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Molecular detection and phylogenetic analysis of *Mycoplasma bovis* in cattle in Nineveh governorate, Iraq

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Abstract

This study targeted to determine the prevalence of *Mycoplasma bovis* in cattle in Nineveh Governorate, Iraq, based on three genes, including *16S rRNA*, *uvr*C, and *gap*A using polymerase chain reaction (PCR) techniques, and to investigate the phylogenetic analysis of *M. bovis* diagnose in the study. Various samples, including 352 blood samples, 352 nasal swabs, 40 ocular swabs, 65 synovial fluid, and 30 milk samples, were randomly obtained from 352 cattle. Based on the amplified *16S rRNA* gene, the prevalence of *Mycoplasma* spp was 38.63% in cattle using the c-PCR technique. At the same time, there was no significant in the prevalence of *M. bovis* in cattle based on amplified *uvr*C and *gap*A genes, which were 30.68% and 28.69%, respectively, using the m-PCR technique. No significant difference was found between the types of samples for detecting *M. bovis*. The phylogenetic analysis for ten local sequences of the *uvr*C (5 sequence) and *gap*A (5 sequence) that were deposited in the NCBI GenBank under the accession numbers OR784598.1-OR784602.1 and OR792211.1-OR792215.1, with highly related 99.13-100% identity and 99.81% identity, respectively, to the other sequences that registered in the GenBank from different countries, including Canada, Egypt, Iran, Poland, and Switzerland. This study concludes that *M. bovis* is widespread in Nineveh Governorate, Iraq. This first study highlights the phylogenetic analysis of *M. bovis* in Nineveh Governorate, Iraq.

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Introduction

Mycoplasma bovis is a bacteria classified as belonging to the family Mycoplasmataceae, and it is characterized by a small genome, a lack of a cell wall, and high nutritional requirements for in vitro growth (1). *Mycoplasma bovis* has been isolated for the first time from a case of epidemic mastitis in cows in 1961 in the state of California in America (2). In terms of risk, it is classified in List B, which infects cows and calves (3). It is highly adapted in ruminants, especially cattle and even humans, who have continuous contact with infected cows (4). *Mycoplasma bovis* can be spread between animals in herds either directly or indirectly; direct transmission between cows can occur during milking or through nose-to-nose contact, but indirect transmission can also occur through shared drinking and feeding troughs (5). Furthermore, it spread from calves suffering from respiratory illnesses and cows with clinical mastitis, suggesting that the pathogen may spread from dairy cows to their young through contaminated milk (6). Several infectious diseases in cattle, including bovine mycoplasmosis, are caused by *M. bovis* in calves and cows (6,7). These diseases include vasculitis and keratoconjunctivitis (8), otitis media and decupital abscesses (9), endocarditis, which has been documented (10), chronic bronchopneumonia, polyarthritis, contagious mastitis (11), subclinical mastitis (12), and abortion and genital problems (13). Depending on the severity of clinical signs, the disease's morbidity rate might range from 20 to 80%, or its mortality rate could range from 3 to 50% as a result of persistent

chronic pneumonia and secondary bacterial infections (14). *Mycoplasma bovis* is globally spread, especially in North America, Australia, Europe, and Asia (15). It was detected in cows and calves infected with pneumonia, polyarthritis, and mastitis in Nineveh Governorate, Iraq, using an indirect enzyme-linked immunosorbent assay (i-ELISA) (16). Moreover, using the PCR technique, Hamad *et al*. (17) diagnosed *M. bovis* in calves infected with pneumonia in Mosul City, Iraq. There are different genes of *M. bovis* that have been used for the detection of *M. bovis*, such as *uvrC* and *gap*A (18,19), *16S rRNA*, *oppD*, and *oppF* genes (20), *gyrA*, *gyrB*, and *parC* genes (21,22), *polC* and *16S-23S rRNA ITS* genes (23,24), *ma-mp81* and *mb-mp81* genes (25,26), and *gltX* gene (27). Moreover, Okella *et al*. (28) stated that *uvrC* is the most commonly used gene, followed by *oppD* and *16S-23S rRNA ITS* gene. Numerous laboratory methods have been used to detect *M. bovis* in infected cattle, such as isolating bacteria and elector microscopy (7). Additional serological tests are employed, including indirect ELISA (29), immunohistochemistry (IHC) (23), and the polymerase chain reaction (PCR) technique (30). Due to the problematic processing, which was time-consuming, and the identification of Mycoplasma at the genus level only using the culture method, further cross-reactivity of *M. bovis* with other pathogens was observed in the serological tests (28). Therefore, PCR techniques are the most commonly used for the specific and rapid detection of *M. bovis* (31,32).

This study aimed to determine the prevalence of *M. bovis* in cattle using PCR techniques and, for the first time, investigate the phylogenetic analysis of *M. bovis* in Nineveh governorate, Iraq.

Materials and methods

Ethical approval

The institutional animal care and use committee in the College of Veterinary Medicine, University of Mosul, ethically permitted this study (UM.VET. 2022.085) on February 15, 2022.

Animals and sampling collection

This study was conducted on 352 cattle of different ages, sexes, origins, and regions of Nineveh Governorate. During the period from March 2022 to February 2023, various samples, including blood sample (n=352), nasal swab (n=352), ocular swabs (n=40), synovial fluid (n=65), and milk sample (n=30), were randomly collected from 352 cattle (14,30,33-35). These samples were kept at -20°C until performed for the conventional PCR (c-PCR) and Multiplex PCR (m-PCR) techniques to detect Mycoplasma spp. and *M. bovis*, respectively.

DNA extraction for *Mycoplasma* detection

The DNA of *Mycoplasma spp.* was extracted from all the above samples using the commercial PrimePrep Genomic DNA Extraction Kit from tissues (GeNetBio, Korea). The DNA was extracted according to the manufacturer's instructions. Using the Nanodrop (BioDrop, Germany), the concentration of extracted DNA was estimated at wavelength 260nm, while the purity of extracted DNA was assessed by calculating the ratio of (A260 nm to A280 nm) as described by Morais *et al*. (36).

Amplification of the *Mycoplasma* DNA

The c-PCR technique was used to amplify the 16S ribosomal RNA (*16S rRNA*) gene of Mycoplasma spp., in cattle's nasal swabs using ‘universal’ primers; M.Genus-F (5’-GGG AGC AAA CAC GAT AGA TAC CCT-3’) and M.Genus-R (5’-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3’), that was designed by Botes *et al.* (37). In addition, the m-PCR technique was used to amplify the deoxyribodipyrimidine photolyase (*uvrC*) gene and glyceraldehyde-3-phosphate dehydrogenase GAPDH (*gap*A) gene of *Mycoplasma bovis* in cattle cultured nasal swabs that positive in the c-PCR technique, also the nasal swab samples negative to the c-PCR technique**.** using specific primers: uvr-F(5’-TTA CGC AAG AGA ATG CTT CA-3’), uvr-R (5’-TAG GAA AGC ACC CTA TTG AT-3’), that was designed by Subramaniam *et al*. (38) and gap-F (5’-ATA GGA GGA TCC AAA AGA GTC GCT ATC AAT GGT TTT GGA CG-3’), gap-R (5’-GGA AAT GGT ACC TTA CTT AGT TAG TTT AGC AAA GTA TGT TAA TG-3’), that was designed by Perez-Casal and Prysliak, (39), respectively. All these primers were provided by Macrogen Inc., South Korea. The positive bands were at approximately 270bp, 1626bp, and 1007bp. For *16S rRNA*, *uvr*C, and *gap*A genes, respectively. Furthermore, a clinically and laboratory-positive cow's DNA was used as a positive control, while all PCR components except DNA were used as a negative control.

The PCR reactions for detecting the Mycoplasma spp. and *M. bovis* were performed in a total volume of 25µl for each one. Furthermore, the thermocycler was set with some modifications in steps according to Botes *et al*. (37), Subramaniam *et al*. (38), and Perez-Casal and Prysliak (39) (Table 1). The Midori green-stained (Axon Scientific Sdn Bhd, Malaysia) and 1.5% agarose gel were used to electrophorese the PCR products and to visualize the resultant bands, UV transillumination (BIO-RAD/USA) was utilized.

Sequencing and phylogenetic analysis of *Mycoplasma bovis* DNA

The DNA PCR products (n=15) for the *uvr*C gene out of 108 positive samples of *M. bovis* in cattle from various samples, including blood sample (n=3), nasal swab (n=3), ocular swabs (n=3), synovial fluid (n=3), and milk sample (n=3). Furthermore, the DNA products (n=15) for the *gap*A gene out of 101 positive samples of *M. bovis* in cattle from various samples, including blood sample (n=3), nasal swab

(n=3), ocular swabs (n=3), synovial fluid (n=3), and milk sample (n=3), were sent to Macrogen Inc., South Korea for sequencing. The retrieved *uvr*C and *gap*A genes local sequences were analyzed using different online programs such as NCBI Blastn (to determine the similarity between obtained sequences and other sequences in the NCBI GenBank), multiple sequence alignments (CLUSTALW) program (to determine the alignment scores (within and between) obtained sequences), and BankIt tool program (to deposited five sequences for each *uvr*C gene and *gap*A gene of *M. bovis* in the NCBI GenBank). Moreover, MEGA11 software was used to create phylogenetic trees with the outgroup sequence (U00089.2)-*Mycoplasma pneumoniae* strain M129, Germany (40,41).

Statistical analysis

To calculate the prevalence of *M. bovis*, descriptive statistics on the Excel program 2010 was used, and to determine the significant differences between sample types and types of genes, an *X*-Square 2x2 table in IBM SPSS Version 22 (Inc., Chicago, USA) was used. Values were considered significant at P<0.05.

Table 1: The PCR program for DNA samples subject to conventional PCR and multiplex PCR techniques

Steps	Temperature	Time	No. of cycles
Initial denaturation	95ºC	5 min.	1
Denaturation	94ºC	45 sec.	35
Annealing of primers	59ºC (*16S rRNA*) 55ºC (*uvr*C & *gap*A genes)	45 sec. 30 sec.	
Extension	72ºC	2 min.	
Final extension	72ºC	5 min.	1
Cooling	4ºC	∞	1

Results

In this study using Nanodrop, the concentration of DNA extracted from the blood samples, nasal swabs, ocular swabs, synovial fluid, and milk samples ranged between 60.5 - 327.5 ng/µl, and the purity of the extracted DNA varied between 1.7 - 1.9. Based on the amplified *16S rRNA* gene, the prevalence of Mycoplasma spp. was 38.63% (136 out of 352) in cattle cultured nasal swabs Nineveh Governorate using the c-PCR technique (Table 2), and the positive bands were approximately at 270 bp. (Figure 1). Further, there was no significant prevalence of *M. bovis* in the cattle's cultured nasal swabs, which was positive in the culture method. Also, the nasal swab samples were negative to the culture method based on amplified *uvr*C and *gap*A genes, which was 30.68% (108 out of 352) and 28.69% (101 out of 352 cattle), respectively, using the m-PCR technique, and the positive bands were approximately at 270 bp (Figures 1 and 2). In addition, the result also revealed that there was no statistically significant difference in the prevalence of *M. bovis* in various types of samples from 352 cattle that were examined using the m-PCR technique based on amplified *uvr*C and *gap*A genes, including blood samples, nasal swabs, ocular swabs, synovial fluid, and milk samples, which were 27, 30.68, 22.50, 27.69, and 23.33%, respectively (Table 3).

Table 2: Prevalence of *Mycoplasma spp. and Mycoplasma bovis* in cattle using conventional PCR and multiplex-PCR techniques using cultured nasal swab

Type of PCR	Primers/Gene	Genotypes	Product size (bp)	No. positive (%)
Conventional	Universal/ *16S rRNA*	Mycoplasma Spp.	285	136 (38.63)
Multiplex	Specific/ *uvr*C	*M. bovis*	1626	108 (30.68)[a]
	Specific/ *gap*A	*M. bovis*	1007	10١ (28.69)[a]

Vertical different letters (a or b) means that the values are significantly different P<0.05. Number of samples testes 352 samples.

In the present study, based on the multiple sequence's alignment program, the alignment score within the local sequences of *the uvr*C gene (n=15) and *gap*A gene (n=15) was 98.41-100 and 98.83-100, respectively. In contrast, there was 23.12 - 25.78 between the sequences of *uvr*C and *gap*A genes (Table 4). In addition, for the first time, five sequences from different samples, one for each (blood sample, nasal swab, ocular swab, synovial fluid, and milk sample), out of 15 local sequences of the *uvr*C gene of *M. bovis* in cattle in Nineveh governorate, were deposited at the NCBI-American GenBank with the accession numbers OR784598.1, OR784599.1, OR784600.1, OR784601.1 and OR784602.1 (Table 5). Moreover, for the first time, five sequences from different samples, one for each (blood sample, nasal swab, ocular swab, synovial fluid, and milk sample), out of 15 local sequences of the *gap*A gene of *M. bovis* in cattle in Nineveh governorate, were deposited at the NCBI-American GenBank with the accession numbers OR792211.1,

OR792212.1, OR792213.1, OR792214.1 and OR792215.1 (Table 5).

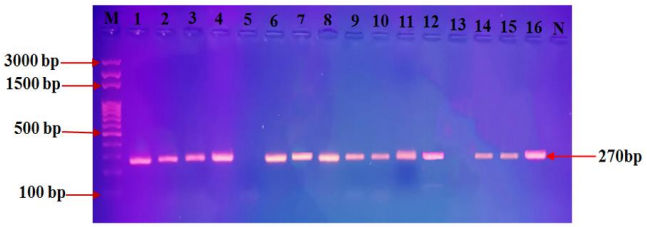

Figure 1: Conventional PCR technique detected *16S rRNA* gene of the Mycoplasma spp. in approximately band size 270bp; Lane M) DNA ladder; Lane N) Negative control.

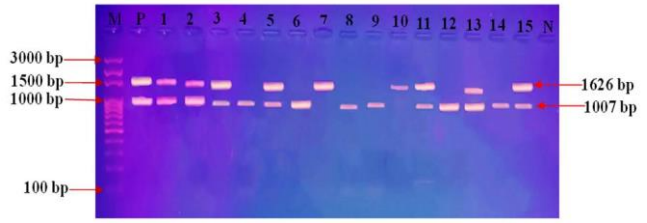

Figure 2: Multiplex PCR technique detected *uvr*C and *gap*A genes of the *Mycoplasma bovis* in approximately band size 1626bp. and 1007bp. respectively; Lane M) DNA ladder; Lane P) Positive control; Lane N) Negative control.

Table 3: Prevalence of *Mycoplasma bovis* based on the type of samples using conventional PCR technique

Type sample	No. of the tested sample	c-PCR No. positive	Prevalence %
Blood	352	97	27.55 [a]
Nasal swab	352	108	30.68 [a]
Ocular swab	40	9	22.50 [a]
Synovial fluid	65	18	27.69 [a]
Milk	30	7	23.33 [a]

Vertical different letters (a or b) means that the values are significantly different ($P < 0.05$).

Table 4: Alignment score within and between local sequences of the *uvr*C and *gap*A genes of *Mycoplasma bovis* using multiple sequence alignment program

Genotype	*M. bovis*	Alignment score
Within	*uvr*C gene	98.41-100
	*gap*A gene	98.83-100
Between	*gap*A gene: *uvr*C gene	23.12 - 25.78

Table 5: The type of samples and the GenBank accession numbers of local sequences for *uvr*C and *gap*A genes of *Mycoplasma bovis* in cattle

Type	Accession numbers	
	*uvr*C gene	*gap*A gene
Blood	OR784600.1	OR792211.1
Nasal swab	OR784598.1	OR792212.1
Ocular swab	OR784602.1	OR792214.1
Synovial fluid	OR784601.1	OR792215.1
Milk	OR784599.1	OR792213.1

In the present study, individual sequencing analysis for the local sequences (OR784598.1, OR784599.1, OR784600.1, OR784601.1, and OR784602.1) of the *uvr*C gene for *M. bovis* was observed to be highly similarity (99.81%–100% identity) to those sequences in the GenBank of various countries such as Canada (CP042938.1, CP042939.1), Iran (KX772803.1, KX772803.1, KP795974.1), Poland (KU168342.1), Switzerland (AF003959.1, LT578453.1), and Egypt (KP099618) using the NCBI Blastn program (Table 6). Furthermore, individual sequencing analysis for the local sequences (OR792211.1, OR792212.1, OR792213.1, OR792214.1, and OR792215.1) of the *gap*A gene for *M. bovis* was observed to be highly similar (99.13%–100% identity) to those sequences in the GenBank of different countries such as Canada (EF436267.1) and Egypt (KU559606, KP099616) using the same program NCBI Blastn (Table 7).

Table 6: Similarity between the local sequences of the *uvr*C gene for *Mycoplasma bovis* and other sequences of the same pathogen in the GenBank using NCBI BLASTn

Accession No. (Local)	Query Cover %	Similarity %	GenBank Accession Number	Country identification
OR784598.1	100	99.81	CP042939.1	Canada
	100	99.81	CP042938.1	Canada
OR784600.1	100	99.81	KX772803.1	Iran
	100	99.81	KX772801.1	Iran
OR784601.1	100	99.81	KU168342.1	Poland
	100	99.81	KP795974.1	Iran
OR784602.1	100	99.81	AF003959.1	Switzerland
	100	99.81	LT578453.1	Switzerland
OR784599.1	100	99.81	KP099618	Egypt

Table 7: Similarity between the local sequences of the *gapA* gene for *Mycoplasma bovis* and other sequences of the same pathogen in the GenBank using NCBI BLASTn

Accession No. (Local)	Query Cover %	Similarity %	GenBank Accession Number	Country identification
OR792211.1	99	100	EF436267.1	Canada
OR792212.1	100	99.13	KU559606	Egypt
OR792213.1	99	99.88	KP099616	Egypt
OR792214.1	99	99.88	KP099617	Egypt

The phylogenetic analysis of the local partial sequences of the *uvr*C for *M. bovis* OR784598.1, OR784599.1, OR784600.1, OR784601.1 and OR784602.1 revealed highly phylogenetic properties and an extremely close evolutionary relationship 80-99% to other global sequences of the same pathogen that have been recorded in the Genbank for different countries, such as Canada, Iran, Poland, Switzerland, and Egypt, after performing 1000 nucleotide sequence reconstruction using MEGA 11-Bootstrap analysis (Figure 3). In addition, the phylogenetic analysis of the local partial sequences of the *gap*A for *M. bovis* OR792211.1, OR792212.1, OR792213.1, OR792214.1 and OR792215.1 revealed highly phylogenetic properties and an extremely close evolutionary relationship 84-100% to other global sequences of the same pathogen that have been recorded in the Genbank for different countries, such as Canada and Egypt, after performing 1000 nucleotide sequence reconstruction using MEGA 11-Bootstrap analysis (Figure 4).

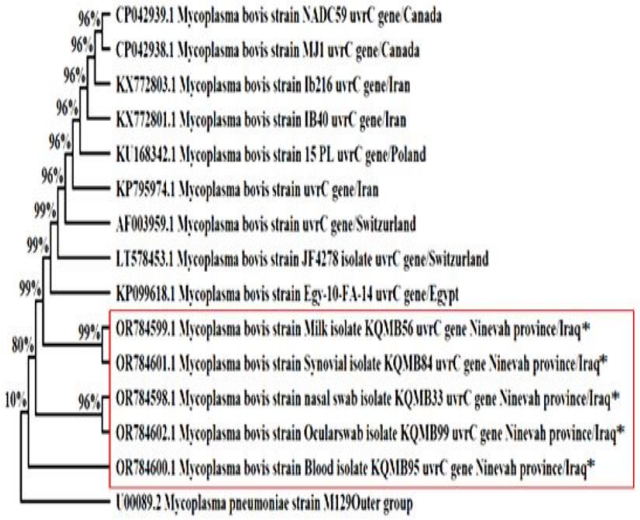

Figure 3: Phylogenic tree of the partial sequences of the *uvr*C gene of *Mycoplasma bovis* in Nineveh Governorate, Iraq (*), with the outgroup *Mycoplasma pneumoniae* strain M129 (U00089.2), Germany.

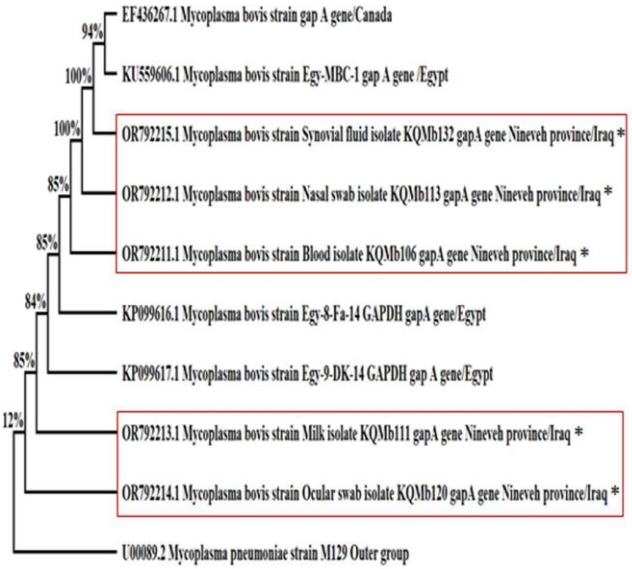

Figure 4: Phylogenic tree of the partial sequences of the *gap*A gene of Mycoplasma bovis in Nineveh Governorate, Iraq (*), with the outgroup Mycoplasma pneumoniae strain M129 (U00089.2), Germany.

Discussion

In the current work, the prevalence of *M. bovis* in cattle in Nineveh Governorate was 30.68% using the c-PCR technique. This finding was lower when compared with other previous reports that mentioned the prevalence of *M. bovis* in Iraq. Mahmood and Rhaymah (16) and Hamad *et al*. (17) stated that the prevalence of *M. bovis* in the calves in Mosul city was 76.09% and 86.5% using i-ELISA and PCR techniques, respectively. Furthermore, different studies indicated the prevalence of *M. bovis* in cattle in various countries using various diagnostic laboratory methods such as in Iran was 8.8% using nested PCR (n-PCR) technique (42), in Jordan was 27.3% using conventional PCR (c-PCR) technique (43), in Saudi Arabia was 24% using c-PCR technique (44), in Turkey was 23.3% using direct fluorescent antibody test (DFAT) (45), in China was 48.7% using i-ELSA (46), in Egypt was 67.5% and 8.3% using culture method and c-PCR technique respectively (26,47), in Sudan was 7.2% using i-ELISA (48), in United States of America

was 100% and 87.5% using LAMP and real-time PCR (RT-PCR) technique respectively (27), in Brazil was 91.4%, 1.1% and 62.3% using (IHC), RT-PCR technique, and i-ELISA respectively (19,49,50), and in Australia was 42.5% using i-ELISA (51). The differs in the prevalence of *M. bovis* among counties may be due to different management approaches, environmental conditions, efficient diagnostic techniques used in other studies, the types of samples that were tested, and the presence or absence of additional factors, such as the host's age, physical characteristics, and immunological status (22,23,28,52-54).

In this study, there was no significant difference in the prevalence of *M. bovis* in cattle based on amplified *uvr*C and *gap*A genes, with alignment scores within each gene of 98.41-100 and 98.83-100 respectively. These results agree with Abdeen *et al*. (18), who stated that there was no significant difference in the prevalence of *M. bovis* based on *uvr*C and *gap*A genes using the PCR technique, and the similarity within each gene was 95.3% and 100%, respectively. The *uvr*C gene and *gap*A were selected for the PCR technique to detect *M. bovis* in cattle because they are the most commonly used in epidemiology, sequencing, and phylogenic analyses studies, and they are available in molecular databases (18,19,39,55,56).

The current study used PCR techniques to detect *M. bovis* in cattle. According to the types of PCR techniques, the sensitivity and specificity differed for the detection of *M. bovis* when compared with the culture method, such as 97.2% sensitivity and 90.9% specificity of the LAMP PCR technique and 86.1% sensitivity and 92.9% specificity of the c-PCR technique (57). Moreover, Parker *et al*. (1) and Scott *et al*. (32) noted that PCR techniques have greater efficiency, specificity, and sensitivity for laboratory detection of *M. bovis*.

In addition, the result showed no significant difference in the prevalence of *M. bovis* in various types of samples (blood samples, nasal swabs, ocular swabs, synovial fluid, and milk samples) when tested using the m-PCR technique. This result corresponds to Parker *et al*. (1), Clothier *et al*. (58), Jain *et al*. (59), Parker *et al*. (60), and Zhao *et al*. (61) they mentioned that there was no difference in the percentage of *M. bovis* among the types of specimens using different diagnostic tools. The interpretation for the reasons is that *M. bovis* is present in various secretions and causes different diseases in infected cattle, which explains why it can be isolated from multiple organs and samples (1,7,28).

Results concerning the sequencing and phylogenetic analysis of the PCR products (n= 15) for the *uvr*C gene and (n=15) for the *gap*A gene of *M. bovis* obtained from cattle's blood samples, nasal swabs, ocular swabs, synovial fluid, and milk samples, were sequenced. Five sequences from each gene were deposited in the NCBI GenBank OR784598.1-OR784602.1 and OR792211.1- OR792215.1 of the *uvr*C and *gap*A genes, respectively, for the first time in Nineveh governorate. These sequences were observed to have phylogenetic characteristics and a very tight evolutionary relationship with the other *M. bovis* sequences in the NCBI GenBank of different countries such as Canada (24,38), Iran (62,63), Poland (64), Switzerland (39,65), and Egypt (26,66), with high similarity 99.13%-100% after 1000 replications using MEGA11 software (41).

Conclusions

This study concludes that *M. bovis* is widely distributed and circulating among cattle in Nineveh governorate, Iraq. There was no significant difference between the *uvr*C and *gap*A genes, also among various samples, in detecting *M. bovis* using the m-PCR technique. Furthermore, sequencing and phylogenetic analysis of *M. bovis* play essential roles in the study areas' strategic control of *M. bovis*.

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Conflict of interest

The authors claim no conflicts of interest.

References

1. Parker AM, Sheehy PA, Hazelton MS, Bosward KL, House JA. Review of mycoplasma diagnostics in cattle. J Vet Intern Med. 2018;32(3):1241-1252. DOI: 10.1111/jvim.15135
2. Hale HH, Helmboldt CF, Plastridge WN, Stula EF. A Mycoplasma species cause bovine mastitis. Cornell Vet. 1962;52:582-591. [available at]
3. OIE. Animal diseases. [available at]
4. Pereyre S, Tardy F. Integrating the human and animal sides of mycoplasmas resistance to antimicrobials. Antibiotics, 2021;10(10):1216-1239. DOI: 10.3390/antibiotics10101216
5. Biesheuvel MM, Ward C, Penterma P, van Engelen E, van Schaik G, Deardon R, Barkema HW. Within-herd transmission of *Mycoplasma bovis* infections after initial detection in dairy cows. J Dairy Sci. 2024;107(1):503-516. DOI: 10.3168/jds.2023-23407
6. Timonen AA, Autio T, Pohjanvirta T, Häkkinen L, Katholm J, Petersen A, Kalmus P. Dynamics of the within-herd prevalence of *Mycoplasma bovis* intramammary infection in endemically infected dairy herds. Vet Microbiol. 2020;242:108608. DOI: 10.1016/j.vetmic.2020.108608
7. Dudek K, Nicholas RA, Szacawa E Bednarek D. *Mycoplasma bovis* infections-occurrence, diagnosis and control. J Pathog. 2020;9(8):640-653. DOI: 10.3390/pathogens9080640
8. Alberti A, Cacciotto C. Eating the enemy: Mycoplasma strategies to evade neutrophil extracellular traps (nets) that promote bacterial nucleotide uptake and inflammatory damage. Int J Mol Sci. 2022;23(23):15030. DOI: 10.3390/ijms232315030
9. Faburay B, McVey DS. Mollicutes. In: McVey DS, Kennedy M, Chengappa MM, Wilkes R, editors. Veterinary Microbiology. 4th ed. USA: John Wiley & Sons, Inc.; 2022. 364-376 p. DOI: 10.1002/9781119650836
10. Kanda T, Tanaka S, Suwanruengsri M, Sukmawinata E, Uemura R, Yamaguchi R, Sueyoshi M. Bovine endocarditis associated with *Mycoplasma bovis*. J Comp Pathol. 2019;171:53-58. DOI: 10.1016/j.jcpa.2019.07.003

11. Hermeyer K, Peters M, Brügmann M, Jacobsen B, Hewicker-Trautwein M. Demonstration of *Mycoplasma bovis* by immunohistochemistry and in situ hybridization in an aborted bovine fetus and neonatal calf. J Vet diagn Invest. 2012;24(2):364-369. DOI: 10.1177/1040638711435145
12. Sadoon AS. Clinical and subclinical mastitis in buffalo in Mosul area, Iraq. Iraq J Vet Sci. 2022;36(1):177-186. DOI: 10.33899/ijvs.2021.129644.1671
13. Priyantha MR, Perera GS, Liyanagunawardana N, Ranatunga AD. Overview of *Mycoplasma bovis* infection in dairy and beef cattle. Wayamba J Anim Sci. 2021;1859-1873. DOI: 10.4038/wjas.v13i0.24
14. Vähänikkilä N, Pohjanvirta T, Haapala V, Simojoki H, Soveri T, Browning GF, Pelkonen S, Wawegama NK, Autio T. Characterisation of the course of *Mycoplasma bovis* infection in naturally infected dairy herds. Vet Microbiol. 2019;231:107-115. DOI: 10.1016/j.vetmic.2019.03.007
15. Klein U, de Jong A, Youala M, El Garch F, Stevenin C, Moyaert H, Rose M, Catania S, Gyuranec M, Ayling RD. New antimicrobial susceptibility data from monitoring of *Mycoplasma bovis* isolated in Europe. Vet Microbiol. 2019;238:108432. DOI: 10.1016/j.vetmic.2019.108432
16. Mahmood K, Shayal M. Diagnosis of arthritis in calves resulting from infection with *Mycoplasma bovis* by using indirect ELISA test. Al-Qadisiyah J Med Sci. 2012;11(1):115-122. [available at]
17. Hamad MA, AL-Jumaa ZM, Al-Aalim AM, Jaber Mayahi MT. Detection of *Mycoplasma bovis* in pneumonic calves. J Pure Appl Microbiol. 2019;13(4):2437-2443. DOI: 10.22207/JPAM.13.4.59
18. Abdeen EE, Mousa WS, Suelam II. Genotyping of *Mycoplasma bovis* isolated from cattle suffering from respiratory manifestation in Menofia province, Egypt. Pak Vet J. 2017;37(1):69-72. [available at]
19. Salina A, Timenetsky J, Barbosa MS, Azevedo CM, Langoni H. Microbiological and molecular detection of *Mycoplasma bovis* in milk samples from bovine clinical mastitis. Pesqui Vet Bras. 2020;40:82-87. DOI: 10.1590/1678-5150-PVB-6259
20. Maya-Rodríguez LM, Carrillo-Casas EM, Rojas-Trejo V, Trigo-Tavera F, Miranda-Morales RE. Prevalence of three *Mycoplasma sp.* by multiplex PCR in cattle with and without respiratory disease in central Mexico. Trop Anim Health Prod. 2022;54:394-402. DOI: 10.1007/s11250-022-03398-y
21. Ashraf A, Imran M, Yaqub T, Tayyab M, Shehzad W, Mingala CN, Chang YF. Development and validation of a loopmediated isothermal amplification assay for the detection of *Mycoplasma bovis* in mastitic milk. Folia Microbiol. 2018;63:373-380. DOI: 10.1007/s12223-017-0576-x
22. Ammar AM, Abd El-Hamid MI, Mohamed YH, Mohamed HM, Al-khalifah DM, Hozzein WN, Selim S, El-Neshwy WM, El-Malt RS. Prevalence and antimicrobial susceptibility of bovine Mycoplasma species in Egypt. Biol. 2022;11:1083-1101. DOI: 10.3390/biology11071083
23. Oucheriah Y, Heleili N, Colin A, Mottet C, Tardy F, Becker CM. Prevalence of *Mycoplasma bovis* in Algeria and characterisation of the isolated clones. Front Vet Sci. 2022;9:910799. DOI: 10.3389/fvets.2022.910799
24. Cantón G, Llada I, Margineda C, Urtizbiría F, Fanti S, Scioli V, Fiorentino MA, Louge Uriarte E, Morrell E, Sticotti E, Tamiozzo P. *Mycoplasma bovis*-pneumonia and polyarthritis in feedlot calves in Argentina: First local isolation. Rev Argent Microbiol. 2022;54:299-304. DOI: 10.1016/j.ram.2022.02.005
25. García-Galán A, Seva J, Gómez-Martín Á, Ortega J, Rodríguez F, García-Muñoz Á, De la Fe C. Importance and Antimicrobial Resistance of Mycoplasma bovis in Clinical Respiratory Disease in Feedlot Calves. Animals. 2021;11(5): 1470-1484. DOI: 10.3390/ani11051470
26. Hashem YM, Mousa WS, Abdeen EE, Abdelkhalek HM. Prevalence and molecular characterization of Mycoplasma species, *Pasteurella multocida*, and *Staphylococcus aureus* isolated from calves with respiratory manifestations. Anim. 2022;12(3):312-324. DOI: 10.3390/ani12030312
27. Appelt S, Aly SS, Tonooka K, Glenn K, Xue Z, Lehenbauer TW, Marco ML. Development and comparison of loopmediated isothermal amplification and quantitative polymerase chain reaction assays for the detection of *Mycoplasma bovis* in milk. J Dairy Sci. 2019;102(3):1985-1996. DOI: 10.3168/jds.2018-15306
28. Okella, H, Tonooka K, Okello E. A systematic review of the recent techniques commonly used to diagnose *Mycoplasma bovis* in dairy cattle. Pathog. 2023;12(9):1178-1195. DOI: 10.3390/pathogens12091178
29. Andersson AM, Aspán A, Wisselink HJ, Smid B, Ridley A, Pelkonen S, Autio T, Lauritsen KT, Kenso J, Gaurivaud P, Tardy F. A European inter-laboratory trial to evaluate the performance of three serological methods for diagnosis of *Mycoplasma bovis* infection in cattle using latent class analysis. BMC Vet Res. 2019;15(369):1-10. DOI: 10.1186/s12917-019-2117-0
30. Wisselink HJ, Smid B, Plater J, Ridley A, Andersson AM, Aspán A, Tardy F. A European interlaboratory trial to evaluate the performance of different PCR methods for *Mycoplasma bovis* diagnosis. BMC Vet Res. 2019;15(1):86-98. DOI: 10.1186/s12917-019-1819-7
31. Hirose K, Kawasaki Y, Kotani K, Tanaka A, Abiko K, Ogawa H. Detection of Mycoplasma in mastitic milk by PCR analysis and culture method. J Vet Med Sci. 2001;63(6):691-693. DOI: 10.1292/jvms.63.691
32. Scott D, Kennedy M, Chengappa MM, Wikes R. Veterinary microbiology. 4th ed. UK: John Wiley and Sons.; 2022.
33. Al-AAlim AM, Sheet OH, Al-Jumaa ZM, Hamad MA. Molecular detection of *Mycoplasma spp*. from camel's milk. Iraq J Vet Sci. 2023;37(2):333-337. DOI: 10.33899/IJVS.2022.134635.2388
34. Esmaeel SA, Albadrani BA. Prevalence and some risk factors of bovine heamotropic Mycoplasma in Nineveh province-Iraq. Iraq J Vet Sci. 2019;33(2):427-431. DOI: 10.33899/IJVS.2019.163170
35. Nishi K, Hirano Y, Sato A, Eguchi A, Matsuda K, Toda M, Watanabe T, Iwasaki T, Takahashi N, Hosotani M, Watanabe R, Kato T, Ohtsuka H, Gondaira S, Higuchi H. Effects of intra-articular inoculation with *Mycoplasma bovis* on immunological responses in calf joints. Vet Immunol Immunopathol. 2022;244:110364. DOI: 10.1016/j.vetimm.2021.110364
36. Morais AD, Pires DR, Cunha ND, Machado LS, Helayel MA, Mendonça JD, de Souza GN, Barreto ML, Nascimento, ED. Risk factors associated with intramammary colonization with Mollicutes in dairy cattle from southeast Brazil. Ciênc Rural. 2021;51:e20200694. DOI: 10.1590/0103-8478cr20200694
37. Botes A, Peyrot BM, Olivier AJ, Burger WP, Bellstedt DU. Identification of three novel Mycoplasma species from ostriches in south Africa. Vet Microbiol. 2005;111(3-4):159-169. DOI: 10.1016/j.vetmic.2005.10.017
38. Subramaniam S, Bergonier D, Poumarat F, Capaul S, Schlatter Y, Nicolet J, Frey J. Species identification of *Mycoplasma bovis* and *Mycoplasma agalactiae* based on their genes by PCR. Mol Cell Probes. 1998;12(3):161-169. DOI: 10.1006/mcpr.1998.0160
39. Perez-Casal J, Prysliak T. Detection of antibodies against the *Mycoplasma bovis* glyceraldehyde-3-phosphate dehydrogenase protein in beef cattle. Microb Pathog. 2007;43(5-6):189-197. DOI: 10.1016/j.micpath .2007.05.009
40. Himmelreich R, Hilbert H, Plagens H, Pirkl E, Li BC, Herrmann R. Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. Nucleic Acids Res. 1996;24(22):4420-4449. DOI: 10.1093/nar/24.22.4420
41. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022-3027. DOI: 10.1093/molbev/msab120
42. Bagheri Z, Mohammadzadeh A, Bahari AA, Koohi PM, Sharifi AA. A molecular survey for detecting *Mycoplasma bovis* in bovine bulk milk samples from dairy farms in Hamedan, Iran. Iranian Vet J. 2023;19(3):14-22. DOI: 10.22055/IVJ.2023.389326.2568
43. Hananeh WM, Al Momani WM, Ababneh MM, Abutarbush SM. *Mycoplasma bovis* arthritis and pneumonia in calves in Jordan: An emerging disease. Vet World. 2018;11:1663-1668. DOI: 10.14202/vetworld.2018.1663-1668
44. Al-Arfaj AA, Hessan AM, Al-Doss AA, Mohamed IA, Moussa IM. Molecular Identification of the false negative Mycoplasma isolates

from bovine mastitis infections. J Pure Appl Microbiol. 2013;7(3):2149-2154. DOI: 10.5555/20133 417313
45. Altun S, Ozdemir S. Detection of *Mycoplasma bovis* Infection in cattle mammary tissue by immunofluorescence and QRT-PCR methods. Kocatepe Vet J. 2019;12:110–115. DOI: 10.30607/kvj.493259
46. Niu J, Wang D, Yan M, Chang Z, Xu Y, Sizhu S, Li Z, Hu S, Bi D. Isolation, identification and biological characteristics of *Mycoplasma bovis* in yaks. Microb Pathog. 2021;150:104691. DOI: 10.1016/j.micpath.2020.104691
47. Abd El-Tawab A, Elhofy F, Hassan N, Ramadan M. Identification and genetic characterization of Mycoplasma species affecting respiratory system in Egyptian cattle. Benha Vet Med J. 2021;40:21–26. DOI: 10.21608/bvmj.2021.67213.1361
48. Onsa RH, Aldeen H, Abdelrazig M, Kit E. *Mycoplasma bovis* seroprevalence in Khartoum state-Sudan. Asian J Res Anim Vet Sci. 2022;9:15-19. [available at]
49. Oliveira TS, Pelaquim IF, Flores EF, Massi RP, Valdiviezo MJ, Pretto-Giordano LG, Alfieri AA, Saut JE, Headley SA. *Mycoplasma bovis* and viral agents associated with the development of bovine respiratory disease in adult dairy cows. Transbound Emerg Dis. 2020;67:82-93. DOI: 10.1111/tbed.13223
50. Pires DR ,Morais AN, Cunha NC, Machado LS, Barbosa LC, Mendonça JM, Balaro MA, Santos JC, Souza GN, Barreto ML. Proposal of an IELISA for *Mycoplasma bovis* diagnosis in dairy cattle and associated risk factors. Arq Bras Med Vet Zootec. 2021;73:293-301. DOI: 10.1590/1678-4162-12140
51. Gogoi-Tiwari J, Tiwari HK, Wawegama NK, Premachandra C, Robertson ID, Fisher AD, Waichigio FK, Irons P, Aleri JW. Prevalence of *Mycoplasma bovis* infection in calves and dairy cows in western Australia. Vet Sci. 2022;9(7):351-361. DOI: 10.3390/vetsci9070351
52. Penterman PM, Holzhauer M, van Engelen E, Smits D Velthuis AJ. Dynamics of *Mycoplasma bovis* in Dutch dairy herds during acute clinical outbreaks. Vet J. 2022;283:105841. DOI: 10.1016/j.tvjl.2022.105841
53. Sultana R. Prevalence and antimicrobial susceptibility of *Mycoplasma bovis* and *Pasteurella multocida* isolated from Albertan feedlot cattle [Ph.D. dissertation]. Canada: University of Lethbridge; 2023.
54. Alvarez I, Ducatez M, Guo Y, Lion A, Widgren A, Dubourdeau M, Hägglund S. Proteomic and lipidomic profiling of calves experimentally co-infected with Influenza D virus and *Mycoplasma bovis*: Insights into the host–pathogen interactions. Viruses. 2024;16(3):361-372. DOI: 10.3390/v16030361
55. Behera S, Rana R, Gupta PK, Kumar D, Sonal, Rekha, V Arun TR, Jena D. Development of real-time PCR assay for the detection of *Mycoplasma bovis*. Trop Anim Health Prod. 2018:875-882. DOI: 10.1007/s11250-018-1510-1
56. Li R, Wang J, Sun X, Liu L, Wang J, Yuan W. Direct and rapid detection of *Mycoplasma bovis* in bovine milk samples by recombinase polymerase amplification assays. Front Cell Infect Microbiol. 20211;1:639083. DOI: 10.3389/fcimb.2021.639083
57. Higa Y, Uemura R, Yamazaki W, Goto S, Goto Y, Sueyoshi M. An improved loop-mediated isothermal amplification assay for detecting *Mycoplasma bovis*. J Vet Med Sci. 2016;78(8):1343-1346. DOI: 10.1292/jvms.15-0459
58. Clothier KA, Jordan DM, Thompson CJ, Kinyon JM, Frana TS, Strait EL. *Mycoplasma bovis* real-time polymerase chain reaction assay validation and diagnostic performance. J Vet Diagn Investig. 2010;22(6):956-960. DOI: 10.1177/104063871002200618
59. Jain U, Verma AK, Pal BC. PCR based detection of *Mycoplasma bovis* from bovine clinical specimens. Indian Vet J. 2012;89:61-63. [available at]
60. Parker AM, House JK, Hazelton MS, Bosward KL, Sheehy PA. Comparison of culture and a multiplex probe PCR for identifying Mycoplasma species in bovine milk, semen, and swab samples. PLoS One. 2017;12:e0173422. DOI: 10.1371/journal.pone.0173422
61. Zhao G, Hou P, Huan Y, He C, Wang H, He H. Development of a recombinase polymerase amplification combined with a lateral flow dipstick assay for rapid detection of the *Mycoplasma bovis*. PLoS One. 2018;14:412. DOI: 10.1371/journal.pone.0278869
62. Dudek K, Bednarek D, Ayling RD, Kycko A, Szacawa E, Karpińska TA. An experimental vaccine composed of two adjuvants gives protection against *Mycoplasma bovis* in calves. Vaccine. 2016;34:3051-3058. DOI: 10.1016/j.vaccine.2016.04.087
63. Gou H, Zhang J, Liao M. Point-of-care testing for infectious and foodborne pathogens. Front Cell Infect Microbiol. 2022;12:975449. DOI: 10.3389/fcimb.2022.975449
64. Szacawa E, Szymańska-Czerwińska M, Niemczuk K, Dudek K, Woźniakowski G., Bednarek D. Prevalence of pathogens from Mollicutes class in cattle affected by respiratory diseases and molecular characteristics of *Mycoplasma bovis* field strains. J Vet Res. 2016;60(4):391-397. DOI: 10.1128/MRA.00838-19
65. Morimoto M, Kenri T, Ohmori T, Teshima K, Shibuya K, Sasakawa C, Suzuki M. Complete genome sequence of *Mycoplasma bovis* strain KG4397, isolated from cattle in Japan. Microbiol Resour Announc. 2019;8(40):1128-1132. DOI: 10.1128/MRA.00838-19
66. Eissa S, Hashem Y, Shaker M. Sequence analysis of three genes of *Mycoplasma bovis* isolates from Egyptian cattle and buffaloes. Br Microbiol Res J. 2016;14(3):1-10. DOI: 10.9734/BMRJ/2016/25014

الكشف الجزيئي وتحليل النشوء الجيني للمفطورات البقرية في الأبقار في محافظة نينوى، العراق

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الخلاصة

هدفت الدراسة الحالية تحديد مدى انتشار المفطورات البقرية في الأبقار في محافظة نينوى، العراق، اعتمادا على ثلاث جينات تضمنت *16S rRNA* و *uvr*C و *gap*A باستخدام تقنيات تفاعل البلمرة المتسلسل، والتحقيق من تحليل النشوء الجيني للمفطورات البقرية. تشخيص في الدراسة. تم جمع عينات مختلفة تضمنت ٣٥٢ عينة الدم و ٣٥٢ مسحات الأنف و ٤٠ مسحات من العين و٦٥ السائل المفصلي و٣٠ عينة الحليب وبشكل عشوائي من ٣٥٢ رأسا من الأبقار. اعتمادا على تضخيم الجين *16S rRNA*، بلغت نسبة انتشار جنس المفطورات ٣٨,٦٣% في الأبقار باستخدام تقنية تفاعل البلمرة المتسلسل التقليدي. في حين لم يلاحظ وجود فرق معنوي في نسبة انتشار المفطورات البقرية في الأبقار اعتماداً على تضخيم الجين *uvr*C و*gap*A والتي بلغت ٣٠,٦٨ و٢٨,٦٩% على التوالي باستخدام تقنية تفاعل البلمرة المتسلسل المتعدد. كما لوحظ عدم وجود فرق معنوي بين نوع العينات في الكشف عن المفطورات البقرية. خضعت التسلسلات الجينية المحلية (١٠ تسلسلات) للتحليل النشوئي تضمنت (٥ تسلسلات للجين *uvr*C) و (٥ تسلسلات للجين *gap*A) والتي تم تسجيلها في بنك الجينات المركز الوطني لمعلومات التكنولوجيا الحيوية بأرقام تسلسلية OR784602.1-OR784598.1 و OR792215.1-OR792211.1، مع نسبة تشابه عالية ٩٩,١٣-١٠٠% و٩٩,٨١%، على التوالي، بتلك التسلسلات المسجلة في بنك الجينات العالمي ومن بلدان مختلفة، مثل كندا ومصر وإيران وبولندا وسويسرا. استنتج من هذه الدراسة إلى أن المفطورات البقرية منتشرة على نطاق واسع في محافظة نينوى، العراق. كما سلطة الدراسة الضوء ولأول مرة على التحليل النشوئي الجيني للمفطورات البقرية في محافظة نينوى، العراق.