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Detect of the eggs of *P. equorum* in the feces of horses by traditional method and molecular techniques in Baghdad, Iraq

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Abstract

The risk of *P. equorum* infection in horses remains critical. Little studies were conducted to investigate prevalence and molecular analysis of *P. equorum* in Baghdad city, Iraq. In this study traditional detection followed by molecular technique depending on ITS-2 region were used as an attempt to evaluate this nuclear region as a genetic marker to diagnose the parasitic infection. One hundred and thirty-eight fecal samples of horses were collected and examined by direct wet mount smears, floatation method by NaCl. Extraction of genomic material, nested PCR was done followed by phylogenic analysis depending on ITS-2 region performed for the first time in Iraq and genetic substitutions to analyze Iraqi horses. Nested PCR were done after determining the total infection rate 6.52% by conventional technique, including 3.84% in males and 10% in females with significant difference. Highly infection rate 11.42% was recorded in the age group under 2 years and the lower infection rate 4% was found in the age group between 2-4 years with significant difference. The Iraqi isolates were recorded in the Gen Bank under the accession numbers MZ400507.1, MZ400508.1, MZ400509.1, and MZ4005010.1; while, phylogenetic analysis recorded an identity range between 97-100% with China, Australia and USA isolates. *P. equorum* is more distributed in younger horses than elderly in Baghdad city and ITS-2 region is a certain molecular marker for detection *P. equorum* isolates in Iraqi horses.

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Introduction

P. equorum is a member in the phylum Nematoda, a roundworm which predominantly affecting the health of foals and yearling horses and decreased in elderly with a wide geographical distribution, this parasite starts its life cycle in small intestine of the host during meet and mate between adult worms, then female adult worms produce huge number of eggs which laid in host small intestine then exit with feces or hatches to larval stage which migrate to liver and lung (1-6). Worm burdens can achieve high numbers (7). The infection is manifested clinically as nasal discharge, ill thrift (8), ceased growth, inappetence, rough hair coat, lethargy (9). In severe cases, other signs detected like pneumonia respiratory, bronchial hemorrhage, intestinal impaction, colic or even liver and lungs damage were diagnosis during migration larval phase (1). Many authors detected *P. equorum* as main species of *Parascaris* spp. eggs in feces of horse (10,11). Other study detected *P. univalens* mitochondrial genome with high sequences similarity with *P. equorum* and identical genetic maps (12). Due to scarce of data and the importance of *P. equorum* in horses, this study was designed to estimate the prevalence and molecular detection and some risk factors (sex and age) on the infection rate.

Materials and methods

Ethical approval

Samples were collected and transferred after obtaining approval from the regulations of Iraqi Ministry of Agriculture. The research protocols for the study were approved by the College of Veterinary Medicine, University of Baghdad, and were included in laboratory standards after animal utilization protocol certification No. P. G. 1904 in 2/10/2022.

Samples collection

One hundred and thirty-eight horse fecal samples were collected from different areas in Baghdad city during the period from the beginning of December till the end of October 2022. The fecal samples of 25 g were divided into two parts, the first one 10 g for fecal analysis by using direct wet mount smears and flotation method by using NaCl (13) and the other part 15 g for the molecular study.

Statistical Analysis

The Chi-square was used for assess the significant differences among factors at $P \leq 0.01$ (14).

DNA extraction

DNA extraction from *P. equorum* using G- spin DNA extraction kit (Intron, Korea) was done according to the manufacturer´s recommendation. Estimation of Genomic DNA was detected by Nanodrop spectrophotometer to estimate the concentration and its purity at absorbance 260-280 nm.

Primers

Two sets of primers were designed targeting the ITS-2 region; the first PCR run with ITS-2 first run PCR primer; PE1F5'-GCTGCGTTCTTCATC GAT-3' and PE1R5'-TTAGTTTCTTTT CT CCG CT- 3' at 400 bp PCR product depend on (15), as well as the second PCR run with ITS-2 second PCR run primer; PE2F5'-AAT GCC ATA TAT GAA ATA TAT ACG-3' and PE2R5'-TTA GTT TCT TTT CCT CCG CT-3' at 290 bp PCR product depend on (16).

PCR mix and amplification profile

The DNA was amplified by using nested PCR (first and second) reaction and for gene diagnosis a mixture of the specific interaction components volume include Master mix-Maxime Pre-Mix Kit (i-Taq), template DNA 1.5µl, 1µl forward primer (10pmo); 1µl reverse primer (10pmo) and 16.5µl Free nuclease water to maintain total PCR reaction volume 20 µl.

The optimum thermocycler condition for gene detection was run under the condition (Table 1). Agarose gel Electrophoresis 1.5% had been done to determine DNA pieces and to detect the result of PCR interaction and compare it with marked standard DNA to distinguish the bundles size (17).

Table 1: Thermocycler conditions targeting ITS-2 region for 1st and 2nd PCR reaction

Condition	1st			2nd		
	Temperature	Time	Cycle	Temperature	Time	Cycle
Initial denaturation	95ºC	5 min	1	95ºC	5 min	1
Denaturation	95ºC	30 sec		95ºC	30 sec	
Annealing	52ºC	35 sec	35	58ºC	30 sec	35
Extension	72ºC	1 min		72ºC	30 sec	
Final extension	72ºC	5 min	1	72ºC	5 min	1
Hold	4ºC	-	-	4ºC	-	-

Sequencing and phylogenic tree analysis

Approximately 290 bp Amplicons from four worm samples were sequenced using Sanger sequencing and the resulting sequences were submitted on NCBI and analyzed using MEGA6.0 (18).

Results

Morphology of parasite eggs

The morphological appearance of twenty-five eggs of the parasite from different samples revealed a spherical to sub spherical shape and their measurements were 95-120µm length and 90-110 µm width (Figure 1).

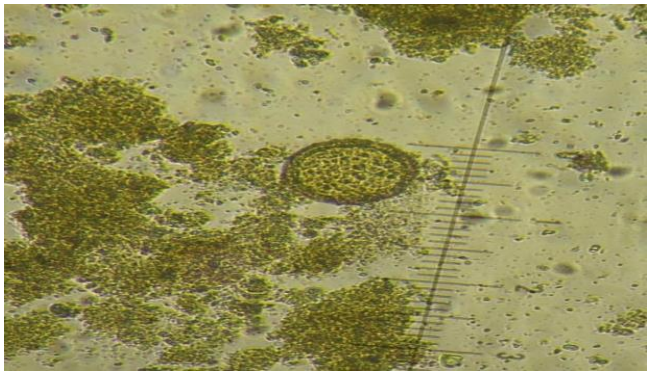

Figure 1: *P. equorum* egg by direct wet mount smears (X10).

Total infection rate

The total infection rate of *P. equorum* in horses was 6.52% (9/138) with a significant difference between males 3.84% and females 10% (Table 2). The rate of infection was the highest 11.42% in age group under 2 years, followed by the age group4 years old 5.66% and the lower infection rate 4% occurred in the age group between 2-4 years with significant difference (Table 3).

Table 2: Distribution of *P. equorum* infection according to sex of study animals

Sex	Total No.	Positive	
		No.	%
Males	78	3	3.84
Females	60	6	10.00
Total	138	9	6.52
χ^2			71.76

Table 3: Prevalence of *P. equorum* infection according to age of horses

Age (Year)	Total No.	Positive	
		No.	%
< 2	35	4	11.42
>2-4	50	2	4.00
> 4	53	3	5.66
Total	138	9	6.52
χ^2			52.39

Molecular analysis

Molecular findings detected that DNA purity and concentration were 1.8 and 300 ng/ µl respectively. Nested PCR technique was done for 10 randomly collected samples, using two primers for ITS-2 region. Results of amplification of 5 samples with 400 bp in first PCR run and 290 bp in second run were showed by electrophoresis of 1.5% agarose (Figures 2 and 3).

Four positive local isolates were sequencing and submitted in NCBI with accession numbers MZ400507.1; MZ400508.1; MZ400509.1 and MZ4005010.1 then phylogenic analysis indicated that Iraqi isolates were closely to Australia, China and USA isolates with very low genetic diversity at 0.0035 (Figure 4).

Genetic substitutions for Iraqi isolate compared with China isolate ID: MK209647.1. Results indicated transition in location 742 A/G with isolate MZ400507.1. Transition and trans-version with isolate MZ4005010 in the locations 495 C/T,501G/T,709 T/C and 719 T/C (Transition) and 550 A/C,692 G/C and 707A/T (Trans-version) while other isolates showed no genetic substitution (Table 4).

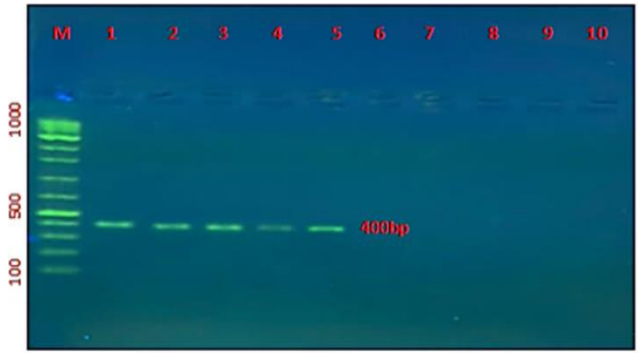

Figure 2: Nested 1st PCR reaction targeting the ITS-2 region at 400 bp. Lane (M): Ladder marker (1500-100 bp); Lanes (1-5): Positive PCR products.

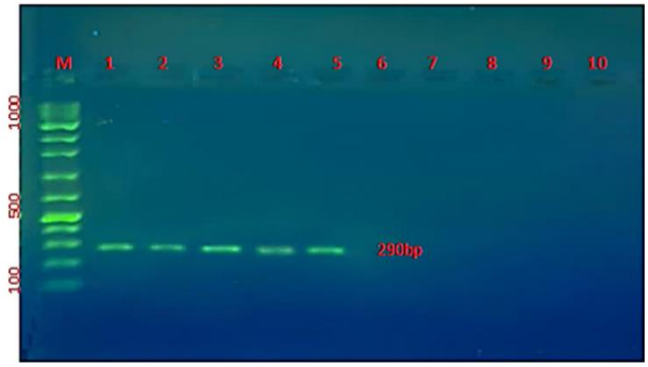

Figure 3: Nested 2nd. PCR reaction targeting the ITS-2 region at 290 bp. Lane (M): Ladder marker (1500-100 bp); Lanes (1-5): Positive PCR products.

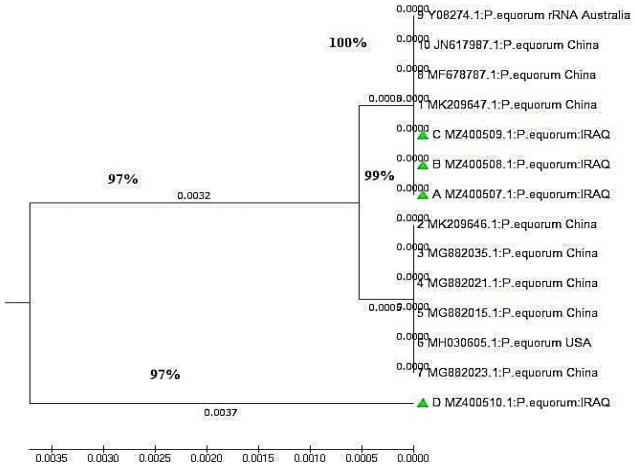

Figure 4: Phylogenetic tree analysis of local and global *P. equorum* isolates.

Table 4: *P. equorum* ITS-2 region substitutions Iraqi isolates

No.	Type	Location	Nucleotide	Sequence ID	Source	Identify
1	Transition	742	A/G	MK209647.1	*P. equorum*	99%
2	-	-	-	MK209647.1	*P. equorum*	100%
3	-	-	-	MK209647.1	*P. equorum*	100%
4	Transition	719	T/C			
	Transition	709	T/C			
	Trans-vertion	707	A/T			
	Trans-vertion	692	G/C	MK209647.1	*P. equorum*	97%
	Trans-vertion	550	A/C			
	Transition	501	G/A			
	Transition	495	C/T			

Discussion

P. equorum is a nematode roundworm of young horses with wide geographical range (19). This study describes the results of detection of the infection in horses in Baghdad city by using microscopic examination; direct wet mount smears and flotation methods of fecal samples and molecular confirmative diagnosis by Nested PCR. The results of the present study show spherical to sub spherical shape of *P. equorum* eggs and their measurements between 10^4×98.2 µm, length and width respectively. This agreed with Zajac and Conboy (20) who found that eggs of the parasite spherical in shape with diameter 90-100 µm. The total infection rate of *P. equorum* was 6.52%. This result disagreed with previous studies in some countries such as Lind and Christensson (6) who found 48% positive eggs samples for *P. equorum.* Also, Romero *et al.* (21) analyzed fecal samples by coproparasitological concentration-flotation technique of 218 horses found 11.93% were infected with *Parascaris spp*.

According to age, *P. equorum* infections occur commonly in foals, yearlings and seldom harbored in horses older than 4 years (6). Regarding gender, Nielsen *et al.* (13) reported that the equine ascarid occurred predominantly in foals and this is in agreement with the results of the present study. Due to resistant of eggs to the environmental conditions, this leads to increasing the infection rate of *P. equorum* and this agreed with Reinemeyer (9) who indicated that the survival of larvae eggs up to 10 years and the highly development of infective stage (3^{rd} larvae) within 10 days potentiate the survival of the parasite and increased the infection in the field (22). Breed with place of origin were significantly associated with helminth infection (21). Nielsen *et al.* (13) stated that no studies in naturally infected horses have detected occurrence of the two *Parascaris* species.

The molecular results of the present study indicated that ITS-2 region was good genetic marker for detecting different nematode parasites, *P. equorum* for the first time in Iraq, that agreement with other studies which detected parasites infection and manifested dependent of genetic marker on ITS region as good molecular marker (23-30), due to difficulty of diagnosis of parasite by traditional methods (30). The four sequences were fell into same cluster and aligned with other 10 sequences of ITS-2 region isolate from different countries depend on final aligned sequence which closely related taxa and only one Iraqi isolate was located outside main cluster as a highly supported sister cluster rooted in a basal position to the main group. Phylogenetic tree-maximum-likelihood was generated from final alignment sequences and showed the main four Iraqi isolate of *P. equorum* and eight isolates of *P. equorum* identified in China and one from each Australia and USA. Most isolates of Iraq connected with main clades with (97-100%) identity, coinciding with other studies (16,23,31). Many studies used phylogenic tree comparisons with molecular parasites genome revealed high genetic similarities among local isolates and the globally registered sequences (31-39). Molecular detection and Phylogeny one of identification methods used in many studies (40-43).

Comparing local ITS-2 region sequences of *P. equorum* with accession MK209647.1, results showed many genetic substitutions (transition and trans-version) which indicated that Nested-PCR technique followed by phylogenic and substitution analysis to ribosomal rDNA ITS-2 region was suggested for estimation of genetic diversity which considered the main genetic line study to detect genetic homology (23,24).

Conclusion

P. equorum occurs in a high percentage in horses at Baghdad city. ITS-2 region is a good molecular marker for diagnosis the infection of *P. equorum.* Molecular detection with nested PCR flowed by phylogenic tree analysis were very useful to detection and diagnosis parasite *P. equorum* in horses.

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Conflict of interests

The author has no conflict of interest.

References

1. Burk SV. Detection of antibodies against *P. equorum* excretory-secretory antigens (Ph.D. dissertation). UK: College of Agriculture at the University of Kentucky; 2013. https://uknowledge.uky.edu/animalsci_etds/21
2. Hautala KN, Areaho A, Kauppinen O, Nielsen MK, Sukura A, Rajala-Schultz PJ. Risk factors for equine intestinal parasite infections and reduced efficacy of pyrantelembonate against *Parascaris sp*. Vet Parasitol. 2019;273:52-59. DOI: 10.1016/j.vetpar.2019.08.004
3. Saeed MA, Beveridge I, Abbas G, Beasley A, Bauquier J, Wilkes E. Systematic review of gastrointestinal nematodes of horses from Australia. Parasites Vectors. 2019;12:188. DOI: 10.1186/s13071-019-3445-4
4. Fritzen B, Rohn K, Schnieder T, Von SG. Endoparasite control management on horse farms-lessons from worm prevalence and questionnaire data. Equine Vet J. 2010;42(1):79-83. DOI: 10.2746/042516409X471485
5. Khan A, Khan MS, Avais M, Mahmood AK, Ijaz M. Prevalence and chemotherapy of *P. equorum* in equines in Pakistan. J Equine Vet Sci. 2010;30(3):155-158. DOI: 10.1016/j.jevs.2010.01.057
6. Al-Sultan I I, Youkhana O S and Mahran M O. Study on the pathology and parasitic affection of liver in sheep and cattle at Mosul area. The Iraqi J Vete. Med. 1999; 23(1), 50–58. https://doi.org/10.30539/ijvm.v23i1.1191.
7. Nielsen MK. Evidence-based considerations for control of *Parascaris spp.* infections in horses. Equine Vet Edu. 2016;28(4):224-231. DOI: 10.1111/eve.12536
8. Lloyd S, Soulsby EJ. Is anthelmintic resistance inevitable: back to basics?. Equine Vet J. 1998;30:280-283. DOI: 10.1111/j.2042-3306.1998.tb04097.x
9. Reinemeyer CR. Diagnosis and control of anthelmintic-resistant *P. equorum*. Parasit Vectors. 2009;2(2):S2-S8. DOI: 10.1186/1756-3305-2-S2-S8
10. Armstrong SK, Woodgate RG, Gough S, Heller J, Sangster NC, Hughes KJ. The efficacy of ivermectin, pyrantel and fenbendazole against *P. equorum* infection in foals on farms in Australia. Vet Parasitol. 2014;205:575-580. DOI: 10.1016/j.vetpar.2014.08.028
11. Beasley A, Coleman G, Kotze AC. Suspected ivermectin resistance in a southeast Queensland *P. equorum* population. Aust Vet J. 2015;93:305-307. DOI: 10.1111/avj.12352
12. Jabbar A, Littlewood DT, Mohandas N, Briscoe AG, Foster PG, Müller F. The mitochondrial genome of *Parascaris univalens*-implications for a "forgotten" parasite. Parasit Vector. 2014;7:428. DOI: 10.1186/1756-3305-7-428
13. Soulsby EJ. Helminthes, arthropods, protozoa of domesticated animals.7th ed. London: Baillier Tindal; 1982.
14. SAS. Statistical analysis system, user's guide. Statistical version 9. 1st ed. USA: SAS Inst Inc; 2012.
15. Zhu X, Gasser RB, Jacobs DE, Hung GC, Chilton NB. Relationship among some ascaridoid nematodes based on ribosomal DNA sequencing data. Parasitol Res. 2000;86:738-744. DOI: 10.1007/PL00008561
16. Tydén E, Morrison DA, Engström A, Nielsen MK, Eydal M, Höglund J. Population genetics of *P. equorum* based on DNA fingerprinting. Infect Genet Evol. 2013;13:236-241. DOI: 10.1016/j.meegid.2012.09.022
17. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A Laboratory Manual. 2nd ed. USA: Cold Spring Harbar Laboratory Press; 1989.
18. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9. DOI: 10.1093/molbev/mst197
19. Hade B F, Abdulameer M, May H. Direct Amplification of B1 gene of *Toxoplasma gondii* DNA using nested polymerase chain reaction following microwave treatment for whole blood samples. The Iraqi J Vete. Med.2015; 39(1): 23-27. https://www.iasj.net/iasj/download/c0e302aa5dd643a3.
20. Faraj A A and May H K. Detection of *Sarcocystosis* in some wild birds. The Iraqi J Vete. Med. 2012; 36(2), 65–70. https://doi.org/10.30539/iraqijvm.v36i2.448.
21. Romero C, Heredia R, Miranda L, Arredondo M. Prevalence of gastrointestinal parasites in horses of central Mexico. Open J Vet Med. 2020;10(8):117-125. DOI: 10.4236/ojvm.2020.108010.
22. Premaalatha B, Kartiyayini S, Selvi V, Sohayati AR. *P. equorum* in a thoroughbred horse in Perak Turf club. Mala J Vet Res. 2018;9(2):175-177.(http://www.dvs.gov.my/dvs/resources/u.
23. Georg SH, Jana I, Janssen I, Sabrina R, Clara G, Fernando AB, Bretislav K, Artur N, Krzysztof T, Maria BS, Slawomir K, Jürgen K. Very low intraspecific sequence variation in selected nuclear and mitochondrial *Parascaris univalens* genes. Infect Genet Evol. 2021;95:105035. DOI: 10.1016/j.meegid.2021.105035
24. Hade BF, Al-Biatee ST, Al-Rubaie HM. Traditional and molecular diagnosis of H*aemonchus contortus* in sheep in Babylon province, Iraqi J Vet Sci. 2022;36(2):479-481. DOI: 10.33899/ijvs.2021.130533.1842
25. Monroy-Ostria A, Nasereddin A, Monteon M, Guzmán-Bracho C, Charles L. ITS1 PCR-RFLP diagnosis and characterization of leishmania in clinical samples and strains from cases of human cutaneous leishmaniasis in states of the Mexican southeast. Interdiscip Perspect Infect Dis. 2014;2014:607287. DOI: 10.1155/2014/607287
26. Hitakarun A, Tan-ariya P, Siripattanapipong S, Mungthin M, Piyaraj P, Naaglor T, Siriyasatien P, Tiwananthagorn S, Leelayoova S. Comparison of PCR methods for detection of *Leishmania siamensis* infection. Parasit Vectors. 2014;7:458. DOI: 10.1186/s13071-014-0458-x
27. Liu Y, Zheng G, Alsarakibi M, Zhang X, Hu W, Lu P, Lin L, Tan L, Luo Q, Li G. Molecular identification of *Ancylostoma caninum* isolated from cats in southern China based on complete ITS sequence. BioMed Res Int. 2013;2013:868050. DOI: 10.1155/2013/868050
28. Toz S, Culha G, Zeyrek F, Ertabaklar H, Alkan M, Vardarl A, Gunduz C, Ozbe Y. A real-time ITS1-PCR based method in the diagnosis and species identification of Leishmania parasite from human and dog clinical samples in Turkey. PLoS Negl Trop Dis. 2013;7(5):e2205. DOI: 10.1371/journal.pntd.0002205
29. Gasser RB, Chilton NB, Hoste H, Beveridge I. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. Nucleic Acids Res. 1993;21(10):2525-2526. DOI: 10.1093/nar/21.10.2525
30. Bowman DD. The anatomy of the third-stage larva of *Toxocara canis* and *Toxocara cati*. Toxocara Toxocariasis. 2020;39-61. DOI: 10.1016/bs.apar.2020.03.002
31. Al-Musawi AM, Abdul Hussein HA, Mansoor JA, Molecular analysis of Cryptosporidium species in domestic goat in central Iraq. Iraqi J Vet Sci. 2022;36(4):1041-1045. DOI: 10.33899/ijvs.2022.132974.2155
32. Hade BF, Al-Biatee ST, Al-Rubaie HM. Molecular detection and phylogenetic tree analysis of ß-tubulin and nad-4 genes of Haemonchus species. Biochem Cell Arch. 2021;21(1):2813-2817. DocID: https://connectjournals.com/03896.2021.21.2813.
33. Al-Rubaie M, Al-Biatee S, Al-Saffar N. Molecular diagnosis of *Haemoproteus columbaein* local domestic pigeons (*columba livia domestica*) in Baghdad city. Plant Arch. 2020;20(1):195-198. (available at)
34. Hade, B. F., A. M. Al-Amery, Z. Ibrahim and S. Saadedin. Histopathological study of different VLM Stages *Toxocara canis* infection in liver of rabbits. Iraqi J. Biotecnol. 2018: 17(2):47-56. https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/53.
35. George N, Bhandari V, Reddy P, Sharma P. Molecular and Phylogenetic analysis revealed new genotypes of *Theileria annulata* parasites from India. Parasit Vectors. 2015;8:468. DOI: 10.1186/s13071-015-1075-z
36. Hade, B. F., S. Saadedin and A. M. Al- Amery. Sequencing and phylogenic variation of ITS-2 region and *rrnL* gene in *Toxocara canis* of Iraqi isolation. Journal of Biodiversity and Environmental Sciences.

الكشف عن بيوض طفيلي إسكارس الخيول في عينات براز الخيول باستخدام الطرق التقليدية والجزيئية في مدينة بغداد، العراق

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الخلاصة

يؤدي الخمج بديدان إسكارس الخيول الى حدوث أذى في الخيول، توجد دراسات جزيئية قليلة لتحديد نسبة انتشار الطفيلي في مدينة بغداد، العراق. اعتمدت الدراسة الحالية على الطرائق التقليدية لتشخيص الطفيلي ويتبعه الكشف الجزيئي لتأكيد الاصابة اعتمادا على منطقة فاصل نسخ داخلي-٢ كمحاولة لتقييمها كعلامة وراثية لتشخيص العدوى بالطفيلي. تم جمع مائة وثمانية وثلاثين عينة براز من الخيول مع تحديد عمر وجنس الحيوانات. تم فحص عينات البراز بعمل مسحات مباشرة، طريقة التطويف بواسطة كلوريد الصوديوم. تم استخلاص المادة الوراثية وتبعه أجراء تفاعل البلمرة المتسلسل المتداخل من ثم تحديد الشجرة التطورية لتحليل عزلات الطفيلي التي تصيب الخيول ولأول مرة في العراق. تم إجراء تفاعل البلمرة المتسلسل المتداخل بعد تحديد معدل الإصابة الكلي ٦,٥٢٪ بالطرائق التقليدية، وسجلت اقل نسبة إصابة ٣,٨٤٪ في الذكور والأعلى ١٠٪ في الإناث مع اختلاف معنوي. تم تسجيل ارتفاع معدل إصابة ١١,٤٢٪ في الفئة العمرية أقل من عامين، بينما وجد أقل معدل الإصابة ٤٪ في الفئة العمرية بين ٢-٤ سنوات مع وجود فرق معنوي. تم تسجيل العزلات المحلية في بنك الجينات العالمي؛ بينما سجل تحليل الشجرة التطورية تطابق ٩٧-١٠٠٪ مع عزلات الصين وأستراليا والولايات المتحدة الأمريكية. ويعد طفيلي إسكارس الخيول الأكثر انتشارا في الخيول ذات الأعمار الصغيرة في مدينة بغداد، وتعد فاصل نسخ داخلي-٢ منطقة وراثية يمكن اعتمادها بشكل مؤكد في تشخيص الخمج بالطفيلي في الخيول.

2018. 13: 71-82. file:///C:/Users/SamaOffice/Downloads/jcoagri,+17.pdf.

37. Salloum T, Khalifeh I, Tokajian S. Detection, molecular typing and phylogenetic analysis of Leishmania isolated from cases of leishmaniasis among Syrian refugees in Lebanon. Parasite Epidemiol Control. 2016;1(2):159-168. DOI: 10.1016/j.parepi.2016.02.002
38. Verma C, Chaudhary A, Singh A. PCR-based molecular characterization, phylogenetic analysis and secondary structure of the 28S rDNA of *Thaparocleidus wallagonius* (Monogenea: Dactylogyridae) - the most primitive species of this genus from India. Biomed Inform. 2012;8(17):816-819. DOI: 10.6026/97320630008816
39. Faraj A A, Hade B F, AlAmery A M. Conventional and molecular study of Babesia spp. natural infection in dragging horses at some areas of Baghdad city, Iraq. I J Ag Sci. 2019; 50 (3):909-915. DOI: https://doi.org/10.36103/ijas.v50i3.707.
40. ShathaA W, H A Nada. Prevalence of Blastocystis hominis and Giardia lamblia parasites in patients of four regions in east – south Baghdad. The Iraqi J Vete. Med. 2011; 35 (2): 74 – 84. https://doi.org/10.30539/iraqijvm.v35i2.579.
41. Hade B F. Molecular sequencing and phylogenic analysis to virulence *nmuc-1* gene in visceral larvae migrance. I J Ag Sci. 2020; 51(3):894902. DOI: https://doi.org/10.36103/ijas.v51i3.1044.
42. Yousra A, Al-Sanjary RA. The molecular identification of diarrheagenic *Escherichia coli* (DEC) isolated from meat and meat products. Iraqi J Vet Sci. 2023;37(1):9-15. DOI: 10.33899/ijvs.2022.133244.2192
43. Ameer IA, Abdullah HB. Molecular and serological detection of *Toxoplasma gondii* in three species of wild birds of Babylon province, middle Iraq. Iraqi J Vet Sci. 2023;37(1):39-44. DOI: 10.33899/ijvs.2022.133394.2219