Research Article

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Molecular and Genetic Diversity of *Ovine hemotropic* Mycoplasma in Nineveh, Iraq

Sufyan Safar AL Shamo, S.A. Esmaeel.

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul-Iraq.

Corresponding Author Email Address: Sufyan.23vmp22@student.uomosul.edu.iq

ORCID ID: https://orcid.org/0009-0001-7683-2562

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Abstract

*Ovine hemotropic Mycoplasma (Ohm)*are zoonotic pathogens that are emerging and are responsible for severe hemolytic anemia and substantial financial losses. The purpose of this study was to use the conventional polymerase chain reaction (C-PCR) method to molecularly detect *Ovine hemotropic Mycoplasma spp.* in infected sheep in Nineveh Province, Iraq. Additionally, the phylogenetic analysis of *Ohm spp.* diagnosed in this study was investigated. Sheep from various regions of Nineveh province were collected to provide a total of 241 blood samples (3 ml). Results revealed that the infection rates of *Mycoplasma ovis and Candidatus Mycoplasma haematovis* were 52.6% and 23.2%, respectively, using C-PCR with a primer specific to the species. Four 16S rRNA gene sequences were extracted from sheep blood for individual sequencing analysis. The NCBI GenBank contains the sequences of *Ohm* under the accession numbers PV273224 and PV273225 *(Mycoplasma ovis*) and PV273226 and PV273227 (*Candidatus Mycoplasma haematovis*). These sequences were identical (100%) to those associated with *Mycoplasma ovis* found in the following databases: NCBI GenBank (GU230142 and AF338268 in the United States), MH379799 in Brazil, MF377458 in Turkey, JF931138 in Japan, MW547439 in Poland, ON202709 in Germany, and EU828582 in Switzerland. Additionally, OQ310852 was identified in Egypt as *Candidatus Mycoplasma haematovis*. *Mycoplasma ovis* and *Candidatus M. haemovis*, two ovine hemotropic mycoplasmas, were first detected in sheep in the Nineveh province in this study. It was observed that they are ubiquitous, and this discovery may prove advantageous for future research and strategic management of this mycoplasma species in the study region

Key words: Ovine hemotropic Mycoplasma, PCR, Genetic Diversity, Nineveh, Iraq.

Introduction

Hemoplasmas, although never cultivated in vitro, are diminutive epierythrocytic microorganisms that, like other mycoplasmas, lack a cell wall and exhibit heightened susceptibility to tetracyclines. Animals can develop hemolytic anemia as a result of infections; however, the lack of appropriate diagnostic techniques has hindered veterinary research. Although the majority of research uses cytological identification on blood smears to check for these organisms, this method has poor diagnostic sensitivity and can't tell distinct species apart (1). Furthermore, because hemoplasmas and Howell-Jolly bodies contain DNA, are often seen after splenectomy, and are associated with anemia, this diagnostic technique may mistakenly identify the hemoplasmas as the latter. There currently exist only two hemoplasma species known to exist in sheep (Ovis aries): *Mycoplasma ovis* (formerly Eperythrozoon ovis) was categorized (2) and the "*Candidatus Mycoplasma haemovis*" was studied by (3,4) The earliest known *Ovine hemotropic Mycoplasma* organisms in small ruminants were *Candidatus Mycoplasma haemovis and Mycoplasma ovis*, which was formerly known as *Eperythrozoon ovis* (5). The identity of the strain or species represented by these hemoplasmas remains unknown. There is some doubt around the identification of *M. ovis* strain Michigan due to the fact that its complete genome sequence contains two sets of 16S rRNA genes that are identical to those of *M. ovis and "Ca. M. haemovis*" (6). Many different clinical presentations, including asymptomatic courses, moderate hemoglobin deficiency, poor performance, reproductive abnormalities, or even fatal anemia, can result from hemoplasma infections. Before molecular research reclassifies the organism as a *Mycoplasma*, it is vital to include the hemoparasite *Mycoplasma ovis* -formerly known as *Eperythrozoon ovis* and classified as a Rickettsia in the Anaplasmataceae family in the differential diagnosis of anemia in small ruminants (2). Global populations of small ruminants, humans, and reindeer are infected with the pathogen *Mycoplasma ovis* (7). Infections caused by dirty sharp objects and bites from blood-sucking insects that are drawn to open wounds are the ways that field studies show *Mycoplasma ovis* spreads. Severe hemolytic anemia and mortality are common outcomes of acute mycoplasmosis in young animals. Factors like age, nutrition, immune system strength, gender, and other infections can influence how serious mild anemia and other symptoms are in animals with chronic *Mycoplasma ovis* infections (7). In areas where sheep production is high, *hemotropic Mycoplasma ovis* is often assumed to be endemic, even though there is a lack of data on the disease's prevalence and social and economic impacts (8). It's noteworthy that more and more cases of hemoprotozoa infections in humans are being reported, especially in immunocompromised patients, pregnant women, and those who frequently interact with animals and arthropods. Furthermore, relying solely on microscopic examination

of Giemsa-stained smears of blood remains diagnostically challenging, even though *M. ovis* often disappears in animals with severe anemia. There are not enough reliable published studies on whole genome and phylogenetic tree analysis, despite the growing importance of PCR diagnostics. Finally, the potential transboundary spread of *Mycoplasma ovis* and other parasites, germs, and viruses from infected small ruminants to uninfected regions is a threat to the expanding global trade in these animals (9). It is appropriate to review the information currently available on *Mycoplasma ovis* as an illness that affects small ruminants and potentially other kinds of animals. It is being considered a newly discovered pathogen that deserves more study. Since the disease is linked to other blood diseases in sheep, including trypanosomiasis, babesiosis, and theileriosis, clinical signs are rarely useful in diagnosing it (10,11). Therefore, three studies were conducted in Mosul by blood smears, Basrah Governorate using ELISA, and in the city of Diwaniyah using PCR in southern and central Iraq (12,13,14). No research has been done on northern Iraq, more especially the Nineveh province. Consequently, the purpose of the present research was to examine the phylogenetic analysis of *Mycoplasma ovis and Candidatus Mycoplasma haemovis*, both of which were identified in the present study. Additionally, for the first time ever, in Nineveh Province, Iraq, the C-PCR technique was used to identify *ovine hemotropic mycoplasma* species in sheep.

Material and Methods

Ethical approval

Permission to conduct the study was granted by the Institutional Animal Care and Use Committee of the University of Mosul's College of Veterinary Medicine on July 9, 2024 (UM.VET.2024.04).

Animals and collections of samples

The study included 241 sheep, consisting of both males and females, with ages ranging from 1 to over 3 years, different breeds, and management approaches. These sheep were clinically suspected of being infected with *ovine hemotropic Mycoplasma spp.* 241 sheep had their blood drawn from the jugular vein between July 2024 and January 2025; the samples were then preserved in tubes containing the anticoagulant ethylenediamine acetic acid (EDTA). Before being tested using the C-PCR method, the tubes were kept at -20°C (15,16,17).

DNA extraction for C-PCR technique

The DNA from 241 sheep blood samples was extracted using the AddPrep DNA Genome Extraction Kit (Add Bio, Korea) as directed by the manufacturer. The concentration of the extracted DNA varied between 80.9 and 370.5 ng/µl, as determined by a Nanophotometer (BioDrop, Germany). The A260/A280 nm ratio, which ranged from 1.7 to 1.9, was used to measure the DNA purity (18,19).

Amplification of DNA

The objective is to enhance the C-PCR amplification of the ovine hemotropic mycoplasma sixteen-small subunit rRNA gene, which is highly conserved. The DNA

sample from a sheep that showed positive results for *ovine hemotropic mycoplasma* in both clinical and laboratory tests was used as a reference sample. Aside from DNA, the negative control included all PCR-competent samples (except DNA) and DNA isolated from healthy samples. Hampel *et al.* (20) generated the oligonucleotides of the primers that were prescribed. In order to improve the ovine hemotropic mycoplasma 16S rRNA gene, primers were donated by Macrogen Inc. of South Korea. A C-PCR reaction was performed with particular primers (F 5′ ACG AAA GTC TGA TGG AGC AAT A 3′ and R 5′ ACG CCC AAT AAA TCC GRA TAA T 3′) to identify hemoplasma species in sheep who tested positive. *Mycoplasma ovis* has a band size of 193 base pairs, and *Ca. Mycoplasma haemovis* has a band size of 176 base pairs. The following components were needed for the traditional PCR procedure, which called for a volume of 25 µl: The required components for the traditional PCR procedure were eight and a half microliters of PCR-grade water, twelve and a half microliters of 2X AddBio Master Mix, ten micromoles of each primer (H16S-F and H16S-R), two microliters of DNA (150 ng/L), and one microliter of each primer. Another group served as a control; they also had all the necessary components, but the template DNA was omitted. Here is the setup of the thermocycler (BIO-RAD/USA): When the temperature is 95 degrees Celsius, the polymerase activation phase lasts for 10 minutes, according to Hampel *et al*. (20). After that, there will be a 45-second annealing phase at 55°C, a 1-minute extension phase at 72°C, and a 45-second denaturation phase at 95°C. The procedure goes on for 35 cycles, with the last extension phase lasting five minutes at 72°C. Three microliters of GelRed dye and 1.5% agarose (AddBio, Korea) were used to separate the amplification outputs. Each PCR product was added to the agarose gel in five microliters. The electrophoresis was carried out at 75 V for one hour using a genotyping container (Bio-Rad, United States of America) and a 300-mA power supply with a cycle TBE buffer (GeNetBio, Korea). The hundred base-pair DNA marker (6 µL) was derived from GeneDirex H3 in Korea and functioned similarly to the standard molecular mass biomarker.

Sequencing of DNA

From sheep blood samples that tested positive for *Mycoplasma ovis and Candidatus Mycoplasma haematovis* using the C-PCR technique, four PCR fragments were sent to the Macrogen Company (South Korea) to be purified and sequenced. An online software program called CLUSTALW GenomeNet was used to align the 16S rRNA sequences before they were compared with other mycoplasma sequences in GenBank. Following that, we used NCBI BLAST (BLASTn) from http://www.ncbi.nlm.nih.gov to compare the findings. Using MEGA12 software and bootstrap analysis with 1000 resamplings, neighbor-joining on the Tamura-Nei model was conducted (21). And using its 16s rRNA gene sequence, the phylogenetic tree used *Mycoplasma bovoculi* (GenBank: NR122008) as the outgroup.

Statistical Analysis

The data used for this study were analyzed using the Chi-square test in IBM-SPSS Version 22 (Inc., Chicago, USA). If the P value was less than 0.05, the data was deemed to have statistical significance.

Results

Depending on C-PCR results, the overall prevalence of *ovine hemotropic mycoplasma* was 75.9% (*Mycoplasma ovis* was 52.6% (127 of 241), with positive bands at about 193 bp) and (the incidence of *Candidatus Mycoplasma haematovis* was 23.2% (56 of 241), with positive bands at about 176bp) (Figure 1, Table 1).

Four 16S rRNA gene sequences were subjected to individual sequencing analysis (BLASTn) in this study. One of these sequences was taken from sheep blood. The *ovine hemotropic Mycoplasma* sequences under accession numbers PV273224, PV273225 (Mycoplasma ovis), and PV273226, PV273227 (*Candidatus Mycoplasma haematovis*) are accessible in the NCBI GenBank (Table2). These sequences were 100% identical to those found in the NCBI GenBank, including (GU230142.1, AF338268.1) in the USA, (MH379799.1) in Brazil, (MF377458.1) in Turkey, (JF931138.1) in Japan, (MW547439.1) in Poland, (ON202709.1) in Germany, and (EU828582.1) in Switzerland with *Mycoplasma ovis*, and (OQ310852.1) in Egypt with *Candidatus Mycoplasma haematovis.* (Table 3,4). Furthermore, the MEGA12 program's neighbor-joining phylogenetic tree analysis proved that Ca. M. haemovis and M. ovis native sequences were 100% similar to the GenBank sequences of the same species. As an outgroup, *Mycoplasma bovoculi* (GenBank: NR122008) was used to root the tree (Figure 2).

Table 1: The prevalence of the ovine heamotropic Mycoplasma spp. type in sheep in the Nineveh region (n=241) was determined using the C-PCR technique.

Type of pathogen	C-PCR method Positive number	Percentage (%)
Mycoplasma ovis	127	52.6 [a]
Candidatus Mycoplasma haematovis	56	23.2 [b]
Total	183	75.9

Different superscript letters (a, b) were used to indicate values that showed significant differences (P < 0.05).

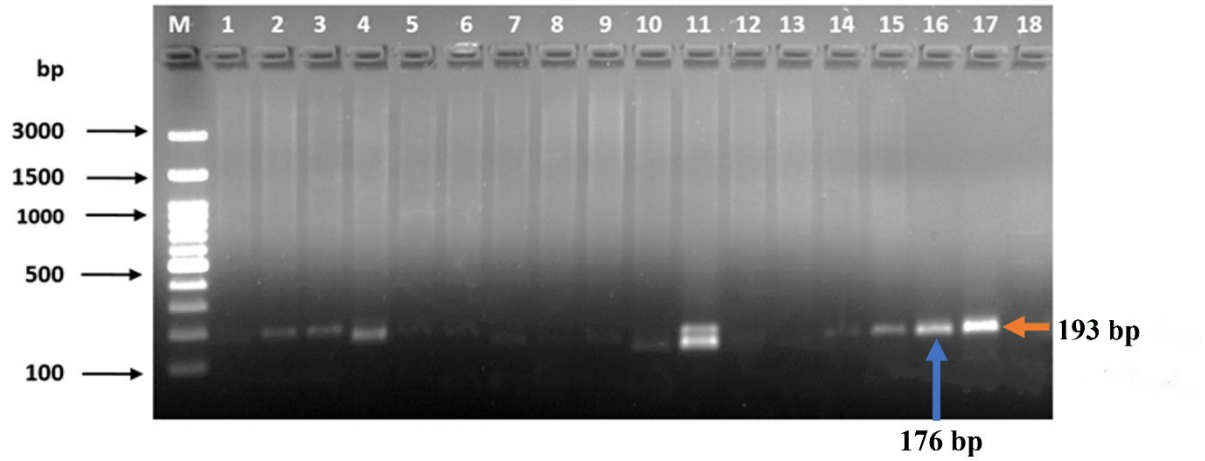

Figure 1: PCR of sheep blood samples for *Mycoplasma spp.* 16SrRNA gene with HO primer produced 176 bp for *Ca. M. haematovis* and 193 for *M. ovis*. Lane M: 100-bp DNA ladder. Lanes 2, 3, 9, 11, and 17 are 193 bp positives. Lanes 4, 7,10, 11, 14,15,16 are 176 bp positives. Lanes 1,5,6,8,12,13 are negative. Lane 18 control-negative.

Discussion

The use of C-PCR in conjunction with phylogenetic analysis to detect infections in sheep from various parts of Nineveh Province with the species "*Mycoplasma ovis, Candidatus M. haematovis*" has never been done before. The overall prevalence in this study was 75.9%. In the Nineveh province, the incidence rate of *Mycoplasma ovis* was 52.6%, and *Candidatus M. haematovis* was 23.2% when C-PCR was used. In previous studies in Iraq Using Microscopic examination of blood-stained smears, (12) noticed that 40% of sheep in Mosul, Iraq, had an infection. Using C-PCR (14) observed a 25.5% the rate in sheep in Al-Diwaniyah, Iraq, while (13) found that the prevalence of *Ovine hemotropic mycoplasma (Ohm)* in Basrah, South Iraq, was 100% based on ELISA and stained blood smears viewed under a microscope in 2017, According to (13), there are a number of possible explanations for the regional variation in sheep infection rates of *Ovine hemotropic mycoplasma.*

Table 2: Genomic DNA for ovine hemotropic mycoplasma isolates that were entered into the gene bank, including the 16S ribosomal RNA gene sequence.

Accession No. of 16S rRNA gene	Pathogen	Local Strain
PV273224	*Mycoplasma ovis*	Mycoplasma ovis isolate SSM1
PV273225		Mycoplasma ovis isolate SSM2
PV273226	*Candidatus Mycoplasma haematovis*	Candidatus Mycoplasma haematovis isolate SSM3
PV273227		Candidatus Mycoplasma haematovis isolate SSM4

Table 3: Using NCBI, compare the genomes of local Mycoplasma ovis strains to those in GenBank BLASTn that have the same disease.

Name of isolate	Accession no.	Name of gene	Country name	Percent identity
Mycoplasma ovis	GU230142.1	16SrRNA partial gene	USA	100%
	MH379799.1	16SrRNA partial gene	Brazil	100%
	AF338268.1	16SrRNA partial gene	USA	100%
	MF377458.1	16SrRNA partial gene	Turkey	100%
	JF931138.1	16SrRNA partial gene	Japan	100%
	MW547439.1	16SrRNA partial gene	Poland	100%
	EU828582.1	16SrRNA partial gene	Switzerland	100%
Uncultured Mycoplasma sp.	ON202709.1	16SrRNA partial gene	Germany	100%
Candidatus Mycoplasma haemocervae	KF306249.1	16SrRNA partial gene	Japan	99%

Table 4: According to NCBI, there is a high degree of similarity between the initial Ca. M. haemovis genotypes and additional sequences of the same infection that can be obtained in GenBank.

Name of isolate	Accession no.	Name of gene	Country name	Percent identity
Uncultured Mycoplasma sp. clone Haemovis1	OQ310852.1	16S rRNA partial gene	Egypt	100%
Mycoplasma ovis	EU165509.1	16S rRNA partial gene	Switzerland	99%
Uncultured Mycoplasma sp.	OP860306.1	16S rRNA partial gene	Sweden	99%
Mycoplasma ovis	MF377460.1	16S rRNA partial gene	Turkey	99%

These include differences in breeding practices, testing protocols, the prevalence of tick carriers, the quantity of specimens collected, and environmental factors that influence tick populations (22) Additional global research has demonstrated that varying experimental approaches reveal varying levels of *hemoplasma spp*. Prevalence in sheep is similar to other livestock HM species (7) Instances include Tunisia 6% (23), the Philippines 36% (24), Argentina 81% (25), Hungary 52%(3), Turkey 54% (26), USA 69-79% (8), Brazil 79% (27), Malaysia 50.7% (28), Japan 50% (29) and China 45% (30). The prevalence of *Hemoplasma species* can differ from continent to continent depending on some factors, including ecology, management practices, diagnostic effectiveness, tick prevention strategy effectiveness, and the presence of efficient tick vectors (30,31,32).

After 1000 generations, the evolutionary tree of local ovine hemotropic mycoplasma sequences was created using the bootstrap technique and the neighbor-joining method based on the Tamura-Nei model in the MEGA12 program (21) revealed that it shares common phylogenetic traits and an exceptionally tight progressive relationship with the remaining DNA sequences of *ovine hemotropic mycoplasma* documented in the GenBank database of the NCBI for different nations, including the USA. (2, 33), Brazil (27), Turkey (26), Japan (29, 34), Poland (35), Germany (36), Switzerland (3), Sweden (37), and Egypt (38), with the 100% Identity, the reason for this may be attributed to the local adaptation of mycoplasma and genetic branching in different parts of the world as contributing factors to this (39).

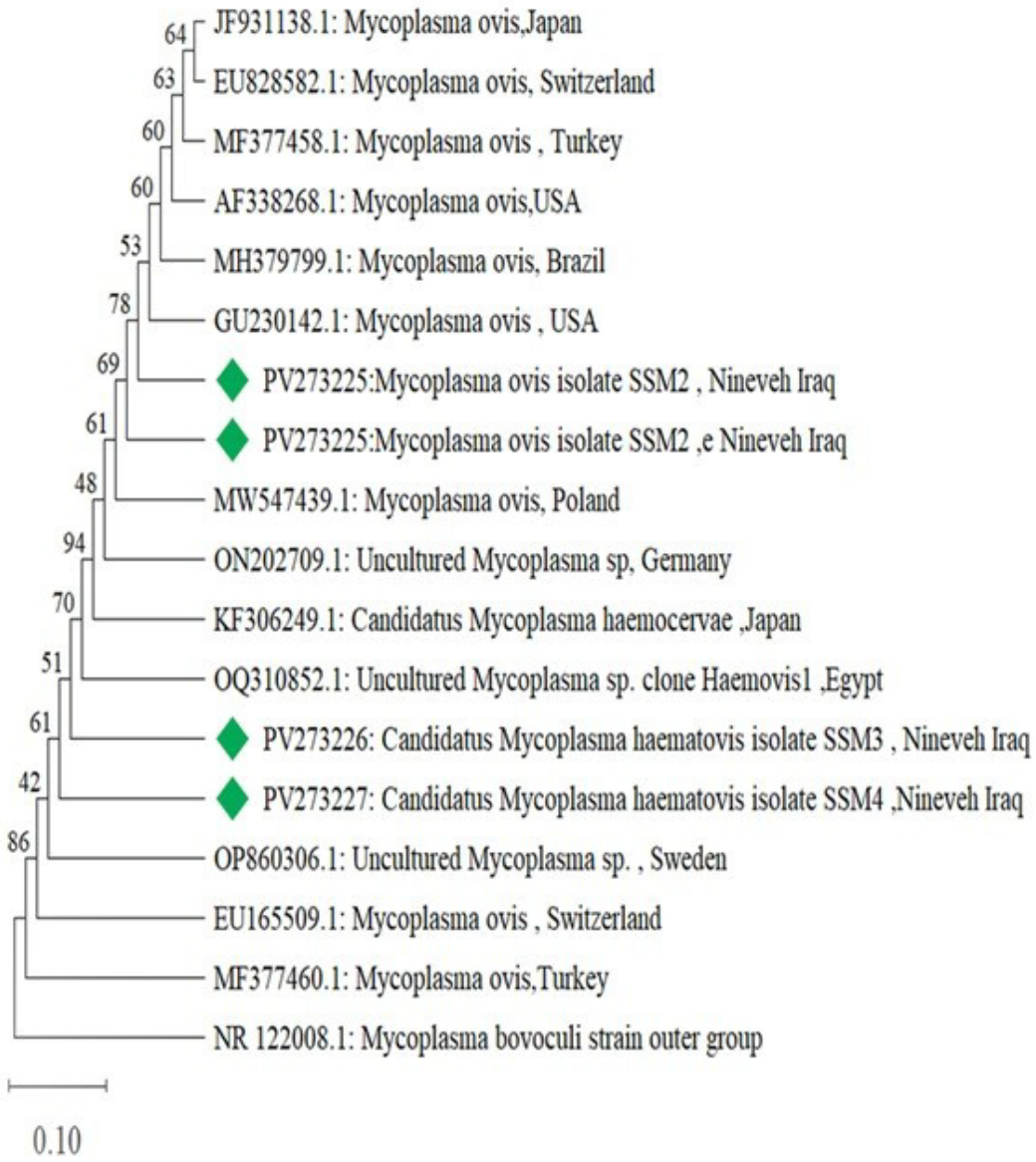

Fig. (2): The neighbor-joining 16S rRNA gene phylogenetic tree of *Mycoplasma species* recovered for this investigation is shown here. Branch numbers indicate bootstrap support (1000 replicates). Outgroup: *Mycoplasma bovoculi* (GenBank: NR122008). Diamond ( ) indicate *Mycoplasma ovis and Candidatus Mycoplasma haemovis* sequences.

Conclusions

We need more research to assess the pathogenicity and transmission of sheep hemoplasmas in the New World. Extensive research is required to confirm its presence in other parts of Iraq. Based on phylogenetic studies, the four patterns of *Candidatus Mycoplasma haematovis* and *M. ovis* that were produced for this work clustered with the *M. ovis and Candidatus Mycoplasma haematovis* 16S rRNA sequences that were available in GenBank. Iraq has little data on cases of *Mycoplasma ovis* and *Candidatus*

Mycoplasma haematovis infections, and nothing is known about how these illnesses affect the country's economy or the health of animals. This study found that within sheep populations, *Candidatus Mycoplasma haematovis and Mycoplasma ovis* are common. Whether this infection is present alone or in conjunction with other diseases, it nevertheless causes substantial anemia and economic losses for sheep breeders. This finding should raise serious concerns.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

References

1- Messick, J.B. (2004). *Hemotrophic mycoplasmas* (hemoplasmas): a review and new insights into pathogenic potential. *Veterinary Clinical Pathology*, *33*(1), 2-13. https://doi.org/10.1111/j.1939-165x.2004.tb00342.x

2- Neimark, H., Hoff, B. and Ganter, M. (2004). *Mycoplasma ovis* comb. Nov. (formerly *Eperythrozoon ovis)*, an epierythrocytic agent of haemolytic anaemia in sheep and goats. *International journal of systematic and evolutionary microbiology*, *54*(2), 365-371. https://doi.org/10.1099/ijs.0.02858-

3- Hornok, S., Meli, M.L., Erdős, A., Hajtós, I., Lutz, H. and Hofmann-Lehmann, R. (2009). Molecular characterization of two different strains of haemotropic mycoplasmas from a sheep flock with fatal haemolytic anaemia and concomitant *Anaplasma ovis* infection. *Veterinary Microbiology*, *136*(3-4), 372-377. https://doi.org/10.1016/j.vetmic.2008.10.031.

4- Suzuki, J., Sasaoka, F., Fujihara, M., Watanabe, Y., Tasaki, T., Oda, S., Kobayashi, S., Sato, R., Nagai, K. and Harasawa, R. (2011). Molecular identification of *Candidatus Mycoplasma haemovis'* in sheep with hemolytic anemia. *Journal of Veterinary Medical Science, 73*(8), 1113-1115. https://doi.org/10.1292/jvms.11-0113

5- Hornok, S., Hajtós, I., Meli, M., Farkas, I., Gönczi, E., Meili, T. and Hofmann-Lehmann, R. (2012). First molecular identification of *Mycoplasma ovis and 'Candidatus M. haemoovis'* from goat, with lack of haemoplasma PCR-positivity in lice. *Acta Veterinaria Hungarica*,*60*(3), 355-360. https://doi.org/10.1556/avet.2012.030.

6- Deshuillers, P.L., Santos, A.P., do Nascimento, N.C., Hampel, J.A., Bergin, I.L., Dyson, M.C. and Messick, J.B. (2014). Complete genome sequence of *Mycoplasma ovis* strain Michigan, a hemoplasma of sheep with two distinct 16S rRNA genes. *Genome*

Announcements, *2*(1),10-1128. https://doi.org/10.1128/genomeA.01235-13.

7- Paul, B.T., Jesse, F.F.A., Chung, E.L.T., Che-Amat, A., Mohd Lila, M.A., Hashi, H.A. and Norsidin, M.J. (2020). Review of clinical aspects, epidemiology and diagnosis of *haemotropic Mycoplasma ovis* in small ruminants: Current status and future perspectives in tropics focusing on Malaysia. *Tropical animal health and production*, 52, 2829-2844. https://doi.org/10.1007/s11250-020-02357-9.

8- Urie, N.J., Highland, M.A., Knowles, D.P., Branan, M.A., Herndon, D.R. and Marshall, K.L. (2019). *Mycoplasma ovis* infection in domestic sheep (Ovis aries) in the United States: Prevalence, distribution, associated risk factors, and associated outcomes. *Preventive veterinary medicine*, *171*, 104750. https://doi.org/10.1016/j.prevetmed.2019.104750.

9- Windsor, P.A., Nampanya, S., Tagger, A., Keonam, K., Gerasimova, M., Putthana, V., Bush, R.D. and Khounsy, S. (2017). Is orf infection a risk to expanding goat production in developing countries? A study from Lao PDR. *Small Ruminant Research*, *154,* 123-128. https://doi.org/10.1016/j.smallrumres.2017.08.003.

10- Costa, R.V., Abreu, A.P.M., Thomé, S.M., Massard, C.L., Santos, H.A., Ubiali, D.G. and Brito, M.F. (2020). Parasitological and clinical-pathological findings in twelve outbreaks of acute trypanosomiasis in dairy cattle in Rio de Janeiro state, *Brazil. Veterinary Parasitology: Regional Studies and Reports,* *22*, 100466. https://doi.org/10.1016/j.vprsr.2020.100466.

11- Windsor, P.A. (2022). Anaemia in lambs caused by *Mycoplasma ovis*: global and Australian perspectives. *Animals*, 12(11), 1372. https://doi.org/10.3390/ani12111372.

12- Hassan SD. (2021). Prevalence of border disease virus in sheep and goats in Mosul, Iraq. *Iraqi Journal of Veterinary Sciences. 35*(2): 257-262. http://www.doi.org/10.33899/ijvs.2020.126758.1372.

13- Abed, F.A., Alsaad, K.M. (2017). Clinical, hematological and diagnostic studies of hemomycoplasma infection (*Mycoplasma ovis*) in sheep of Basrah Governorate. *Basrah Journal of Veterinary Research,* *16*(2), 284-301. http://dx.doi.org/10.33762/bvetr.2017.143551.

14- Kshash, Q.H. (2017). Molecular detection of haemotropic mycoplasma infection in sheep. *Kufa Journal for Veterinary Medical Sciences, 8*(1), 120-129. http://dx.doi.org/10.36326/kjvs/2017/v8i14317.

15- Aghwan, S.S., Hussein, E.S., Esmaeel, S.A. (2025). Microscopic and molecular detection of *Cytauxzoon spp*. in cats in Mosul city, Iraq. *Iraqi Journal of Veterinary Sciences*, *39*(1), 135-141. http://dx.doi.org/10.33899/ijvs.2024.151520.3759.

16- Abdulazeez, A., Esmaeel, S. (2024). Molecular Detection of Bovine Herpes Virus-1 Among Cattle in Mosul City, Iraq.

Bulgarian Journal of Veterinary Medicine, *27*(2), 190-195. http://dx.doi.org/10.15547/bjvm.2022-0047.

17- Sheet, O.H., Hussien, S.A., Alchalaby, A.Y. (2021). Detection of methicillin-resistant *Staphylococcus aureus* from broiler carcasses in Mosul city. *Iraqi Journal of Veterinary Sciences*, 35(3), 2021 (489-493) http://dx.doi.org/10.33899/ijvs.2020.127052.1451.

18- Abd-Esmaeel, S., Albadrani, B.A. (2019). *Mycoplasma wenyonii*: a causative agent of new mastitis in dairy cows. *Advances in Animal and Veterinary Sciences*,*7*(6), 480-483. http://dx.doi.org/10.17582/journal.aavs/2019/7.6.480.483.

19- Al-Obaidii, W.A., Al-Obaidi, Q.T., Hasan, S.D. (2021). Detection of Trichomoniasis in cattle in Nineveh province. *Iraqi Journal of Veterinary Sciences*, *35*(2): 287-290. http://dx.doi.org/10.33899/ijvs.2020.126790.1380.

20- Hampel, J.A., Spath, S.N., Bergin, I.L., Lim, A., Bolin, S.R. and Dyson, M.C. (2014). Prevalence and diagnosis of hemotrophic mycoplasma infection in research sheep and its effects on hematology variables and erythrocyte membrane fragility. *Comparative Medicine, 64*(6),478-485.

21- Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., and Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution*, *41*(12), 263. https://doi.org/10.1093/molbev/msae263.

22- Abdullah, D.A., Ali, M.S., Omer, S.G., Ola-Fadunsin, S.D., Ali, F.F. and Gimba, F.I. (2019). Prevalence and climatic influence on hemoparasites of cattle and sheep in Mosul, Iraq. *Journal of Advanced Veterinary and Animal Research,* *6*(4), 492. https://doi.org/10.5455/javar.2019.f373.

23- Rjeibi, M.R., Darghouth, M.A., Omri, H., Souidi, K., Gharbi, M. and Rekik, M. (2015). First molecular isolation of Mycoplasma ovis from small ruminants in North Africa. *Onderstepoort Journal of Veterinary Research,* *82*(1), 1-5. https://doi.org/10.4102/ojvr.v82i1.912.

24- Galon, E.M.S., Moumouni, P.F.A., Ybanez, R.H.D., Macalanda, A.M.C., Liu, M., Efstratiou, A., Ringo, A.E., Lee, S.H., Gao, Y., Guo, H. and Li, J. (2019). Molecular evidence of *hemotropic mycoplasmas* in goats from Cebu, Philippines. *Journal of Veterinary Medical Science,* *81*(6), 869-873. https://doi.org/10.1292/jvms.19-0042.

25- Aguirre, D.H., Thompson, C., Neumann, R.D., Salatin, A.O., Gaido, A.B. and de Echaide, S.T. (2009). Clinical mycoplasmosis outbreak due to *Mycoplasma ovis* in sheep from Shalta, Argentina. Clinical, microbiological, and molecular diagnosis. *Revista Argentina de Microbiología*, *41*(4), 212-214.

26-Aktas, M., Ozubek, S. (2017). A molecular survey of small ruminant hemotropic mycoplasmosis in Turkey, including first laboratory confirmed clinical cases caused by *Mycoplasma ovis*.

Veterinary Microbiology, 208, 217-222. https://doi.org/10.1016/j.vetmic.2017.08.011 .

27- Souza, U.A., Oberrather, K., Fagundes-Moreira, R., Almeida, B.A.D., Valle, S.D.F., Girotto-Soares, A. and Soares, J.F. (2019). First molecular detection of *Mycoplasma ovis* (Hemotropic mycoplasmas) from Sheep in Brazil. *Revista Brasileira de Parasitologia Veterinária*, *28*, 360-366. https://doi.org/10.1590/s1984-29612019022.

28- Paul, B.T., Jesse, F.F.A., Lim Teik Chung, E., Che-Amat, A. and Mohd-Azmi, M.L. (2021). Prevalence and risk factors of *Haemotropic Mycoplasma ovis* infection in selected smallholder sheep and goat flocks in Malaysia. *The Thai Journal of Veterinary Medicine*, *51*(2), 259-266. https://doi.org/10.56808/2985-1130.3117.

29- Tagawa, M., Takeuchi, T., Fujisawa, T., Konno, Y., Yamamoto, S., Matsumoto, K., Yokoyama, N. and Inokuma, H. (2012). A Clinical Case of Severe Anemia in a Sheep Coinfected with *Mycoplasma ovis and Candidatus Mycoplasma haemovis'* in Hokkaido, Japan. *Journal of Veterinary Medical Science, 74*(1), 99-102. https://doi.org/10.1292/jvms.11-0296.

30- Wang, X., Cui, Y., Zhang, Y., Shi, K., Yan, Y., Jian, F., Zhang, L., Wang, R. and Ning, C. (2017). Molecular characterization of hemotropic mycoplasmas (*Mycoplasma ovis and 'Candidatus Mycoplasma haemovis*') in sheep and goats in China. *BMC Veterinary Research*, *13*, 1-8. https://doi.org/10.1186/s12917-017-1062-z.

31- Walker, A.R. (2003). Ticks of domestic animals in Africa: a guide to identification of species (Vol. 74). Edinburgh: *Bioscience Reports.*

32- Estrada-Peña, A.J.R.S.T. (2015). Ticks as vectors: taxonomy, biology and ecology. *Revue scientifique et technique (International Office of Epizootics)*, *34*(1), 53-65. https://doi.org/10.20506/rst.34.1.2345 .

33- Sykes, J.E., Lindsay, L.L., Maggi, R.G. and Breitschwerdt, E.B. (2010). Human coinfection with Bartonella henselae and two *hemotropic mycoplasma* variants resembling *Mycoplasma ovis. Journal of Clinical Microbiology,* 48(10), 3782-3785. https://doi.org/10.1128/JCM.01029-10.

34- Tagawa, M., Matsumoto, K., Yokoyama, N. and Inokuma, H. (2014). Prevalence and molecular analyses of *hemotrophic Mycoplasma spp*.(hemoplasmas) detected in sika deer (Cervus nippon yesoensis) in Japan. *Journal of veterinary medical science*, *76*(3), 401-407. https://doi.org/10.1292/jvms.13-0486.

35- Gałęcki, R., Jaroszewski, J., Bakuła, T., Galon, E.M., Xuan, X. (2021). Molecular detection of selected pathogens with zoonotic potential in deer keds (Lipoptena fortisetosa). *Pathogens, 10*(3), 324. https://doi.org/10.3390/pathogens10030324.

36- Unterköfler, M.S., Harl, J., Barogh, B.S., Spergser, J., Hrazdilová, K., Müller, F., Jeschke, D., Anders, O., Steinbach, P., Ansorge, H. and Fuehrer, H.P. (2022). Molecular analysis of blood-associated pathogens in European wildcats (Felis silvestris silvestris) from Germany. International Journal for Parasitology: *Parasites and Wildlife*, 19, 128-137.

https://doi.org/10.1016/j.ijppaw.2022.08.012 .

37- Persson Waller, K., Dahlgren, K., Grandi, G., Holding, M.L., Näslund, K., Omazic, A., Sprong, H., Ullman, K. and Leijon, M. (2023). A disease outbreak in beef cattle associated with Anaplasma and Mycoplasma infections. *Animals, 13*(2), 286. https://doi.org/10.3390/ani13020286.

38- Eissa, S.I., Hassan, A.M., Mohamed, Y.H., Ouda Ahmed, S.E.S. (2024). Molecular detection and characterization of haemoplasmas in different animal species in Egypt. *Egyptian Journal of Veterinary Sciences*, *55*(3), 851-861. https://doi.org/10.21608/ejvs.2023.245264.1658.

39-Benedetti, F., Curreli, S., Zella, D. (2020). Mycoplasmas–host interaction: mechanisms of inflammation and association with cellular transformation. *Microorganisms*, *8*(9), 1351. https://doi.org/10.3390/microorganisms8091351.

التنوع الجزيئي والوراثي للميكوبلازما الدموية في الأغنام في نينوى، العراق

سفيان سفر ال شمو,سلام عبد إسماعيل

فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل- العراق.

الخلاصة

المايكوبلازما الدمية في الأغنام هي احدى المسببات المرضية حيوانية المنشأ تؤدي إلى فقر دم انحلالي شديد وخسائر مالية كبيرة الهدف من هذه الدراسة الكشف الجزيئي للمايكوبلازما الدمية في الاغنام المصابة حيث كانت هذه الدراسةالأولى في محافظة نينوى، العراق باستخدام تقنية تفاعل البلمرة المتسلسل التقليدي، وللتحقق من تحليل الشجرة الجينية للمايكوبلازما الدمية في الاغنام المشخصة في هذه الدراسة. تم جمع 241 عينة دم (3 مل) من الاغنام من مناطق مختلفة من محافظة نينوى. وأظهرت النتائج أن معدل الإصابة *بالمايكوبلازما اوفيس* كان 52.6٪ والكانديديدتس مايكوبلازما هيمواوفيس كان 23.2٪ باستخدام تفاعل البلمرة المتسلسل التقليدي باستخدام بادئ خاص بالنوع. خضعت التسلسلات الجينية للتحليل الفردي (أربعة تسلسلات المايكوبلازما الدمية في الاغنام من الجين 16S rRNA والتي شملت أربعة عينات مستخلصة من الاغنام ، تم تسجيلها في بنك الجينات المركز الوطني لمعلومات التكنولوجيا الحيوية بأرقام تسلسلية(PV273224 PV273225,للمايكوبلازما اوفيس)و(PV273227, PV273226 للكانديديدتس مايكوبلازما هيمواوفيس) و كانت هذه التسلسلات مطابقة للغاية (100٪) لتلك التسلسلات المسجلة في بنك جينات NCBI مثل GU230142.1 ، AF338268.1 في الولايات المتحدة الأمريكية، (MH379799.1)في البرازيل، (MF377458.1)في تركيا، (JF931138.1)في اليابان، (MW547439.1)في بولندا، (ON202709.1)في ألمانيا و (EU828582.1) سويسرا على التوالي مع المايكوبلازما اوفيس، و (OQ310852.1) في مصر مع الكانديديدتس مايكوبلازما هيمواوفيس. بينت هذه الدراسة عن تسجيل أولى الإصابات بماكوبلازما الدم الغنمية (اوفيس وهيمواوفيس في الأغنام محافظة نينوى. وقد لوحظ شيوع هذه الأنواع وانتشارها، وقد يُفيد هذا الاكتشاف الدراسات المستقبلية والمكافحة الاستراتيجية لهذه الأنواع من الميكوبلازما في منطقة الدراسة.

الكلمات المفتاحية: المايكوبلازما الدمية الضانية، تقنات تفاعل البلمرة المتسلسل التقليدي،التنوع الجيني, نينوى- العراق.