32(4): 317-321. Available at: https://europepmc.org/article/med/19156603

Soulsby EJL (1982). Helminths, arthropods, and protozoa of domesticated animals, 7th Edition. Bailliere Tindall., London. p. 809. DOI: https://www.doi.org/10.1016/0035-9203(84)90110-X

Ullah R, Shams S, Khan MA, Ayaz S, Akbar NU, Din QU, Khan A, Leon R, and Zeb J (2021). Epidemiology and molecular characterization of *Theileria annulata* in cattle from central Khyber Pakhtunkhwa, Pakistan. PloS one, 16(9): e0249417. DOI: https://www.doi.org/10.1371/journal.pone.0249417

Wheeler TJ and Kececioglu JD (2007). Multiple alignment by aligning alignments. Bioinformatics, 23(13): 559-568. DOI: https://www.doi.org/10.1093%2Fbioinformatics%2Fbtm226

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