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Detection of *Theileria equi* in Baghdad Racing Horses Using Hematological and Molecular Assay

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A B S T R A C T

The aim of this study was to investigate the prevalence of *Theileria equi* infection in racing horses in Baghdad governorate, utilizing clinical signs, microscopic examination, molecular assays, and hematological tests to identify significant differences between infected and non-infected horses. Between January and December 2021, a total of 160 racing horses from three locations in Baghdad governorate (Al-Amiriya Equestrian Club, Arabian horses in Alzwraa Zoo, and Iraqi Equestrian School) were randomly selected for examination. Microscopic examination of blood smears revealed 11 positive samples for *Theileria equi* (6.875%), while the results of the polymerase chain reaction assay targeting the 18S ribosomal RNA gene confirmed 26 positives (16.25%). Sequenced amplicons and phylogenetic tree analysis revealed a genetic similarity of 93.03–100% and 94-100% site coverage compared to many global countries' isolates. Interestingly, only 16 (61.5%) of the 26 infected horses showed mild to subclinical symptoms or were carriers of the disease without tick infestation. Hematological blood parameters showed non-significant differences between infected and non-infected horses, except for a decrease in packed cell volume (PCV) and hemoglobin (Hb) concentration, which caused anemia in 12 horses among the 26 molecularly positive cases (46.15%). Of these cases, 6 horses (23.08%) had normocytic normochromic anemia, while 3 (11.54%) cases each had normocytic hypochromic and microcytic hypochromic anemia. Notably, young horses (two years old) were more susceptible to infection (odds ratio 15.4) than those over six years old. Additionally, sex and breed did not show any significant correlation with equine theileriosis. In conclusion, this study detected *Theileria equi* infection in young racing horses in Baghdad. Clinically, most infected cases showed mild to asymptomatic signs accompanied by anemia. Molecular investigation revealed high genetic similarity to isolates reported globally. These findings highlight the importance of implementing measures to control and prevent the spread of *Theileria equi* in racing horses in Baghdad and other regions. Further studies are warranted to better understand the epidemiology, pathogenesis, and risk factors associated with equine theileriosis.

Keywords: *Theileria equi*, racehorses, Baghdad, molecular, hematology

INTRODUCTION

Haemoprotozoan *Theileria equi* is a tick-borne disease that infects all horse breeds and Equidae species (1, 2). Clinical signs include fever, icteric and pale mucous membranes, and anemia (3). Theileriosis affects different breeds of horses and is considered an endemic illness in most regions of the globe (4, 5). Several investigations have shown that the disease is prevalent in Iraq (6-14). Numerous infected horses carry parasites without displaying symptoms of sickness, posing a serious threat of transmitting protozoa (15). This has economic

consequences associated with restrictions on equine travel between endemic and non-endemic areas, the diminished performance of racehorses (16, 17), and treatment expenses in the absence of available treatment (18).

Ixodid ticks are the primary source of transmitted *Theileria equi* (19), and the distribution of many tick species in Iraq has been documented (20). The infection is transmitted by tick bites, where sporozoites invade equine lymphocytes, replicate to merozoites, invade red blood cells (RBCs), multiply by asexual division, reinvade RBCs, multiply again several times, and form gametocytes. Ticks feed on horse blood, and the protozoa complete their life cycle in the tick's body (1). Another way of transmission may occur iatrogenically by contaminated needles or surgical instruments (21). Díaz-Sánchez et al. (2018) (4) evaluated the hematology picture in horses infected with theileriosis and found intra-erythrocytic Theileria equi formation in 13.8% of horses with association low hematocrit values.

Diagnosis of theileriosis includes clinical signs and several diagnostic techniques, such as serological tests, light microscopy, clinical pathology changes, and polymerase chain reaction (22). Due to the lack of specificity of the clinical symptoms, horse piroplasmosis is difficult to diagnose clinically, and the reduced parasitaemia seen in the latent phase of theileriosis complicates microscopic identification (23, 24). It usually appears as 2-3 µm polymorph merozoites within RBCs in Giemsa-stained blood film, with mostly occurring as a "characteristic Maltese cross formation" (25). Molecular detection must be the standard diagnostic procedure since it is more accurate and sensitive than other techniques for diagnosing chronic theileriosis infection (26).

This research aims to investigate the spread of *Theileria equi* in Baghdad racing horses based on clinical symptoms, microscopic appearance, molecular evidence, and hematological results. The study aims to screen the spread of *Theileria equi* in Baghdad racing horses by finding significant differences among infected and non-infected horses according to clinical symptoms, microscopic appearance, molecularly proven, and hematological results. This study will provide valuable insights into the prevalence and transmission of *Theileria equi* in Baghdad racing horses and contribute to the development of effective management and control strategies for this disease.

MATERIALS AND METHODS

Ethics

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq reviewed and approved all procedures involved in the current study.

Horse Groups

A total of 160 racing horses (75 Crossbreds, 60 Arabians, and 25 Thoroughbreds) were checked out between January to December 2021. The age range for the 91 mares and 69 stallions in the research was 2–25 years according to (27). The vital signs including breath, heart rate, body temperature and other signs were reported for each animal to investigate the occurrence of Equine theileriosis.

Blood samples were collected from the jugular vein of each animal and kept into 2 different tubes, including 3 ml in EDTA anticoagulant for hematological investigation, (28, 29); while the other sample was frozen for molecular investigations, the PCR assay.

Hematological assessments

The morphological evaluation of anemia (established by the Wintrobe Index) includes hemoglobin, hematocrit measurements, and erythrocyte counts. Blood film observation, counts of thrombocytes and leukocytes (30).

Molecular Assay

The Promega, USA, ReliaPrepTM Blood gDNA Miniprep System kit was utilized to extract horse genomic DNA from blood (31). The purity of DNA and its concentration were recognized by the NanoDrop technique, One µl of each DNA elution and elution buffer (blank) was loaded to quantify DNA in each extraction (32). The pairs of primers "Equi Merozoite Antigen-1 (EMA-1)" used to recognize T. equi were obtained through conventional PCR by pursuing "The18S ribosomal RNA gene" in length 750Bp which were Ema-1 forward "5'-GCATCCATTGCCATTTCGAG-3'" and Ema-1 reverse "5'-TGCGCCATAGACGGAGAAGC-3'" by (15). Promega Corporation's 2018 guidelines recommend that volume of PCR reaction 25µl should contain 12.5µl of "(Promega, USA, 2X GoTaq® G2 Green Master Mix) that consist of Buffer (pH 8.5), 3 mM MgCl2, mixture of 400 M each of dATP, dTTP, dCTP, and dGTP, and 50 units/mL of TaqDNA polymerase", plus Three microliters of template DNA, 7.5 µl of nuclease-free water, 1 µl in concentration 10 pmol of each T. equi primers . A protocol of thermo cycling starts with a ten-minute initial denaturation heat-up to 94 °C, followed by forty cycles (45 seconds denaturation heat-ups at 94 °C and 45 seconds annealing at 51.5°C, then 45 seconds extension heat-ups at 72 °C, and ten minutes final extension at 72 °C) and holding at 4 °C last for a few hours. After undergoing (1.5% concentration) agarose gel electrophoresis using Promega, USA, Diamond™ Nucleic Acid Dye), and visualized DNA amplified fragments against UV. Lights (33).

Sequencing, Phylogenetic Analyses

Theileria equi 18S rRNA gene was sequenced by (Macrogen lab, Korea) using Ema-1 primers; the outcomes

were analyzed according to BLAST of "the NCBI database [http:// www.ncbi.nlm.nih.gov/BLAST]" (34), and recorded in an international gene bank. The "Molecular Evolutionary Genetics Analysis (MEGA 11)" software was applied to create a phylogenetic tree, which was compared with identical worldwide results.

Statistical Analysis

Significant Results (at levels p0.05) suggested by statistical analysis of data using the application "Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Chicago, USA)" (36).

RESULTS

Clinical Signs

Theileriosis symptoms occurred among 16 horses (61.5%) from 26 infected horses according T. equi PCR results, signs were documented in Table (I), which listed the main signs as pale mucus membrane, icterus, and emaciation. Examination of body temperature was 37.93C0 (ranging 37.1-38.90 C) with no fevered case, respiratory rate was 17.5 (ranging 14 - 36 breath/minute), and heart rate was 40.35 (28 – 52 beats/minute).

Table 1. The Clinical signs of racing horses Infected with *Theileria equi*

Clinical signs	(26) infected with horses *Theileria equi* (No.) %	Clinical signs	(26) infected with horses *Theileria equi* (No.) %
Anorexia	(2) 7.7	Lameness	(1) 3.8
Congestion Conjunctiva	(2) 7.7	Legs Swelling	(1) 3.8
Dehydration	(1) 3.8	Pale Mucous Membrane	(6) 23.1
Emaciation	(5) 19.2	Polyurea	(2) 7.7
Icterus	(5) 19.2	Petechial hemorrhage in 3rd eyelid	(1) 3.8

Microscopic Examination

Theileria equi was seen microscopically in 11 blood smears out of 160 horses at percentage (6.875%) as small Maltese cross form of quadrate merozoites bodies inside erythrocytes or blue inclusion body inside lymphocytes cytoplasm (Figure 1).

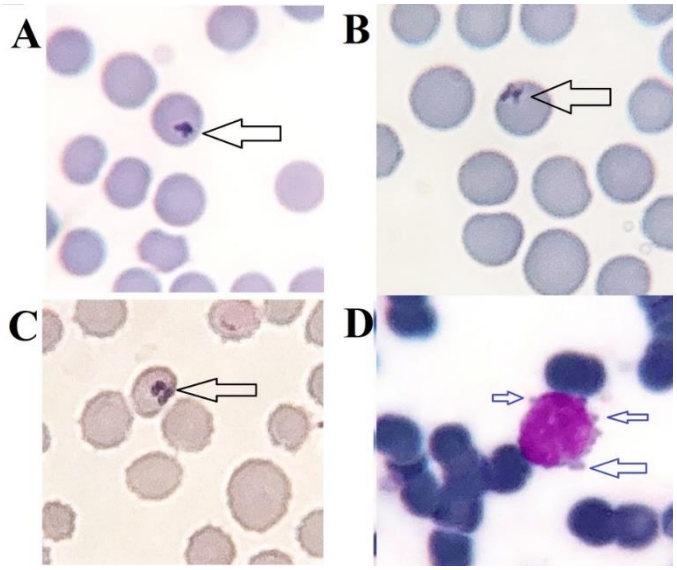

Figure 1. *Theileria equi* detection in X100, Romanowsky_stained blood smears. A and B: black arrow pointed to Maltese cross appearance of merozoites. C: black arrow pointed to quadrate merozoite bodies. D: blue arrows pointed to blue inclusion bodies of schizonts intra lymphocytes cytoplasm at 100X

Results of PCR Assay

Polymerase chain reaction assay on 160 horse samples revealed that (26) 16.25% horses had positive 750bp amplicons of "The18S ribosomal RNA gene" (Figure 2).

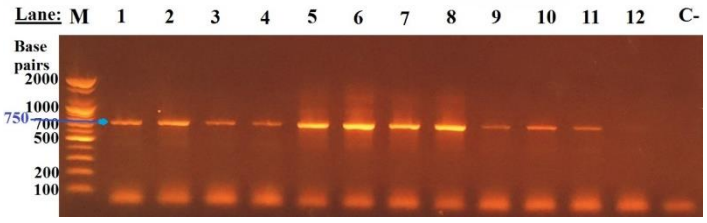

Figure 2. Agarose gel cast loaded with PCR amplicons of *Theileria equi* 18S ribosomal RNA genes, at 750Bp, Stained by "DiamondTM Nucleic Acid Dye", isolated from horse blood. Lane M: ladder 100 - 2000 bp, Lane 1-11: T equi Positives amplicons, , and Lane 12: Negative sample, Lane C-: control Negative

There was a significant association between infection, microscopic and molecular results, Chi-square (X2) = 6.876; df = 1; P value = 0.008735431; (P ≤ 0.05). Sequenced and BLAST results are recorded in "the National Center for Biotechnology Information (NCBI) Gene bank" as T equi in Baghdad racing horses, in identification numbers: ON641879.1 to ON641891.1.

Drawing The phylogenetic tree in (Figure 3) observed our isolates had highest similarity of 93.03-100% with 94-100% site coverage with Brazil, China, Jordan, India, Iran, Japan, Scotland, Morocco, United states of America, and Thailand isolates.

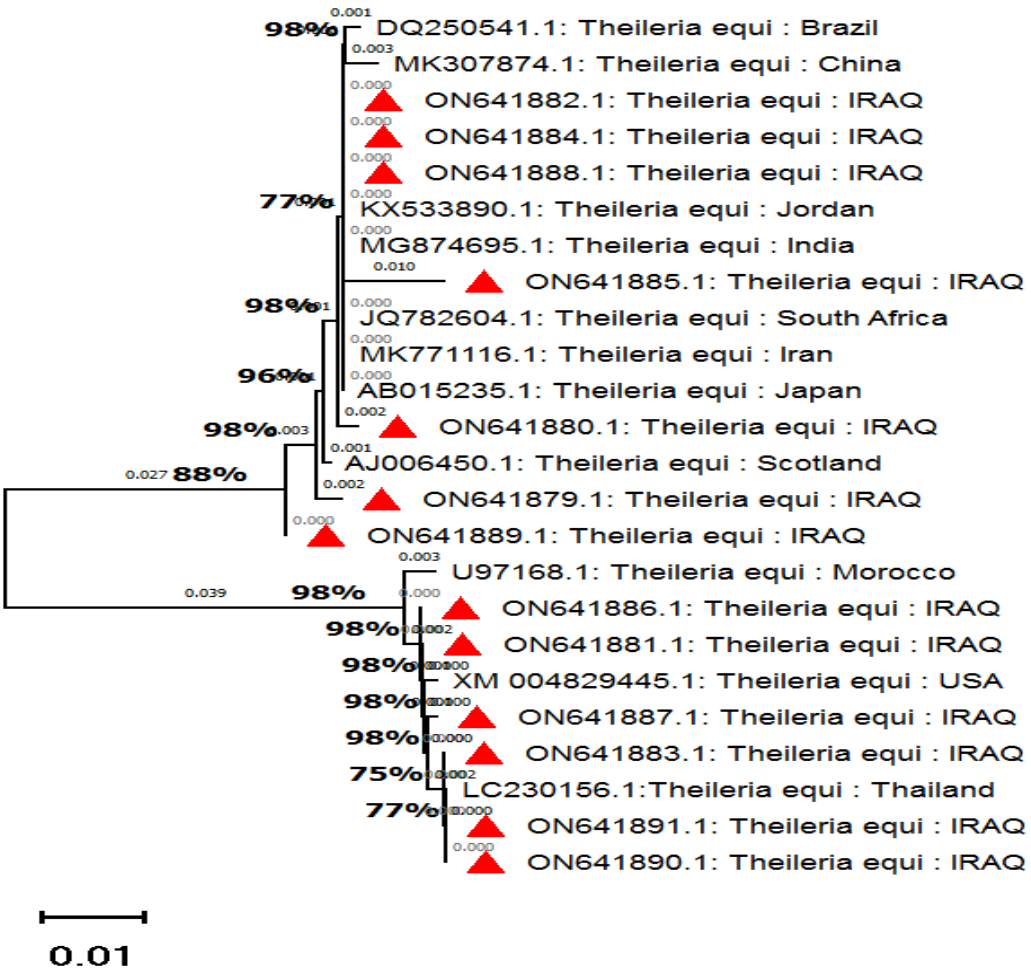

Figure 3.Phylogenetic tree of sequenced "The18S ribosomal RNA gene" of *Theileria equi* isolates, orange Triangles marked the Baghdad racing horses blood samples

Table 2. Erythrogram Values in Molecular Positive and Negative Horse

Parameter	*Positive with Theileria equi* (26)	Negative Results (134)	Total Horses (160)
RBCs count (10^{12}/L)	8.69±0.354 (5.29 – 11.74)a	9.23 ±0.183 (4.9 - 13.41)a	9.14 ±0.164 (4.9 - 13.41)
PCV (L/L)	32.73±1.164 (18 - 44)b	36.4±0.538 (24 - 52)a	35.82 ±0.499 (18 - 52)
Hb (g/dL)	10.8 ±0.399 (7.92-114.84)b	12.18± 0.203 (7.16- 18.16)a	11.95 ±0.185 (7.16 -18.16)
MCV (fl)	38.17 ±1.032 (29.31 -57.14)a	40.32±0.493 (24.88 -59.41)a	39.97 ±0.449 (24.9 - 65.2)
MCH (pg)	12.64 ± 0.38 (9.47 -18.06)a	13.45 ±0.177 (9.4 - 20.03)a	13.32 ±0.162 (9.4 - 19.25)
MCHC (g\dL)	32.81±0.437 (28.76-38.36)a	33.4 ±0.205 (24.9 -39.55)a	33.37 ±0.208 (24.9 - 39.55)
Platelets count (10^9/L)	259.01±14.652 (146 - 410)a	279.8±7.167 (128 - 642)a	275.9 ±6.545 (128 - 642)
Total WBC count (10^9\L)	11.91 ±0.658 (7.25 – 20.5)a	10.92 ±0.294 (5 – 25.4)a	11.08 ±0.269 (5 - 25.4)
Horizontally Results took same small letters weren't had significant differences at level (P ≤ 0.05), **Results showed by: Mean, ±Standard Error, and (Range)			

Hematological study

Table 2 illustrated significant decrease in packed cell volume and hemoglobin concentration comparable non infected group, that lead to (12) 46.15% cases had anemia divided to (6) 23.08% cases Normocytic Normochromic anemia, and (3) 11.54% cases for each Normocytic Hypochromic and Microcytic Hypochromic anemia, otherwise other blood parameters didn't show any significant differences in 26 infected horse.

Predisposing factors affect infection rate with theileriosis

The young two years old horses showed highest susceptible to infection 31.82% comparing to 2.94% in aged horses over six years old, and statistically considered as predisposing factor for horse to infected by T. equi (Odds Ratio 15.4) Confidence interval 95% was 1.7366 to 136.562. Sexes and breeds of horses didn't considered as predisposing factor of equine thelieriosis as demonstrated in Table 3.

Table 3: Predisposition of age, breed, and sex of race horses to be infected by *Theileria equi*

Factor		Total Horses (160)	26 infected horses *i*	percentage	(Odds Ratio) Confidence interval 95%
Sex	**Stallions**	69	10	14.49%	(0.7945) 0.3361 to 1.8786
	Mares	91	16	17.58%	
Breed	**Arabian Horses**	60	8	11.59%	(1.3629) 0.5246 to 3.5408
	Thoroughbred	25	5	20 %	
	Crossbreds	75	13	17.33 %	
Age	**2 years**	22	7	31.82% a	(15.4) 1.7366 to 136.562
	3 years	**24**	5	20.83%	
	4 years	**32**	8	25%	
	5 years	**25**	5	20%	
	6 -10 years	**34**	1	2.94% b	
	<11 years	**23**	0	0%	

DISCUSSION

The presence of *Theileria equi* merozoites in Romanowsky-stained horses blood films was showed in 11 horses (6.875%) in this study, there is significant increased positive results in PCR method revealed by 26 horses (16.25%), this increase difference between microscopic and molecular to the results of (37) who found that 6.25% occurred microscopically as 1.14-1.88µm sized body inside RBCs and 10.83% PCR, Kumar et al., (2020) detect 6.97% microscopically compared to 10.46% by PCR, Soliman et al., (2021) record 8.9% appeared as single or double, or Maltese cross shapes and 23.4% by PCR, other studies reports 11.4% and 36.4% in PCR (39), Camino et al., (2019) found small round to ovoid or pyriform intra erythrocytes in 9.3% and 39.3% by PCR, and 8.33% and 50% by PCR; also 20.2% and 31.9% in PCR founded by (40) and (12) respectively. This result agreed with the average of 34.6% positive PCR found by twenty years of 25 molecular studies in 18 worldwide regions (17). Parallel confirmation was perceived by sequencing isolates and BLAST in NCBI had the highest similarity of 93.03-100% with 94-100% site coverage with Jordan, Iran, Morocco, China, India, Japan, Thailand, Scotland, The United States of America, and Brazil isolates in the phylogenetic tree. PCR proved in this study as best technique to diagnosis *Theileria equi* in our Racing horses.

Mild signs occurred in16 (61.5%) positive horses (showed in table 1) without fever or significant increase in respiratory and heart rates in randomly selected horses survey, also mild symptoms found in 42.9% by (3), and disagreed with significant increased body temperature, heart and respiratory rates in two studies in south and central of Iraq (7,9). Our results suggested mild to subclinical and carrier state infection due to absence of the transported tick infestation, this agrees with the term "lack of disease" in Italy positive horses who suggested iatrogenic transmission (41).

Hematologically, there is a significant decrease in packed cell volume and hemoglobin concentration and a non-significant decrease in RBC and platelet count, as well as MCH and MCV; however, non-significant increase in WBC count and MCHC. Similar results with addition significant decrease in Platelets count (39,41)(29), Disagreed with results of (7,9,42) they found significant decrease in all hematological parameters, in contrast only significant increase in WBC count and didn't find other parameter significance by (3). These variable results documented in different studies revealed that the hemogram is not a good diagnostic tool for equine theileriosis. On the other hand all researchers concurrent on association of presence of anemia in infected animals, our results found 6 cases (23.08%) had Normocytic Normochromic anemia and three cases 11.54% for each Normocytic Hypochromic and

Microcytic Hypochromic anemia. Similar Normocytic Normochromic anemia recorded by (9,41), while (7,39) were recorded Macrocytic Hypochromic anemia. These outcomes illustrated the necessity of periodical investigation of theileriosis infection to early treatment and avoid decreased performance due to anemia in race horses in Baghdad.

Mares showed more susceptible to infection than stallions but statistically nonsignificant, as well as Thoroughbred horse more susceptible than other breeds but also statistically non significant, but the young horses (2 years) revealed significant increased infection rate than aged horses (>6 years) in Baghdad race horses, that resemble previous study in Iraq were the females and young horses showed significant elevation than other groups (6). However Soliman et al., (2021) found males are more prevalent but insignificant and horses (<5years) was significant higher than oldest age groups, mares were more significant as recorded by (43), However, the results of the present study disagree with (5,11–13,37) who reported there was significant differences in in percentage of infection between male and female and different age

We used molecular methods to look for the equine hemoprotozoan *Theileria equi* in Baghdad racehorses. The polymerase chain reaction (PCR) is the best way to diagnose it, and our phylogenetic analysis showed the greatest resemblance to the global gene bank. Infections occurring as a result of carrier status or subclinical illness. These findings highlighted the importance of routine theileriosis screenings in Baghdad's racing horses to improve the treatment and control measures of this pathogen. and prevent anemia from affecting their performance and costing the industry money.

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N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. 1. Mehlhorn H, Schein E. Redescription of Babesia equi Laveran, 1901 as *Theileria equi* Mehlhorn, Schein 1998. Parasitol Res [Internet]. 1998;84(6):467–75. Available from: https://doi.org/10.1007/s004360050431
2. 2. Soliman AM, Elhawary NM, Helmy NM, Gadelhaq SM. Molecular and microscopic detection of Babesia caballi and *Theileria equi* among working horses and donkeys in Cairo and Giza provinces of Egypt. 2021;1–14. Available from: https://doi.org/10.21203/rs.3.rs-757240/v1
3. 3. Camino E, Dorrego A, Carvajal KA, Buendia-Andres A, de Juan L, Dominguez L, et al. Serological, molecular and hematological diagnosis in horses with clinical suspicion of equine piroplasmosis: Pooling strengths. Vet Parasitol [Internet]. 2019;275(June):108928. Available from: https://doi.org/10.1016/j.vetpar.2019.108928
4. 4. Díaz-Sánchez AA, Fonseca-Rodríguez O, Luis Del Castillo-Domínguez S, Alfonso-Dorta Y, Lobo-Rivero E, Corona-González B, et al. Hematological alterations found in horses (Equus caballus) infected with Babesia caballi and *Theileria equi*. Rev Salud Anim [Internet]. 2018;40(1):2224–4700. Available from: https://www.cabdirect.org/cabdirect/abstract/20183221658
5. 5. Giubega S, Ilie MS, Morariu S, Dărăbuș G, Luca I, Florea T, et al. Seroprevalence of Anti-*Theileria equi* Antibodies in Horses from Three Geographically Distinct Areas of Romania. Pathogens [Internet]. 2022;11(6). Available from: https://doi.org/10.3390/pathogens11060669
6. 6. Sray A, AL-SHabbani A, GHarban H. Serological and Molecular Estimation of *Theileria equi* Infections in Horses of Baghdad, Al-Qadisiyah, and Wasit Provinces / Iraq. J Res Lepid [Internet]. 2019;50(4):391–404. Available from: https://doi.org/10.36872/LEPI/V50I4/201103
7. 7. AlSaad KM, ALMola GM. Clinical And Pathological Study Of Equine Babesiosis In Draught Horses In Mosul. Iraqi J Vet Sci [Internet]. 2006;20(1):89–101. Available from: http://dx.doi.org/10.33899/ijvs.2006.45787
8. 8. Alsaad KM, Hassan SD, Al-Obaidi QT. Detection of Babesia equi and Babesia caballi antibodies in horses and donkeys in Mosul, Iraq. Res Opin Anim Vet Sci [Internet]. 2012;2(4):291–4. Available from: https://www.cabdirect.org/cabdirect/abstract/20133085653
9. 9. Alsaad KM, Alsaad EA, Al-Derawie HA. Clinical and Diagnostic Study of Equine Babesiosis in Drought Horses in Some Areas of Basrah Province [Internet]. Vol. 4, Research Journal of Animal Sciences. 2010. p. 16–22. Available from: https://medwelljournals.com/abstract/?doi=rjnasci.2010.16.22
10. 10. Aziz KJ, Al-Barwary LTO. Epidemiological study of equine piroplasmosis (*Theileria equi* and Babesia caballi) by microscopic examination and competitive-ELISA in Erbil Province North-Iraq. Iran J Parasitol [Internet]. 2019;14(3):404–12. Available from: https://www.readcube.com/articles/10.18502%2Fijpa.v14i3.1479
11. 11. Aziz KJ, Al-Barwary LTO, Mohammed ZA, Naqid IA. Molecular identification and phylogenetic analysis of *Theileria equi* and babesia caballi infections in equids from erbil province, north of Iraq. Adv Anim Vet Sci [Internet]. 2019;7(12):1060–6. Available from: http://dx.doi.org/10.17582/journal.aavs/2019/7.12.1060.1066
12. 12. Saleem HD, Al-Samarai FR. Prevalence of *Theileria equi* in horses in central Iraq determined by microscopy and PCR. Online J Vet Res [Internet]. 2018;22(4):273–80. Available from: http://onljvetres.com/theileriaabs2018.htm
13. 13. Faraj AA, Hade BF, Al-Amery AM. Conventional And Molecular Study Of Babesia Spp. Of Natural Infection In Dragging Horses At Some Areas Of Baghdad City, Iraq. Iraqi J Agric Sci [Internet]. 2019;50(3):909–15. Available from: http://blast.ncbi.nlm.nih.gov
14. 14. Al-Rammahi HM, Hatem AA, Al-Atabi AC. Molecular detection and occurrence of equine theileriosis in arabian horses in al-najaf province/Iraq. Brazilian J Vet Res Anim Sci [Internet]. 2020;57(3):1–5. Available from: https://doi.org/10.11606/issn.1678-4456.bjvras.2020.166996
15. 15.Idoko IS, Tirosh-Levy S, Mazuz ML, Adam BM, Garba BS, Nafarnda DW, et al. Genetic characterization of piroplasms in donkeys and horses from Nigeria. Animals [Internet]. 2020;10(2):1–9. Available from: https://doi.org/10.3390/ani10020324
16. 16. Camino E, Cruz-Lopez F, de Juan L, Dominguez L, Shiels B, Coultous RM. Phylogenetic analysis and geographical distribution of *Theileria equi* and Babesia caballi sequences from horses residing in Spain. Ticks Tick Borne Dis [Internet]. 2020;11(6):101521. Available from: https://doi.org/10.1016/j.ttbdis.2020.101521
17. 17. Tirosh-Levy S, Gottlieb Y, Fry LM, Knowles DP, Steinman A. Twenty years of equine piroplasmosis research: Global distribution, molecular diagnosis, and phylogeny. Pathogens [Internet]. 2020;9(11):1–32. Available from: https://doi.org/10.3390/pathogens9110926
18. 18. Bishop RP, Kappmeyer LS, Onzere CK, Odongo DO, Githaka N, Sears KP, et al. Equid infective Theileria cluster in distinct 18S rRNA gene clades comprising multiple taxa with unusually broad mammalian host ranges. Parasites and Vectors [Internet]. 2020;13(1):1–7. Available from: https://doi.org/10.1186/s13071-020-04131-0

19. 19. Romiti F, Magliano A, Antognetti V, Manna G, Cersini A, Scicluna MT, et al. Investigation of Ixodid ticks as vectors of Babesia caballi and *Theileria equi* (Protozoa: Apicomplexa) in central Italy. J Vector Ecol [Internet]. 2020;45(1):25–31. Available from: https://doi.org/10.1111/jvec.12370
20. 20. Makawi ZA, Jassim SY, Hasson KI. Review on Spread of Parasitic Hard Ticks on Field Animals in Iraq. Glob J Public Heal Med [Internet]. 2019;1(2):39–43. Available from: https://doi.org/10.37557/gjphm.v1i2.13
21. 21. Short MA, Clark CK, Harvey JW, Wenzlow N. Outbreak of equine piroplasmosis in Florida. J Am Vet Med Assoc [Internet]. 2012;240(5):588–595. Available from: https://doi.org/10.2460/javma.240.5.588
22. 22. Wise LN, Kappmeyer LS, Mealey RH, Knowles DP. Review of equine piroplasmosis. J Vet Intern Med [Internet]. 2013;27(6):1334–46. Available from: https://doi.org/10.1111/jvim.12168
23. 23. Salinas-Estrella E, Ueti MW, Lobanov VA, Castillo-Payró E, Lizcano-Mata A, Badilla C, et al. Serological and molecular detection of Babesia caballi and *Theileria equi* in Mexico: A prospective study. PLoS One [Internet]. 2022;17(3 March 2022):1–14. Available from: https://doi.org/10.1371/journal.pone.0264998
24. 24. Qablan MA, Oborník M, Petrželková KJ, Sloboda M, Shudiefat MF, Hořín P, et al. Infections by Babesia caballi and *Theileria equi* in Jordanian equids: Epidemiology and genetic diversity. Parasitology [Internet]. 2013;140(9):1096–103. Available from: https://doi.org/10.1017/S0031182013000486
25. 25. de Waal DT. Equine piroplasmosis: A review. Br Vet J [Internet]. 1992;148(1):6–14. Available from: https://doi.org/10.1016/0007-1935(92)90061-5
26. 26.Onyiche TE, Suganuma K, Igarashi I, Yokoyama N, Xuan X, Thekisoe O. A review on equine piroplasmosis: Epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. Int J Environ Res Public Health [Internet]. 2019;16(10). Available from: https://doi.org/10.3390/ijerph16101736
27. 27. Khazaeel K, Pesarakli H, Mashhadi AG, Borujeni MP. Age estimation of Arabian mares by incisors morphometry and dentition changes. Vet Res Commun [Internet]. 2022;46(2):405–17. Available from: https://doi.org/10.1007/s11259-021-09865-5
28. 28. Sultan AS. A study on some normal hematological values in foals and arabian horses at different ages. Iraqi J Vet Med [Internet]. 2001;25(1):125–32. Available from: https://doi.org/10.30539/ijvm.v25i1.1154
29. 29. Habasha FG, Abaas WM, GhaniTahaYassin. Hematological changes of Anemia`s in Horses of Equestrian club in Baghdad. Iraqi J Vet Med [Internet]. 2012;36(0A):92–7. Available from: https://doi.org/10.30539/iraqijvm.v36i0A.361
30. 30. Harvey JW. Veterinary Hematology: A Diagnostic Guide and Color Atlas. Missouri: Saunders, Elsevier Inc.; 2012. 329.368.
31. 31. Badawi NM, Yousif AA. Survey and Molecular Study of Babesia gibsoni in Dogs of Baghdad Province, Iraq. Iraqi J Vet Med [Internet]. 2020;44((E0)):34–41. Available from: https://doi.org/10.30539/ijvm.v44i(E0).1019
32. 32. Al-Ani AN, Al-Badrawi TY, Hussein ZS. Toxoplasmosis in Cats : Serological and Molecular Study in Baghdad Province. Ann Trop Med Public Heal [Internet]. 2020;23(2):110–9. Available from: https://www.scilit.net/article/74b0f0cf021359f825bb6cc4d324a857
33. 33. Green MR, Sambrook J. Analysis of DNA by agarose gel electrophoresis. Cold Spring Harb Protoc [Internet]. 2019;2019(1):6–15. Available from: https://doi.org/10.1101/pdb.top100388
34. 34. Hall T. BioEdit:a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT [Internet]. Vol. 41, Nucleic Acids Symp Serie. 1999. p. 95–8. Available from: https://cir.nii.ac.jp/crid/1572261549883338880
35. 35. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol [Internet]. 2013;30(12):2725–9. Available from: https://doi.org/10.1093/molbev/mst197
36. 36. Bluman AG. Elementary statistics : a step by step approach. 8th ed. New Yourk: McGraw-Hill; 2012. 942 p.
37. 37. Malekifard F, Tavassoli M, Yakhchali M, Darvishzadeh R. Detection of *Theileria equi* and Babesia caballi using microscopic and molecular methods in horses in suburb of Urmia, Iran. Vet Res forum an Int Q J [Internet]. 2014;5(2):129–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25568706%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4279624
38. 38. Kumar S, Sudan V, Shanker D, Devi A. Babesia (Theileria) equi genotype A among Indian equine population. Vet Parasitol Reg Stud Reports [Internet]. 2020;19(June):100367. Available from: https://doi.org/10.1016/j.vprsr.2019.100367
39. 39. Mahmoud MS, Abu El-Ezz NT, Abdel-Shafy S, Nassar SA, El Namaky AH, Khalil WKB, et al. Assessment of *Theileria equi* and Babesia caballi infections in equine populations in Egypt by molecular, serological and hematological approaches. Parasites and Vectors [Internet]. 2016;9(1):1–10. Available from: http://dx.doi.org/10.1186/s13071-016-1539-9
40. 40. Derinbay Ekici Ö, Ceylan O, Sönmez G, Dik B, Ceylan C, Semassel A. Molecular detection and phylogenetic analysis of *Theileria equi* and babesia caballi in wild horses in konya province of turkey. Ankara Univ Vet Fak Derg [Internet]. 2021;68(3):275–81. Available from: https://doi.org/10.33988/auvfd.708329
41. 41. Padalino B, Rosanowski SM, Di Bella C, Lacinio R, Rubino GTR. Piroplasmosis in Italian Standardbred Horses: 15 Years of Surveillance Data. J Equine Vet Sci [Internet]. 2019;83:102813. Available from: https://doi.org/10.1016/j.jevs.2019.102813
42. 42.Ahmadpour S, Esmaeilnejad B, Dalir-Naghadeh B, Asri-Rezaei S. Alterations of cardiac and renal biomarkers in horses naturally infected with *Theileria equi*. Comp Immunol Microbiol Infect Dis [Internet]. 2020;71(May):101502. Available from: https://doi.org/10.1016/j.cimid.2020.101502
43. 43. Davitkov D, Vucicevic M, Stevanovic J, Krstic V, Slijepcevic D, Glavinic U, et al. Molecular detection and prevalence of *Theileria equi* and Babesia caballi in horses of central Balkan. Acta Parasitol [Internet]. 2016;61(2):337–42. Available from: https://doi.org/10.1515/ap-2016-0044.

الكشف عن طفيلي الثاليريا في خيول السباق في بغداد بواسطة الفحص الدموي والجزيئي

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الخلاصة

هدفت هذه الدراسة إلى الكشف عن انتشار الثايليريا في خيول السباق في بغداد ، من خلال حساب الفروق المعنوية ما بين الخيول المصابة وغير المصابة ، بالاعتماد على العلامات السريرية ، والفحص المجهري ، والاختبارات الجزيئية ، والفحوصات الدموية. تم فحص ١٦٠ حصانًا بشكل عشوائي للفترة من كانون الثاني الى كانون الاول ٢٠٢١, واللتي وجدت في مناطق (نادي الفروسية في العامرية , تجمع الخيول العربية في حديقة حيوانات الزوراء, والمدرسة العراقية للفروسية) في العاصمة بغداد. اظهر الفحص المجهري لعينات الدم ١١ عينة إيجابية (٦,٨٧٥%) للثايليريا الخيلية الثايليريا ، بينما أكدت نتائج "تفاعل انزيم البوليميراز المتسلسل على جين الحمض النووي الريبي S١٨" وجود ٢٦ عينة إيجابية (١٦,٢٥٪). بعد تسجيل تسلسل القطع الجينية ورسم شجرة النشوء والتطور تم الكشف عن تشابه ٩٣,٠٣-١٠٠٪ مقارنة بالعديد من عزلات دول العالم وبنسبة تغطية ٩٤-١٠٠٪. أظهر ١٦ (٦١,٥٪) حصان فقط من الخيول المصابة أعراضًا سريرية خفيفة الى تحت سريرية او حاملة للمرض مع عدم وجود اصابة بالقراد. أوضحت الفحوصات

الدموية عدم وجود فروق معنوية في فحوصات الدم باستثناء انخفاض تركيز خضاب الدم وحجم الخلايا المرصوص ، مما تسبب ظهور ١٢ حالة فقر دم ما بين ٢٦ حصان موجب بالفحص الجزيئي (٤٦,١٥٪)، ٦ خيول (٢٣,٠٨٪) أظهرت فقر دم من النوع سوي الكرية سوي الصباغ, و٣ (١١,٥٤٪) أظهرت فقر دم لكل من النوعين سوي الكرية قليل الصباغ وصغير الكرية قليل الصباغ. كانت الخيول الصغيرة بعمر(سنتان) أكثر عرضة للإصابة (نسبة الأرجحية ١٥,٤) أكثر من الخيول بعمر ست سنوات. إضافة لذلك, لم تظهر فئات الجنس أوالسلالة نتائج فارقة معنوياً لتعتبرعوامل خطورة للإصابة بالثايليريا الخيلية. بينت هذه الدراسة اصابة خيول السباق في بغداد بطفيلي الثايليريا وبالاخص الامهار, علاوة على ذلك سريرياً, أظهرت اغلب الحالات المصابة اعراضاً طفيفة الى تحت السريرية متصاحبة مع فقر دم. كشف الاختبارالجزيئي عن تشابه جيني عالي مع العزلات المسجلة حول العالم.

الكلمات المفتاحية: طفيلي , الثايليريا , خيول السباق , الدموي, الجزيئي