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Microscopic and molecular detection of *Cytauxzoon* spp. in cats in Mosul city, Iraq

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Abstract

The study aims to diagnose *Cytauxzoon* spp. in affected stray cats in Mosul. To achieve this goal, microscopic and conventional polymerase chain reaction technology was used, and the genetic analysis of *Cytauxzoon* spp. was verified. Blood samples were collected from 50 stray cats in various city areas. The infection rate of *Cytauxzoon spp.* among cats in Mosul city was 76% (38/50). Using single sequence analysis, a single *Cytauxzoon felis* sequence was discovered in Nineveh Governorate for the first time. One of these sequences was submitted to GenBank with the entry number LC775709.1. The results indicate that some of the tested samples contain DNA from the Cytauxzoon genus, based on the presence of bands at the expected 1600 bp size. The local genome sequence of *Cytauxzoon felis* Sesa LC775709.1 is highly similar to sequences found in geographically diverse regions such as Switzerland, Italy, France, and the USA. According to the study, the infection rate of cats with *Cytauxzoon* spp parasites in Mosul city was significant, reaching 76%. The results of genetic sequencing were presented for the first time. One novel isolate was discovered: the *Cytauxzoon felis* Sesa gene LC775709.1 for 18S rRNA. The phylogenetic tree was constructed, and it was discovered that the local sequence LC775709.1 is very closely related genetically to the sequences from Europe and the USA while showing some genetic differences with the sequences from Brazil and South Africa.

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Introduction

Cytauxzoonosis is an emerging tick-borne disease (1). These parasites, caused by *Cytauxzoon felis* (*Theileria felis*), are classified as Phylum Apicomplexa, Class Aconoidasida, and Family Theileriidae (2). Amblyomma americanum and Dermacentor variabilis ticks transmit the disease to both domestic and wild cats (3). Cytauxzoonosis, is a quickly progressive systemic disease with a high mortality rate, as well as mechanical restriction of blood flow across numerous organs, resulting in a shock-like state and intravascular and extravascular hemolysis due to merozoite invasion of erythrocytes (2). Moreover, there are other symptoms such as inappetence, lethargy, anorexia, weight loss, depression, dehydration, diarrhea, vomiting (4), dyspnea, tachycardia, hemolytic crisis, jaundice, recumbency, and death (5,6). Ketosis can be diagnosed clinically by observing *C. felis* in infected tissues or microscopic examination of thin blood smears stained with Wright-Giemsa for *C. felis* piroplasms. These approaches are frequently utilized but are less sensitive to detecting *C. felis* infection (7). The Polymerase Chain Reaction is an accurate technique (8-11). Creating and implementing a sensitive and specific PCR technique to detect *C. felis* in feline blood samples will aid in diagnosing feline cytopathic illness. Furthermore, this assay can study the spread of *C. felis* in domestic and wild cats (12). Several real-time or nested PCR experiments have targeted the *C. felis* 18S ribosomal RNA gene or its internal transcribed spacer 2 (ITS2) region (13). A recently designed real-time PCR test that targets the *C. felis* mitochondrial cox3 gene has

demonstrated improved diagnostic sensitivity (14). However, this test uses double-stranded DNA molecules (SYBR Green I or EvaGreen), which may lead to the detection of specific and non-specific amplification products during the PCR reaction, increasing the likelihood of false positives (15). Cytauxzoon manul has been detected in naturally infected Pallas' cats (*Otocolobus manul*) from Mongolia; an unidentified kind of Cytauxzoon has been discovered in Spanish household cats throughout Europe (16). Wild cats in Spain, Romania, Italy, and Bosnia and Herzegovina (Hodžic) have also been shown to carry the infection (17). In addition, it includes France, Italy, Portugal, and Switzerland (18). The cat is lovely animals (19-24).

Therefore, the study aimed to identify the types of Cytauxzoonosis infecting cats in Mosul using the microscopic and molecular diagnosing (PCR, sequence, and phylogenetic study) to improve disease control due to a lack of research on the feline Cytauxzoonosis parasite.

Materials methods

Ethical approval

This analysis was accepted by the Academic Committee of the Microbiology Department of the College of Veterinary Medicine, University of Mosul, in October 2023.

Animals and Blood sample collection

Blood samples were gathered from 50 stray cats randomly collected from Mosul city. The blood collection location was thoroughly sanitized with 70% ethyl alcohol to disinfect the area before inserting the needle and drawing blood from peripheral and saphenous veins using sterile syringes. Three ml of blood samples from each cat were stored in tubes containing EDTA. The specimen was then transported to the College of Veterinary Medicine, University of the Mosul, Analysis Laboratory.

Microscopically examination

A slim blood smear was produced, stained by Giemsa stain, and inspected using an optical microscopic examination. Thin blood smears were utilized to assess the morphology, and in measurements, the Ocular micrometer was used for the laboratory diagnosis of *Cytauxzoon felis* in cats (25). Parasitism ratio was calculated using the following formula:

The number of RBCs affected is divided by the total number of RBCs counted multiplied by 100.

Hematological parameters

A digital blood analyzer subjected the feline blood sample to a complete blood count.

DNA extraction

DNA was isolated from five Cytauxzoon-infected blood sampling using the DNA extraction kit from (Qiagen). To rehydrate the DNA pellet, add 100 µl of hydrate solution. Store at -20ºC until a Genomic DNA Estimation.

Polymerase chain reaction

Table 1 shows the sequences of primers used in PCR to diagnose *Cytauxzoon felis*, where specific primers are used to amplify the DNA. The second table illustrates the PCR program used to amplify the DNA of *Cytauxzoon felis* using the primers (Table 1). This program includes the steps of denaturation, primer annealing, and extension, facilitating accurate and rapid diagnosis. PCR reactive mixing was prepared in a 20 µl vessel containing 10 µl of the master mix, 1 µl of primers, four µl of template DNA, and four µl of PCR-grade water. Following the completion of the PCR with a thermocycler (Optimum 96 G Germany), the multiplication reaction was carried out using the bespoke software mentioned in table 2.

Table 1: Types of the primers and their sequence (26)

Primer	Sequence
Forward	CCTGGTTGATCCTGCCAG
Revers	CGACTTCTCCTTCCTTTAAG

Table 2: Steps of the conventional PCR scheme

Stage	ºC	Time	Cycle number
Initial denaturation	95	6 min.	1
Denaturation	95	45 sec.	
Annealing	56	1 min.	35
Extension	72	1 min.	
Final extension	72	5 min.	1

Sequencing and phylogenetic analysis

PCR amplicons were sent to Macrogen Company (South Korea) for purification and sequencing after they tested positive for *Cytauxzoon felis* using the PCR technique. The 18S rRNA sequences were subjected to multiple sequence alignment using the GenomeNet online tool. Following this, the NCBI BLAST from NCBI was used to compare the sequences with other sequences available in GenBank. With MEGA11 software, the Likelihood method on the Tamura-Nei model and bootstrap analysis with 1000 resampling (16). In addition, the constructed phylogenetic tree used the 18S rRNA gene sequence of MF536661.1 *Theileria bicornis* as an outgroup.

Statistical analysis

Chi-square tests and SPSS version 19 odds ratios were used to calculate differences in *C. felis* prevalence. Differences at $P \geq 0.05$ were considered statistically significant.

Results

Microscopic examination of infected blood revealed no substantial difference in the form or size of red blood cells, indicating a strong intracellular parasitization of merozoites. It is a limited peripheral region of red blood cells with a blue nucleus shaped like a stage or nucleus and a small amount of cytoplasm. The parasite has an average length of 1.2±3 micrometers and a width of 1.2±1 micrometer. The parasite was evaluated using its morphological phases in red blood cells (Figure 1). Table 3 shows the infection rate of *Cytauxzoon felis* among cats in Mosul city. Out of 50 cats examined, 38 (76%) were infected, while 12 (24%) were not infected (Table 3). Table 4 illustrates the intensity of *Cytauxzoon felis* infection among infected cats in Mosul city. Out of 38 infected cats, 21 (55.3%) had a chronic infection, 10 (26.3%) had a subclinical infection, and 7 (18.4%) had an acute infection (Table 4).

Table 5 shows the differences in Complete blood count values between healthy cats and cats infected with Cytauxzoonosis. The letters 'a' and 'b' indicate significant differences between the two groups, and the different values reflect the impact of the disease on various blood parameters. In this study, cats infected with *Cytauxzoon felis* showed significant differences in hematological parameters, including cell volume, platelet count, hemoglobin Hb, and total erythrocyte count (TEC), compared to the healthy group. The study found a significant rise in MCV and a significant drop in MCH and MCHC, indicating macrocytic hypochromic anemia in cats infected with *C. felis*. Furthermore, cats infected with *Cytauxzoon felis* had significantly lower total and differential leukocyte counts., resulting in a considerable drop in the percentages of lymphocytes and neutrophils but no significant difference in monocytes, eosinophils, or basophils. Compared to the healthy group (Table 5).

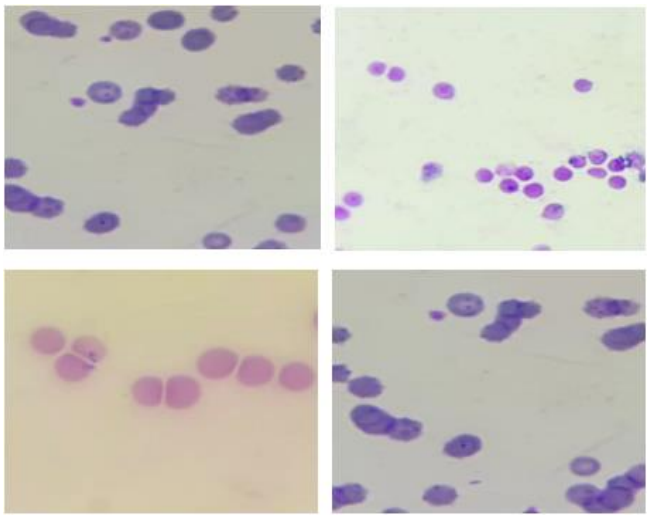

Figure 1: *Cytauxzoon felis* was discovered in thin blood smears stained with Giemsa and photographed at 100X.

Table 3: The infection rate with *Cytauxzoon felis* in cat

Type of infection	Number (total)	Infection (%)
Infected	38 (50)	76%

Table 4: The intensity of infection and percentage of *Cytauxzoon felis* in cats in Mosul city

Intensity of infection	Chronic	Subclinical	Acute
Total infected (38)	21	10	7
Percentage %	55.3	26.3	18.4

Table 5: Complete blood count associated with Cytauxzoonosis

Parameters	Range (mean±SE)	
	Healthy group	Infected Cats with Cytauxzoonosis
TRBC (10^{12}/ML)	4.5-9.44 (6.9±0.41^a)	3.1-8.00 (5.55±0.27^b)
HB (g/L)	88.00-145.00 (116.5±4.41^a)	59.00-110.00 (84.5±3.77^b)
PCV (%)	20.1-44.2 32. (15±1.72^a)	17.00-34.50 (25.75±1.11^b)
MCV (FL)	44.6– 46.82 (45.71±1.28^a)	43.12-54.8 (48.8±1.57^b)
MCH (pg)	15.4-19.5 (17.4±0.89^a)	13.75-19.0 (16.3±0.35^b)
MCHC (g/L)	328.00-437.80 (382.9±21.08^a)	318.8-347,0 (332.0±10.34^b)
PLT (10^9/L)	310.00-960.00 (635.00±40.05^a)	202.00-758.00 (480.87±31.64^b)
TWBC (10^9/ML)	11.20-24.40 (17.8±1.30^a)	6.40-16.30 (11.35±0.48^b)
LYM (%)	16.30-45.20 (30.75±2.31^a)	12.30-36.40 (24.35±1.23^b)
NEUT (%)	38.20-78.10 (58.15±2.54^a)	28.50-70.20 (49.35±1.93^b)
MON (%)	0.20-4.60 (2.4±0.32^a)	0.00-4.90 (2.45±0.31^a)
EOSI (%)	1.11-11.90 (6.5±0.89^a)	0.00-10.50 (5.25±0.51^a)
BASO (%)	0.00-1.70 (0.85±0.16^a)	0.00-1.60 (0.8±0.11^a)

Different letters in each variable mean statistically significant difference at P<0.05.

The bright bands in the figure represent amplified DNA fragments. Each band represents one amplified DNA sample from a single cat blood sample. There are five clear bands indicating the presence of *Cytauxzoon* spp. DNA in 5 blood samples. Using single sequence analysis, a single *Cytauxzoon felis* sequence was discovered in Mosul city from five cat blood samples for the first time. One of these sequences (n=1) was submitted to GenBank with the entry number LC775709.1 (Figure 2).

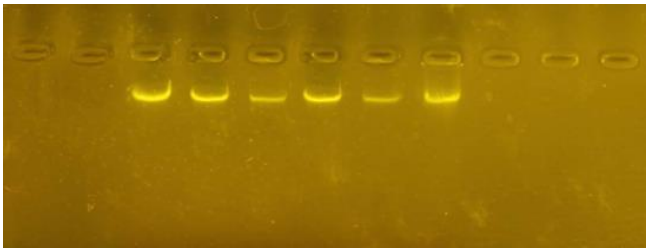

Figure 2: DNA bands extracted from *Cytauxzoon* spp.

The bands present at 1600 bp indicate that the samples contain DNA from the Cytauxzoon genus, which was confirmed by amplifying the targeted 18s rRNA region. The absence of bands in some wells (empty wells) means that those samples do not contain Cytauxzoon DNA or that the amplification process was unsuccessful for those samples. The results indicate that some of the tested samples contain DNA from the Cytauxzoon genus, based on the presence of bands at the expected 1600 bp size. This demonstrates the effectiveness of using PCR to diagnose the presence of Cytauxzoon in the tested samples (Figure 3).

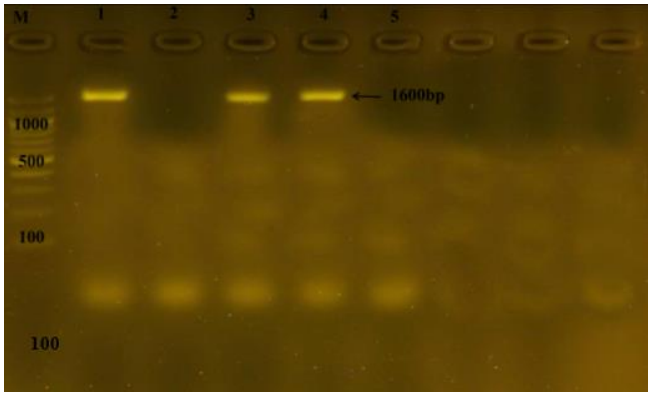

Figure 3: PCR reaction of Cytauxzoon genus based on the 18s rRNA region and amplification product of 1600 bp.

This table presents the nucleotide sequence of the ITS1 and ITS2 regions for the local *Cytauxzoon felis* isolate Sesa (GenBank accession number LC775709.1). The table presents the sequence details of *Cytauxzoon felis* a genotype discovered in an area. It displays the sequences for ITS1 and ITS2 which are utilized in research and molecular testing. The sequence consists of nucleotides essential for genetic identification and analysis. This information has been formally submitted to GenBank. Table 6 shows that the local genome sequence of *Cytauxzoon felis* Sesa (LC775709.1) is highly similar to sequences found in geographically diverse regions such as Switzerland, Italy, France, and the USA. However, there are notable genetic differences compared to sequences from Brazil and South Africa.

Table 6: Homology using BLAST n between the local sequence (LC775709.1) of *C. felis* and other genotypes

Name of strains	GenBank-NCBI No.	Query cover	Sequence Identity	Country
Cytauxzoon felis isolate 303	KU306946.1	100%	100%	Switzerland
Cytauxzoon spp. voucher 40429	OM004053.1	100%	100%	Italy
Cytauxzoon spp. cat/France 1/2008	EU622908.1	100%	99.9%	France
Cytauxzoon spp. Aliso	EF094468.1	100%	99.7%	USA
Cytauxzoon europaeus isolate WC_L68	MT904044.1	87%	99.92%	Czech Republic
Cytauxzoon spp.	GU903911.1	100%	96.2%	Brazil
Cytauxzoon felis	L19080.1	100%	96%	South Africa

Interpretation of the phylogenetic tree

The figure shows a phylogenetic tree illustrating the genetic relationships between the local sequence (LC775709.1) of the Sesa gene of *Cytauxzoon felis* from Mosul/Iraq and other sequences of the same parasite from different countries (Figure 4).

Discussion

A stained peripheral blood film revealed that 76 percent of the cats were infected with Cytauxzoon felis, as evidenced by piroplasma between red blood cells. The low identification of intracellular piroplasma during early acute-phase microscopic examination contributes to high infection rates (16). The abundance of *Amblyomma americanum* and *Dermacenter variabilis* is also important for the sample approach (27). Also, due to tick vectors' high adaptability to different species, their geographical range is projected to expand ecology and host species (28-30). An 80% prevalence of bobcats in Oklahoma (31), the positive rate 78% was comparable to that observed in domestic cats from the United States and can be quite high 79% (32).

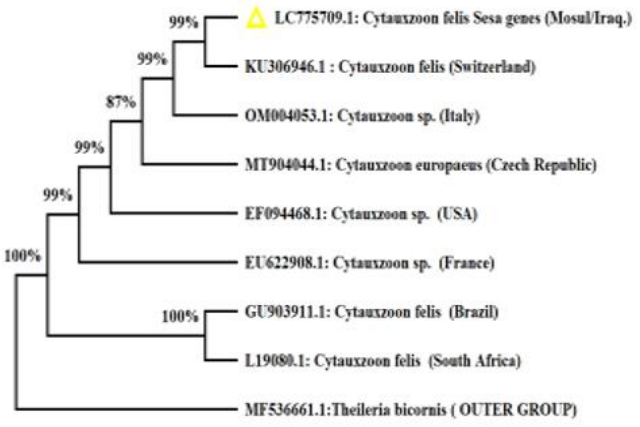

Figure 4: the partial sequence of the 18D rRNA.

Giemsa stain approach resulted in a reduced infection rate 41.5% and 33% (33-35). Other clinical trials have found modest levels of parasitemia (25-37). The current study found that cats infected *with C. felis* have hypochromic macrocytic anemia and a significant drop in PCV, platelet count, hemoglobin, and total red blood cell count, indicating anemia. This result was consistent with research conducted in Iran (38), which also demonstrated that PCV was 31.8% in lynx with spontaneous infection of *Cytauxzoon felis*

Our findings show that a considerable drop in MCH and MCHC in *C. felis*-infected cats indicates macrocytic hypochromic anemia. Iron deficiency and hemolysis may cause a relative drop in MCH and MCHC levels. MCH and MCHC were lower in the naturally infected group (39,40). The most common clinical signs in cats with hemoplasmosis include anemia (41-43).

In addition, cats infected with *C. felis* have a considerable drop in total and differential white blood cell counts. Low white blood cell counts may indicate a bad prognosis (44). Anemia, leukopenia with left shift, toxic neutrophil alterations, and thrombocytopenia are frequently identified in the late stages of the disease (45). Neutropenia and lymphocytosis were described in a mountain lion (*Puma concolor*) with Cytauxzoon-like piroplasma in its blood sample (46), a moderate reduction in red blood cells (anemia), an increase in lymphocyte counts 68% and a decrease in the number of neutrophils 14%, increased vacuolation and toxic changes in neutrophils, resulting in an increase in monocytes 12%, eosinophils 2%, and basophils 0% were in the normal range (47). Microscopic examination of Giemsa-stained blood smears must be more comprehensive to discover and identify difficult-to-classify parasite species (48).

Conclusion

The study's conclusion showed that the rate of *Cytauxzoon* spp parasite infection in cats in Mosul, Iraq, reached 76%. Genetic sequencing results revealed the identification of a new isolate, *Cytauxzoon felis* Sesa (LC775709.1) 18S rRNA gene, partial sequence. The phylogenetic tree revealed that the local sequence (LC775709.1) of the *Cytauxzoon felis* Sesa ITS1, ITS2 genotype is very closely related genetically to the sequences from Europe and the USA while showing some genetic differences with the sequences from Brazil and South Africa. This may reflect genetic branching and local adaptation of the parasite in different geographical regions.

Acknowledgments

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Conflict of interest

None.

Reference

1. Reichard MV, Van Den Bussche RA, Meinkoth JH, Hoover JP, Kocan AA. A new species of Cytauxzoon from Pallas' cat caught in Mongolia and comments on the systematic and taxonomy of piroplasmids. Parasitol. 2005;91:420-7. DOI: 10.1645/GE-384R
2. Taylar MA, Coop RL, Wall RL. Veterinary parasitology. 4th ed. USA: Wiley Blackwell; 2016. DOI: 10.1002/9781119073680
3. Davies S, Abdullah S, Helps C, Tasker S, Newbury H, Wall R. Prevalence of ticks and tick-borne pathogens: Babesia and Borrelia species in ticks infesting cats of Great Britain. Vet Parasitol. 2017;244:129-135. DOI: 10.1016/J.vetpar.2017.07.033
4. Legroux JP, Halos L, René-Martellet M, Servonnet M, Pingret JL, Bourdoiseau G. First clinical case report of Cytauxzoon infection in a domestic cat in France. BMC Vet Res. 2017;13:81. DOI: 10.1186/s12917-017-1009-4
5. Duran DM. Determination of Ownership Practices and *Cytauxzoon felis* Prevalence in Domestic Felines of Northwest Arkansas [Bachelor of Science thesis]. USA: University of Arkansas, College of Agricultural, Food & Life Sciences, Animal Science Undergraduate Honors Thesis; 2021. [available at]
6. Al-Abdaly YZ, Alfathi MY, Al-Mahmood SS. Comparison of azithromycin toxicity in chickens and quails. Iran J Vet Med. 2023;17(4):321-32. DOI: 10.32598/IJVM.17.4.1005354
7. Kier A, Wagner J, Kinden D. The pathology of experimental Cytauxzoonosis. J Comp Pathol. 1987;97:415-432. DOI: 10.1016/0021-9975(87)90020-X
8. Sinjari D, Abdullah S, Jubrael J, Musa V. Morphological and molecular characterization of postharvest Fungal pathogen (*Penicillium chrysogenum*) in pomegranate fruits (*Punica granatum* L.) in Duhok Provence–Iraq. Mesopotamia J Agric. 2024;52(3):106-117. DOI: 10.33899/mja.2024.148152.1400
9. Ami SN, Guri RS. Existence, molecular identification and genetic variation of new isolates of seed gall nematode parasitizing on wheat a barely in Iraq. Mesopotamia J Agric. 2023;51(1):32-47. DOI: 10.33899/magrj.2023.1350991197
10. Ami SN, Taher IE. Survey, races identification and host range of wheat seed gall nematode *Anguina tritici* in Duhok province, Kurdistan region– Iraq. J Agric Vet Sci. 2014;7:44-48. [available at]
11. Alshatty HR, Alkero AN. The use of solar sterilization of soil prepared for forest seedling and its effect in inhibiting the growth of soil fungi and molecular diagnostics by using PCR technique fungi. Mesopotamia J Agric. 2024;52(1):170-181. DOI: 10.33899/mja.2024.145974.1345

12. Birkenheuer AJ, Marr H, Alleman AR, Levy MG, Breitschwerdt EB. Development and evaluation of a PCR assay for the detection of *Cytauxzoon felis* DNA in feline blood samples. Vet Parasitol. 2006;137:144-149. DOI: 10.1016/j.vetpar.2005.12.007
13. Brown HM, Lockhart JM, Latimer KS, Peterson DS. Identification and genetic characterization of *Cytauxzoon felis* in asymptomatic domestic cats and bobcats. Vet Parasitol. 2010;172:311-316. DOI: 10.1016/j.vetpar.2010.05.001
14. Schreeg ME, Marr HS, Tarigo JL, Cohn LA, Levy MG, Birkenheuer AJ. Rapid high resolution melt analysis of *Cytauxzoon felis* cytochrome b to aid in the prognosis of cytauxzoonosis. J Clin Microbiol. 2015;53(8):25172524. DOI: 10.1128/JCM.00635-15
15. Nentwig A, Meli ML, Schrack J, Reichler IM, Riond B, Gloor C, Willi B. First report of *Cytauxzoon sp.* infection in domestic cats in Switzerland: Natural and transfusion-transmitted infections. Parasit Vectors. 2018;11(1):1-13.DOI: 10.1186/s13071-018-2728-5
16. Díaz-Regañón D, Villaescusa A, Ayllón T, Rodríguez-Franco F, García-Sancho M, Agulla B. Epidemiological study of hemotropic mycoplasmas (hemoplasmas) in cats from central Spain. Parasit Vectors. 2018;11(1):140. DOI: 10.1186/s13071-018-2740-9
17. Sherrill MK, Cohn LA. Cytauxzoonosis: Diagnosis and treatment of an emerging disease. J Feline Med Surg. 2015;17:940-8. DOI: 10.1177/1098612X15610681
18. Zenad MM, Radha AM. Clinical, serological, and antigenic study of feline panleukopenia virus in cats in Baghdad, Iraq. Iraqi J Vet Sci. 2020;34(2):435-439. DOI: 10.33899/ijvs.2019.125960.1201
19. Hamdan AK, Al-Baroodi SY. Molecular investigation of feline calicivirus in cats in Mosul city, Iraq. Iraqi J Vet Sci. 1993;36(3):731-735. DOI: 10.33899/ijvs.2022.131706.1993
20. Rashid ZM, Aziz SA, Ali OJ, Kakarash NA, Marif HF. Coprological detection of Toxocariasis in domicile and stray dogs and cats in Sulaimani province, Iraq. Iraqi J Vet Sci. 2022;36(4):1047-1051. DOI: 10.33899/ijvs.2022.132976.2157
21. Elseddawy FD, Beher AE, Hendy EA, Ezzeldein SA. Dental disorders in dogs and cats: A retrospective study. Iraqi J Vet Sci. 2023;37(1):245-251. DOI: 10.33899/ijvs.2022.133750.2289
22. Ali NA, Ali MJ. Molecular detection of *Cryptosporidium spp.* in stray cats in Al-Qadisiyah governorate, Iraq. Iraqi J Vet Sci. 2023;37(2):369-373. DOI: 10.33899/ijvs.2022.133893.2317
23. Abbas LA, Rady AM. Isolation and molecular detection of *Staphylococcus aureus* from feline otitis in Baghdad. Iraqi J Vet Sci. 2023;37(III):141-146. DOI: 10.33899/ijvs.2023.137556.2696
24. Rad NH, Ghaemi P, Shayan Eckert B, Shirazi NS. Detection of native carrier infected with *Theileria annulata* by semi-nested PCR and smear method in Golestan province of Iran. World App Sci J. 2011;12(3):317-323. [available at]
25. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022-3027. [available at]
26. Weerarathne P, Sanders TL, Kao YF, Cotey SR, Place JD, Fairbanks WS, Miller CA, Reichard MV. High prevalence of *Cytauxzoon felis* in bobcats (*Lynx rufus*) across Oklahoma and occurrence in West Texas, USA. J Wildl Dis. 2023;59(3):432-441. DOI: 10.7589/JWD-D-22-00152
27. Rizzi TE, Reichard MV, Cohn LA, Birkenheuer AJ, Taylor JD, Meinkoth JH. Prevalence of *Cytauxzoon felis* infection in healthy cats from enzootic areas in Arkansas, Missouri, and Oklahoma. Parasit Vectors. 2015;8:13. DOI: 10.1186/s13071-014-0618-z
28. Shock BC, Birkenheuer AJ, Patton LL, Olfenbuttel C, Beringer J, Grove DM, Peek M, Butfiloski JW, Hughes DW, Lockhart JM, Cunningham MW, Brown HM, Peterson DS, Yabsley MJ. Variation in the ITS-1 and ITS-2 rRNA genomic regions of *Cytauxzoon felis* from bobcats and pumas in the eastern United States and comparison with sequences from domestic cats. Vet Parasitol. 2012;190:29-35. DOI: 10.1016/j.vetpar.2012.06.010
29. Panait LC, Stock G, Globokar M, Balzer J, Groth B, Mihalca AD, Pantchev N. The first case of feline Cytauxzoonosis in Germany: Clinical description and molecular confirmation. Res Sq. 2020. DOI: 10.21203/rs.2.21005/v1
30. Luaces I, Aguirre E, García-Montijano M, Velarde J, Tesouro MA, Sánchez C. First report of an intraerythrocytic small piroplasm in wild *Iberian lynx* (*Lynx pardinus*). J Wildl Dis. 2005;41:810-5. DOI: 10.7589/0090-3558-41.4.810
31. Carli E, Trotta M, Bianchi E, Furlanello T, Caldin M, Pietrobelli M. Cytauxzoon infection in two free-ranging young cats: Clinicopathological findings ,therapy and follow up. Turk Parazitol Derg. 2014;38:185-9. DOI: 10.5152/tpd.2014.3540
32. Rassouli M, Sabouri S, Goudarzi A, Parsa M. *Cytauxzoon felis* in a stray cat in Iran. Comp Clin Pathol. 2013:75-7. DOI: 10.1007/s00580-013-1858-6
33. Reichard MV, Sanders TL, Weerarathne P, Meinkoth JH, Miller CA, Scimeca RC. Cytauxzoonosis in North America. Pathogens. 2021;10:1170. DOI: 10.3390/pathogens10091170
34. Rotstein DS, Taylor SK, Harvey JW, Bean J. Hematologic Effects of Cytauxzoonosis in Florida Panthers and Texas Cougars in Florida. J Wildl Dis. 1999;35(3):613-617. DOI: 10.7589/0090-3558-35.3.613
35. Do T, Kamyingkird K, Chimnoi P, Inpankaew T. Evaluation of hematological alteration of vector-borne pathogens in cats from Bangkok, Thailand. BMC Vet Res. 2021;17:28. DOI: 10.1186/s12917-020-02737-1
36. Díaz-Regañón D, Villaescusa A, Ayllón T, Rodríguez-Franco F, Baneth G, Calleja-Bueno L, Sainz Á. Molecular detection of *Hepatozoon spp.* and *Cytauxzoon sp.* in domestic and stray cats from Madrid, Spain. Parasit Vectors. 2017;10:1-9. DOI: 10.1186/s13071-017-2056-1
37. Raimundo JM, Guimaraes A, Botelho CF, Peixoto MP, Pires MS, Machado CH. Hematological changes associated with hemoplasma infection in cats in Rio de Janeiro, Brazil. Rev Bras Parasitol Vet. 2016;25(4):441-449. DOI: 10.1590/s1984-29612016086
38. Spada E, Proverbio D, Galluzzo P, Della Pepa A, Bagnagatti De Giorgi G, Perego R. Prevalence of haemoplasma infections in stray cats in northern Italy. ISRN Microbiol. 2014;298352. DOI: 10.1155/2014/298352
39. Cohn LA, Birkenheuer AJ, Brunker JD. Efficacy of atovaquone and azithromycin or imidocarb dipropionate in cats with acute cytauxzoonosis. J Vet Intern Med. 2010;25:55-60. DOI: 10.1111/j.1939-1676.2010.0646
40. Meinkoth JH, Kocan AA. Feline cytauxzoonosis. Vet Clin North Am Small Anim Pract. 2005;35(1):89-101. DOI: 10.1016/j.cvsm.2004.08.003
41. Soares JR, Souza AI, Netto NT. *Cytauxzoon felis*-like in mountain lion (*Puma concolor*): A case report. J Anim Vet Adv. 2004;3(12):820-823. [available at]
42. Pulford DJ, Gias E, Bueno IM, McFadden A. Developing high throughput quantitative PCR assays for diagnosing Ikeda and other *Theileria orientalis* types common to New Zealand in bovine blood samples. N Z Vet J. 2016;64(1):29-37. DOI: 10.1080/00480169.2015.1089798
43. Abdelmonem GJ, Amer AM, Hussein EA, Aboezz ZR, Habashi AR, Sharawi SS. Assessment of multiplex PCR for detection of FMDV, BVDV, BTV, and possible coinfection with *Pasteurella multocida* in cattle. Iraqi J Vet Sci. 2022;36(4):1053-9. DOI: 10.33899/ijvs.2022.132983.2158
44. Sayhood MH, Mohammed AL, Abdulhameed MF, Jori MM. Classical and molecular identification of *Staphylococcus aureus* isolated from infestation cattle wounds with myiasis in Basrah governorate, Iraq. Iraqi J Vet Sci. 2022;36(3):641-646. DOI: 10.33899/ijvs.2021.131196.1930
45. Brown HM, Berghaus RD, Latimer KS, Britt JO, Rakich PM, Peterson DS. Genetic variability of *Cytauxzoon felis* from 88 infected domestic cats in Arkansas and Georgia. J Vet Diagn Invest. 2009;21:59-63. DOI: 10.1177/104063870902100109
46. Wang JL, Li TT, Liu GH, Zhu XQ, Yao C. Two tales of *Cytauxzoon felis* infections in domestic cats. Clin Microbiol Rev. 2017;30:861-885. DOI: 10.1128/CMR.00010-17
47. Shock BC, Murphy SM, Patton LL, Shock PM, Olfenbuttel C, Beringer J, Prange S, Grove DM, Peek M, Butfiloski JW, Hughes DW, Lockhart JM, Bevins SN, Vande Woude S, Crooks KR, Nettles VF, Brown HM,

الكشف المجهري والجزئي عن طفيلي سايتاوكسزون في القطط في مدينة الموصل، العراق

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الخلاصة

هدفت الدراسة الى التشخيص المجهري والجزيئي لطفيلي سايتاوكسزون في القطط السائبة المصابة في مدينة الموصل. ولتحقيق هذا الهدف، تم استخدام التقنيات المجهرية تقنية تفاعل السلسلة المتبلمرة المعتادة، فضلا عن التحقق من التحليل الجيني الى سايتاوكسزون تم جمع عينات الدم من ٥٠ قطة سائبة من مناطق متنوعة من مدينة الموصل. بلغ معدل الخمج بـالطفيلي بين قطط مدينة الموصل ٧٦% حيث كانت ٣٨ قطة خمجة. باستخدام تحليل التسلسل الفردي، تم اكتشاف تسلسل واحد لسايتوزوون فيليس في محافظة نينوى لأول مرة، وتم تقديم أحد هذه التسلسلات إلى بنك الجينات تحت رقم الإدخال LC775709.1 وتشير النتائج إلى أن بعض العينات المختبرة تحتوي على الحامض النووي الرايبوزي منقوص الأوكسجين من جنس سيتوكسون، استنادا إلى وجود نطاقات في الحجم المتوقع ١٦٠٠ قاعدة نيوكليوتيدية. تُظهر تسلسل الجينوم المحلي للستوكسون فيليسSesa LC775709.1-تشابها عاليا مع التسلسلات الموجودة في مناطق جغرافية متنوعة مثل سويسرا وإيطاليا وفرنسا والولايات المتحدة. من خلال الدراسة، يظهر أن معدل إصابة القطط بالطفيلي كان مهما، حيث بلغ ٧٦% في الموصل. تم عرض نتائج التسلسل الجيني لأول مرة، وتم اكتشاف عزلة جديدة واحدة: جين سايتوكسون فيليس. تم بناء شجرة تطورية وتم اكتشاف أن التسلسل المحلي LC775709.1 مرتبط جينيا بشكل كبير مع التسلسلات من أوروبا والولايات المتحدة، مع وجود بعض الاختلافات الجينية مع التسلسلات من البرازيل وجنوب أفريقيا.

Peterson DS, Yabsley MJ. Distribution and prevalence of *Cytauxzoon felis* in bobcats (*Lynx rufus*), the natural reservoir, and other wild felids in thirteen states. Vet Parasitol. 2011;175: 325-330. DOI: 10.1016/j.vetpar.2010.10.009

48. Dantas-Torres F, Otranto D. Dogs, cats, parasites, and humans in Brazil: Opening the black box. Parasit Vectors. 2014;7:22. DOI: 10.1186/1756-3305-7-22