University of Mosul

College of Veterinary Medicine

Molecular and Epidemiological Study of *Mycoplasma bovis* in Cattle in Nineveh Governorate, Iraq

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Ph.D. / Dissertation
Veterinary Medicine / Internal and Preventive Veterinary Medicine

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2024 A.D. **1446 A.H**

Molecular and Epidemiological Study of Mycoplasma bovis in Cattle in Nineveh Governorate, Iraq

A Dissertation submitted
By

Khoder Ahmad Mahmood AL-Azow

To
The Council of the College of Veterinary Medicine
University of Mosul
In
Partial Fulfillment of the Requirements
For the degree of Doctor of Philosophe
In
Veterinary Medicine / Internal and Preventive Veterinary Medicine

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2024 A.D. **1446 A.H**

بسم الله الرحمن الرحيم

فأما الزبد فيذهب جفاء وأما ما

ينفع الناس فيمكث في الأرض

صدق الله العظيم

سورة الرعد آية 17

Supervisor's Certification

We certify that this dissertation entitled **"Molecular and Epidemiological Study of Mycoplasma bovis in Cattle in Nineveh Governorate, Iraq"** was prepared under my supervision at the College of Veterinary Medicine, University of Mosul, Mosul, Nineveh, Iraq as a partial fulfillment of the requirements for the degree of Ph.D. in Veterinary Medicine/ Internal and Preventive Veterinary Medicine.

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Acknowledgment

First, thanks to The Almighty Allah the most merciful for enabling me to accomplish this study.

After completing my study, I must extend my sincere thanks and gratitude to my honorable supervisors, **Professor Dr. Qaes Talb Shukur & Professor Dr. Mohammad Ali Hamad** for their valuable guidance and careful practical observation that influenced this study.

I also extend my thanks and gratitude to the Head of the Internal and Preventive Medicine and Department, for his assistance in collecting samples, as well as for the scientific and moral support throughout the study period.

I also extend my thanks and appreciation to Dean of the College of Veterinary Medicine - for his continuous encouragement to me, and Associate Dean for Scientific Affairs and Assistant Dean for Administrative Affairs. And to all my distinguished professors in the Internal and Preventive Medicine Department who had the credit for completing this study.

I extend my special thanks and gratitude to my family, my dear supporter wife, my dear children, my dear father my dear sisters and my dear brother's companions, for their good influence in urging me to continue my studies.

Khoder

ABSTRACT

The present study aimed at: determining the prevalence of *Mycoplasma bovis* in cattle in Nineveh Governorate, Iraq, using different diagnostic methods: culture method, indirect enzyme immunosorbent assay (i-ELISA), and polymerase chain reaction (PCR) techniques, evaluating the efficiency of these laboratory methods; investigate some of the epidemiological risk factors associated with the prevalence of the microorganism, recording the clinical manifestations in infected animals, and investigating the phylogenetic analyses of *M. bovis* detected in this study.

The study included 352 cattle with different ages, sexes, breeds, herd sizes, health states, pregnancy states, regions of samples collection, and seasons. These cattle comprising 15 cattle which were apparently healthy and tested negative for *M. bovis*, were used as a control group. In the period from (March 2022) to (February 2023), a unique clinical examination card was used to clinical signs record, and other information of epidemiology. Different samples were collected from 352 cattle, including blood samples (n=352), nasal swabs (n=352), ocular swabs (n=40), synovial fluid (n=65), and milk samples (n=30), that were obtained from different regions in Nineveh Governorate. The nasal swabs were used for the culture method and conventional PCR (c-PCR) technique based on the 16S rRNA gene for Mycoplasma spp., and using nasal swabs and the other samples mentioned above for multiplex PCR (m-PCR) technique based on the *uvr*C and *gap*A genes for *M. bovis*. Furthermore, using serum samples for the indirect ELISA (i-ELISA) test to detect the antibody against *M. bovis*. The agreement between the involved diagnostic techniques was assessed according to the Kappa value.

Additionally, computed the accuracy, specificity, and sensitivity of the m-PCR technique and i-ELISA compared to the culture method. A total of 30 PCR positive products for *M. bovis* DNA amplicons of the *uvr*C gene (n=15) and *gap*A gene (n=15) from various cattle samples, three products for each type of sample, respectively, were sent to a commercial company for purification and sequencing. The phylogenetic and trees for the local *M. bovis* sequences were constructed using different online programs. In the current study, the overall prevalence of *M. bovis* in cattle from Nineveh Governorate was 23.57%, 47.15%, and 30.68% using the culture method, i-ELISA, and m-PCR technique, respectively. Based on the kappa value (0.807), there was perfect agreement between m-PCR technique and the culture method, with sensitivity 98.79%, specificity 90.33%, and accuracy 92.32%, for m-PCR technique. Whereas, based on kappa (0.444), there was a moderate agreement between i-ELISA and culture method, sensitivity 89.15%, specificity 65.79%, and accuracy 71.30%, for i-ELISA. According to the m-PCR technique, there was 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, which were 27.55%, 30.68%, 22.50%, 27.69%, and 23.33%, respectively. In addition, there were significant risk factors associated with a higher prevalence of *M. bovis* recording in calves more than 6 months to 2 years old, ox, imported animals, pregnant cows, large herd sizes, indoor and outdoor management, the eastern regions of Nineveh Governorate, and the autumn and winter seasons. Clinical examination of cattle observed that were suffering from fever, loss of appetite, depression, emaciation, bronchopneumonia, keratoconjunctivitis, arthritis, and mastitis. Significantly increased in body temperature, respiratory rate, and heart rate in clinically infected cattle compared to clinically healthy animals. The phylogenetic analysis for the first time, 5 local sequences of the *uvr*C gene

for *M. bovis* in Nineveh Governorate that were deposited in the NCBI GenBank under the accession numbers (OR784598.1, OR784599.1, OR784600.1, OR784601.1, OR784602.1) were highly related (99.13% - 100% identity) to other sequences that were registered in the GenBank from different countries, including Canada, Iran, Poland, Switzerland, and Egypt. Moreover, for the first time, 5 local sequences of the *gap*A gene for *M. bovis* in Nineveh Governorate that were deposited in the NCBI GenBank under the accession numbers (OR792211.1 - OR792215.1) were highly related (99.81% -100% identity) to other sequences that were registered in the GenBank from different countries, including Canada and Egypt. In conclusion, *M. bovis* is widely spread in cattle in Nineveh Governorate, Iraq; the m-PCR technique is more efficient than ELISA for diagnosing *M. bovis*; there are several risk factors associated with the higher prevalence of *M. bovis*. Furthermore, the phylogenetic analysis of the local sequences that were first reported in Nineveh Governorate is important for the strategic control of *M. bovis* in the study regions.

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List of abbreviations

Amplified fragment length polymorphism analysis	AFLP
Base pair	Bp
Bovine respiratory disease	BRD
Bovine respiratory syncytial virus	BRSV
Bovine viral diarrhea virus	BVDV
Conjugate solution	CS
Conventional PCR	c-PCR
Culture method	CM
Diluted solution	DS
Ethylenediaminetetraacetic acid	EDTA

Hydrogen peroxide	H2O2
Immunohistochemistry	IHC
Indirect enzyme-linked immunosorbent assay	i-ELISA
Infectious bovine rhinotrachitis virus	IBRV
Insertion sequence typing	IS
Interferon	IFN-γ
Interleukin 4	IL4
Loop-Mediated Isothermal Amplification- polymerase chain reaction	LAMP-PCR
Mass spectrometry	MS
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	MALDI-TOF MS
Multilocus sequence typing	MLST
Multiple locus variable tandem repetition analysis	MLVA
Multiplex PCR	m-PCR
Mycoplasma bovis	*M. bovis*
Nested- polymerase chain reaction	n-PCR
Odds ratio	OR

Parainfluenza type 3 virus	PI-3
Polymerase chain reaction	PCR
Pulsed-field gel electrophoresis analysis	PFGE
Quantitative polymerase chain reaction	q-PCR
Random amplified polymorphic DNA analysis	RAPD
Real-time polymerase chain reaction	r-PCR
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis	SDS-PAGE
Somatic cell counts	SCC
Tumor necrosis factor alpha	TNF-α
Variable surface lipoproteins	VSPs

CHAPTER ONE:

INTRODUCTION

Chapter One:

Introduction

1.1 Introduction:

Bovine mycoplasmosis are among the important diseases that affect livestock herds and lead to economic losses, with high mortality in fattening calves (Peel, 2020). Mycoplasma microorganism are infecting animals, including ruminants, especially cows and calves, that were caused many infectious diseases, including respiratory diseases (Chloé *et al.,* 2021).

There are more than 125 species in the Mycoplasma genus (Salina *et al.,* 2020). The *Mycoplasma bovis* is a bacteria classified as belonging to the family Mycoplasmataceae, and it is possessing a tiny genome, a doesn't has of a cell wall, motile organism, have variable forms and high nutritional needed for in vitro growth (Parker *et al.,* 2018; Dudek *et al.,* 2020).

In terms of risk, *M. bovis* classified in List B, which infects cows and calves (OIE, 2021; Gelgie *et al.,* 2022). It is highly adapted in ruminants, especially cattle and even humans, who have immunocompromised and close contact with infected cattle or their products such as infected milk (Calcutt *et al.,* 2018; Pereyre *et al.,* 2021).

Mycoplasma bovis is disseminated by direct or indirect animal-to-animal contact in herds; the direct transmission between cows can occur during milking or through nose-to-nose contact, but indirect transmission can also happen through sharing drinking and feeding troughs (Biesheuvel *et al.,* 2024). Furthermore, Gille *et al.* (2020) and Timonen *et al.* (2020) they stated that certain strains of *M. bovis* were isolated from calves that

had respiratory illnesses and cows with clinical mastitis, suggesting a potential pathogen transfer via tainted milk between dairy cows and calves.

A number of infectious diseases in cattle, are caused by *M. bovis* called bovine mycoplasmosis (Dudek *et al.,* 2020; Timonen *et al.,* 2020). These diseases include vasculitis and keratoconjunctivitis (Alberti *et al.,* 2022), otitis media and decubital abscesses (Faburay *et al.,* 2022), Meningoencephalitis (Suwanruengsri *et al.,* 2022), endocarditis, which has been documented (Kanda *et al.,* 2019), chronic bronchopneumonia, polyarthritis, contagious mastitis (Hermeyer *et al.,* 2012), subclinical mastitis (Gelgie *et al.,* 2024), and abortion and genital problems (Priyantha *et al.,* 2021). Depending on how serious the clinical symptoms are, 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 (Vähänikkilä *et al.,* 2019).

Mycoplasma bovis is globally spread, especially in North America, Australia, Europe, and Asia (Klein *et al.,* 2019). In Iraq, it was detected in cows and calves infected with pneumonia, polyarthritis, and mastitis in Nineveh Governorate (Mahmood and *Rhaymah*, 2012; Hamad *et al.,* 2019). Moreover, Al-Alo *et al.* (2020) were detected of cases of keratoconjunctivitis infected with *M. bovis* of cattle in Najaf, Iraq.

The prevalence of the *M. bovis* have several risk factors associated with cattle such as age of animals, calving, herd size, overcrowding, infected animals in nearby barns, introduction of cattle from outside of the herd, water sources, rotting feed transport of animals, and changes in the climatic and season (Aebi *et al.,* 2015; Calcutt *et al.,* 2018; Gille, 2018; Haapala *et al.,* 2021)

Several *M. bovis* genes, including *uvrC* and gapA, have been utilized for *M. bovis* detection (Abdeen *et al.,* 2017; Salina *et al.,* 2020), 16S rRNA, oppD, and oppF genes (Maya-Rodríguez *et al.,* 2022), gyrA, gyrB, and parC genes (Ashraf *et al.,* 2018; Ammar *et al*., 2022), polC and 16S-23S rRNA ITS genes (Cantón *et al.,* 2022; Oucheriah *et al.,* 2022), ma-mp81 and mb-mp81 genes (García-Galán *et al.,* 2021; Hashem *et al.,* 2022), and gltX gene (Appelt *et al.,* 2019).

The clinical manifestations and pathological alterations of the cattle infected with *M. bovis* diseases are not definitive enough to confirm the presence of the disease because they interfere and confuse with other respiratory diseases such as influenza D virus and pasteurellosis (Caswell *et al.,* 2007; Lion *et al.,* 2021). Therefore, there are several laboratory methods have been used to detect *M. bovis* in infected cattle, such as the isolation of bacteria and elector microscopy (Dudek *et al.,* 2020), serological tests are employed, including indirect ELISA (Andersson *et al.,* 2019; Talvitie, 2021), immunohistochemistry (IHC) (Oucheriah *et al.,* 2022), and the polymerase chain reaction (PCR) technique (Wisselink *et al.,* 2019). Due to the difficult processing, time-consuming, and identified 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 (Okella *et al.,* 2023). Hence, PCR techniques are the most commonly used for the specific and rapid detection of *M. bovis* (Scott *et al.,* 2022).

Till now the reasons behind the prevalence of its risk factors, and phylogenetic analysis are unknown in Nineveh Governorate in Iraq. The veterinary authority is unable to apply *M. bovis* disease control methods without epidemiological data. Therefore, the study was conducted to aiming at:

1. Recording the clinical manifestations in infected cattle.
2. Identifying the prevalence of *Mycoplasma bovis* in cattle in Nineveh Governorate, Iraq using different diagnostic methods: culture method, indirect ELISA test, and PCR techniques,
3. Investigating some of epidemiological risk factors related to the prevalence of disease: cattle factors, regions, managements, and seasons.
4. Assessing the efficiency of various laboratory techniques for detection. *M. bovis*
5. Analyzing the phylogenetic of *M. bovis* detected in this study.

CHAPTER TWO:

LITERATURES REVIEW

Chapter Two:

Literatures review

2.1 Bovine Mycoplasmosis Definition:

Bovine mycoplasmosis acts a highly infectious disease, it can produce a number of clinical forms in cattle, the most common including mastitis and pneumonia in adult cattle, and chronic pneumonia, arthritis, and otitis in calves (Maunsell *et al.,* 2011; Salina *et al.,* 2020; Salina *et al.,* 2020; Ambroset *et al.,* 2022), eye infection (Raman *et al.,* 2024), as well as endometriosis, oviductitis, abortion in cows, seminal vesicle inflammation, and infertility in bulls (Buchenau *et al.,* 2010; Pohjanvirta *et al.,* 2023). Through experimental studies on calves infected with *M. bovis* bacteria found that it causes meningitis, as well as subcutaneous abscesses (Maunsell *et al.,* 2009).

2.2 Economic losses caused by *Mycoplasma bovis* infection

The *Mycoplasma bovis* is one of the important epidemic bacteria because it causes great economic losses in herds of fattening calves, as well as adult cows, that were cause many diseases including respiratory disorders, polyarthritis, epidemic mastitis, reproductive problems, and abortion in adult cows (Gonçalves, 2022). Moreover, *M. bovis* is responsible for a quarter to a third of economic losses in the cattle industry that are associated with bovine respiratory disease (BRD) (Sulyok *et al.,* 2014).

In Europe, *M. bovis* causes significant economic losses to ruminants, amounting to 576 million euros annually (Nicholas and Ayling, 2003). In

the United States, the cost of economic losses due to infection with *M. bovis* in herds of ruminants suffering from pneumonia amounts to 140 million dollars annually, as well as losses as a result of mastitis in cows due to these germs amounting to 108 million dollars (McAuliffe *et al.,* 2006). In addition, *M. bovis* is associated in California with up to 52.2% of Mycoplasmal mastitis (Okella *et al.,* 2023). In Canada, 36.5% of calves die due to respiratory diseases, including pneumonia caused by *M. bovis* (Menghwar and Perez-Casal, 2022). A British dairy consultancy company stated about 25–50 % of dairy farms were positive for *M. bovis*, and estimated costs for the dairy industry at up to EUR 17 million a year. (Hennessey *et al.,* 2020). In New Zealand, just over 1,800 farms were destroyed, leading to the approximately 160,000 animals being killed at a total price of 203 million dollars (Boyce *et al.,* 2021).

Moreover, Suzuki *et al.* (2024) noted that economic lost could be significant for individual farmers due to serious outbreak of *M. bovis* mastitis in herds. Other economic losses caused by *M. bovis* include a decrease in milk production in subclinical mastitis (Timonen *et al.,* 2017), and fertility problems due to *M. bovis*-positive semen (Suzuki *et al.,* 2024). The reasons for these economic losses are the lack of response to antibiotics, the length of the treatment period, and the lack of appropriate vaccine, and consequently, this will lead to loss of appetite, decrease in milk production and weight loss, and then the death of animals as a result of chronic disease (Rossetti *et al.,* 2010; Gille, 2018).

2.3 *Mycoplasma bovis* Background:

The first infection of the genus Mycoplasma, causing clinical symptoms, was discovered in 1816 in the city of Metaxas, Italy, and it was known as “infectious agalactiae” by Brusasco Zavagli, 1951 (Jaÿ and

Tardy, 2019). In 1920, the first type of Mycoplasma was diagnosed as *Mycoplasma mycoides sub. mycoides* cause epidemic bovine pleural pneumonia, later in 1961, *Mycoplasma bovis* was first isolated in cows infected with epidemic mastitis in California, USA (Hale *et al.,* 1962). It was known as *M. bovimastids*, then it was called *Mycoplasma agalactiae sub. bovis* being clinically similar to *M. agalactiae* that causes epidemic mastitis in sheep and goats, and later it was differentiated from *M. agalactiae* by 16S rRNA assay, which showed little difference in 8 nucleotides from that of *M. bovis agalactiae*, later learning the name *M. bovis* (Askaa and Erno, 1976).

There are many countries diagnosed the disease as a result of animal movements, as in Palestine in 1964, Australia in 1970, France in 1974, Great Britain in 1975, Slovakia in 1975, Spain in 1976, Germany in 1977, Denmark in 1981, Switzerland in 1983, Morocco in 1988 and South Korea in 1989, Brazil in 1989, Northern Ireland in 1993, Southern Ireland in 1994, and Chile in 2000 (Nicholas *et al.,* 2000). Also, it was diagnosed in the Czech Republic in 2005 (Zendulkova, 2007), and Nigeria in 2009 (Tambuwal *et al.,* 2011). In Iraq, *M. bovis* was first reported in Mosul in 2012 (Mahmood and Rhaymah, 2012). Recently, *M. bovis* entering Finland (Vähänikkilä *et al.,* 2019), and New Zealand in 2019 (Boyce *et al.,* 2021). Some author's as Semmate *et al.* (2023) noted that *M. bovis* was spreading to Europe, China, Australia, the Middle East, and African countries.

2.4 Classification and Characteristics of *Mycoplasma spp*.

Mycoplasmas are phenotypically distinct from other organisms due to their minuscule size and complete absence of a cell wall. Taxonomically in 1960, mycoplasmas were classified as "Mollicutes" (mollis, soft; cutis, skin) because they lack cell walls, which distinguish them another

microorganism (Brown *et al.,* 2007; Brown *et al.,* 2018; Talaro and Chess, 2018). More than 143 species have been identified in the Mollicutes class; there are four orders in this class, five families (Three of them contain the pathogenic bacteria for animals and humans) and 3 important genera in animals and humans (Figure 2-1). According to Jambhekar *et al.* (2019), Mycoplasmas were first described as commensal or opportunistic infections 100 years ago.

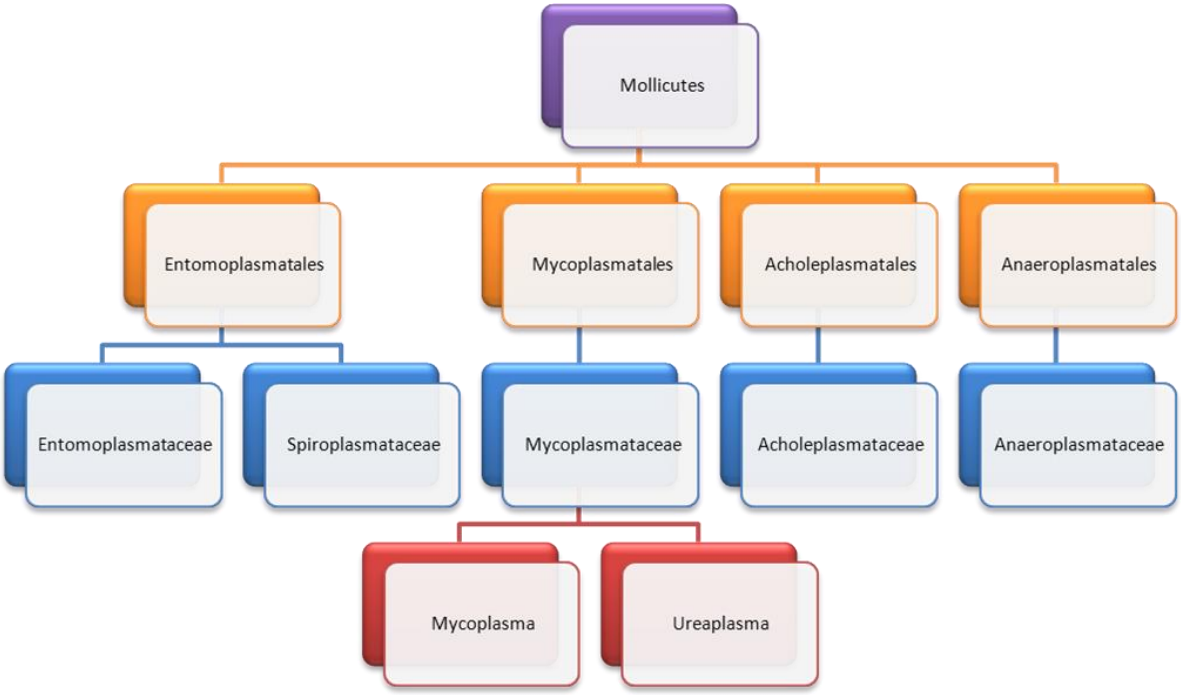

Figure (2.1): Taxonomy of class Mollicutes (Krieg *et al*., 2010; Quinn *et al.,* 2016)

The taxonomy of the genus Mycoplasma is a delicate and complex issue, along with the new nomenclature recommended for Mycoplasma (Gupta *et al.,* 2018).

Mycoplasmas bovis is considered a Gram-negative bacterium, as this bacterium consists of tiny prokaryotic cells that possess the capacity for self-replication, and they have pleomorphic organisms with variable shapes, including spherical in size 0.3-0.9 nm /diameter and others

filamentous in shape longer than 1.5 nm and does not contain a cell wall due to the inability of these organisms to manufacture collagen peptides or one of its derivatives (Quinn *et al.,* 2016; Gonçalves, 2022). With a size of 0.22-0.45 nm, it is one of the bacteria resistant to penicillin due to its loss of cell wall, some of these organisms are picky by nature and require urea, sterol, or cholesterol to thrive for growing, they need enriched media supplemented with 20% horse serum and yeast extract that provides vitamins and amino acids, in addition, use penicillin to inhibit Gram-positive bacteria (Calcutt *et al.,* 2018).

Thallus acetate is also used to inhibit Gram-negative bacteria and fungi. As result of the unique structure of mycoplasma and its inability to manufacture fatty or amino acids, the growth medium used in its cultivation must be rich in peptone, serum, yeast extract, and beef heart infusion, with a final pH range of 7.3 to 7.8. Although the majority of them were facultative anaerobes, some thrive in environments with 5–10% CO2 and develop distinctive umbonate microcolonies when lit obliquely, as well as having a "fried egg appearance" in reflected light (Faburay and McVey, 2022; Willey *et al.,* 2023).

There are many liquid culture media suitable for isolation such as Medium B, flick's medium, and Friis medium. These media contain a rich protein source such as horse and pig serum, glucose, yeast extract, pyruvate, and selective materials such as (Ampicillin, Thallous acetate, Amphotericin- B) (Nicholas *et al.,* 2008; Rifatbegović *et al.,* 2024).

Depending on the type, level of moisture, and temperature, *Mycoplasma bovis* can live outside the host for varying amounts of time. The majority of *Mycoplasma bovis* are dry-condition stable at 4°C, while many are unstable at higher temperatures. Mycoplasma spp is better

preserved when there is organic material present, such as host tissues or body fluids. However, after being lyophilized, Mycoplasma spp can endure storage at 4°C, or -70°C for 10 years or longer, making it vulnerable to heat, desiccation, detergents, and disinfectants (Procop *et al.,* 2017; Tille, 2022).

2.5 Characteristic Genomic and Genotyping:

Mycoplasma bovis have very small genomes with a size of ~953 kbp, DNA molecules containing 480 genes, and the genome has a low guanine-cytosine (G+C) content, 80% of the codons have an Adenine or a Thiamine (A+T) (Li *et al.,* 2011). Although this genome size is sufficient for their extracellular survival, their metabolic capacity is limited and they rely on a nutrient-rich environment for feeding (Kinnear *et al.,* 2021). Despite its small genome size, Mycoplasma shows great genetic flexibility, allowing it to modulate the appearance of key surface antigenic proteins to evade host immune surveillance (Scott *et al.,* 2022). More than 20 Mycoplasma genomic sequences have been published as of 2010 (Li *et al.,* 2011). The conventional PCR technique, including sequence-specific oligonucleotide primers and thermostable polymerase enzymes, are used in the in vitro amplification of unique specific DNA targets (Gonçalves, 2022). Some DNA targets of *M. bovis* have been recorded, and they involve *uvrC*, 16S rRNA, gyrB, polC, 16S-23S rRNA ITS, oppD, gapA, vspB, and gltX, among others. Furthermore, other genes of *M. bovis* have been recorded in studies that have used conventional single-plex PCR to diagnose bovine mastitis with success (Wilson *et al.,* 2009; Jain *et al.,* 2012; Pothmann *et al.,* 2015; Sayin *et al.,* 2016; El-Tawab *et al.,* 2021; Penterman *et al.,* 2022).

Several techniques are used to ascertain the evolutionary relationships between the isolates of *M. bovis* and their phylogenetic

structure (McAuliffe *et al.,* 2004; Pinho *et al.,* 2012; Nicholas *et al.,* 2016). Some examples of such techniques are amplified fragment length polymorphism (AFLP) analysis, random amplified polymorphic DNA (RAPD) analysis, pulsed-field gel electrophoresis (PFGE) (McAuliffe *et al.,* 2004). Insertion sequence typing (IS typing) is another more discriminating typing technique using mobile genetic elements (insertion sequences) present in *M. bovis* DNA (Aebi *et al.,* 2012; Gille, 2018). Many new techniques were created based on the partial genome sequence of *M. bovis*, but all of these techniques are difficult to replicate and lab-specific, making comparisons challenging (Pinho *et al.,* 2012). In order to compare and determine the relationship between *M. bovis* strains, one method for detecting single-point mutations in house genetics is Multilocus sequence typing (MLST) (Register *et al.,* 2015). In addition, another technique based on the complete genome of *M. bovis* is multiple locus variable tandem repetition analysis (MLVA) (Rosales *et al.,* 2015).

2.6 Epidemiology of bovine Mycoplasmosis:

2.6.1 Occurrence:

Bovine mycoplasmosis is a highly contagious and severe pathogenic disease (Pardon *et al.,* 2020). In particular, it can be zoonotic, especially in immunosuppressed patients, and sometimes causes systemic disease (Pitcher and Nicholas, 2005). *Mycoplasma bovis* affects several age groups (pre-birth, post-birth, newborn, and adult) including beef and dairy cattle (Maunsell *et al.,* 2011; Biesheuvel *et al.,* 2024). Vähänikkilä *et al.* (2019) indicated that fattening calves were infected with this type of bacteria a few weeks after their arrival and entry into the field after the weaning period, and the morbidity rate of the disease ranged between 20%-80%, or the mortality rate due to chronic pneumonia from 3% to 50% depends on

severity of signs and secondary bacterial infections, further *M. bovis* has a long duration survive in the flock and pathogens may be released by the infected animals; can release pathogens in a period of weeks to months (Maunsell *et al.,* 2011; Okella *et al.,* 2023). The high mortality rate with outbreaks in the herd is associated with mixed livestock; buffalo, bison, and camels (Bras *et al.,* 2016; Calcutt *et al.,* 2018). Furthermore, Pardon *et al.* (2020) commented that chronic bronchiolitis-infected calves are related to a variety of causes, including *Histophilus somni*, *Mannheimia haemolytica*, *Pasteurella multocida*, all of which are isolated from pneumonia-infected calves caused by *M. bovis*, resulting in increased incidence and mortality of pneumonia-infected calves.

Cows infected with mastitis excrete a large number of these organism, estimated at (105-108 cfu/ml) during the milking process and before the appearance of clinical signs (Gonçalves, 2022; Nobrega *et al.,* 2024). *Mycoplasma bovis* is highly adapted to ruminants and was isolated from buffalo, small ruminants, and this bacterium even isolated from humans (Nicholas *et al.,* 2008; Dawood *et al.,* 2022).

2.6.2 Sources of infection and transmission of the *M. bovis*:

The *M. bovis* isolate from different animals that were act as reservoirs such as sheep and goats (Egwu *et al.,* 2001), rabbits (Rougier *et al.,* 2006), buffalo and camels (Jabbar *et al.,* 2023; Al-AAlim *et al.,* 2023). In Turkey, these bacteria were isolated from the trachea of broilers that were clinically infected with these bacteria that live together with cows (Ongor *et al.,* 2008), as well as humans (Pitcher and Nicholas, 2005). Furthermore, many sources of infection for *M. bovis* include, people working with ruminants are considered an effective source of infection (Dudek *et al.,* 2020). Materials used in sperm collection, artificial

insemination, and freezing of sperm are the main sources of infection (Bagheri *et al.,* 2020). In general, subclinical animals or carrier animals of *M. bovis* is considered the main source of livestock infections (Maunsell *et al.,* 2011). as well as Gille *et al.* (2020) noted that fomites and food and water contamination are sources of infection of the bacteria.

Mycoplasma bovis is transmitted directly or indirectly between animal herds, and is transmitted directly between cows through nose-to-nose contact and milking (Punyapornwithaya *et al.,* 2011). The infection occurs by inhaling small droplets from an infected animal, which is released by coughing, and the sudden occurrence of respiratory diseases, which leads to the infection being transmitted very quickly in the group (Nicholas and Ayling, 2003). The *M. bovis* is excreted into the nasal secretion within 24 hours of infection and can be grown from infected animals seven days later (Caswell *et al.,* 2010). These microorganisms are greatly expelled when cows are infected with lung disease (Deeney *et al.,* 2021). According to Biesheuvel *et al.* (2024), *M. bovis* is quickly spread to all ages when entering a herd. Furthermore, Aebi *et al.* (2015) indicated that the colonization of clinically healthy animals continued and concluded that young animals should be considered as reservoirs. Timsit *et al.* (2012) reported horizontal transmission from old animals to adjacent nests, and the newborn calves can receive infections from old animals and maintain the infection chain of the herd (Haapala *et al.,* 2021).

Gille *et al.* (2020) and Haapala *et al.* (2021) referred to that *M. bovis* enters the udder through the nipples resulting from the contamination of the udder by vaginal secretion, which caused contagious mastitis. Moreover, infected cows can spread billions of organisms in raw milk during clinical outbreaks, which is one of the main sources of suckling-calf infection (Bagheri *et al.,* 2020). Recently, *M. bovis* has also suggested

that colostrum is a source of infection, especially those from cows that have obtained positive results from *M. bovis* DNA (Gille *et al.,* 2020).

Male genitals are also infected by *M. bovis*, either through contamination of the external environment or direct contact with infected females, resulting in orchitis, seminal vesicular inflammation, reduced sperm amount, and excretion (Polo *et al.,* 2023). Female reproductive systems naturally infected with *M. bovis* through an outer environment or artificial insemination by contaminated sperm, and was one of the major problems with adult cow disease transmission (Haapala *et al.,* 2018).

Shastin *et al.* (2022) mentioned that in experimental study, it has been proven that transmission of the disease occurs vertically from the dam to the fetus during gestation. Calcutt *et al.* (2018) stated that the hematogenic spread of *M. bovis* causes respiratory diseases and arthritis in young calves. While Indirect transmission can also occur through public drinking water, feeding consumption, and fomites contaminated by *M. bovis* (Penterman *et al.,* 2022).

2.6.3 Geographical distribution of *Mycoplasma bovis* in Iraq and other countries of the world:

Mycoplasma bovis is a worldwide disease that spreads in North America, South America, Australia, Europe, Africa, and Asia; as a result of animal movements between these countries, the incidence of *M. bovis* varies significantly depending on the livestock and the country (Pardon *et al.,* 2020).

The disease is worldwide geographically distributed and endemic in many countries in Asia such as Iraq (Mahmood and Rhaymah, 2012; Hamad *et al.,* 2019; Al-Alo *et al.,* 2020), Iran (Imandar *et al.,* 2018; Bagheri *et al.,* 2020; Mashhour *et al.,* 2020), Saudi Arabia (Al-Abdullah *et*

al., 2006), Turkey (Akan *et al.,* 2014; Altun *et al.,* 2019), Jordan (Hananeh *et al.,* 2018), India (Jain *et al.,* 2012; Hananeh *et al.,* 2018; Goswami *et al.,* 2019), Japan (Higa *et al.,* 2016; Itoh *et al.,* 2020) and China (Niu *et al.,* 2021) (Table 2-1).

The *M. bovis* is reported in European countries such as Finland (Haapala *et al.,* 2018), Belgium (Bokma *et al.,* 2020), Spain (García-Galán *et al.,* 2021), Sweden (Hurri *et al.,* 2022) and Netherlands (Veldhuis *et al.,* 2023). Furthermore, *M. bovis* is also present in African countries like Sudan (Onsa and Bilal, 2022), Egypt (El-Tawab *et al.,* 2021; Hashem *et al.,* 2022;), and Algeria (Oucheriah *et al.,* 2022). It is documented in some countries in the Americas, such as the USA (Appelt *et al.,* 2019; Thompson *et al.,* 2023) Argentina (Neder *et al.,* 2022), Brazil (Oliveira *et al.,* 2020; Pires *et al.,* 2021) and Mexico (Maya-Rodríguez *et al.,* 2022). The disease is also recorded in Australia (Hazelton *et al.,* 2018; Hazelton *et al.,* 2020) (Table 2-1).

Table 2-1: Prevalence of *Mycoplasma bovis* in different countries with the references

Continent	Country-regions	Number of cattle	Clinical form	Type of sample	Prevalence	Type of tests	References
Asia	Iraq- Mosul	46	Polyarthritis (calves)	Serum	(35/46) 76.09%	i-ELISA	Mahmood and Rhaymah, 2012
	Iraq- Mosul	42	pneumonia (calves)	Lung tissue	(32/42) 86.5%	c-PCR	Hamad *et al.*, (2019)
	Iraq- Al-Najaf	116	Keratoconjunctivitis (calves)	Eye swab	(26/116) 22.4%	PCR	Al-Alo *et al.*, 2020
	Iran- Hamedan	118	pneumonia (calves)	Lung tissue	(9/118) 8.33%	PCR	Mashhour *et al.*, 2020
	Iran	328	Mastitis (dairy cows)	Milk sample	(58/328) 17.69% (97/328) 29.57%	CM PCR (16sRNA)	Imandar *et al.*, 2018
	Iran- Hamedan	125	Mastitis (dairy cows)	Milk sample	(11/125) 8.8 %	n-PCR	Bagheri *et al.*, 2020
	Turkey	127	Broncho-pneumonia calves	tracheal swab	(16/127)12.6% (45/127)35.4%	c-PCR i-ELISA	Akan *et al.*, 2014
	Turkey	120	Mastitis (dairy cows)	Milk sample	(28/120) 23,3%	q-PCR	Altun *et al.*, 2019
	Saudi Arabia	1350	Mastitis (dairy cows)	Milk sample	(20 /1350) 1.48%	CM	Al-Abdullah *et al.*, 2006
	Jordan	220	arthritis and pneumonia calves	Synovial fluid and lung tissue	(60/220)27.27% (136/220)61.7%	PCR	Hananeh *et al.*, 2018
	India	384	Mastitis and bronchopneumonia	Milk, semen, nasal, and vaginal dis.	26.3% (101/384)	PCR	Jain *et al.*, 2012

	India	51	Healthy cattle	Lung tissue and milk	(1/51) 2%	RT- PCR	Behera *et al*., 2018
	India	87	Bronchopneumonia calves	Lung tissues	(16/87) 18.3%	PCR	Goswami *et al.*, 2019
	Japan	55	Bronchopneumonia (calves)	Nasal swab	(33/55) 60%	LAMP-PCR	Higa *et al.*, 2016
	Japan	16	Mastitis	Milk sample	(8/16) 50%	LAMP-PCR	Itoh *et al.*, (2020)
	China	959	Pneumonia (calves)	Serum	(467/959)48.7%	i-ELISA	Niu *et al.*, 2021
Europe	Finland	75	Mastitis	Milk	(3/30) 10.0% (4/45)8.9%	CM, m-PCR	Haapala *et al.*, 2018
	Belgium	100	Pneumonia (calves)	Bronchoalveolar lavage fluid	(38/100) 38%	CM	Bokma *et al.*, 2020
	Spain	23	Pneumonia (calves)	Lung swab	(20/23) 86.9%	PCR	García-Galán *et al*., 2021
	Ireland	1313	Mastitis	Milk sample	(588/1,313) 44.78%	i-ELISA	McAloon *et al*., 2022
	Swed	3,144	Bronchopneumonia calves Mastitis cows	Nasal swab, milk	(147 /3,144) 4.8% (0/3,144) 0%	i- ELISA RT- PCR	Hurri *et al*., 2022
	Netherlands	415	Mastitis	Serum	(290/415)69.9%	i-ELISA	Veldhuis *et al*.,2023

Africa	Egypt	200	Bronchopneumonia and keratoconjunctivitis (calves)	nasal swabs, oral and conjunctival swabs	(40/200) 20%	PCR	Abdeen *et al.*, 2017
	Egypt	305	Bronchopneumonia (cattle)	Nasal, trachea swabs and lung tissues	(206/305)67.5% (7/20)35.0%	CM c-PCR	El-Tawab *et al*., 2021
	Egypt	60	Bronchopneumonia (calves)	Nasal swabs	(5/60)8.33%	PCR	Hashem *et al*., 2022
	Sudan	180	Bronchopneumonia (cattle)	Serum	7.2% (13/180)	ELISA	Onsa and Bilal, 2022
	Algeria	351	Bronchopneumonia (calves)	serum and Bronchoalveolar lavage fluid	(241/351)69.0% and (102/351)58.0%	i-ELISA and RT-PCR	Oucheriah *et al*., 2022
America	USA	23	Mastitis dairy cattle	Milk	(13/13)100% and (14/16)87.5%	LAMP And q-PCR	Appelt *et al.,* 2019
	Brazil	35	Bronchopneumonia Adult cattle	Lung tissue	(16/35) 45.71%	IHC	Oliveira *et al.,* 2020
	Brazil	400	Bronchopneumonia cows	Serum	(249/400) 62.3%	i-ELISA	Pires *et al*., 2021
	Argentina	35	Mastitis cows	Milk	(3/35) 7.9%	m-PCR	Neder *et al*., 2022
	Mexico	335	with and without respiratory disease dairy cows	Nasal swabs	(43/101) 42.57%	m-PCR	Maya-Rodríguez *et al*., 2022

	USA	226	mastitis dairy cows	Milk	63%	MALDI-TOF MS	Thompson *et al.*, 2023
Australia	Australia-western	474	Sub clinically carrier cattle	Milk, eye, and vaginal swabs	93.8% (15/16) and 18.8% (3/16)	CM and PCR	Hazelton *et al.*, 2018
	Australia	16	Sub clinically carrier cattle	serum, eye, nose, and vaginal swabs	23.0% (111/474) and 27.0% (102/474)	i-ELISA and PCR	Hazelton *et al.*, 2018

(i-ELISA) Indirect Enzyme-linked immunosorbent assay; (c-PCR) Conventional polymerase chain reaction; (qPCR) Quantitative polymerase chain reaction; (RT-PCR) real-time polymerase chain reaction; (m-PCR) Multiplex polymerase chain reaction; (N-PCR) nested- polymerase chain reaction; (LAMP-PCR) Loop-Mediated Isothermal Amplification- polymerase chain reaction; (MS) mass spectrometry; (CM) Culture method; (IHC) immunohistochemistry; (MALDI-TOF MS) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

2.6.4 Risks factors of *Mycoplasma bovis*:

The risk factors in livestock farms are usually predisposing factors for disease transmission on the farm, such as herd size, age, sex, and older animals in the neighboring barn (Timsit *et al.,* 2012). In addition, risk factors that influence the entry of diseases into agriculture, such as imported animals, transportation, management practices, and environmental and climate factors (Gaudino *et al.,* 2022). Both Schibrowski *et al.* (2018) and Aebi *et al.* (2015) referred to water sources and feeding between pens as a risk factor, stress factors such as feeding rot and overpopulation are related to the outbreak of *M. bovis*. A combination of risk factors can increase animal sensitivity to bovine respiratory diseases (BRDs), while a single risk factor cannot lead to BRDs (Sultana, 2023).

2.6.4.1: Risk factors related to the cattle:

Several studies indicated that cattle age plays an important role in increasing the sensitivity of livestock to *M. bovis* infections (Nicholas, 2004; Maunsell *et al.,* 2011; Askar et al., 2021; Szacawa *et al*., 2016; Suzuki *et al.,* 2024). Niu *et al.* (2021) and Rifatbegović *et al.* (2024) stated that the high incidence of calves (6-24 months) infection is possibly due to dropping maternal immunity, the introduction of new calves from previously infected farms, overcrowding, and strong stress due to transportation. Haapala *et al.* (2021) added that the overcrowding of the farm's livestock barns, visitors, and veterinarians contributed to the increase in the rate of infection. On the other hand, Soehnlen *et al.* (2012), Akan *et al.* (2014), and Prysliak *et al.* (2018) mentioned that bovine mycoplasmosis is a particularly important cause of newborn pneumonia under the age of 6 months due to vertical transmission of infection, feed of infected cow colostrum, contaminated milk substitutes, indicating a

continued infection. Further, small calves can be infected by older calves in the grazing areas and by adults in the calving areas. Moreover, Dudek and Szacawa et al., (2016), and Suzuki *et al.* (2024) stated that older cattle over 3 years of age, it is observed that the incidence of *M. bovis* is higher, possibly due to chronic infections, teat-contaminated infections or imported bovine semen, which can be the origin of *M. bovis* infection. While Aebi *et al.* (2015) found that all ages of cattle are susceptible to *M. bovis* infection.

Some studies have shown that ox are thought to be more likely to have an infection with *M. bovis* than females, this might be due to high trade, a lack of strict quarantine, and drug resistance, which is growing worldwide and few commercial vaccines have been developed to protect against *M. bovis*, and it has been difficult to eradicate it (Hazelton *et al.,* 2018; McAloon *et al.,* 2022). Whereas Gogoi-Tiwari *et al.* (2022) mentioned that the spread of *M. bovis* is significantly higher in females than in males, possibly due to low resistance during pregnancy and birth associated with immunological and hormonal changes. In addition, increasing the number of females in the herd in comparison to males in reproduction and milk production, as well as staying the longer duration of females in the herd and the more stress of reproduction, calving, and lactation, can increase the prevalence of *M. bovis* in cows (Tambuwal *et al.,* 2011; Francis *et al.,* 2015). On the contrary, Niu *et al.* (2021) found that there was no Significant variation in the spread of *M. bovis* between the male and female cattle. In addition, other studies found no significant difference between pregnant and non-pregnant cows infected with *M. bovis* (Fu *et al.,* 2011; Hazelton *et al.,* 2020).

The detection of the source of infection in calves and contagious mastitis in cows could be related to the importation and frequent

introduction of new animals of unknown health status into the country or farms, leading to a high risk of spread of the disease from endemic countries (fox, 2012; Dudek and Szacawa *et al*., 2016). On the contrary, imported species have a significant risk factor compared to local breed (Siugzdaite *et al.,* 2012; Haapala *et al.,* 2021).

2.6.4.2 Risk factors related to countries or regions, management, and seasons:

The prevalence of *M. bovis* in cattle differs depending on the country or region, which can be due to the presence of additional factors such as different management approaches, environmental conditions, efficient diagnostic techniques, types of samples tested, host age, physical characteristics, and immune status (Calcutt *et al.,* 2018; Ammar *et al.,* 2022; Oucheriah *et al.,* 2022; Penterman *et al.,* 2022; Okella *et al.,* 2023; Sorin-Dupont *et al.,* 2023).

In addition, there is a correlation between the size of the herd and the risk of infection by *M. bovis*; large herds are more susceptible to infection than small herds, and the spread of large herds may be due to overpopulation, poor management practices, and the arrival of new infected calves in the herd. (McAloon *et al.,* 2022; Lysnyansky *et al.,* 2016; Nicholas *et al.,* 2016). On the other hand, Rifatbegović *et al.* (2024) found that the infection by *M. bovis* in smaller herds is greater than in large herds may be because farmers often buy less productive cows from larger farms and lack quarantine or other prevention and control measures. Regardless of the size of the herd, outbreaks of bovine mycoplasmosis are more common in herds that buy new bulls and cows, use shared pastures, livestock movements, and neglect vaccines that have recently been acquired (Haapala *et al.,* 2018; Wennekamp *et al.,* 2021).

There are several environmental risk factors affecting *M. bovis* infection, such as weather conditions, temperature difference, rainfall, moisture, airflow, and dust particles containing Mycoplasma (Snowder, 2007). Zinabu *et al*. (2018) mentioned that climatic play important role of prevalence of *M. bovis* disease. Furthermore, changes in wind and temperature during September and November were associated with a higher prevalence of bovine mycoplasmosis. Furthermore, Gille *et al*., (2020) reported that temperatures below 0 to 15 °C in the low-temperature range are the most predisposing risk factors for getting sick or transmitting *M. bovis*. However, when temperatures drop, younger animals under the age of one month have a limited range of comfort (Calcutt *et al.,* 2018).

Livestock may become stressed as the surrounding temperature falls especially in the colder months, depending on additional climatic factors including relative humidity, wind speed, rainfall, and wind (Zinabu *et al.,* 2018). In addition, Hashem *et al.* (2022) reported in their study the highest incidence of *M. bovis* in calves between December and March, with infected calves showing signs of respiratory manifestations. Jordan *et al.* (2021) found that the spread of *M. bovis* was significantly higher in the autumn and winter of each year and then declined in other months of the year.

2.7 Immunity against *Mycoplasma bovis*:

Mycoplasma bovis causes host immunosuppression, destroying neutrophils and lymphocytes, and causing apoptosis by producing special proteins called p26, which distinguishes the bacteria from other species of the genus Mycoplasma. (Maina, 2019; Démoulins *et al.,* 2024). *M. bovis* affects the immune system by changing the function of neutrophils, lymphocytes, and macrophages, and increasing the secretion of cytokines,

in spite of this, cows are able to produce cellular and humoral immunity against these bacteria; as they produce IgG1 antibodies for the specific antigen of these bacteria mainly as well as produce IgG2 antibodies in smaller quantities (Gonçalves, 2022). Askar *et al.* (2021) added that several important factors affecting the capability of *M. bovis* can also avoid host immune defense mechanisms**.** *M. bovis* has different surface antigens that may affect immune responses; in addition, exposure to specific antibodies can lead to the choice of new resident surfaces lipoproteins and biofilm production (Maunsell and Chase, 2019).

2.8 Relationship between *Mycoplasma bovis* and other pathogenic organisms:

Mycoplasma bovis is usually associated with other microorganisms such as bovine viral diarrhea virus (BVDV), infectious bovine rhinotrachitis virus (IBRV), Parainfluenza type 3 virus (PI-3), bovine adenovirus, bovine respiratory syncytial virus (BRSV), *Pasteurella multocida*, *Mannheimia haemolytica*, *Arcanobacterium pyogenes*, *Haemophilus somni*, *Ureaplasma diversum*, *Mycoplasma canis*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Mycoplasma dispar*, all these bacteria commonly change the clinical symptoms of infections caused by *M. bovis* (Pardone *et al.,* 2020; Hashem *et al.,* 2022). The Major bacteria are present in the upper airway of livestock, and further stress or viral infection reduces the animal's immune response, allowing opportunist germs to be colonized in the pulmonary tract and inhaled into the lungs, and lead to bronchopneumonia (Hashem *et al.,* 2022).

2.9 Pathogenesis of *Mycoplasma bovis* infection:

In adult ruminants, generally, *M. bovis* is normally present on the mucous membrane of the conjunctiva, nasal cavity, trachea, oropharynx, urinary and genital tracts of both sexes, and the digestive system without clinical signs or antibodies being excreted through nasal secretions; however, they can become infectious and cause major diseases in dairy herds (Maunsell *el al.,* 2011; Munoz, 2020; Torres et al, 2022). The pathogenicity of *M. bovis* is known to be accountable for critical complications and economic losses to livestock because it has many virulence properties, including variable surface lipoproteins (Vsps), adhesion, invasion of host cells, modulation of host immune systems, production of minor metabolites, and the production of biofilms (Bürkie *et al.,* 2015; Pereze-Casale, 2020). One of the first steps in the infection of *M. bovis* is bound to tracheobronchial epithelial cells of bovine cattle and facilitates pulmonary colonization, it is mediated by membrane proteins, including Vsps, the family of immune-resident lipoproteins on the bacterial surface, and non-relative proteins such as P26 and pMB67 (Thomas, 2003; Guo *et al.,* 2017). Being colonized, *M. bovis* invades host immunity and distributes the infection to different host sites, like the pulmonary tract, the mid-ear, joints, the lymph node, and the mammary glands (Ammar *et al.,* 2021; Gelgie et al., 2024). Variable surface lipoproteins (VSPs) are accountable for the very variable antigenic characteristics of *M. bovis*. (Bürki *et al.,* 2015; Khan *et al.,* 2017). Furthermore, Variations in antigens (VSPs) by phenotypic modulation of primary surface lipid proteins in the immune system that possibly result in changes in host immune reactions can cause the survival of *M. bovis* and developing chronic infection (Gagea, 2006; Wang *et al.,* 2023). *M. bovis* also produces minor metabolites, like hydrogen peroxide (H2O2), which destroy host cells, the

quantity of (H2O2) production differs depending on the isolate (Khan *et al.,* 2017). Finally, the biofilm produced by *M. bovis* contributes to the persistence of this pathogen and increases its resistance to dehydration and heat pressure (Ammar *et al.,* 2021).

2.10 Clinical forms of bovine Mycoplasmosis:

Several factors influence the severity of clinical forms of bovine mycoplasmosis such as bacterial virulence, host age, overcrowding, weaning, immune status, and presence of concurrent infections of other enteric viral, bacterial, and parasitic infections, as well as environmental factors, seasons, all of these factors play an important role in the spread, occurrence, and severity of clinical forms of cattle (Gille, 2018; Loy *et al.,* 2021; Ferrulli, 2023).

There are various clinical forms of bovine Mycoplasmosis, including bronchopneumonia, polyarthritis, contagious mastitis, keratoconjunctivitis, otitis media, and reproductive disorders (Ammar *et al.,* 2021; Priyantha *et al.,* 2021; Shil *et al.,* 2022). These forms range from mild to severe, depending on the development of clinical signs (Deeney *et al.,* 2021).

2.10.1 Respiratory form:

The respiratory form of *M. bovis* can progress in livestock at all ages and can affect newborns up to 5 days after birth (Prysliak *et al.,* 2023). This illness is marked by fever, loss of appetite, dyspnea, depression, cough, and nasal discharge (Cantón *et al.,* 2022). In most cases, *M. bovis* causes bronchopneumonia in newborn calves between 14 to 42 days of age (Stipkovitse *et al.,* 2000; Maunselle and Donovan, 2009; Gelgie *et al.,* 2024). The correlation with the bovine viral diarrhea virus can aggravate

clinical symptoms (Shahriare *et al.,* 2002; Gageae *et al.,* 2006). *M. bovis* can reduce immune reactions and can also work as predisposition factors for infecting or worsening symptoms of other respiratory pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, Bovine respiratory syncytial virus. Also, *M. bovis* is part of the bovine respiratory disease complex (BRD) (Caswelle and Archambaulte, 2008; Lion *et al.,* 2021). Pneumonia caused by *M. bovis* is related to four principal kinds of lesion types: caseonecrotic bronchopneumonia, suppurative bronchopneumonia without necrosis, bronchopneumonia with foci of coagulation necrosis, and chronic bronchopneumonia with subsequent abscessation (Hananeh *et al.,* 2018; Cantón *et al.,* 2022).

2.10.2 Keratoconjunctivitis form:

Keratoconjunctivitis is primarily caused by *M. bovis* in cattle (Kuibagarov *et al.,* 2023). It is associated with other Mycoplasma species, such as *M. conjunctivae* (Loy *et al.,* 2021). *M. bovis* is a predisposing factor for the rapid spread of keratoconjunctivitis in the herd in association with *Moraxella bovis* and the infectious bovine rhinotrachitis virus (IBRV) that increases the severity of the disease (O'Connor *et al.,* 2019). Furthermore, there are many clear clinical signs of keratoconjunctivitis in cows including bilateral conjunctivitis, tearing, photophobia, and keratitis (Loy *et al.,* 2021; Gupta *et al.,* 2023). Keratoconjunctivitis is often called ‘‘Pinkeye’’ because of the characteristic reddening and inflammation of the conjunctiva of the eyelid and eyeball, predisposing causes such as intense sunlight, dust, and grass seeds are factors in the infection (Raman *et al.,* 2024).

2.10.3 Polyarthritis form:

Arthritis related to *M. bovis* is more common in calves, and this form has also been recognized in adult cows ((Maunselle and Donovane, 2009; Mahmood and Rhaymah, 2012; Gogoi-Tiwari *et al.,* 2022). Animals affected have acute lameness with enlarged joints and tendons sheaths and fever (Mahmood and Rhaymah, 2012; Cantón *et al.,* 2022). Large rotator joints such as shoulders, elbows, and knees are often affected (Maunsell and Donovan, 2009). At opening, necrofibrinous to granulomatous synovitis, bursitis, and tenosynovitis can be noted in arthritis of different degrees of severity markedly swelling in both limbs (Hananeh *et al.,* 2018). Under experimental situations, ulcerations of the knee distal planum and secondary synovial sac ruptures were observed (Desrochers and Francoz, 2014). As a result, affected calves by *M. bovis* arthritis generally develop lesions in other organs, like the lungs or udders (Cantón *et al.,* 2022). Furthermore, Hananeh *et al.* (2018) reported that *M. bovis* could persist in the joints for as long as four weeks.

2.10.4 Mastitis form:

Mycoplasma bovis is the main reason for highly contagious mastitis in adult cows, causing a reduction in milk yield, mainly in big dairy farms (Rifatbegović *et al.,* 2024). In infected animals, the incubation period usually takes between 2 and 10 days before mastitis is observed, through this time shedding can actually happen (Al-Farha *et al.,* 2017). Mycoplasmal mastitis is clinically characterized by a change in the consistency of milk, a sharp decrease in milk yield, and resistance to therapy (Rifatbegović *et al.,* 2024). Infection can affect several quarters because the bacteria are able to spread hematogenously (Okella *et al.,* 2023). Suzuki *et al.* (2024) noted that the consistency of the milk can differ

from watery to purulent, although a watery milk with some sandy fibrinous sediments is highly characteristic of the disease. Furthermore, affected animals may appear clinically normal, although the udder is seriously damaged, and can remain a sub-clinical holder of the bacteria for longer time intervals, as released the *M. bovis* is intermittently (Nicholase *et al.,* 2016; Timonene *et al.,* 2017). Increased somatic cell counts (SCC) accompanied by subclinical carriers are manifested by decreased milk yield, and decreased milk fat content (Al-Farhae et al., 2017; Timonene *et al.,* 2022). It's interesting, that *M. bovis* has also been shown to induce mastitis in pre-pubertal heifers, resulting in udder nodules and dehydration in one quarter or more at the first lactation (Foxe *et al.,* 2008). Gogoi-Tiwari *et al.* (2017) stated that histologically acute *M. bovis* mastitis is manifested by alveolar epithelial degradation with the Leukocyte exudation. whereas, in chronic cases, plasma cells begin to invade the interalveolar space, with gradual fibrous hyperplasia of the milk canals and alveoli atrophy of the udder or possibly the presence of an abscess.

2.10.5 Genital form of *Mycoplasma bovis*:

Mycoplasma bovis was isolated from the semen of the bulls infected with these bacteria, as it caused major seminal vasculitis, epididymitis, and orchitis (Oksay and Diker 2023). In addition, *M. bovis* combined with *M. bovigenitalium,* it also causes reproductive disorders in cows such as hysteritis, oviductitis, and vulvovaginitis, which also cause abortion (Pohjanvirta *et al.,* 2023). Trautwein *et al.* (2012) mentioned that *M. bovis* was isolated from the genital tracts of cows infected with vulvovaginitis and uterine inflammation. Clinically infected cows are characterized by increased granules or papules and abnormal purulent secretions in the lining of the vagina and uterus, and the infection usually lasts 3 to 10 days, after which it disappears spontaneously (Ghanem *et al.,* 2013). These

bacteria also cause abortion at the last stages of pregnancy. The cause of abortion is either from infection of the placenta or from infection of the foetus with pneumonia (Guo *et al.,* 2014). Infected cows and bulls shed these germs intermittently, so it is difficult to diagnose them by isolation (Guo *et al.,* 2017).

2.10.6 Otitis media form of *Mycoplasma bovis*:

Mycoplasma bovis is a reason of otitis media in so many cases that the disease has been called a "*Mycoplasma bovis* - related disease", as well as arthritis and pneumonia, and may be an index of the presence of *M. bovis* on the field (Maunsell and Donovan, 2009; Lotfollahzadeh *et al.,* 2024). Clinically infected Calves are most often, lie down more frequently, and usually show a unilateral or bilateral ear prolapse with a head tilting, fever, and epiphora (Foster *et al.,* 2009). In addition, it causes chronic weight loss, and waste and can lead to internal otitis and meningitis with temporal bone abscess if not treated. (Peek *et al.,* 2018; Maedae *et al.,* 2003). Histologically, fibrinosuppurative to caseous exudate is seen in the affected tympanic bullae (Gille, 2018).

2.11 Diagnosis:

In the diagnosis of Mycoplasma, a comprehensive physical examination and collection of medical history must be performed. These are the first steps in the diagnosis of Mycoplasma, although transmission of infection can occur in the herd often through inhalation of respiratory drops, so it is common to have a history of exposure to livestock. In addition, most cattle will show mild clinical symptoms (Schwartz, 2023). Gütgemann *et al.* (2023) stated that the detection of Mycoplasma is based on the golden standard; cultured and isolation of this bacterium from mucous surfaces, secretions, or tissues (Scott *et al.,* 2022). Followed by

serological tests such as enzyme-linked immunosorbent assay (ELISA) (Francis *et al.,* 2015), immunohistochemistry (IHC) (Hermeyer *et al.,* 2012), direct fluorescent antibody technique (DFAT) (Dudek *et al.,* 2022), immunobinding test (IBT) (Okella *et al.,* 2023) and immunoblotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Xu *et al.,* 2022). Furthermore, molecular identification using several molecular techniques for *M. bovis*, including conventional polymerase chain reaction (c-PCR) technique (Molla Kazemiha *et al.,* 2016), real-time PCR (qPCR) technique (Naikare *et al.,* 2015), nested PCR (Molla Kazemiha *et al.,* 2016) and isothermal amplification, loop-mediated (LAMP), multiplex PCR (mPCR) (Ashraf *et al.,* 2018).

2.11.1 Bacterial Isolation (Culture):

Symptoms and lesions are the result of infection with *M. bovis* that is not sufficiently differentiated, so laboratory tests such as bacterial isolation and immunological tests must be performed to confirm infection (Priyantha *et al.,* 2021). Sampling sites are chosen according to the changes observed in the diseased animal, and swabs that are taken from the conjunctiva, genital, nasal secretions, milk samples, and joint fluids must be taken in a liquid nutrient medium that provides the needs for its sufficient growth. While milk samples, synovial fluid, semen, and other liquid samples can be grown on solid culture media in the laboratory. All samples obtained should be taken in the early stages of disease and directly transferred to the lab using Mycoplasma transport media (Petersen *et al.,* 2020). Bacterial isolation is an important method for the detection of Mycoplasma infection, as it is confused in its symptoms with many other causes of respiratory diseases in cattle (Yatoo *et al.,* 2018). The cultures of upper respiratory tract samples can contain growth from normal flora of healthy animals and should be evaluated with caution (Woolums, 2019). In

cattle with more severe clinical symptoms or evidence of a tracheal wash or bronchoalveolar lavage samples are recommended (Petersen *et al.,* 2020).

There are many culture media suitable for isolation, such as (Medium B, Hayflick's medium, Friis medium, and PPLO medium). These media contain a rich protein source such as horse serum, heart infusion brain, glucose, yeast extract, pyruvate, and selective substances such as (Ampicillin, Thallous acetate, and Amphotericin -B). Inoculated Mycoplasma medium is incubated at 37℃ with 5% Co2 in a candle jar for up to 14 days (Willey *et al.,* 2023).

Colonies typically form inclined microcolonies when indirectly lighted and microcolonies with a "fried egg appearance " in reflected light (Figure 2.2), and the thickened center area results from the stretching of the microcolonies in the agar (Figure 2.3). Therefore, recognition of microcolonies was facilitated when stained with Dienes stain, which stained the central zone dark blue and lighter blue in the peripheral (Quinn *et al.,* 2016).

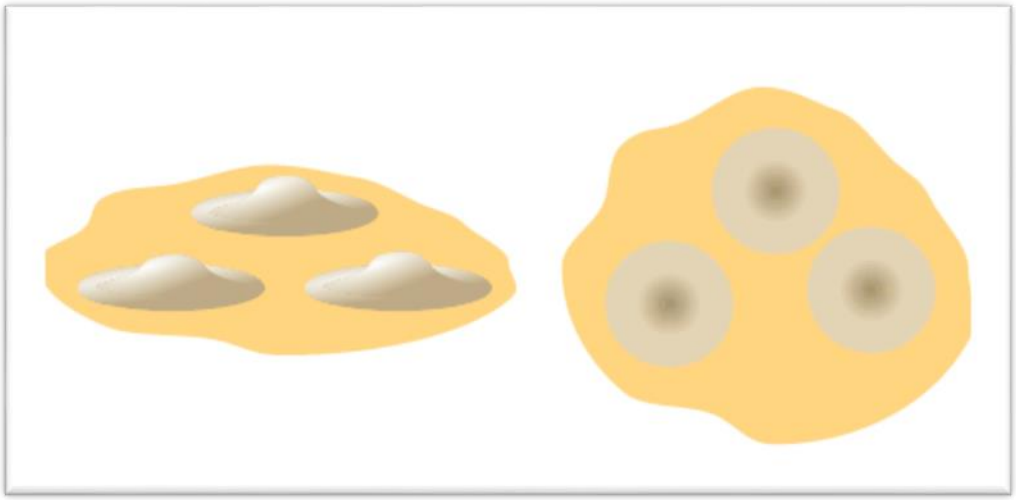

Figure (2.2): The appearance of Mycoplasma microcolonies as oblique illumination, have an umbonate, and a "fried egg" in transmitted light. (Quinn *et al.,* 2016).

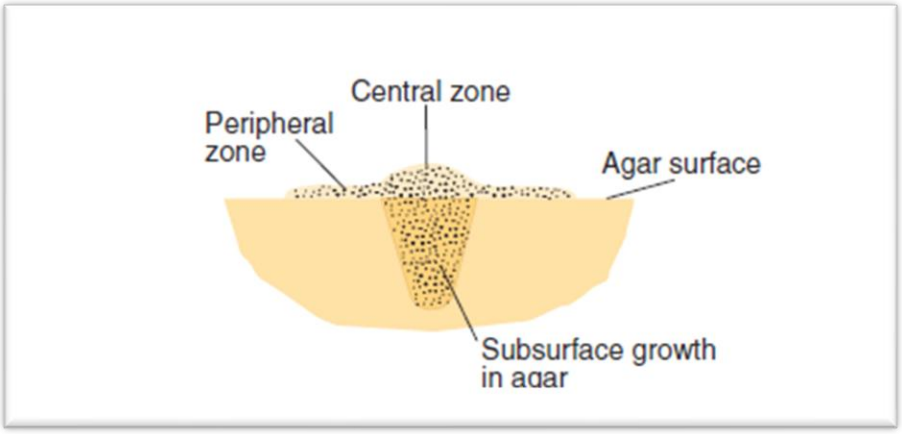

Figure (2.3): A section through a Mycoplasma microcolony on agar showing surface and subsurface growth. (Quinn *et al.,* 2016).

The culture method is more complex and time-intensive, shows low specificity, and, in some cases, low sensitivity, and freezing stored significantly reduces its sensitivity (Biddle *et al.,* 2004; Pinnow *et al.,* 2001). Detection of *M. bovis* pathogens using isolation is tedious, time-intensive, and difficult to accomplish. Furthermore, detecting *M. bovis* by serologic approaches is a challenge as a result of potential cross-reactivity (Okella *et al.,* 2023).

2.11.2 Microscopic examination:

Since direct microscopy is not commonly used for making a preliminary diagnosis of *Mycoplasma spp.*, the examination of direct smears from the Mycoplasma broth medium after incubation were stained with Gram's stain resulting in Mycoplasma appearing as small polymorphic Gram-negative bacteria (Tille, 2022).

2.11.3 Enzyme-linked immunosorbent assay (ELISA test):

Serological techniques are used to detect *M. bovis* antibodies in milk, plasma, or serum samples two weeks' post-infection, as the antibodies remain high for several months (McAloon *et al.,* 2022). Serology is a credible approach in flocks that make high use of antibiotics, while chronic infection often requires the isolation of *M. bovis* (Okella *et al.,* 2023). Two various serological tests are available, including the enzyme-linked immunosorbent test (ELISA) for antigen detecting, which is a less expensive, fast, and accurate diagnostic technique for identifying the occurrence of *M. bovis* in clinical specimens, so that the enzyme is released after specific antibodies used in this test are bound and recognized to specific antigens on the surface of Mycoplasma (Scott *et al.,* 2022). The second one is Antibody- ELISAs are also available but primarily detect antibodies after any exposure, instead of indicating present or active infection (Szacawa *et al.,* 2016). Furthermore, Petersen *et al.* (2020) mentioned that during an outbreak, individual antibody responses are dynamic, vary between cows, and generally decrease between 8 and 9 weeks after the onset of clinical symptoms. However, infected cattle can shed *M. bovis* for several months (Punyapornwithaya *et al.,* 2011; Hazelton *et al.,* 2018). Herd-level diagnosis requires serial tests on several cattle by

using serology and polymerase chain reaction (PCR) (Szacawa *et al.,* 2016; Vähänikkilä *et al.,* 2019).

2.11.4 Polymerase Chain Reaction (PCR) techniques:

DNA-based methods are becoming the diagnostic method of choice; DNA-based assays, specifically polymerase chain reaction (PCR), provide fast and more specific detection of *M. bovis* when compared to culture and serological approaches, and results are obtained in just a few hours (Thézé *et al.,* 2023). Therefore, allow for immediate procedures, such as the removal of affected cattle from a herd. Furthermore, PCR approaches can accurately amplify *M. bovis* DNA, which promotes the determination of Mycoplasma species (Wisselink *et al.,* 2019). Furthermore, when using the conventional PCR technique, it is possible to detect multiple species as well as uncultivable or unknown species (McAuliffe *et al.,* 2005).

However, the disadvantage related to the PCR technique is the expensive cost, which includes conventional PCR, multiplex PCR, nested PCR, and real-time PCR (Hazelton *et al.,* 2020). The PCR techniques are still used for the diagnosis of *M. bovis* (Timonen *et al.,* 2020). Furthermore, multiplex PCR variants of conventional PCR, it utilizes multiple paired primers for the simultaneous amplification of multiple target sequences in a single reaction tube (Shang *et al.,* 2020). However, the *uvr*C gene was the most favorable goal for the detection of *M. bovis*, followed by the 16S rRNA gene showing a small level of variability to distinguish between the most closely related Mycoplasma species such as *Mycoplasma bovis* and *Mycoplasma agalactiae* (Motaung *et al.,* 2017; Green and Sambrook, 2019).

2.12 Advantages and disadvantages of *Mycoplasma bovis* diagnostic tools:

Parker *et al.* (2018) mentioned that there are several advantages and disadvantages to each method (culture method, PCR technique, and antibody ELISA test) to diagnose *M. bovis* in terms of accuracy, speed of completion, and cost (Table 2-2).

Table 2-2: Advantages and disadvantages of *Mycoplasma bovis* diagnostic tools (Parker *et al*., 2018)

	Culture	**PCR**	**Antibody ELISA**
Advantages	Inexpensive (costs may vary between countries and laboratories)	Organism does not have to be viable as it targets the DNA of the organism	Measures antibody response, therefore animal does not need to be shedding the organisms at the time of sample collection
	Can detect most Mycoplasma species	Quick diagnosis turnaround of several hours	Only blood or milk sample required to assess immune response
		Can discriminate between different Mycoplasma spp.	Longevity of antibody expression is possibly several months
		Can discriminate Mycoplasma spp. from Acholeplasma spp.	
Disadvantages	Fastidious growth requirements	Higher cost	Uncertainty around cross-reactivity with other organisms
	Diagnosis turnaround of up to 10 days	Many mycoplasma PCRs are species specific, therefore eliminating the detection of other species	Seroconversion may take 2–3 weeks before antibodies can be detected
	Unable to discriminate between Mycoplasma spp. and Acholeplasma spp., which may lead to false positives	Animal must be shedding the organism at the time the sample was taken	Suggestions of poor sensitivity
	Unable to discriminate between different Mycoplasma species19	Identification of non-viable organisms may lead to insignificant positive results	
	Organism must be viable, therefore storage and handling of the sample is important		
	Animal must be shedding the organism at the time of sampling		

2.13 DNA sequencing and phylogenetic analysis:

Discovering the nucleic acid sequence, or the arrangement of nucleotides in DNA, is the process of DNA sequencing. The four bases—adenine, guanine, cytosine, and thymine—are arranged in this way using any technique or technology (Rizzo and Buck, 2012). Studies in the fields of biology and medicine have been significantly advanced by the development of rapid DNA sequencing techniques (Behjati and Tarpey, 2013; Tille, 2022). The information necessary for living to survive and reproduce is encoded in the DNA sequence. Therefore, understanding exactly the sequence is helpful for both applied and basic studies of the causes and mechanisms of living (Heather and Chain, 2016). Given the central role that DNA plays in all living things, knowing DNA sequences is helpful in almost every field of biological research, DNA genographic projects, and a wide range of applied fields; for instance, it can be applied to medicine to identify, diagnose and virology, forensic biology, and possibly even create treatments for genetic diseases (Henkin and Peters, 2020). Similar to this, the study of pathogens may help develop drugs to treat contiguous disorders. A growing field, biotechnology has the potential to produce a wide range of beneficial products and services (Singh and Singh, 2023). According to Chmielecki and Meyerson (2014), comparing DNA sequences of healthy and mutant DNA can be used to identify a variety of diseases, including various malignancies, characterize an antibody repertoire, and even help patients receive treatment (Pekin *et al.,* 2011). Quick DNA sequencing enables the identification and description of more organisms, as well as allowing faster and more specific medical care (Abate *et al.,* 2013; Straiton *et al.,* 2019).

A phylogenetic tree, also known as an evolutionary tree, describes the evolutionary relationships between many biological species or living things that are believed to have originated from a single common ancestor (DeSalle *et al.,* 2021). The terms "phylogenetic" and "phylogeny" are derived from the ancient Greek words "phûlon" and "genesis," respectively, which signify "race, lineage," "origin," and "source." (Staton, 2015). Also, it's a branching diagram or tree demonstrating the evolutionary relations among distinct biological species or other things based on similarities and differences in their physical or genetic traits. Furthermore, one evolutionary tree represents all life on earth and shows their shared ancestry (Xia and Sun, 2023). The lengths of the branches in the phylogenetic tree represent estimates of time, and each node and its branches in the tree represent the most recent common ancestor of its offshoot (Hoffmann *et al.,* 2021). A taxonomic unit is the term used to describe each node. Because they cannot be directly viewed, internal nodes are frequently referred to as hypothetical taxonomic units (HTUs) (Baum, 2008; Scott and Baum, 2016). Three kingdoms of life, are represented by a phylogenetic tree based on rRNA genes: bacteria, archaea, and eukaryotic (Choudhuri, 2014). Biology disciplines such as bioinformatics, systematics, and phylogenetics all benefit from the use of trees (Xia and Sun, 2023).

2.14 Treatment of *Mycoplasma bovis*:

In the past, Lysnyansky and Ayling (2016) and Ammar *et al.* (2022) have reported that *M. bovis* is resistant to therapy with any chemotherapeutic drug. In particular, Mycoplasmal mastitis is considered to be a cause of culling, however, it is not recommended for treatment (Nicholas *et al.,* 2016). Until vaccines are ready, the utilization of

antibiotics is the only potential interference after introducing *M. bovis* into the flock (Gelgie *et al.,* 2022).

Certain categories of antimicrobial agents, particularly macrolides and phenolic derivatives, have been proven to have a significant impact in reducing respiratory disease caused by *M. bovis* (Bartram *et al.,* 2016; Lysnyansky and Ayling, 2016). In addition, antimicrobial therapy for otitis media has been proven to lead to a 75% clinical cure rate (Gosselin *et al.,* 2012). Only a few types of antimicrobials, namely fluoroquinolone and aminoglycosides like neomycin and gentamicin, have known bactericidal actions on *M. bovis* (Ammar *et al.,* 2022). According to recent guidelines on antimicrobial overuse in veterinary practice, fluoroquinolones must be utilized sparingly and only wherever bacteria are resistant to a particular antimicrobial, considering their great importance in human medicine (WHO, 2017; AMCRA, 2018). All other groups of antimicrobials are bacteriostatic slow down *M. bovis* and allow the host to kill the bacteria (Lysnyansky and Ayling, 2016). In addition, Cai *et al.* (2019) mentioned that in experimental or field studies, Spectinomycin, florfenicol, and tulathromycin have been found to be effective on *M. bovis* pneumonia. As such, this agent should be used first, with chlortetracycline, doxycycline, oxytetracycline, tilmicosin, tulathromycin, or tylosin as second choice (AMCRA, 2018).

2.15 Prevention and control of *Mycoplasma bovis*:

Treatment of diseases caused by *M. bovis* is hard and mostly unsuccessful. Therefore, preventing the illness and controlling its prevalence in affected fields must be the prime concern (Calcutt *et al.,* 2018). Multiple scientific teams have attempted, with limited success, to develop an active vaccine to prevent or minimize the risk of disease caused

by *M. bovis* in flocks (Mulongo *et al.,* 2013; Dudek *et al.,* 2016). Differences in strain and altered expressions of Vsps can explain this limited potency (Dudek *et al.,* 2016). The variation in the expression of Vsp-based vaccines is a major difficulty. On the one hand, they are highly immunogenic, which makes them an optimal vaccine, but on the other hand, due to their altered expressions, Vsp-based vaccines may not stay effective for a long time (Perez-Casal *et al.,* 2017). Self-vaccination, where the herd-specific strain is inactivated and used in the same herd, maybe a low-level solution in closed herds, but will have limited success in highly mixed herds such as fattening herds and its effectiveness may be short-lived, due to frequent changes in the Vsps, moreover live attenuated vaccines have also shown promising results, but will need more extensive research before they can be widely applied (Perez-Casal *et al.,* 2017).

As long as vaccine-based prevention is not provided, present *M. bovis* management programs must be dependent on control of current infections and preventing their transmission within and among flocks (El-Sayed and Kamel, 2021).

CHAPTER THREE:

METHODOLOGY

Chapter Three:

Methodology

3.1 Instruments and Equipment:

The instruments and equipments that were used in this study are given in (Table 3.1) below.

Table (3-1): Instrument and equipment used in this study and their manufacture origin

Instrument and Equipment	Manufacture	Country
Bacteriological cabinet	HYSC	Korea
Autoclave	Sugold	China
Refrigerator	Beko	Turkey
Candle jar	Citotest	China
Benzen burner	Local	Iraq
Sensitive electronic balance	Want	China
Deep freezer (-80˚C)	Arctiko	Denmark
Digital camera	Sony	Japan
Cotton	Ajanta	India
Electrophoresis system	BioRad	USA
Eppendorf tubes 1.5ml	Citotest	China
Eppendorf centrifuge	Wisd	Germany
Electric centrifuge	Want	Korea
Gel tube without anticoagulant for serum collection	AFCOVAC	Jordan
Incubator	Memmert	Germany
Hot plate stirrer	Daihan LAB TECH	Korea
Microwave oven	LG	Korea
Micropipette (different sizes)	Dragon	China

Light Microscope	SWIFT	USA
Dissecting Microscope	SMZ-161/Motic	China
Nanodrop	Biodrop	Germany
Disposable Petri dish	Citotest	China
PCR tubes 0.2ml	Citotest	China
Plane tubes	FACO	Jordan
Tube with anticoagulant	FACO	Jordan
Disposable syringe (different sizes)	Changzhou Yuekang Medical Applicance	China
Thermal cycler	T100 BioRad	UAS
Electrophoresis tank	BioRed	USA
Vortex mixer	Digisystem	Taiwan
Water bath	Kottermann	Germany
Gel documentation system	Gel Doc EZ Image, Bio-Rad	USA
Water distillator	H2O Lab	China
L-shaped glass spreader	Citotest	China
Standard wire loop	Himedia	India
Rack	Citotest	China
Transport swab	AFCO	Jordan
Conical flask	Citotest	China
Tips (different sizes)	Citotest	China
Conical beaker	Citotest	China
Membrane filters	Falcon	UAE
Gloves	Wally plastic	China
Aluminum Foil	Falcon	UAE
ELISA reader	BioSan.IV	Latavia

3.2 Stains and chemicals:

The chemical substances that were used in this study are given in (Table 3.2) below.

Table (3.2): Stains and Chemical substances used in this study and their manufacturing origin

No.	Chemicals	Manufacture	Country
1	Methylene Blue stain	Thomas Barker	India
2	Gram stain	Atom Scientific	UK
3	Dienes Stain	Beijing Solarbio Science & Technology Company	China
4	Ethyl Alcohol (95%)	Scharlaus	Spain
5	HS Prime Taq Premix (2X) (Extraction kit)	GeNetBio	Korea
6	BioX Mycoplasma bovis	BioX Diagnostics	Belgium
7	AddBio master mix 2x	AddBio	Korea
8	Primer for the *Mycoplasma* genus	Macrogen	Korea
9	Primer for *Mycoplasma bovis*	Macrogen	Korea
10	Midori green-stained	Axon scientific Sdn Bhd	Malaysia
11	Loading Dye	GeNetBio	Korea
12	10X TBE-Buffer	GeNetBio	Korea
13	Absolute Ethanol	Scharlau	Spain
14	Nuclease-free water (dH_2o)	AddBio	Korea
15	Diluted solution 5x (SD) Sample dilution buffer.	BioX Diagnostics	Belgium
16	- Washing solutions.	BioX Diagnostics	Belgium
17	- Conjugate solution (CJ).	BioX Diagnostics	Belgium
18	Substrate solution color reagent.	BioX Diagnostics	Belgium
19	Stop solution.	BioX Diagnostics	Belgium

20	Positive control serum.	BioX Diagnostics	Belgium
21	Negative control serum.	BioX Diagnostics	Belgium
22	Adhesive films.	BioX Diagnostics	Belgium
23	Diluted solution 5x (SD) Sample dilution buffer.	BioX Diagnostics	Belgium
24	Penicillin	KPI	USA
25	Sulfadiazine-trimethoprim	KPI	USA
26	Nystatin solution	Prolife Pharma	Netherlands
27	Clotrimazole solution	Pure Health pharma	Jordan

3.3 Microbiological Medias:

The microbiological medias that were used in this study are shown in (Table 3.3).

Table (3.3): Microbiological media used in this study and their manufacturing origin

No.	Microbiological media	Manufacture	Country
1	Agar-agar	Acumedia/Lab/Neogen culture media	USA
2	Agarose gel	GeNetBio	Korea
3	Brain heart infusion broth	Acumedia/Lab/Neogen culture media	USA
4	Brain heart infusion agar	Acumedia/Lab/Neogen culture media	USA
5	Yeast extract	Acumedia/Lab/Neogen culture media	USA
6	pleuropneumonia like organism (PPLO)	Himedia	India

3.4 Ethical approval:

The institutional animal care and use committee in the college of veterinary medicine, University of Mosul, was ethically permitted for this study (UM.VET. 2022.085) on February 15, 2022.

3.5 Study areas:

The study was covered different areas of Nineveh Governorate, Iraq; they included: **North region**, which included TelKaif district (Wana subdistrict), and AL Shekhan region, the **Southern regions**, which included Yarmejah, Salamiyah, Gliukhan and Hammam Al-Alil, the **Eastern regions**, which included Hamdanya district (Kolantaba and Bartilla subdistrict and, Goggelli), the **Western regions**, which include Badush and Tal Afar region (Muhallabiya subdistrict), and areas in the **center** of Nineveh Governorate, including Mosul district (Entisar avenue, the Arbachia). Using ArcGIS V.10.6 software (ESRI, Redlands, CA, USA Global Positioning System (GPS) data (35º55'30"N-36º41'30"N latitude - 42º20'0"E-43º52'0"E longitude). A total of 50 fields were visited in the course of the study Figure (3-1).

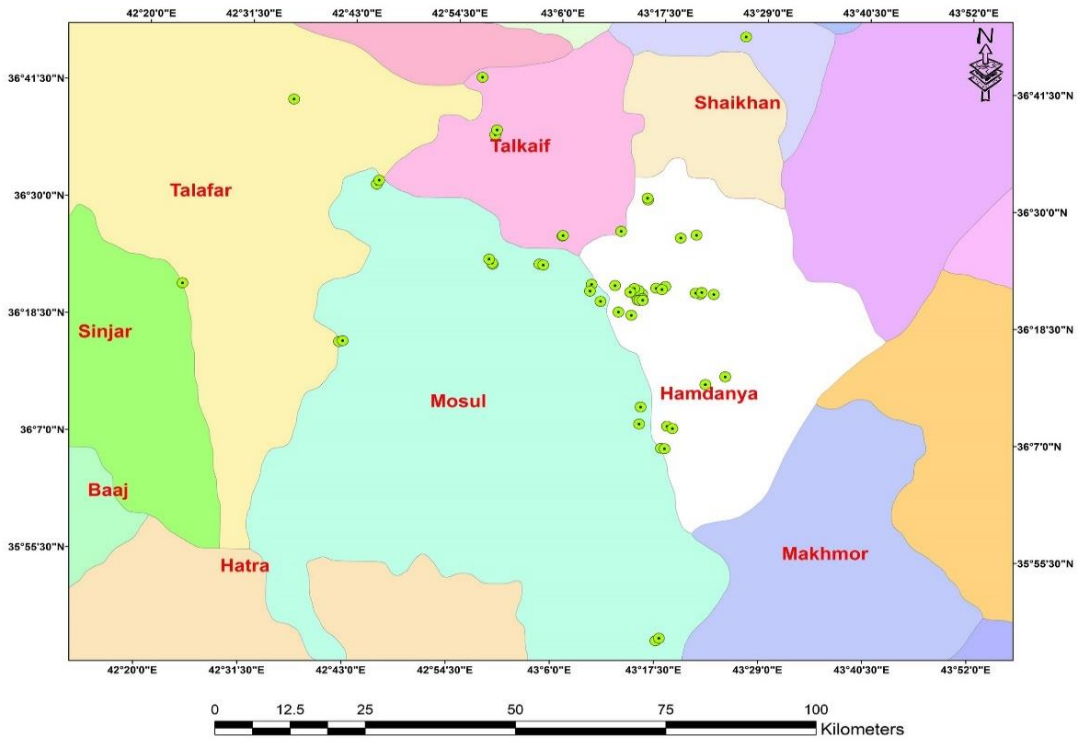

Figure (3.1): Geographical map of Nineveh Governorate showing the locations of cattle sampling fields using ArcGIS V.10.6 software (ESRI, Redlands, CA, USA program).

3.6 Animal of study and sampling size:

This cross-section study was conducted on cattle of various sex, age, breed, and origin, that were obtained from different regions in Nineveh Governorate, Iraq. Furthermore, for calculating the number of animals to be sampled was based on the previous study, the seroprevalence *M. bovis* in Mosul was 76.09% (Mahmood and Rhaymah, 2012). Therefore, with the expected prevalence 70%, a confidence rate was 95 percent and the absolute error rate was 5% (Charan and Biswas, 2013), The equation below was used:

$n = \frac{z^2 p(1-p)}{d^2}$	where: n = number of sampled animals, Z = value of the normal distribution for a 5% confidence level is (1.96), P = expected prevalence, and d = absolute error.

The minimum number of cattle required for this study was at least 322. However, 352 samples were collected from 50 fields.

3.7 Epidemiological data:

As for sample collection, epidemiological data were gathered through interviews with the owners of the farms which include:

A. **Cattle data:** Sex of the animals (males or females), age of the animals were categorizing into (young calves <6 month, beef lot calves >6 months - 2 years, cows > 2 years) origin of animals (Native and Imported), Exporting countries (Iranian, Syrian and Turkish…etc.), and Pregnancy (Yes or No).
B. **Herd data:** Management (indoor and outdoor) and Herd size (small ≤10 and large ≥40). Regions (north, southern, eastern, western and center regions), sampling seasons (winter, spring, summer, autumn), from March 2022 to February 2023.
C. **The case history,** inspection of animals, clinical examination of cattle and type of samples (blood samples, nasal swabs, ocular swabs, synovial fluid, and milk samples) were recorded in the clinical examination card as shown in Figure (3.2).

Clinical Examination Card			
Animal Deseription			
Sex :	**Age :**	**Color :**	**Notes :**
Case History			
Total No. of animal :			
Affected No. of the animal :			
Respiratory Abnormal :			
Arthritis	**Swelling :**		
	Supperative :		
	Redness :		
	Pain :		
Mastits :			
Abortion :			
Eye infection :			
Animal Examination			
Animal Inspection		**Physical Examination**	
Condition :		**Heart :**	**H . R :**
Skin :		**Lung :**	**R . R :**
Behavior :		**Mucous Membrane :**	
Posture :		**Temperature :**	
Notes :		**Notes :**	
Diagnosis :			
Treatment :			

Figure (3.2): The clinical examination card

3.8 Types of samples collection:

During the period from March 2022 to February 2023, different samples were collected from cattle includes:

A. Blood samples:

Blood samples (n=352) were collected from Jugular vein of cattle: 8ml from each animal, then divided into two tubes; 3ml placed in tube with anticoagulant ethylenediaminetetraacetic acid (EDTA) for PCR technique and 5ml placed in tube without any anticoagulant, for serum separation using centrifuge at 2500 rpm for 15 min and stored at -20°C until use for indirect ELISA (Willey *et al.*, 2023).

B. Nasal swab:

A nasal swab (n=352) was taken by swabs from nose should be rotated over any inflamed areas, that were visible exudate or rhinorrhea from the anterior nares, then placed in transporting Mycoplasma broth medium, and they were kept in containers containing a sufficient amount of ice until they were delivered to laboratory. These swabs were kept at -20°C until use for PCR technique (Vähänikkilä *et al.*, 2019).

C. Ocular swab:

An ocular swab (n=40) were taken by swabs from infected eye discharge should be rotated over any inflamed areas, then sample transporting in Mycoplasma broth medium and they kept in containers containing a sufficient amount of ice until they were delivered to laboratory. These swabs were kept at -20°C until use for PCR technique (Vähänikkilä *et al.*, 2019).

D. Synovial fluid:

The synovial fluids (n=65) were collected by arthrocentesis (joint aspiration) from the cattle suffering from clinical arthritis. the skin around the affected joint was cleaned using 70% ethyl alcohol. The synovial fluid is normally a thick, straw-colored liquid found in small amounts in joints. The health care provider inserts a sterile needle through the skin and into the joint space, then the fluid drawn through the needle into a sterile syringe and placed in sterile small plastic tubes and kept at -20°C until use for PCR technique (Nishi *et al.*, 2022).

E. Milk samples:

The milk samples (n=30) were collected from cows suffering from clinical mastitis, after washing the udder with water and leaving it to dry, then sterilizing the end of each nipple using 70% ethyl alcohol. Then the milk collected in sterilized container with a cover capacity of 10 ml, after that the milk was centrifuged at a speed of 4000 rpm for 20 minutes, then the milk liquid was withdrawn by 1 ml from the middle layer by sterile Pasteur pipettes after ignoring the upper fatty layer and lower layer containing the precipitate. Finally, the milk liquid placed in sterile small plastic tubes and kept at -20°C until use for PCR technique (Markey *et al.*, 2013).

3.9 Laboratory diagnostic methods for detection *Mycoplasma bovis*:

3.9.1 Culture method:

The nasal swabs (n=352) were placed in mycoplasma broth media and then immediately transferred to the microbiological laboratory.

3.9.1.1 Preparation of Mycoplasma broth medium according to Leber, (2016) and Willey *et al.* (2023):

1. Brain heart infusion broth 3.7g was suspended in 100 ml of distilled water, then boiled and autoclaved at 121°C for 15min. The medium was cooled at 45°C and added the prepared supplement to the medium as below
2. The yeast extract with a serum prepared as 0.5 gm of yeast extract powder suspended in 40 ml of distilled water, boiled, then cooled and pH accurate to 8 and filtered. For prepared yeast extract-serum, 20 ml of filtered calf serum (filtered by 0.22-micron filter paper) was added to 10 ml of autoclaved yeast extract in percentage as 2:1, which were added to 100 ml of prepared medium.
3. A 3ml of filtered Penicillin solution which contain100,000 un/ml at pH=7.8 was added to 100 ml of the prepared medium for inhibiting the growth of contaminated Gram-positive bacteria.
4. A 3ml of filtered Sulfadiazine-Trimethoprim solution was added to 100 ml of the prepared medium to inhibit the growth of contaminated Gram-negative bacteria.
5. A 1ml of Nystatin solution was added to 100 ml of the prepared medium to inhibit the growth of yeast.
6. 1ml of Clotrimazole solution was added to 100 ml of the prepared medium to inhibit fungi.

3.9.1.2 Preparation of Mycoplasma agar medium preparation according to Talaro and chess (2018):

1. A 4.9 g of brain heart infusion agar was suspended in 100 ml of distilled water, boiled and autoclaved at 121 °C for 15min. Then cooled at 45 °C.
2. Added the supplement as above in Mycoplasma broth medium.

3.9.1.3 Procedure of Culture of the samples on Mycoplasma agar medium and pleuropneumonia like organism medium according to Willey *et al.* (2023):

1. Added the nasal swab samples in the Mycoplasma broth.
2. Incubated at 37°C with 5% Co2 for 7-14 days in a candle jar.
3. When turbidity appeared, then inoculum of each broth on Mycoplasma agar medium and PPLO medium.
4. Incubated at 37°C with 5% Co2 for 7-14 days in a candle jar.
5. The culture was checked daily for the first 7 days until 14 days.

3.9.1.4 Confirmation of Mycoplasma by morphological tests:

1. **Microscopic appearance:**

   Smears from Mycoplasma broth medium after the incubation period were prepared and were stained with a Grams' stain, then the slides were examined under the oil lens to note the presence of Mycoplasma polymorphic cells (Tille, 2022).
2. **Colony Morphology:**

   The morphological characteristics of Mycoplasma colonies were studied when the colonies were grown on Mycoplasma agar medium and PPLO agar medium after incubation period by using

Light and Dissecting Microscope (Stereo microscopes) (Leber, 2016).

3. **Staining with modified Dienes' stain:**

   For staining the colonies, the agar dish surface was submerged (Containing the colonies of Mycoplasma) in 1ml of working solution of modified Dienes stain, washed with distilled water, then decolorized with 1ml of 95% ethyl alcohol for 1min., removed the excess alcohol, washed with distilled water, and examined the color of colonies of Mycoplasma (Talaro and chess, 2018).

3.9.1.5 Preservation of bacterial isolates:

The isolates were preserved by replanting the bacteria on Mycoplasm agar PPLO agar medium and used as daily work cultures to perform complementary diagnostic tests for mycoplasma, and after confirming the diagnosis of Isolates were cultured on PPLO broth medium and kept at -20°C for the purpose of confirming the diagnosis Mycoplasma by polymerase chain reaction technique (Quinn *et al.,* 2016).

3.9.2 Indirect enzyme linked immune assay (i-ELISA):

The serum samples (n=352) were tested for detecting the antibodies against *M. bovis*, using indirect ELISA kit BioX Mycoplasma bovis (BioX Diagnostics, Belgium).

3.9.2.1 Kit components of the i-ELISA

- Diluted solution 5x (SD) Sample dilution buffer.
- Washing solutions.
- Conjugate solution (CJ).
- Substrate solution color reagent.

- Stop solution.
- Positive control serum.
- Negative control serum.
- Adhesive films.
- Supplies needed (not provided): Round bottomed microtiter plate, precision pipettes (0.1 - 5 µL, 10 - 200 µL, and 200 - 1000 µL), with the different size pipette tips and clean containers and /or tubes and ELISA plate reader.

3.9.2.2 Procedure of the i-ELISA

This test was conducted according to the instructions of the manufacturer as follows: -

1. The test kit was taken out of the refrigerator to reach room temperature (20-25°C).
2. Take the ELISA plate and place it on a white sheet of paper, taking into account not to touch the plate with your hand from below.
3. The positive and negative control solutions were diluted by adding 0.5 ml of distilled water to each solution, then it was shaken well and placed in the freezer at -20°C until used according to the manufacturer's instructions.
4. A dilution buffer (5X) solution was prepared by adding 10 ml of sample diluent to 40 ml of distilled water.
5. The serum sample was diluted with the previously prepared dilution buffer in a ratio of (100:1) by adding 1000 µl of the sample dilution solution to the test tube and then adding the serum sample in an amount of 10 µl while shaking it well and in the same way the two positive control solutions were diluted and negative.
6. The samples were placed in the pits of the dish at a rate of 100 microliters of the sample for each hole

7. The dish was shaken quietly for 20-25 seconds, then put the lid on it and incubated at 21 °C for an hour.
8. A washing solution was prepared in a ratio (20:1) by withdrawing 10 ml of washing solution and adding 190 ml of distilled water to it.
9. After the incubation period was completed, the contents of the dish were poured out, and then the dish was washed using a diluted washing solution by adding 260 microliters of the solution to each pit and all the pits.
10. 100 microliters of conjugate solution were added to each hole and to all holes, then the lid was placed on it and incubated at 21 °C for one hour.
11. After the incubation period, the contents of the dish were poured out, and then the washing process was repeated three times using diluted washing solution, as described previously.
12. After the washing process was completed, the color reagent Substrate was added in the amount of 100 microliters to each hole and to all the holes and mixed well with a simple movement and incubated at the laboratory temperature of 21 °C for 10 minutes in a dark place.
13. After completing the incubation period, a stop solution was added at the rate of 50 microliters to each pit and to all pits and mixed well with a simple movement avoiding the formation of bubbles in the pits. After that, the plate was read directly using an ELISA device at a wavelength of 450 nm.

3.9.2.3 The measurement made use of equation 2 to Validate and interpreted the test results:

The corrected OD values for the positive and negative controls and the tested samples were found from the following equation:

OD control positive – OD control = OD corrected

OD control negative – OD control = OD corrected

OD sample – OD control sample = OD corrected

Then the (PP) Percent positively values for the samples and controls were calculated from the following equation:

$$PP = \frac{\text{Test sample or neg. (OD corr)}}{\text{Positive control (OD corr)}} \text{ X } 100$$

3.9.3 Molecular techniques for detecting Mycoplasma genus and *Mycoplasma bovis* infection:

3.9.3.1 DNA extraction for Mycoplasma detection:

The DNA of Mycoplasma spp. was extracted from blood sample (n=352), nasal swab (n=352), ocular swabs (n=40), synovial fluid (n=65), and milk sample (n=30) using the commercial Prime Prep Genomic DNA Extraction Kit from tissues (GeNetBio, Korea). It is based on the effective binding of DNA to a microfiber-silica-based membrane through a mix of lysis and binding buffers, then removing the impurities from the membrane away with two different wash buffers. The compounds of the kit are given below

Extraction Kit components:

- Spin columns
- Lysis
- Washing1
- Washing2
- Elution
- Ethanol (not provided)

A. Samples preparation: according to the manufacturer as follows:

1. 1 ml of bacterial broth and blood sample were transferred to 1.5 ml Eppendorf tube, centrifuged for 5 min. at 14000 rpm, then discarded the supernatant.
2. Two hundred 200µl of GT Buffer was added to 1.5 ml centrifuged Eppendorf tubes, then re-suspended the pellet by the vortex.
3. Twenty 20µl of Proteinase K (Dissolved by dH_2o) was added to the bacterial cell and then mixed by the vortex.
4. Incubated at 60°C at 10 min. and inverted the tubes every 3 min. during the incubation period.

B. Lysis:

1. Two hundred 200µl of GB Buffer was added to the sample and mixed with vortex for 10 sec.
2. Incubated at 70°C for at least 10 min. and inverted the tubes every 3 min. during the incubation period. At this time, the required elution buffer was pre-heated at 70°C for the next step (Elution).

C. DNA Binding:

1. Two hundred 200µl of absolute ethanol was added to sample lysate and mixed immediately by shaking vigorously.
2. The mixture was transferred to GD Column which was placed in 2mlcollection tube, and centrifuged at 14000 rpm for 2min. then the 2ml collection tube (containing the flow-through) was discarded and replaced by a new 2ml collection tube.

D. Wash:

1. Four hundred 400µl of W1 Buffer was added to the GD Column, centrifuged at 14000 rpm for 30 sec., then the flow-through was discarded and the GD Column was put back in a new 2ml collection tube.
2. Six hundred 600µl of Wash Buffer diluted with ethanol was added to the CD Column, and centrifuged at 14000 rpm for 30sec., then the flow-through was discarded and placed GD Column back in the 2ml collection tube.
3. The tubes were centrifuged again at 14000 rpm for 3min. to dry the column matrix.

E. Elution:

1. Dried GD Column was transferred to a new 1.5ml Eppendorf tube.
2. One hundred 100µl of pre-heated elution buffer was added into the center of the column matrix and waited for at least 3min. to allow the elution buffer to be completely absorbed.
3. The tubes were centrifuged at 14000 rpm for 1min. to elute the purified DNA.

3.9.3.2 Determination of DNA concentration and purity:

The concentration and purity of the DNA extracted from the nasal, blood, ocular, synovial fluid and milk samples were determined. Using the Nanodrop (BioDrop, Germany), the concentration of extracted DNA was estimated at wavelength 260nm, while the purity of extracted DNA was estimated by calculating the ratio of (A260 nm to A280 nm) as described by Morais *et al.* (2021).

3.9.3.3 Amplification of the Mycoplasma DNA:

Amplify the highly conserved region 16S rRNA, *uvrC* and *gap*A genes of *M. bovis* from blood sample (n=352), nasal swab (n=352), ocular swabs (n=40), synovial fluid (n=65), and milk sample (n=30), as a target using conventional PCR (c-PCR) technique was used to amplify the 16S rRNA gene using universal primers; (M. Genus-F and M. Genus-R) that were designed by Botes *et al*. (2005) (Table 3.4). In addition, multiplex (m-PCR) technique was used to amplify the deoxyribodipyrimidine photolyase (*uvrC*) gene that were designed by Subramaniam *et al*. (1998) and glyceraldehyde-3-phosphate dehydrogenase GAPDH (*gap*A) gene of *Mycoplasma bovis* that were designed by Perez-Casal and Prysliak (2007), using specific primers: (uvr-F and uvr-R) and (gap-F and gap-R) respectively, (Table 3.4). All these primers were provided by Macrogen Inc., South Korea. The positive bands were at approximately 270bp, 1626bp, and 1007bp. for 16S rRNA, *uvrC*, and *gap*A genes respectively (Table 3.4). Furthermore, a clinically and laboratory-positive cow's DNA was used as a positive control, while, all PCR components except DNA used as a negative control.

To prepare these primers for PCR amplification, the primers tubes were centrifuged at 1200 rpm for 3min and stock concentration of 100µM of primer solutions were made, only one decimal point was moved backwards from the 'nMoles' volume in the datasheet of each primer.

For example, the: amount of oligo primer was 30,0 nMoles. The resuspension was calculated thus: 30,0 = 300µL, this amount of nuclease-free water (dH_2o) was added to the primer tube, vortexed for 15 min, followed by a short spin to remove the drops from the lids. The 100µM stock primer solution obtained was stored at -20ºC until use. The

preparation and dilution of primers were made by adding 10µL of stock primer with 90µL of nuclease-free water (dH_2o) and the concentration of primers were 10 pmol.

Table (3.4): The PCR primers used in this study

Primer	Sequence (5´----------3´)		Target gene	Size (bp)	Reference
Detection of *Mycoplasma* genus	F	5'GGGAGCAAACACGATAGA TACCCT-3'	16 rRNA	270	Botes *et al*.(2005)
	R	5'-TGCACCATCTGTCACTCTGT TACCCTC-3'			
M. bovis	F	5'-TTACGCAAGAGAATGCTTCA -3'	*uvrC*	1626	Subramaniam *et al*. (1998)
	R	5'-TAGGAAA GCACCCTATTGAT-3'			
M. bovis	F	5'ATAGGAGGATCCAAAAGA GTCGCTATCAATGGTTTTGG ACG-3'	*gap*A	1007	Perez-Casal and Prysliak, (2007)
	R	5'GGAAATGGTACCTTACTTA GTTAGTTTAGCAAAGTATGT TAATG-3'			

The procedure of c-PCR and m-PCR amplification was as follows:

1. The chemicals and DNA extracted samples were taken out of the freezer and allowed to thaw at room temperature.
2. The c-PCR or m-PCR techniques were carried out in 25µl as a total reaction volume in a PCR Eppendorf tube for each sample, as mentioned in table (3.5).

Table (3.5): The PCR reactions for DNA samples subjected to conventional PCR and multiplex PCR techniques

Type of PCR techniques	Mixtures	One reaction
c-PCR	2x PCR Master Mix	12.5µl
	10µM M.Genus-F primer	1µl
	10µM M.Genus-R primer	1µl
	DNA samples	3µl
	dH_2o	7.5µl
	Total	25µl
m-PCR	2x PCR Master Mix	12.5µl
	10µM uvr -F primer	1µl
	10µM uvr-R primer	1µl
	10µM gap-R primer	1µl
	10µM gap-R primer	1µl
	DNA samples	3µl
	dH_2o	5.5µl
	Total	25µl

3. Each sample mix was vortexed for 15 sec.
4. The tubes were labeled on a set corresponding to the number of samples to be analyzed.
5. 17 µl of the PCR mix was transferred to the labeled thin PCR tubes. Then, 3 µl of each DNA sample was added to the corresponding PCR tube and vortexed for 15 sec.
6. The amount of PCR mix transferred was equivalent to the number of samples (reactions) to be studied plus three more for negative and positive control tubes and pipetting.
7. The reaction tubes were taken to the thermocycler, and the lid was closed. The program was installed (Table 3.6) by pressing the “pause” button.
8. The PCR amplification of the desired gene was done using the following steps: 1cycle of initial denaturation of DNA at 95 ºC for 5 min, followed by 35 cycles of denaturation of DNA at 94 ºC for 45 secound, annealing of the first primers at 59 ºC for 45min (16S rRNA),

the second and third primers at 55 ºC for 30min (*uvrC* and *gap*A genes), and extension at 72 ºC for 2min, and then 1cycle for final extension at 72 ºC for 5 min. The samples were removed at the end of the program when the temperature on the screen showed 4ºC (Table 3.6).

Table (3.6): The PCR program for DNA samples subject to conventional PCR and multiplex PCR techniques

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

9. During the time of the thermocycler run, the gel electrophoresis was prepared by weighing 42.5 g of powder TBE buffer, which was dissolved in 500 ml of distilled water to prepare 5x TBE buffer, and then 100 ml of 1x TBE buffer was prepared by mixing 20 ml of 5x TBE buffer with 80 ml of distilled water.
10. Weighing 0.6 g of agarose and placing it into the 500 ml flask, 40 ml of 1X TBE buffer was added and microwaved for 1min until the solution was melted. Checks were made to ensure that the agarose was completely dissolved. A metal clamp beaker was used to bring out the flask from the microwave.
11. The casting tray was taped on both sides, and the gel comb (16 wells) was inserted into the dedicated notches on the side of the casting tray.
12. When the agarose was cooling until the flask could be touched with bare hands, 1.5 µl of Midori green-stained was placed in it, gently

swirled, and poured into a casting tray, then set aside for 45 min. for the agarose to solidify.

13. The comb and the tape were removed from the casting tray, and the gel was placed in the gel tank of an agarose electrophoresis apparatus. The gel was oriented so that the wells were adjacent to the negative (black) electrode. Then the gel chamber was filled with 1X TBE buffer until it covered the gel by approximately 1cm.
14. 7 µl of the PCR samples were pipetted into comb wells (13 wells), and the pipette tip was changed between samples. Then 7 µl of the DNA ladder (100 - 3000 bp) was loaded into the first well, 7 µl of control positive was loaded into the second well, and 7 µl of control negative was loaded into the last well (well 16).
15. The electrode covers were attached to the gel electrophoresis and then plugged into the power supply, set at 100 volts and 700 mA (milliampere) electrical current, and run for 30 min.
16. After stopping the gel electrophoresis, the gel tray was removed and carefully placed into the auto gel imaging and analyzing imager.
17. Results analysis for the reaction visualized the DNA bands in amplifying sizes 250 bp and 630 bp, meaning the samples were positive for FPV.

3.9.4 Sequencing and phylogenetic analysis of *Mycoplasma bovis* DNA:

The DNA PCR products (n=15) for the *uvrC* gene out of 108 positive samples of *M. bovis* in cattle, comprising various samples; blood sample (n=3), nasal swab (n=3), ocular swabs (n=3), synovial fluid (n=3), and milk sample (n=3), further, the DNA products (n=15) for the *gap*A gene out of 101 positive samples of *M. bovis* in cattle, also comprising various samples; 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 *uvrC* 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 Bank it tool program (to deposited five sequences for the each *uvrC* 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 (Himmelreich *et al.,* 1996; Tamura *et al.,* 2021).

3.9.5 Comparison between the different diagnostic methods used in this study:

The compatibility between the culture and the c-PCR technique and the i-ELISA and the c-PCR technique was checked based on the Kappa value. There was no compatibility between the two tests: if the Kappa value is < 0.00, the compatibility is low; if the Kappa value is ranged 0.0–0.20, the compatibility is fair; if the Kappa value is ranged 0.21–0.40, the compatibility is moderate; if the Kappa value is ranged 0.41–0.60, the compatibility is substantial; if the Kappa value is ranged 0.61–0.80 and the compatibility is almost perfect; if the Kappa value is ranged 0.81–1 (Franco and Di Napoli, 2016).

Moreover, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of the rapid test and i-ELISA were computed and compared to the PCR technique according to the epidemiological table and equations shown in the table below (Table 3.7) (Baratloo *et al.,* 2015).

Table (3.7): Positive and negative predictive value of screening test

Result of Screening test	Actual Health Status		
	Infected (I+)	Not-infected (I-)	
Positive (T+)	a	b	a+b
Negative (T-)	c	d	c+d
	a+c	b+d	n

(a)True positive samples, (b) False positive samples, (c) False negative samples, (d) True negative samples. Sensitivity= a/(a+c)x100, Specificity = d/(b+d)x100, Accuracy =(a+d) /(a+c+b+d) x100.

3.10 Statistical analysis:

The difference in prevalence between the various risk factors was assessed by using the two-sided Chi-square and Fischer's exact tests in the IBM-SPSS Statistics (Version 22) program. If an expected cell value in the Chi-square test is less than 5, Fisher exact test P value results recommend using factors with P values <0.05, which are considered significant. The odds ratio (OR) for the association between risk factors for *M. bovis* and its 95% confidence interval was computed using 2 by 2 tables in Epi-InfoTM 7 software (Version 7).

Data analysis was done using the IBM-SPSS Statistics (Version 22) program to compare the m-PCR technique and the culture method, and the i-ELISA and the culture method were checked for agreement between tests based on Kappa values (Franco and DiNpoli, 2016). Moreover, the accuracy, sensitivity, and specificity of the m-PCR technique and i-ELISA were computed and compared to the culture method (Baratloo *et al.,* 2015) Furthermore, the significance of variations between clinically infected cattle and control group in clinical symptoms was statistically analyzed using independent sample t-tests in the IBM-SPSS Statistics (Version 22) program. All the significant differences were determined at $P < 0.05$.

CHAPTER FOUR:

RESULTS

Chapter Four

Results

4.1 Determining the prevalence of *Mycoplasma bovis* using different laboratory methods:

In the current study, from a total of 352 cattle, 352 nasal swabs were tested using the culture method, and multiplex polymerase chain reaction (m-PCR) technique (*uvrC* gene), also 352 serum samples were tested using the indirect enzyme-linked immunosorbent assay (i-ELISA). The overall prevalence of *Mycoplasma bovis* in Nineveh Governorate was 23.6% (83 out of 352), 30.7% (108 out of 352) and 47.2% (166 out of 352) using culture method, m-PCR technique, and i-ELISA respectively (Table 4.1).

Table (4.1): Determining the overall prevalence of *M. bovis* infection using culture method, multiplex-PCR technique and indirect-ELISA

Type of test	No. of samples	Overall prevalence of *M. bovis* (%)
Culture method	352	83(23.6)
Indirect-ELISA		166 (47.2)
Multiplex-PCR		108 (30.7)

Based on kappa value (0.807), there was perfect agreement between m-PCR technique and culture method, with sensitivity 98.79%, specificity 90.33%, and accuracy 92.32%, for m-PCR technique (Table 4.2). While, based on kappa (0.444), there was a moderate agreement between i-ELISA and culture method, with sensitivity 89.15%, specificity 65.79%, accuracy 71.30%, for i-ELISA (Table 4.3).

Table (4.2): Compatibility between culture and m-PCR technique based on kappa value, with the calculating the ratio of the m-PCR technique sensitivity, specificity, and accuracy for *M. bovis* diagnosis

		Culture		
		Infected	Uninfected	Total No.
m-PCR technique	Infected	82 a	26 b	108
	Uninfected	1 c	243 d	244
Total		83	269	352

(a) True positive samples, (b) False positive samples, (c) False negative samples, (d) True negative samples. Kappa value was (0.807). Sensitivity = a/(a+c) x 100 = 98.79%. Specificity = d/(b+d) x 100 = 90.33%. Accuracy= (a+d)/ (a+c+ b+d) x 100 = 92.32%

Table (4.3): Compatibility between culture and indirect ELISA based on kappa value, with the calculating the ratio of the indirect ELISA sensitivity, specificity, and accuracy for *M. bovis* diagnosis

		Culture		
		Infected	Uninfected	Total No.
i-ELISA	Infected	74 a	92 b	166
	Uninfected	9 c	177 d	186
Total		83	269	352

(a) True positive samples, (b) False positive samples, (c) False negative samples, (d) True negative samples. Kappa value was (0.444). Sensitivity = a/(a+c) x 100 = 89.15%. Specificity = d/(b+d) x 100 = 65.79%. Accuracy= (a+d)/ (a+c+ b+d) x 100 = 71.30%

In addition, the result also revealed 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, including blood, nasal swabs, ocular swabs, synovial fluid, and milk samples, which were 27.6%, 30.7%, 22.5%, 27.7%, and 23.3%, respectively (Table 4.4).

Table (4.4): Prevalence of *Mycoplasma bovis* based on the type of samples using m-PCR technique

Type sample	No. of tested sample	m-PCR No. positive	Prevalence %
Nasal swab	352	108	30.7
Blood	352	97	27.6
Synovial fluid	65	18	27.7
Ocular swab	40	9	22.5
Milk	30	7	23.3

4.2 Determining the prevalence of *Mycoplasma bovis* based on the regions in Nineveh Governorate:

Based on the m-PCR technique, the prevalence of *M. bovis* varied in different regions of Nineveh Governorate, as the highest prevalence of the bacteria in the center regions of Nineveh Governorate was recorded in the Arbachia region was 34.2%, and the lowest prevalence was recorded in the Al Entisar region was 12.5%. While in the eastern regions, the highest prevalence of bacteria in Nineveh Governorate was recorded in the Gogjalee region was 48.3%, and the lowest prevalence was recorded in the Kolantaba region was 20.0% As for the northern regions of Nineveh Governorate, the highest prevalence of the bacteria was recorded in the Wana region was 36.0%, and the lowest prevalence was recorded in the AL Shekhan region was 8.3%. In the western regions of Nineveh Governorate, the highest prevalence of bacteria was recorded in the Badush region was 31.6%, and the lowest prevalence of *M. bovis* was recorded in Tal Aafr region was 16.7%. While in the southern regions of Nineveh Governorate, the highest prevalence was recorded in Yarmejah region was 35.3%, and the lowest prevalence was recorded in the Gliukhan region was 10,5% Figure (4.1), (Appendix 1).

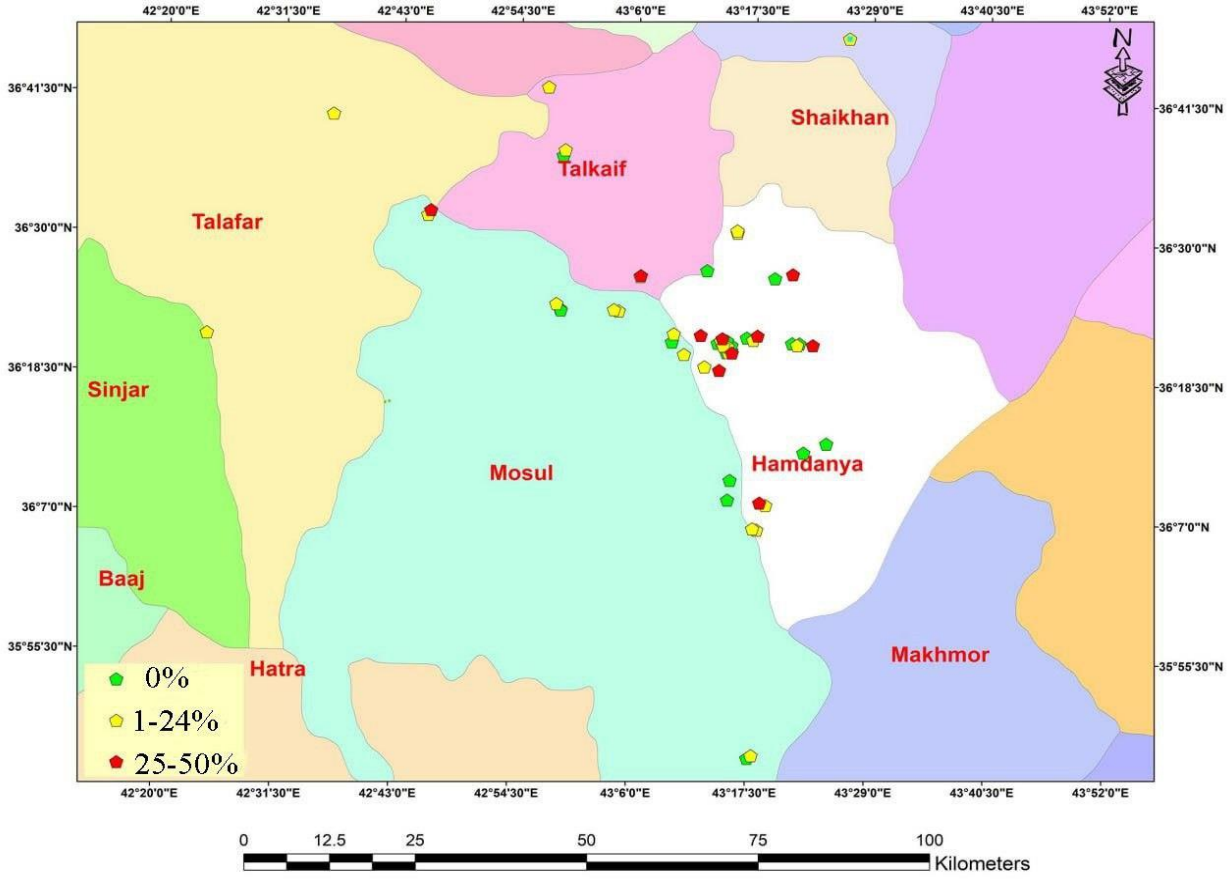

Figure (4.1): Geographical map of Nineveh Governorate in Iraq showing the distribution of *M. bovis*. The different marks show infection rate in each field at different regions using Map Window ArcGIS V.10.6 software.

4.3 Isolation and identification of *Mycoplasma bovis* in cattle:

A. Colonial appearance on solid media:

Based on the culture method of 352 nasal swabs on pleuropneumonia like organism (PPLO) medium, the Mycoplasma colonies appeared as small, pearlescent, with a matte center and a white to colorless, with an egg-like (fried egg) appearance Figure (4.2).

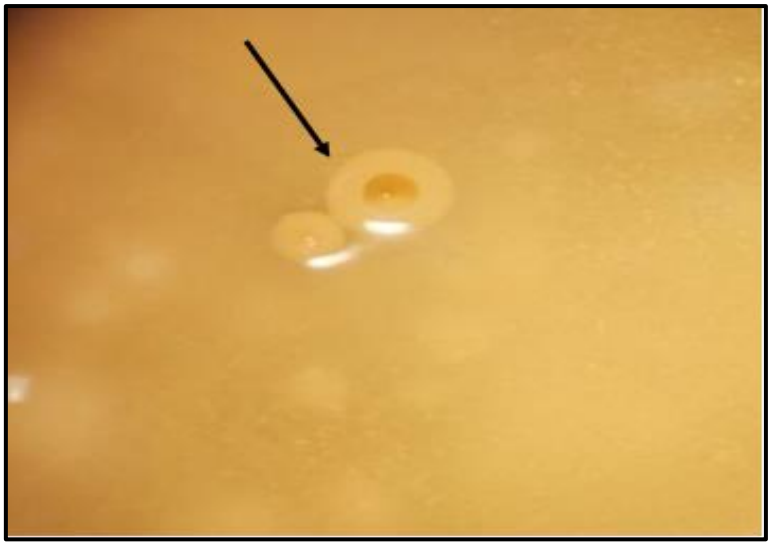

Figure (4.2): Fried egg appearance of Mycoplasma colonies growing on medium (PPLO Medium) using light microscope (40X)

B. Microscopic examination using stains:

The results of the microscopic examination of smears prepared from Mycoplasma broth media of nasal swabs, that were stained with gram stains, the positive samples were characterized by the appearance of Mycoplasma cells as a small in size, purple in color, and appear in various pleomorphic shapes, ranging from spherical Bacillus, filamentous, and branched (Figure 4.3).

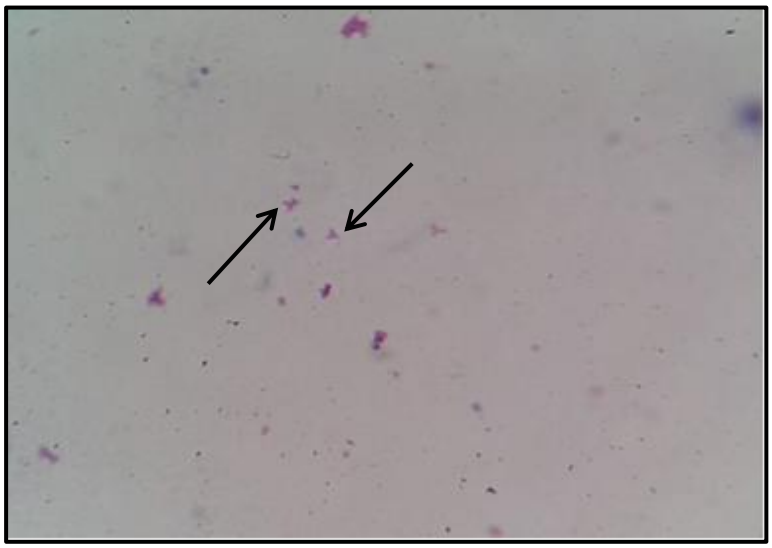

Figure (4.3) pleomorphic of Mycoplasma Cells Gram negative cells in different forms (1000X)

C. Testing the ability to reduce methylene blue dye:

Using Dennis stain, the colonies of Mycoplasma grown on the medium PPLO agar appeared as colorless colonies due to its ability to reduce methylene blue dye, which is one of the components of the main dye Dienes Stain (Figure 4.4).

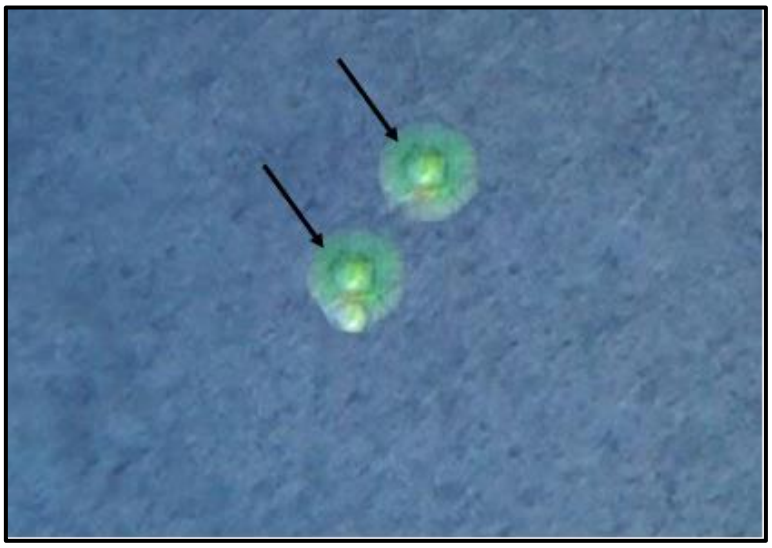

Figure (4.4): Staining Mycoplasma colonies by modified Dienes stain (X400)

4.4: Molecular detection of *Mycoplasma spp.* and *Mycoplasma bovis* in cattle using conventional PCR and multiplex PCR techniques:

The concentration of DNA extracted from Culture of nasal swabs, blood, synovial fluid or ocular swab and milk regarding to wavelength 260nm was ranged between 60.5 - 327.5 ng / µl and the purity of extracted DNA calculated by the ratio of (A260 nm to A280 nm) was between 1.7 - 1.9, using the Nanodrop.

In the current study, results based on conventional PCR technique for amplified 16S rRNA gene of *Mycoplasma spp.*, for cultured nasal swabs that positive in culture method, also the nasal swab samples negative

to the culture method using 'universal' primers (M. Genus-F and M. Genus-R) revealed a positive band approximately at 285 bp (Figure 4.5), with detection rate of 38.6% (136 out of 352 cattle) (Table 4.5).

Results based on multiplex PCR technique for amplified deoxyribodipyrimidine photolyase (*uvrC*) gene and glyceraldehyde-3-phosphate dehydrogenase GAPDH (*gap*A) gene of *Mycoplasma bovis* in the positive and negative nasal swabs to the c-PCR technique, and in the other samples (blood samples, ocular swabs, synovial fluid, and milk samples) using specific primers (gap-F, gap-R and uvr-F, uvr-R), observed that the prevalence of *M. bovis* DNA was 30.7% (108 out of 352 cattle) and 28.7% (101 out of 352 cattle) respectively (Table 4.5). For the *uvrC* gene, the positive bands were at approximately at 1626 bp. (Figure 4.6). While, for the gapA gene, the positive bands were at approximately at 1007 bp. (Figure 4.6). In addition, there was no significant difference in the prevalence of *M. bovis* between *uvrC* gene and gapA gene (Table 4.5).

Table (4.5): Detection rate of *Mycoplasma spp.* and *Mycoplasma bovis* in nasal swab of cattle samples using conventional-PCR and multiplex-PCR techniques

Type of PCR techniques	Primer/ Gene	Genotypes	Product size (bp)	No. of samples tested	No. positive (%)
Conventional-PCR	Universal 16S rRNA	Mycoplasma Spp.	285	352	136 (38.6)
Multiplex-PCR	Specific *uvrC*	*M. bovis*	1626		108 (30.7) [a]
	Specific *gap*A	*M. bovis*	1007		101 (28.7) [a]

Values significantly different ($P < 0.05$) are labelled with different letters (a or b).

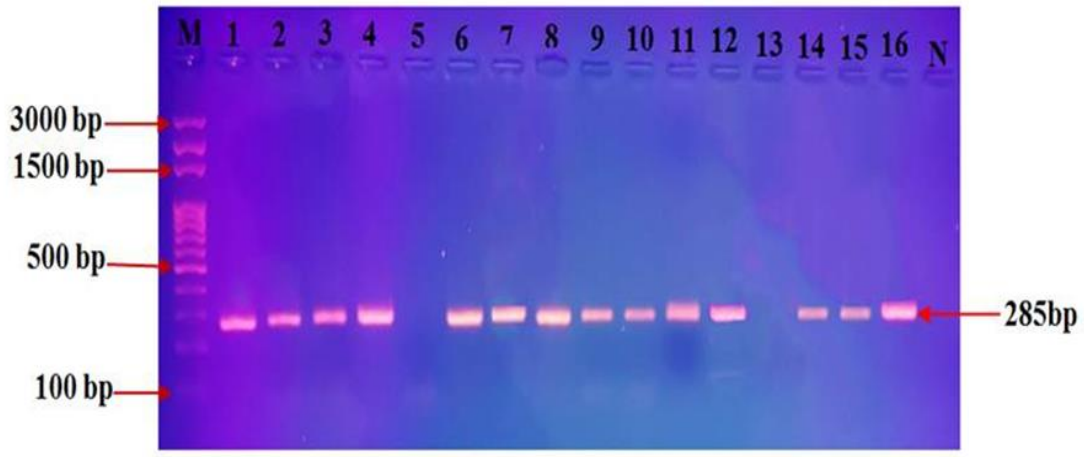

Figure (4.5): Mycoplasma spp., detected by Conventional PCR technique. Gel electrophoresis image showing: lane M) Exact Mark 100-3000bp DNA ladder; Lane 1-4,6-12,14-16) using universal primers in approximately band size 285bp; Lane N: negative control.

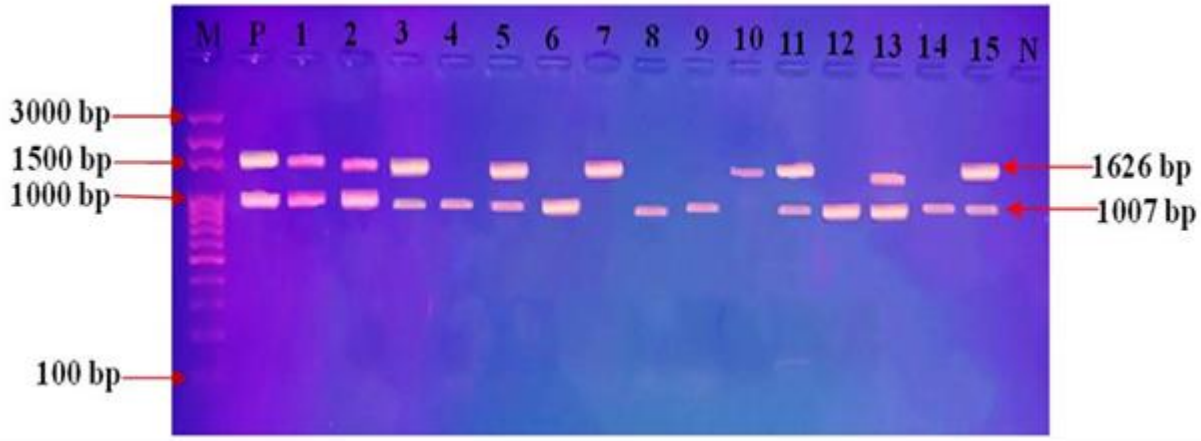

Figure (4.6): Detecting *M. bovis* Multiplex PCR technique Gel electrophoresis image showing: lane M) Exact Mark 100-3000bp DNA ladder; Lanes 1-3,5,7,10,11,13, 15) and Lanes 1-6,8,9,11-15) positive samples for *uvr*C and *gap*A genes in approximately band size 1626bp and 1007 respectively; Lane p) positive control for *M. bovis*; Lane N: negative control.

4.5 Risk factors associated with prevalence of *Mycoplasma bovis*:

Based on m-PCR technique, the study demonstrated significant (P<0.001) differences between the ages of cattle. The prevalence of *M. bovis* was significantly higher in calves aged between > 6 months to 2 years 48.6% (OR: 6.93 times, CI: 3.90-12.30), compared to cattle of > 2 years and low ages < 6 months 12% and 16% respectively (Table 4.6).

In the present study, the prevalence of *M. bovis* showed significant (P<0.001) difference between cattle sex. The prevalence of the *M. bovis* was significantly higher in male 40.6% (OR: 3.26times, CI: 1.96-5.41) compared to female cattle 17.3% (Table 4.6).

This study demonstrates that significant differences between cattle origins, the prevalence of *M. bovis* was significantly (P<0.001) higher in imported cattle 39.3% (OR: 4.42 times, CI: 2.34-8.34), compared to local cattle 12.4%. Furthermore, no significant (P< 1.001) difference between cattle exported countries: Iran, Syria, Georgia, and Turkey (Table 4.6).

The present study showed that the prevalence of *M. bovis* was significantly (P<0.001) higher in pregnant cows 43.6% (OR: 8.76 times, CI: 3.45-22.19), compared to non-pregnant cows 8.1% (Table 4.6).

This study also found that the prevalence of *M. bovis* was significantly (P< 0.001) higher in large herds size 39.1% (OR: 4.06 times, CI: 2.25 - 7.31), compared to small herds 13.7%. Moreover, no significant (P< 0.593) difference between clinically healthy and infected cattle (Table 4.6).

The current study showed that the prevalence of *M. bovis* was significantly (P< 0.001) higher in indoor feeding cattle 37.7% (OR: 3.19 times, CI: 1.81 - 5.62), compared to outdoor feeding cattle 15.9% (Table 4.6).

Table (4.6): Risk factors associated with prevalence of *M. bovis.*

Factors	No. of cattle tested	m-PCR technique			
		No. of positive (%)	OR	95% CI	P value
Age					
> 2 years (Cows)	150	18 (12.0) [a]	1		
< 6 months (small calves)	25	4 (16.0) [a]	0.71	0.22-2.32	0.8159
> 6 months - 2 years (Feedlot calves)	177	86 (48.6) [b]	6.93	3.90-12.30	0.000
Sex					
Females	150	26 (17.3) [a]	1		
Males	202	82 (40.6) [b]	3.26	1.96-5.41	0.000
Origin					
Local	105	13 (12.4) [a]	1		
Imported	247	95 (39.3) [b]	4.42	2.34-8.34	0.000
Exporting countries					
Iran	72	27 (37.5) [a]	1		
Syria	34	13 (38.2) [a]	1.03	044-2.39	1.000
Georgia	81	31 (38.3) [a]	1.03	0.53-1.98	1.000
Turkey	60	24 (40.0) [a]	1.11	0.55-2.24	0.909
Pregnant					
Non	111	9 (8.1) [a]	1		
Yes	39	17 (43.6) [b]	8.76	3.45-22.19	0.000
Herd size					
Small ≤10	117	16(13.7) [a]	1		
Large ≥40	235	92(39.1) [b]	4.06	2.25-7.31	0.000
Health status					
Subclinically	93	26 (27.9) [a]	1		
Clinically infected	259	82 (31.6) [a]	1.19	0.70-2.01	0.593
Management					
Outdoor	113	18 (15.9)	1		
Indoor	239	90 (37.7)	3.19	1.81-5.62	0.000

Values significantly different (P < 0.05) labelled with different letters (a, b or c).
OR (Odds ratio), CI (Confidence interval), P (probability).

The prevalence of *M. bovis* was significantly affected by geographical regions in Nineveh Governorate. The prevalence of the *M. bovis* was significantly (P<0.0001) higher in the Eastern regions of Nineveh Governorate 40.2% (OR: 2.47 times, CI: 1.21-5.04), compared to other regions (Table 4.7).

Table (4.7): Regional factors in Nineveh Governorate associated with prevalence of *M. bovis*

Regional factors	No. of cattle tested	m-PCR technique			
		No. of positive (%)	OR	95% CI	P value
Southern regions of Nineveh Governorate	61	13 (21.3) [a]	1		
Western regions of Nineveh Governorate	60	15 (25) [a]	1.23	0.52-2.87	0.790
Central regions of Nineveh Governorate	54	15 (27.8) [a]	1.42	0.60-3.33	0.556
Northern regions of Nineveh Governorate	55	16 (29.1) [a]	1.51	0.65-3.52	0.452
Eastern regions of Nineveh Governorate	122	49(40.2) [b]	2.47	1.21-5.04	0.017

Values significantly different (P < 0.05) are labelled with different letters (a, b or c).

This study also clarified that the prevalence of *M. bovis* was significantly affected by seasons. The prevalence of *M. bovis* was significantly (P<0.0001) higher in both autumn and winter seasons, with 39.3 % and 42.7% respectively (OR: 4.16 and 3.62 times respectively), compared to the summer season, with 15.2% (Table 4.8).

Table (4.8): Seasonal factors associated with prevalence of *M. bovis*.

Factors	No. of cattle tested	m-PCR technique			
		No. of positive (%)	OR	95% CI	P value
Summer 2022 (June- July-Aug)	79	12 (15.2) [a]	1		
Spring 2022 (Mar-Apr-May)	88	20 (22.7) [a]	1.64	0.74-3.62	0.298
Autumn 2022 (Sep-Oct-Nov)	89	35 (39.3) [b]	3.62	1.71-7.64	0.000
Winter 2022-2023 (Dec-Jan-Feb)	96	41 (42.7) [b]	4.16	1.99-8.68	0.000

Values significantly different ($P < 0.05$) are labelled with different letters (a, b or c).

4-6: Clinical manifestation of *M. bovis* disease:

In the present study, clinically infected cattle (n=82) with *M. bovis* were suffering from fever, loss of appetite, depression, emaciation, bronchopneumonia (coughing, nasal discharge, polypnea and dyspnea) (Figure 4.7), keratoconjunctivitis (congestion and lacrimation) (Figure 4.8), arthritis (swelling, lameness and painful) (Figure 4.9), and mastitis (swelling, redness, hotness, painful, and abnormal milk) (Figure 4-10). These signs have different frequency and percentages (Table 4.9).

Figure 4-7: Cow suffering from bronchopneumonia that positive for *M. bovis*, showed mucous secretions with lethargy of the animal.

Figure 4-8: Cow suffering from keratoconjunctivitis that positive for *M. bovis*, showed congestion and lacrimation of eye.

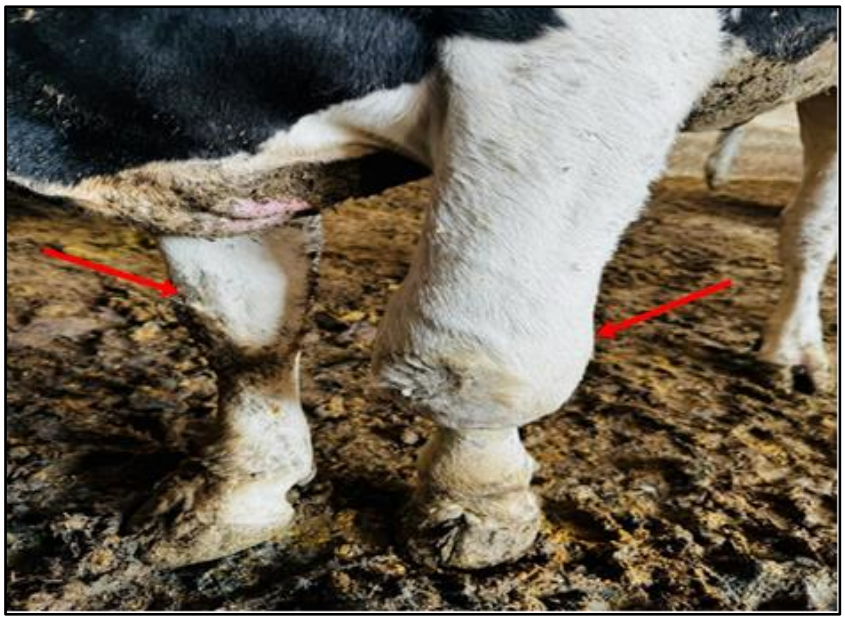

Figure 4-9: Calf suffering from polyarthritis that positive for *M. bovis*, showed swelling of the knee joints.

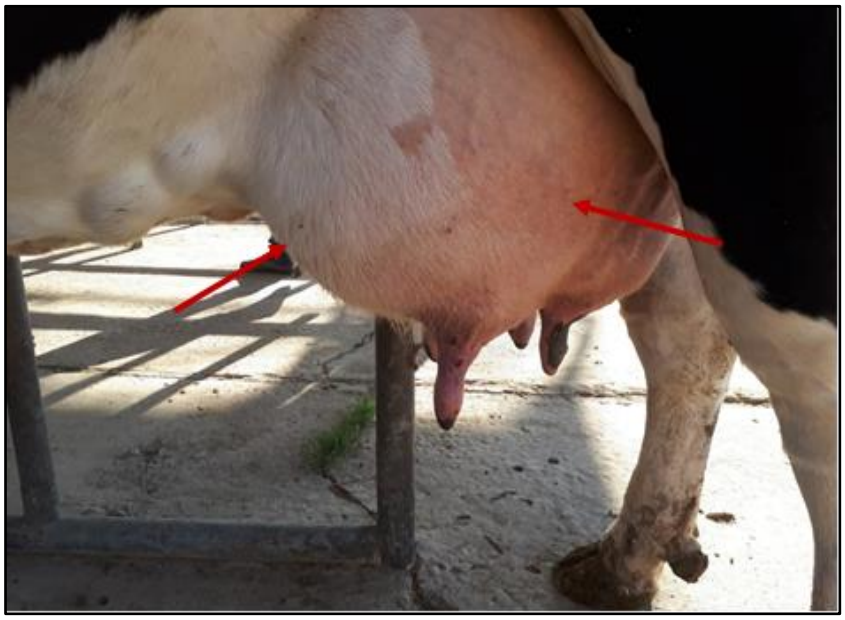

Figure 4-10: Cow suffering from Mastitis that positive for *M. bovis*, showed swelling and redness of udder.

Table (4.9): The frequents and percentages of clinical signs in clinically infected cattle with *M. bovis* (n=82).

Clinical signs	Frequents	Percentages %
Fever	63	76.9
Loss of appetite	72	87.9
Depression	66	80.4
Emaciation	55	67.0
Respiratory signs	76	92.7
Ocular signs	9	10.9
Joint signs	18	21.9
Udder signs	7	8.5

The body temperature (40.5Cº), respiratory rate (57.6 /min), and heart rate (97.8 /min) are significantly increased while rumen contraction (2.71/5min) decreased in cattle infected with *M. bovis* disease compared to the healthy group (P<0.05) (Table 4.10).

Table (4.10): Clinical symptoms of cattle infected with *M. bovis* and healthy group. Data are presented as mean ± standard error of mean.

Parameters	Healthy group (n=15)	Infected animals (n=69)
Body temperature / °C	38.9 ± 0.32	40.5 ± 0.83*
Respiratory rate / min	28.0 ± 3.27	57.6 ± 6.45*
Heart rate / min	58.3 ± 3.341	97.8 ± 5.33*
Rumen contraction / 5min	5.93 ± 0.32	2.71 ± 0.30*

* P<0.05 between infected cattle and control group.

4.7 Sequencing and phylogenetic analysis of *Mycoplasma bovis*:

In the current study, 15Th DNA product out of 108 positive samples for the *uvrC* gene of *M. bovis* in cattle in Nineveh Governorate, were sent to Macrogen Company/South Korea for sequencing. These DNA products

representing different samples (nasal swab=3, blood=3, synovial fluid=3, ocular swab=3, milk=3) (Appendix 2).

In addition, 15Th DNA product out of 101 positive samples for the gapA gene of *M. bovis* in cattle in Nineveh Governorate, were sent to Macrogen Company/South Korea for sequencing. These DNA products representing different samples (nasal swab=3, blood=3, synovial fluid=3, ocular swab=3, milk=3) (Appendix 2).

Using multiple sequences alignment-CLUSTALW (Genome Net) program on the website (http://www.genome.Jp/tools/clustalw/), the alignment within obtained sequences of the *uvrC* gene of *M. bovis* genotype was ranged from 98.41-100 (Table 4.11), (Figure 4.11). While, the alignment within obtained sequences of the *gap*A gene of *M. bovis* genotype was ranged from 98.83-100 (Table 4.11), (Figure 4.12), (Appendix 2). Furthermore, the alignment between the obtained sequences of the *uvrC* gene of *M. bovis* genotype and the obtained sequences of the gapA gene of *M. bovis* genotype were ranged from 23.12-25.78 (Table 4.11), (Figure 4.13), (Appendix 2).

Table (4.11): Alignment score within and between local sequences of the *uvrC* and gapA genes for *Mycoplasma bovis* using multiple sequence alignment program

Category	*M. bovis* Genotypes	Alignment score
Within genotype	*uvrC* gene	98.41-100
	gapA gene	98.83-100
Between genotypes	*uvrC* gene: *gap*A gene	23.12 - 25.78

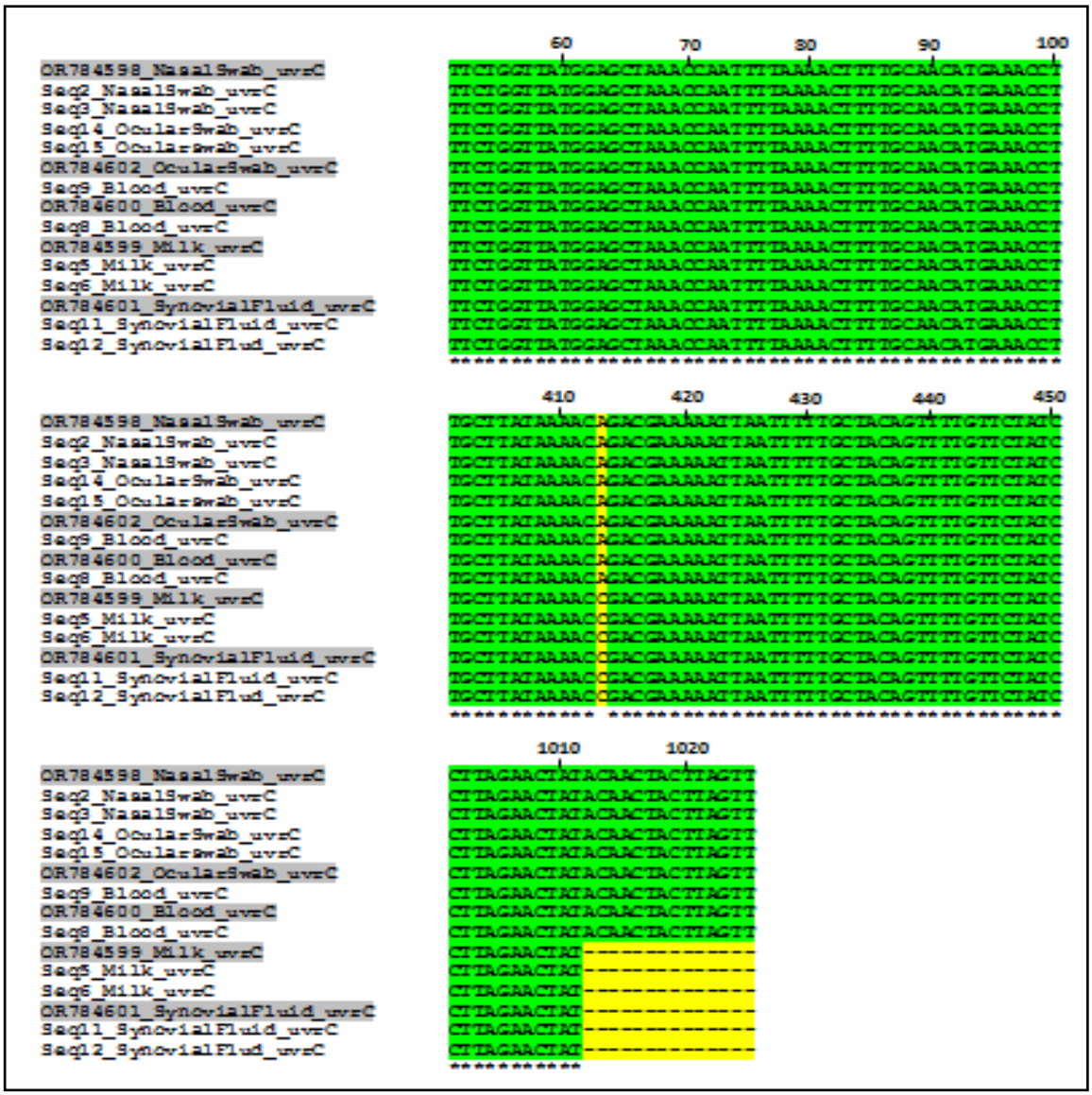

Figure (4.11): Alignment within local multiple sequences of the *uvrC* gene of *Mycoplasma bovis* showed an alignment score of 98.41%-100, using multiple sequence alignment- CLUSTALW

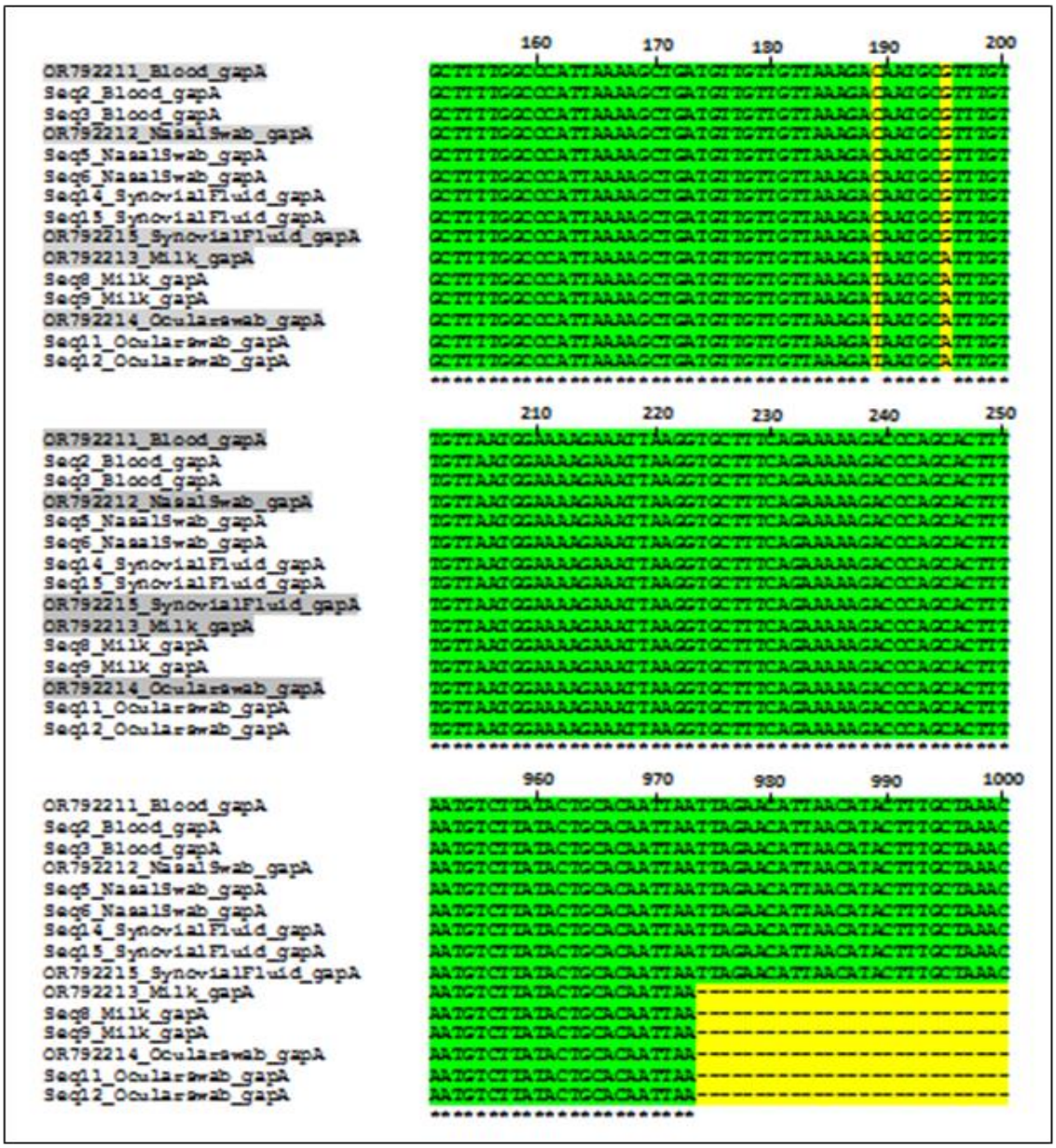

Figure (4.12): Alignment within local multiple sequences of the *gap*A gene of *Mycoplasma bovis* showed an alignment score of 98.83%-100, using multiple sequence alignment- CLUSTALW.

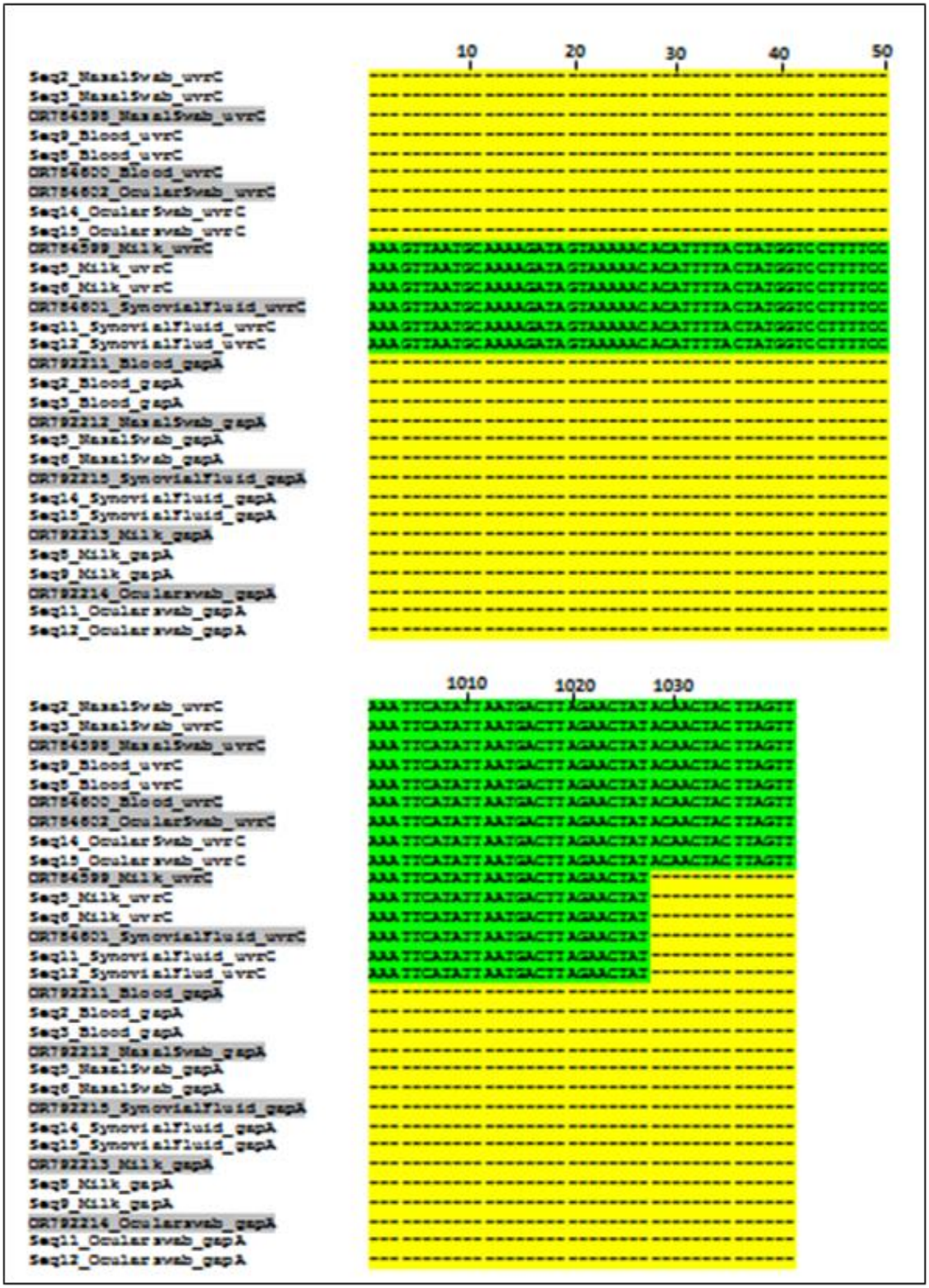

Figure (4.13): Alignment between local multiple sequences of the *uvrC* gene and *gap*A gene of *Mycoplasma bovis* showed an alignment score of 23.12 - 25.78, using multiple sequence alignment- CLUSTALW.

For the first time, five sequences from different samples: nasal swab, blood sample, synovial fluid, ocular swab and milk sample (one for each sample) out of 15 sequences were deposited at the National Centre for Biotechnology Information (NCBI) (American GenBank) and the accession number(s), which were obtained as local genetic sequences of *gap*A gene for *M. bovis* in cattle at Nineveh Governorate, Iraq (OR792211.1, OR792212.1, OR792213.1, OR792214.1, OR792215.1), (Table 4.12). Moreover, for the first time, five sequences from different samples: nasal swab, blood sample, synovial fluid, ocular swab and milk sample (one for each sample) out of out of 15 sequences were deposited at the National Centre for Biotechnology Information (NCBI) (American GenBank) and the accession number(s), which were obtained as local genetic sequences of *uvr*C gene for *M. bovis* in cattle at Nineveh Governorate, Iraq (OR784598.1, OR784599.1, OR784600.1, OR784601.1, OR784602.1), (Table 4.12).

Table (4.12): The type of samples and the GenBank accession numbers of local sequences for gapA and *uvrC* genes of *Mycoplasma bovis* in cattle.

Type of sample	Accession numbers of gapA gene	Accession numbers of *uvrC* gene
Nasal swab	OR792212.1	OR784598.1
Blood	OR792211.1	OR784600.1
Synovial fluid	OR792215.1	OR784601.1
Ocular swab	OR792214.1	OR784602.1
Milk	OR792213.1	OR784599.1

The results of performing the Blastn (Basic Local Alignment Search Tool) process in the (nr/nt) Nucleotide collection database located on the website (www.ncbi.nlm.nih.gor.NCBI) for individual sequencing analysis of local sequences (OR784598.1, OR784599.1, OR784600.1, OR784601.1, and OR784602.1) of the *uvrC* gene for *M. bovis* that have a

highly related (99.81%–100% identity) to those sequences that have accession numbers in the GenBank of different countries, including Canada (CP042938.1, CP042939.1), Iran (KX772803.1, KX772801.1, KP795974.1), Poland (KU168342.1), Switzerland (AF003959.1, LT578453.1), and Egypt (KP099618), (Table 4.13) (Appendix 3).

Table (4.13): Similarity between the local *Mycoplasma bovis* of the *uvrC* gene sequences and other sequences of same pathogen in GenBank using NCBI BLASTn

Local sequences Accession No.	Pathogen Identified	Query Cover %	Identic Number %	GenBank Accession Number	Country Identification
OR784598.1 OR784600.1 OR784601.1 OR784602.1 OR784599.1	***M. bovis* *uvrC* gene**	100	99.81	CP042939.1	Canada
		100	99.81	CP042938.1	Canada
		100	99.81	KX772803.1	Iran
		100	99.81	KX772801.1	Iran
		100	99.81	KU168342.1	Poland
		100	99.81	KP795974.1	Iran
		100	99.81	AF003959.1	Switzerland
		100	99.81	LT578453.1	Switzerland
		100	99.81	KP099618	Egypt

Furthermore, using same program mentioned above (Blastn) for individual sequencing analysis of local sequences (OR792211.1, OR792212.1, OR792213.1, OR792214.1, and OR792215.1) of the gapA gene for *M. bovis* that have a highly related (99.13%–100% identity) to those sequences that have accession numbers in the GenBank of different countries, including Canada (EF436267.1), and Egypt (KU559606, KP099616 and KP099617), (Table 4.14) (Appendix 3).

Table (4.14): Similarity between the local *Mycoplasma bovis* of the gapA gene sequences and other sequences of same pathogen in GenBank using NCBI BLASTn

Local sequences Accession No.	Pathogen Identified	Query Cover %	Identic Number %	GenBank Accession Number	Country Identification
OR792211.1 OR792212.1 OR792213.1 OR792214.1 OR792215.1	***M. bovis* gapA gene**	99	100	EF436267.1	Canada
		100	99.13	KU559606	Egypt
		99	99.88	KP099616	Egypt
		99	99.88	KP099617	Egypt

In the current study, the phylogenetic analysis of the local genetic sequences of *uvrC* for *M. bovis* that were recorded in the NCBI GenBank under the accession numbers (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 4.14). In addition, the phylogenetic analysis of the local genetic sequences of gapA for *M. bovis* that were recorded in the NCBI GenBank under the accession numbers (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 4.15).

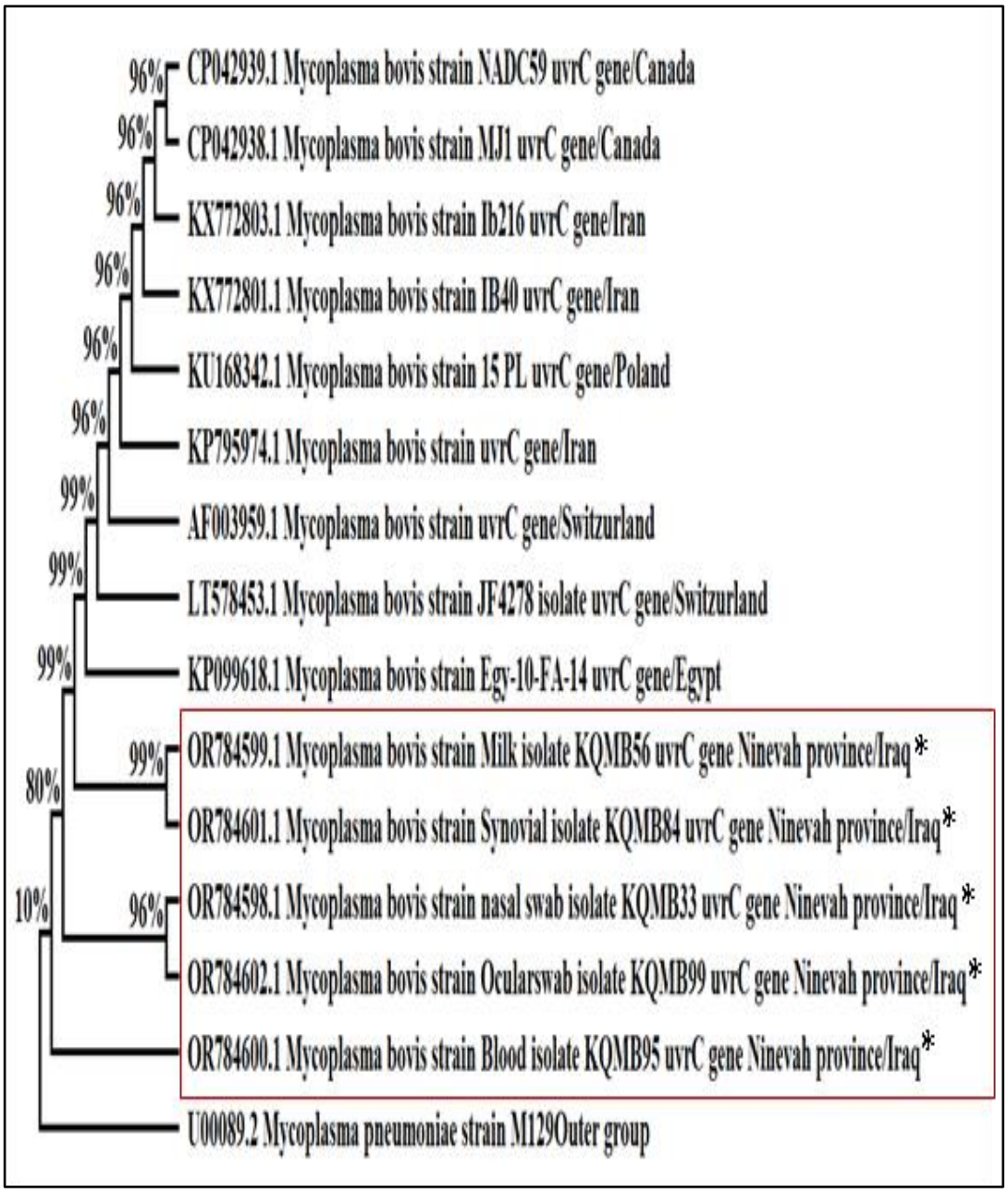

Figure (4.14): Phylogenic tree of *uvr*C gene *Mycoplasma bovis* in Nineveh province, Iraq (*). According to Partial DNA sequences of concatenated partial *uvr*C gene .

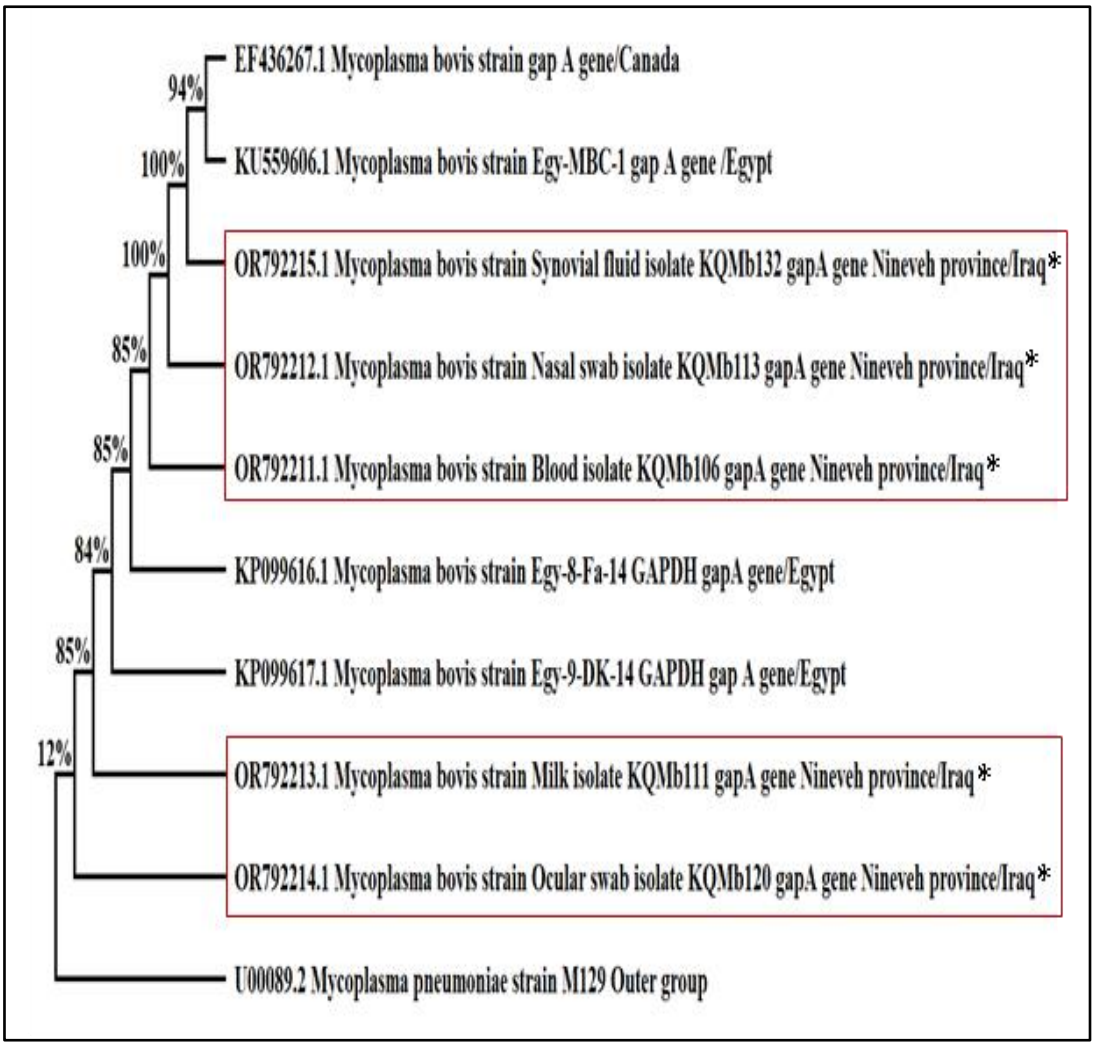

Figure (4.15): Phylogenic tree of *gap*A gene of *Mycoplasma bovis* in Nineveh province, Iraq (*). According to Partial DNA sequences of concatenated partial *gap*A gene .

CHAPTER FIVE: DISCUSSION

Chapter Five:

Discussion

5.1 The prevalence of *Mycoplasma bovis* in Cattle using different diagnostic techniques and compression between them:

It is worth why that, the overall prevalence of the prevalence of *M. bovis* infection in cattle in Nineveh Governorate was 23.6%, 30.7% and 47. 2% using culture method, m-PCR technique and i-ELISA test respectively. These results are lower than other studies addressing the prevalence of *M. bovis* in Iraq, Mahmood and Rhaymah (2012) and Hamad *et al.* (2019) were mentioned that the prevalence of *M. bovis* in calves in Mosul city were 76.09% and 86.5% using i-ELISA test and PCR techniques, respectively. Furthermore, Al-Alo *et al.* (2020) detected cases of keratoconjunctivitis in cattle infected with *M. bovis* by using PCR techniques in Najaf, Iraq, at a rate of 22.4%.

There are many studies that have detected the prevalence of *M. bovis* in cattle in various countries, including Turkey, was 23.3% using direct fluorescent antibody testing (DFAT) (Altun and Ozdemir, 2019), In Iran, was 88% using the nested PCR (n-PCR) technique (Bagheri *et al.,* 2022), in Jordan, was 27.3% using conventional PCR (c-PCR) technique (Hananeh *et al.,* 2018), in Saudi Arabia, was 24% using c-PCR technique (Al-Arfaj *et al.,* 2013), in China, was 48.7% using i-ELSA test (Niu *et al.,* 2021), in Egypt, was 67.5% and 8.3%, using culture method and c-PCR technique respectively (El-Tawab *et al.,* 2021; Hashem *et al.,* 2022), in Sudan, was 7.2% using i-ELISA test (Onsa *et al.,* 2022), in Algeria, was

69.0% and 58.0% using i-ELISA test and Real time PCR technique respectively (Oucheriah *et al.,* 2022), in United States of America, was 100% and 87.5% using LAMP and real-time PCR (RT-PCR) technique respectively (Appelt *et al.,* 2019), in Brazil, was 91.4%, 1.1% and 62.3% using immunohistochemistry (IHC), RT-PCR technique, and i-ELISA test respectively (Salina *et al.,* 2020; Oliveira *et al.,* 2020; Pires *et al.,* 2021), and in Australia, was 42.5% using i-ELISA test (Gogoi-Tiwari *et al.,* 2022).

The difference in the prevalence of *M. bovis* among countries may be due to different management approaches, environmental conditions, efficient diagnostic techniques, 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 (Oucheriah *et al.,* 2022; Ammar *et al.,* 2022; Alvarez *et al.,* 2024).

Based on the culture method of *M. bovis* on PPLO medium, it appeared as small, pearlescent, with a matte center and a white to colorless aura halo, with an egg-like (fried egg) appearance. This result was similar to those of Niu *et al.* (2021); Quinn *et al.* (2016) and Gioia *et al.* (2021). Despite some significant drawbacks with the culture method, such as its ability to identify Mycoplasma organisms to the genus level only, the process is laborious and time-consuming, *M. bovis* cannot be detected at low concentrations of less than 100 cfu/mL (less than one colony in 10ml of milk), and it cannot distinguish between closely related species (Parker *et al.,* 2018; Ismael *et al.,* 2016; Cai *et al.,* 2005; Boonyayatra *et al.,* 2012). The culture method is considered a gold standard method for detection *M. bovis* in this study, which is used for evolution of new techniques, and it is difficult and inexpensive method as well (Parker *et al.,* 2018; Dudek *et al.,* 2020).

In this study, the 16S rRNA and *uvrC* genes were selected for the PCR techniques to detect Mycoplasma genus and *M. bovis* in cattle respectively, because they are most commonly targeted in epidemiology, sequencing, and phylogenic analyses studies, and they are available in various molecular databases (Perez-Casal and Prysliak, 2007; Behera *et al.,* 2018; Salina *et al.,* 2020; Li *et al.,* 2021). Moreover, this study also used *gap*A gene for detection of *M. bovis*. This gene had been used in previously studies for diagnosis of *M. bovis* using different PCR techniques (Eissa *et al.,* 2016; Abdeen *et al.,* 2017).

The current study indicated that perfect agreement was showed between the m-PCR technique and culture method, based on the Kappa value (0.807), with a sensitivity 98.8%, specificity 90.3%, and accuracy 92.3% for the m-PCR technique. According to the type of PCR technique, the agreement, sensitivity, and specificity differed for detection of *M. bovis* when compared with the culture method, such as the perfect agreement between the LAMP PCR technique and the culture method (Kappa value = 0.8231), with 97.2% sensitivity and 90.9% specificity, and the substantial agreement between the c-PCR technique and the culture method (Kappa value = 0.7767), with 86.1% sensitivity and 92.9% specificity (Higa *et al.,* 2016). Moreover, Parker *et al.* (2018) and Scott *et al.* (2022) noted that PCR techniques have greater efficiency, specificity, and sensitivity in laboratory detection of *M. bovis* when compared with traditional culture methods.

The results also showed moderate agreement between the i-ELISA test and the culture method based on Kappa value (0.444), with sensitivity 89.2%, specificity 65.8%, and accuracy 71.3% for the i-ELISA. This finding may be due to the different targets for the two methods in i-ELISA, the target is the antibodies, while in the culture method, the target is the

antigen), cross-reactivity of *M. bovis* with other pathogens, seroconversion, which may take 2-3 weeks before antibodies can be detected, the only detected *M. bovis* in serum, plasma, or milk, and suggestions of poor sensitivity of i-ELISA test (Parker *et al.,* 2018; Appelt *et al.,* 2019; Gioia *et al.,* 2016). Furthermore, Szacawa *et al.* (2016) stated that i-ELISA test had the lowest level of compatibility with antigen ELISA, PCR techniques, and culture method for diagnosing *M. bovis*, but a high degree of correlation between these methods.

In addition, it is shown that there was 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 Clothier *et al.* (2010), Jain *et al.* (2012), Parker *et al.* (2017), and Zhao *et al.* (2018) 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 various organs and samples (Parker *et al.,* 2018; Dudek *et al.,* 2020, Okella *et al.,* 2023).

5.2 Risk factors associated with prevalence of *Mycoplasma bovis* infection based on multiplex-PCR technique:

The current study showed that the prevalence of *M. bovis* infection is significantly higher in calves aged >6 months - 2 years old compared to calves less than 6 months and adult cows. This finding agreed with those of Hazelton *et al.* (2020b) and García-Galán Pérez *et al.* (2021), who mentioned that the prevalence of *M. bovis* was significantly higher in calves than in adult cows and may cause fatal infection in calves, whereas

mild immunosuppressive disease occurs in cows. Further, adult cows may constitute reservoirs for the spread of the disease and are considered a potential risk for calves (Fanelli *et al.,* 2021). Also, calves are more susceptible to *M. bovis* because the bacteria capable of self-replication, such as in lung tissues and lymphoid tissues, and *M. bovis* has strong virulence through variable surface proteins (VSPs) (Liu *et al.,* 2021; Johnson, 2021). Whereas these results were in conflict with Aebi *et al.* (2015) and Haapala *et al.* (2021), whose found that there were no significant differences between calves less than 6 months and cows more than two years old.

The present study revealed a significantly higher in the prevalence of *M. bovis* in male compared to female cattle. This result is in accordance with those Catania *et al.* (2020), and McAloon *et al.* (2022). This may be due to the bull production system as well as the trade of bulls mostly occurs directly between farms. This may explain the risk from purchasing infected animals was high in study, as well as the presence of infected animals in livestock markets or animals carrying the infection after recovering from the disease during the convalescence period (Catania *et al.,* 2020). While, Niu *et al.* (2021) and Gogoi-Tiwari *et al.* (2022) reported a higher prevalence in female than male cattle. This is probably due to the physiological effects of females during pregnancy and lactation that are associated with immunological and hormonal changes (Francis *et al.,* 2015). In this study, the prevalence of *M. bovis* infection was significantly higher in imported compared to local breed cattle. This result was identical to that of Siugzdaite *et al.* (2012) and Haapala *et al.* (2021) they noted that imported cattle have a significant risk compared to local breeds. This may be due to the fact that these imported cattle were exported from endemic countries with *M. bovis* such as Georgia, Iran, and Turkey (Martin *et al.,*

1980; Imandar *et al.,* 2018; Mottaghian *et al.,* 2022; Altun *et al.,* 2019). Furthermore, Fox (2012) and Dudek and Szacawa (2020) mention that the source of *M. bovis* infection in calves and contagious mastitis in cows could be related to the importation and frequent introduction of new animals of unknown health status into the country or farms, leading to a high risk of spread of the disease from endemic countries. On the other hand, Aebi *et al.* (2015), and Niu *et al.* (2021) referred to local breed cattle as a source of *M. bovis* infection in the imported calves introduced to the herd.

Moreover, the prevalence of *M. bovis* infection was significantly higher in larger herd size compared to small herds. This result agrees with the findings of Nicholas *et al.* (2016), Schibrowski *et al.* (2018), and Murai and Higuchi (2019) they indicate that the prevalence of *M. bovis* was significantly higher in the larger herd size farms. This is probably due to overcrowding, poor management practices, and the arrival of new infected calves in the herd (Gaudino *et al*., 2022).

Besides, the prevalence of *M. bovis* was significantly higher in pregnant cattle when compared with non-pregnant animals. The pregnant cattle are more susceptible to diseases and develop severe clinical signs; cattle infected with *M. bovis* during pregnancy are afflicted by reproductive issues, and the embryos are more susceptible to dying (Hazelton *et al.,* 2020). Further, significant reproductive problems are caused by *M. bovis* when it is transported through the placenta and causes infection in the embryo or hardly weakens pregnant cows without congenital infection (Pohjanvirta *et al.,* 2023). The *M. bovis* problems during pregnancy and fertility were reported in infected cattle by Hazelton *et al.* (2020) and Carli *et al.* (2022).

While there was no significant difference in the prevalence of *M. bovis* infection between clinically and subclinically infected cattle. This finding agrees with Hazelton *et al.* (2018) and Hazelton *et al.* (2020). This is due to the fact that the carrier animals and previously infected animals were considered sources of infection in the herd (Maunsell *et al.,* 2011; Vähänikkilä *et al.,* 2019). Moreover, Jordan *et al.* (2021) noted that the subclinical cattle are capable of transmitting the infection, and it may be possible for infection to persist at a very low herd-level prevalence. The current study demonstrated that the prevalence of *M. bovis* was significantly higher in indoor cattle compared to outdoor cattle. This result was agreed upon by Calcutt *et al.* (2018) and Jordan *et al.* (2021). These may be due to stressors that have potential negative effects on feedlot calves indoor feeding, including dust, overcrowding, and poor ventilation (Martin and Grandin, 2018).

The results revealed that the prevalence of *M. bovis* infection in cattle was significantly higher Eastern regions compared to the Northern, Southern, Eastern, and Central regions of Nineveh govermante. This result is comparable to Murai and Higuchi (2019) and Fanelli *et al.* (2021) who found differences in the prevalence of *M. bovis* among cattle in regions within countries such as Japan and Italy respectively. The verity of the prevalence of *M. bovis* among regions may be related to management performance, the type of tests that were used to detect *M. bovis*, sampling size, host immune status, and various climatic factors such as temperature, humidity, and rainfall (Calcutt *et al.,* 2018; Okella *et al.,* 2023). Furthermore, these areas were found to be at higher risk of *M. bovis* infection on feedlot farms than farms just for breeding and production, this probably due to poor management and frequent transportation of cattle within the field. In addition, purchased cattle could be one of the pathways

for introducing *M. bovis* into the herd in these areas (Murai and Higuchi, 2019; Catania *et al.,* 2020). Contrary, Menghwar *et al.* (2017) and Klein *et al.* (2017) they found no significant difference between regions within the country.

According to the seasons, the prevalence of the *M. bovis* infection was significantly higher in the autumn and winter seasons compared to the spring and summer seasons. These findings are in accordance with Rifatbegovic *et al.* (2007), Lubbers *et al.* (2017), Schibrowski *et al.* (2018), and Jordan *et al.* (2021). These results may be due to livestock becoming stressed as the surrounding temperature falls, especially in the colder months, depending on additional climatic factors including relative humidity, rainfall, dust, and wind speed (Zinabu *et al.,* 2018). On the other hand, Fanelli *et al.* (2021) noted that *M. bovis* infection was significantly prevalent in the spring months' peaks in April and then late summer periods. This result might be due to the fact that the infected cattle suffered stress conditions during the spring and late summer periods.

5.3 Clinical findings of *Mycoplasma bovis* infection:

Most of the infected cattle were suffering from the acute form of the disease, which was manifested by various signs such as fever, loss of appetite, depression, emaciation, respiratory signs, ocular signs, joint signs, and udder signs. This finding agrees with Maunsell *et al.* (2011), White *et al.* (2012), Aebi *et al.* (2015), Calcutt *et al.,* (2018), Dudek *et al.* (2020), and Biesheuvel *et al.* (2024). In general, (Ammar *et al.,* 2021; Gelgie *et al.,* 2024) noted that the clinical signs that appeared might be due to the fact that, after being colonized, *M. bovis* invades host immunity and distributes the infection to different host systems, like the pulmonary tract, the mid-ear, joints, the lymph node, and the mammary glands.

The interpretation of these clinical signs is that the ability of the *M. bovis* to move through the lower respiratory tract, and depends on the virulence variable surface lipoproteins (Vsps), adhesion, invasion of host cells, modulation of host immune systems, production of minor metabolites, and the production of biofilms and also produces minor metabolites, like hydrogen peroxide (H2O2), which destroy host cells these responsible about increase body temperatures (Bürki *et al.,* 2015; Khan *et al.,* 2017; Perez-Casal, 2020).

Moreover, infected cattle with *M. bovis* suffering from a loss appetite and decrease in body weight. This might be due to a systemic reaction, or the infected animals are suffering from a stressful cough and restlessness that prevent them from feeding (Peek *et al.,* 2018). Moreover, Maunsell *et al.* (2011) stated that the decrease in body weight has been observed in chronically infected animals with Mycoplasma pneumonia that can be associated with otitis media, arthritis, or both in the same animal or in other animals in the herd, with animals developing arthritis associated with chronic pneumonia, occurring in beef cattle several weeks after entering the feedlot. Sameed *et al.* (2024) and Constant *et al.* (2018) added that the infected cattle's inability to eat food may be due to the animal's inability to reach the places of feeding due to severe pain, illness as a result of chronic pneumonia, lameness resulting from arthritis, and loss of vision due to inflammation of the cornea and conjunctiva of the eye in infected animals, which leads to an inability to move, and the preference of infected animals to lie down for long periods, which causes a loss of body weight.

In addition to, infected cattle showed respiratory signs of pneumonia, such as tachypnea, dyspnea, with or without nasal discharge, coughing, and abnormal lungs sounds such as exaggerated, moist and dry rales, and crackling, according to the stages of the disease. These might be

due to acutely infected animals with *M. bovis* leading to large accumulations of lymphocytes in affected tissues of the lung, and the production of proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), interferon (IFN-γ) and interleukin 4 (IL4) secreted in response to *M. bovis* antigen in lung inflammation (Gabinaitiene *et al.,* 2011; Peek *et al.,* 2018; Pohjanvirta *et al*., 2022).

Cassell *et al.* (2013), Dawood *et al.* (2022), and Vulikh (2023) stated that on auscultation of the lungs of infected animals with *M. bovis*, there were abnormal bronchial sounds consisting of sound amplification, crackles, and wheezes, and in other lung areas, no respiratory sounds were heard. This is probably due to the hardening and narrowing of the bronchial tubes with secretions due to the penetration of these bacteria into the bronchi and alveoli, producing necrotic material and secretions that fill the airways. While, Maunsell *et al.* (2011), and Lopez and Martinson (2017) noted that, the reason for hearing an amplified voice in the early stages of lung inflammation is due to the adhesion of *M. bovis* to the epithelial cells of the lung tissue, which causes large amounts of blood to reach these cells, which leads to their narrowing as a result of congestion of the capillary blood vessels and thus the appearance of an amplified voice when auscultating the lungs, and when the disease progresses, these bacteria penetrate the epithelial cells of the bronchi and alveoli to produce mucous secretions when these secretions move, resulting in a sound of moist rales.

Furthermore, Maunsell *et al.* (2011) stated that, the continuation of inflammation without the use of any treatment for the infected cases with *M. bovis* and reaching chronic cases, which leads to an increase in mucous secretions and becomes more viscous and immobile when air passes, the sound of dry rales, and in the advanced stages of the disease, the inflammatory exudate increases more than normal to fill the pulmonary

alveoli, which leads to their bursting and the exit of these exudates to the outside when air enters the burst alveoli, this leads to a cripitant sound, while in the final stage of pneumonia, no respiratory sounds heard (muffled sound), the reason for this is due to the sclerosis and adhesion in the lung tissue, which causes the death of epithelial cells, so the lung tissue becomes more fibrotic and degraded without the ability to exchange gas due to the great damage to the lung tissue caused by these bacteria.

In addition to, infected calves and cows showed arthritis with pain, swelling, redness in large mobile joints, enlargement of the tendon sheath associated with fibrous synovitis, lameness in acute form of infection, while in the chronic form of the disease, difficulty walking and exudation of synovial fluid from the joint are the characteristic signs, so the animal cannot stand for a long time and the development of decubitus ulcers in the affected joint as a result of lying the animal for long periods of time until weight loss and dehydration of the animal. As a result, affected calves with *M. bovis* arthritis generally develop lesions in other organs, such as the lungs or udders (Cantón *et al.,* 2022). Furthermore, Calcutt *et al.* (2018) stated that the hematogenic spread of *M. bovis* produce products such as phospholipases, hydrogen peroxide, superoxide radicals, biofilm formation, and the production of toxic metabolites that damage host cells of the lung and joints in young calves.

Furthermore, cattle infected with *M. bovis* showed signs of keratoconjunctivitis with or without any prior infection with pneumonia or arthritis. This is attributed to the association of these bacteria with other causative agents such as *Moraxella bovis*, *M. conjunctivae*, *M. bovoculi*, and the virus that causes infectious bovine rhinotracheitis (IBR), which increases the severity of pathogenicity. In addition, there are many factors that interfere with the severity of pathogenicity, such as the environment,

season, previous infection, dust, and the host's immune status. All these factors play an important role in the spread and occurrence of the disease and determine the severity of pathogenicity in bovine keratoconjunctivitis (Maunsell *et al.,* 2011; Bras *et al.,* 2016; Pimenov *et al.,* 2024).

Moreover, cows infected with *M. bovis* showed clinical mastitis with udder swelling and redness, as well as abnormal milk consistency. This might be adhesion of mycoplasmas to host cells by variable surface proteins (VSPs) is regarded as an important virulence mechanism in the *M. bovis* mastitis pathogenesis and lead immune response and destroyed neutrophil and macrophage then production of proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), interferon (IFN-γ), interleukin4 (IL4), and interleukin (IL) − 1β, IL-6, IL-8, are secreted in acute mastitis, also *M. bovis*-induced apoptosis in neutrophil, bovine mammary epithelial cells and embryonic bovine lung cells in chronic mastitis (Jimbo *et al.,* 2017; Liu *et al.,* 2020; Wu *et al.,* 2021; Gelgie *et al.,* 2022).

In the current study, infected cattle with *M. bovis* observed significantly increase in body temperature, heart rate, and respiratory rate. This result agreed with Aebi *et al.* (2015), Pohjanvirta *et al*. (2022), and Biesheuvel *et al.* (2024). The increase of these parameters in the case of pneumonic and arthritic animals is due to the ability of *M. bovis* to penetrate the epithelial cells of lung tissue and enter the bloodstream, resulting in a systemic reaction (Lu and Rosenbusch, 2004; Acuña *et al.,* 2021).

5.4 Sequencing and phylogenic analyses of the *Mycoplasma bovis* in cattle:

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.* (2017) who stated that there was no significant difference in the prevalence of *M. bovis* based on *uvrC* and gapA genes using the PCR technique, and the similarity within each gene was 95.3% and 100% respectively. In the current study, PCR techniques were used for the detection of *M. bovis* in cattle. This because the PCR techniques have greater efficiency, specificity, and sensitivity for laboratory detection of *M. bovis* (Parker *et al.,* 2018; Scott *et al.,* 2022)

Results concerning the sequencing and phylogenetic analysis of the PCR products (n= 15) for the *uvrC* gene and (n=15) for the gapA gene of *M. bovis* obtained from cattle's blood samples, nasal swabs, ocular swabs, synovial fluid, and milk samples, were sequenced and five sequences from each gene were deposited in the NCBI GenBank (OR784598.1-OR784602.1) and (OR792211.1- OR792215.1) of the *uvrC* and gapA 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 (Perez-Casal and Prysliak, 2007; Cantón *et al.,* 2022), Iran (Dudek et al., 2016; Guo et al., 2022), Poland (Szacawa *et al.,* 2016), Switzerland (Subramaniam *et al.,* 1998, Morimoto *et al.,* 2019), and Egypt (Hashem *et al.,* 2022; Eissa *et al.,* 2016), with highly similarity (99.13%-100%) after 1000 replications using MEGA11 software (Tamura *et al.,* 2021).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

Chapter Six:

Conclusions and Recommendation

6.1 Conclusions:

1. The *Mycoplasma bovis* is circulating among cattle, with widely distributed in Nineveh Governorate, Iraq.
2. The m-PCR technique is efficient diagnostic tool for detection of *M. bovis* in compared to i-ELISA.
3. There were several risk factors associated with a higher prevalence of *M. bovis*.
4. Different clinical forms of *M. bovis* infection were recorded according to clinical examination of infected animals
5. Both *uvrC* and gapA genes, for detection *M. bovis* in cattle using m-PCR technique.
6. The local sequences of *M. bovis* obtained were highly similar to the sequences of the same bacteria reported in the NCBI GenBank of different countries such as Canada, Egypt, Iran, Poland, and Switzerland.

6-2: Recommendation:

1. A study is required for the reservoir animals like sheep, goats, camel, horses, etc.
2. Further epidemiological study of *M. bovis* infection in buffaloes in Nineveh Governorate, Iraq.
3. This study recommends *uvr*C and *gap*A genes for detection *M. bovis* by m-PCR technique.
4. Further studies are required on more than genes in *M. bovis* to detect the site of mutation.
5. Establish planned program for controlling *M. bovis*.

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APPENDICES

Appendices

Appendix 1: Local obtained sequences of *uvrC* and gapA genes of *Mycoplasma bovis*

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Appendix 2: Alignment score within and between local sequences of the *uvrC* and gapA genes for *Mycoplasma bovis* using multiple sequence alignment program

CLUSTAL 2.1 Multiple Sequence Alignments gapA + *uvrC

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Seq3_NasalSwab_uvrC               --------------------------------------------------
OR784598_NasalSwab_uvrC           --------------------------------------------------
Seq9_Blood_uvrC                   --------------------------------------------------
Seq8_Blood_uvrC                   --------------------------------------------------
OR784600_Blood_uvrC               --------------------------------------------------
OR784602_OcularSwab_uvrC          --------------------------------------------------
Seq14_OcularSwab_uvrC             --------------------------------------------------
Seq15_Ocularswab_uvrC             --------------------------------------------------
OR784599_Milk_uvrC                AAAGTTAATGCAAAAGATAGTAAAAACACATTTTACTATGGTCCTTTTCC
Seq5_Milk_uvrC                    AAAGTTAATGCAAAAGATAGTAAAAACACATTTTACTATGGTCCTTTTCC
Seq6_Milk_uvrC                    AAAGTTAATGCAAAAGATAGTAAAAACACATTTTACTATGGTCCTTTTCC
OR784601_SynovialFluid_uvrC       AAAGTTAATGCAAAAGATAGTAAAAACACATTTTACTATGGTCCTTTTCC
Seq11_SynovialFluid_uvrC          AAAGTTAATGCAAAAGATAGTAAAAACACATTTTACTATGGTCCTTTTCC
Seq12_SynovialFlud_uvrC           AAAGTTAATGCAAAAGATAGTAAAAACACATTTTACTATGGTCCTTTTCC
OR792211_Blood_gapA                --------------------------------------------------
Seq2_Blood_gapA                    --------------------------------------------------
Seq3_Blood_gapA                    --------------------------------------------------
OR792212_NasalSwab_gapA            --------------------------------------------------
Seq5_NasalSwab_gapA                --------------------------------------------------
Seq6_NasalSwab_gapA                --------------------------------------------------
OR792215_SynovialFluid_gapA        --------------------------------------------------
Seq14_SynovialFluid_gapA           --------------------------------------------------
Seq15_SynovialFluid_gapA           --------------------------------------------------
OR792213_Milk_gapA                 --------------------------------------------------
Seq8_Milk_gapA                     --------------------------------------------------
Seq9_Milk_gapA                     --------------------------------------------------
OR792214_Ocularswab_gapA           --------------------------------------------------
Seq11_Ocularswab_gapA              --------------------------------------------------
Seq12_Ocularswab_gapA              --------------------------------------------------


Seq2_NasalSwab_uvrC               TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq3_NasalSwab_uvrC               TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
OR784598_NasalSwab_uvrC           TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq9_Blood_uvrC                   TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq8_Blood_uvrC                   TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
OR784600_Blood_uvrC               TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
OR784602_OcularSwab_uvrC          TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq14_OcularSwab_uvrC             TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq15_Ocularswab_uvrC             TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
OR784599_Milk_uvrC                TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq5_Milk_uvrC                    TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq6_Milk_uvrC                    TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
OR784601_SynovialFluid_uvrC       TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq11_SynovialFluid_uvrC          TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
Seq12_SynovialFlud_uvrC           TTCTGGTTATGGAGCTAAACCAATTTTAAAACTTTTGCAACATGAAACCT
OR792211_Blood_gapA                ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
Seq2_Blood_gapA                    ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
Seq3_Blood_gapA                    ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
OR792212_NasalSwab_gapA            ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
Seq5_NasalSwab_gapA                ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
Seq6_NasalSwab_gapA                ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
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OR792215_SynovialFluid_gapA      ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
Seq14_SynovialFluid_gapA         ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
Seq15_SynovialFluid_gapA         ----------ATGAAAAGAGTCGCTATCAATGGTTTTGGACGTATAGGCC
OR792213_Milk_gapA               --------------------------------------------------
Seq8_Milk_gapA                   --------------------------------------------------
Seq9_Milk_gapA                   --------------------------------------------------
OR792214_Ocularswab_gapA         --------------------------------------------------
Seq11_Ocularswab_gapA            --------------------------------------------------
Seq12_Ocularswab_gapA            --------------------------------------------------

Seq2_NasalSwab_uvrC              TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq3_NasalSwab_uvrC              TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
OR784598_NasalSwab_uvrC          TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq9_Blood_uvrC                  TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq8_Blood_uvrC                  TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
OR784600_Blood_uvrC              TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
OR784602_OcularSwab_uvrC         TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq14_OcularSwab_uvrC            TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq15_Ocularswab_uvrC            TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
OR784599_Milk_uvrC               TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq5_Milk_uvrC                   TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq6_Milk_uvrC                   TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
OR784601_SynovialFluid_uvrC      TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq11_SynovialFluid_uvrC         TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
Seq12_SynovialFlud_uvrC          TATACGAAAATGGCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATT
OR792211_Blood_gapA               GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
Seq2_Blood_gapA                   GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
Seq3_Blood_gapA                   GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
OR792212_NasalSwab_gapA           GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
Seq5_NasalSwab_gapA               GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
Seq6_NasalSwab_gapA               GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
OR792215_SynovialFluid_gapA       GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
Seq14_SynovialFluid_gapA          GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
Seq15_SynovialFluid_gapA          GTCTCGCCCTTCG-TTACCTTTTAGACACGAATAATAAAGAAGTTGAAAT
OR792213_Milk_gapA                --------------------------------------------------
Seq8_Milk_gapA                    --------------------------------------------------
Seq9_Milk_gapA                    --------------------------------------------------
OR792214_Ocularswab_gapA          --------------------------------------------------
Seq11_Ocularswab_gapA             --------------------------------------------------
Seq12_Ocularswab_gapA             --------------------------------------------------

Seq2_NasalSwab_uvrC              AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq3_NasalSwab_uvrC              AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
OR784598_NasalSwab_uvrC          AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq9_Blood_uvrC                  AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq8_Blood_uvrC                  AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
OR784600_Blood_uvrC              AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
OR784602_OcularSwab_uvrC         AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq14_OcularSwab_uvrC            AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq15_Ocularswab_uvrC            AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
OR784599_Milk_uvrC               AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq5_Milk_uvrC                   AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq6_Milk_uvrC                   AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
OR784601_SynovialFluid_uvrC      AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq11_SynovialFluid_uvrC         AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
Seq12_SynovialFlud_uvrC          AATCAGTTTAATAAAATTAAAGAAATTTTAAGTTTCAAAAACAATAATTA
OR792211_Blood_gapA               TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
Seq2_Blood_gapA                   TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
Seq3_Blood_gapA                   TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
OR792212_NasalSwab_gapA           TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
Seq5_NasalSwab_gapA               TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
Seq6_NasalSwab_gapA               TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
OR792215_SynovialFluid_gapA       TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
Seq14_SynovialFluid_gapA          TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
Seq15_SynovialFluid_gapA          TGTTGCTGTTAATGATTTAACAGAACCAAAAATGTTAGCTCATTTGCTTA
OR792213_Milk_gapA                ----------------------------AAAAATGTTAGCTCATTTACTTA
Seq8_Milk_gapA                    ----------------------------AAAAATGTTAGCTCATTTACTTA
Seq9_Milk_gapA                    ----------------------------AAAAATGTTAGCTCATTTACTTA
OR792214_Ocularswab_gapA          ----------------------------AAAAATGTTAGCTCATTTACTTA
Seq11_Ocularswab_gapA             ----------------------------AAAAATGTTAGCTCATTTACTTA
Seq12_Ocularswab_gapA             ----------------------------AAAAATGTTAGCTCATTTACTTA
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Seq2_NasalSwab_uvrC                TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq3_NasalSwab_uvrC                TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
OR784598_NasalSwab_uvrC            TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq9_Blood_uvrC                    TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq8_Blood_uvrC                    TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
OR784600_Blood_uvrC                TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
OR784602_OcularSwab_uvrC           TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq14_OcularSwab_uvrC              TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq15_Ocularswab_uvrC              TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
OR784599_Milk_uvrC                 TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq5_Milk_uvrC                     TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq6_Milk_uvrC                     TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
OR784601_SynovialFluid_uvrC        TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq11_SynovialFluid_uvrC           TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
Seq12_SynovialFlud_uvrC            TATTAATGAACTAACTAACAAAATGCATCAAGCAGCCAATAATATGCAAT
OR792211_Blood_gapA                AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq2_Blood_gapA                    AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq3_Blood_gapA                    AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
OR792212_NasalSwab_gapA            AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq5_NasalSwab_gapA                AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq6_NasalSwab_gapA                AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
OR792215_SynovialFluid_gapA        AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq14_SynovialFluid_gapA           AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq15_SynovialFluid_gapA           AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
OR792213_Milk_gapA                 AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq8_Milk_gapA                     AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq9_Milk_gapA                     AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
OR792214_Ocularswab_gapA           AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq11_Ocularswab_gapA              AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
Seq12_Ocularswab_gapA              AAT--ATGACACAGCTTTTGGCCCATTAAAAGCTGATGTTGTTGTTAAAG
                                     **  ****   * **            **** *    *  * *  **

Seq2_NasalSwab_uvrC                TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq3_NasalSwab_uvrC                TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
OR784598_NasalSwab_uvrC            TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq9_Blood_uvrC                    TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq8_Blood_uvrC                    TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
OR784600_Blood_uvrC                TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
OR784602_OcularSwab_uvrC           TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq14_OcularSwab_uvrC              TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq15_Ocularswab_uvrC              TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
OR784599_Milk_uvrC                 TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq5_Milk_uvrC                     TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq6_Milk_uvrC                     TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
OR784601_SynovialFluid_uvrC        TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq11_SynovialFluid_uvrC           TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
Seq12_SynovialFlud_uvrC            TTGA-ACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGTT
OR792211_Blood_gapA                ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq2_Blood_gapA                    ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq3_Blood_gapA                    ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
OR792212_NasalSwab_gapA            ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq5_NasalSwab_gapA                ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq6_NasalSwab_gapA                ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
OR792215_SynovialFluid_gapA        ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq14_SynovialFluid_gapA           ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq15_SynovialFluid_gapA           ACAATGCGTTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
OR792213_Milk_gapA                 ATAATGCATTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq8_Milk_gapA                     ATAATGCATTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq9_Milk_gapA                     ATAATGCATTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
OR792214_Ocularswab_gapA           ATAATGCATTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq11_Ocularswab_gapA              ATAATGCATTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
Seq12_Ocularswab_gapA              ATAATGCATTTGTTGTTAATGGAAAAGAAATTAAGGTGCTT-------TC
                                      *  * *   ** **  **    *    ****  *  **       *

Seq2_NasalSwab_uvrC                AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq3_NasalSwab_uvrC                AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
OR784598_NasalSwab_uvrC            AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq9_Blood_uvrC                    AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq8_Blood_uvrC                    AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
OR784600_Blood_uvrC                AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
OR784602_OcularSwab_uvrC           AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq14_OcularSwab_uvrC              AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
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Seq15_Ocularswab_uvrC            AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
OR784599_Milk_uvrC               AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq5_Milk_uvrC                   AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq6_Milk_uvrC                   AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
OR784601_SynovialFluid_uvrC      AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq11_SynovialFluid_uvrC         AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
Seq12_SynovialFlud_uvrC          AAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTAT
OR792211_Blood_gapA               AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
Seq2_Blood_gapA                   AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
Seq3_Blood_gapA                   AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
OR792212_NasalSwab_gapA           AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
Seq5_NasalSwab_gapA               AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
Seq6_NasalSwab_gapA               AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
OR792215_SynovialFluid_gapA       AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
Seq14_SynovialFluid_gapA          AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
Seq15_SynovialFluid_gapA          AGAAAAAGACCCAGCACTTTTGCCTTGGAAAGAACTTAATATTGACAT--
OR792213_Milk_gapA                AGAAAAAGACCCAGCACTTTTGCCTTGAAAAGAACTTAATATTGACAT--
Seq8_Milk_gapA                    AGAAAAAGACCCAGCACTTTTGCCTTGAAAAGAACTTAATATTGACAT--
Seq9_Milk_gapA                    AGAAAAAGACCCAGCACTTTTGCCTTGAAAAGAACTTAATATTGACAT--
OR792214_Ocularswab_gapA          AGAAAAAGACCCAGCACTTTTGCCTTGAAAAGAACTTAATATTGACAT--
Seq11_Ocularswab_gapA             AGAAAAAGACCCAGCACTTTTGCCTTGAAAAGAACTTAATATTGACAT--
Seq12_Ocularswab_gapA             AGAAAAAGACCCAGCACTTTTGCCTTGAAAAGAACTTAATATTGACAT--
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Seq2_NasalSwab_uvrC              TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq3_NasalSwab_uvrC              TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
OR784598_NasalSwab_uvrC          TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq9_Blood_uvrC                  TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq8_Blood_uvrC                  TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
OR784600_Blood_uvrC              TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
OR784602_OcularSwab_uvrC         TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq14_OcularSwab_uvrC            TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq15_Ocularswab_uvrC            TTGCTTATAAAACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
OR784599_Milk_uvrC               TTGCTTATAAAACCGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq5_Milk_uvrC                   TTGCTTATAAAACCGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq6_Milk_uvrC                   TTGCTTATAAAACCGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
OR784601_SynovialFluid_uvrC      TTGCTTATAAAACCGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq11_SynovialFluid_uvrC         TTGCTTATAAAACCGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
Seq12_SynovialFlud_uvrC          TTGCTTATAAAACCGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTAT
OR792211_Blood_gapA               -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq2_Blood_gapA                   -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq3_Blood_gapA                   -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
OR792212_NasalSwab_gapA           -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq5_NasalSwab_gapA               -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq6_NasalSwab_gapA               -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
OR792215_SynovialFluid_gapA       -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq14_SynovialFluid_gapA          -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq15_SynovialFluid_gapA          -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
OR792213_Milk_gapA                -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq8_Milk_gapA                    -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq9_Milk_gapA                    -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
OR792214_Ocularswab_gapA          -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq11_Ocularswab_gapA             -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
Seq12_Ocularswab_gapA             -TGTTTTAGAGTGCACTGGATTTTTCGTTAAAAAGGATCTTGCACACAAA
                                   ** **   *       * *   **  **          **     * *

Seq2_NasalSwab_uvrC              CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq3_NasalSwab_uvrC              CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
OR784598_NasalSwab_uvrC          CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq9_Blood_uvrC                  CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq8_Blood_uvrC                  CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
OR784600_Blood_uvrC              CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
OR784602_OcularSwab_uvrC         CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq14_OcularSwab_uvrC            CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq15_Ocularswab_uvrC            CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
OR784599_Milk_uvrC               CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq5_Milk_uvrC                   CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq6_Milk_uvrC                   CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
OR784601_SynovialFluid_uvrC      CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq11_SynovialFluid_uvrC         CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
Seq12_SynovialFlud_uvrC          CGCTATGGAATATTAATCAACAAGGTTAATTTAACAATTCCACTAGGTTT
OR792211_Blood_gapA               CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq2_Blood_gapA                   CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
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Seq3_Blood_gapA                 CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
OR792212_NasalSwab_gapA         CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq5_NasalSwab_gapA             CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq6_NasalSwab_gapA             CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
OR792215_SynovialFluid_gapA     CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq14_SynovialFluid_gapA        CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq15_SynovialFluid_gapA        CACATAGAGGCAGGCGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
OR792213_Milk_gapA              CACATAGAGGCAGGTGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq8_Milk_gapA                  CACATAGAGGCAGGTGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq9_Milk_gapA                  CACATAGAGGCAGGTGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
OR792214_Ocularswab_gapA        CACATAGAGGCAGGTGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq11_Ocularswab_gapA           CACATAGAGGCAGGTGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
Seq12_Ocularswab_gapA           CACATAGAGGCAGGTGCTAAAAAAGTTATAGTTTCTGCTCCGGCTGGTAA
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Seq2_NasalSwab_uvrC             AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq3_NasalSwab_uvrC             AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
OR784598_NasalSwab_uvrC         AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq9_Blood_uvrC                 AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq8_Blood_uvrC                 AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
OR784600_Blood_uvrC             AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
OR784602_OcularSwab_uvrC        AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq14_OcularSwab_uvrC           AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq15_Ocularswab_uvrC           AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
OR784599_Milk_uvrC              AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq5_Milk_uvrC                  AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq6_Milk_uvrC                  AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
OR784601_SynovialFluid_uvrC     AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq11_SynovialFluid_uvrC        AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
Seq12_SynovialFlud_uvrC         AAGTGTTGATGA-ATCACTTAGAGTTTTCTTTGAACAATTCTA-----TG
OR792211_Blood_gapA              GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq2_Blood_gapA                  GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq3_Blood_gapA                  GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
OR792212_NasalSwab_gapA          GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq5_NasalSwab_gapA              GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq6_NasalSwab_gapA              GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
OR792215_SynovialFluid_gapA      GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq14_SynovialFluid_gapA         GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq15_SynovialFluid_gapA         GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
OR792213_Milk_gapA               GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq8_Milk_gapA                   GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq9_Milk_gapA                   GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
OR792214_Ocularswab_gapA         GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq11_Ocularswab_gapA            GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
Seq12_Ocularswab_gapA            GGATGTTAAAACTATTGTTTATGGTGTTAACCATGAAACCCTTGTTGCTG
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Seq2_NasalSwab_uvrC             AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq3_NasalSwab_uvrC             AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
OR784598_NasalSwab_uvrC         AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq9_Blood_uvrC                 AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq8_Blood_uvrC                 AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
OR784600_Blood_uvrC             AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
OR784602_OcularSwab_uvrC        AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq14_OcularSwab_uvrC           AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq15_Ocularswab_uvrC           AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
OR784599_Milk_uvrC              AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq5_Milk_uvrC                  AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq6_Milk_uvrC                  AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
OR784601_SynovialFluid_uvrC     AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq11_SynovialFluid_uvrC        AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
Seq12_SynovialFlud_uvrC         AGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAAC
OR792211_Blood_gapA              AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq2_Blood_gapA                  AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq3_Blood_gapA                  AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
OR792212_NasalSwab_gapA          AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq5_NasalSwab_gapA              AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq6_NasalSwab_gapA              AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
OR792215_SynovialFluid_gapA      AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq14_SynovialFluid_gapA         AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq15_SynovialFluid_gapA         AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
OR792213_Milk_gapA               AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq8_Milk_gapA                   AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq9_Milk_gapA                   AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
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OR792214_Ocularswab_gapA          AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq11_Ocularswab_gapA             AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
Seq12_Ocularswab_gapA             AAGACGAAATTATTTCTGGTGCTTCA--TGTACTACAA-ACTGCTTGGCT
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Seq2_NasalSwab_uvrC               TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
Seq3_NasalSwab_uvrC               TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
OR784598_NasalSwab_uvrC           TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
Seq9_Blood_uvrC                   TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
Seq8_Blood_uvrC                   TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
OR784600_Blood_uvrC               TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
OR784602_OcularSwab_uvrC          TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
Seq14_OcularSwab_uvrC             TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
Seq15_Ocularswab_uvrC             TTTGATCTAAACCTATCAATTGAATATAAATTTATTAGTCCCAAAATAGG
OR784599_Milk_uvrC                TTTGATCTAAACCTATCAAGTGAATATAAATTTATTAGTCCCAAAATAGG
Seq5_Milk_uvrC                    TTTGATCTAAACCTATCAAGTGAATATAAATTTATTAGTCCCAAAATAGG
Seq6_Milk_uvrC                    TTTGATCTAAACCTATCAAGTGAATATAAATTTATTAGTCCCAAAATAGG
OR784601_SynovialFluid_uvrC       TTTGATCTAAACCTATCAAGTGAATATAAATTTATTAGTCCCAAAATAGG
Seq11_SynovialFluid_uvrC          TTTGATCTAAACCTATCAAGTGAATATAAATTTATTAGTCCCAAAATAGG
Seq12_SynovialFlud_uvrC           TTTGATCTAAACCTATCAAGTGAATATAAATTTATTAGTCCCAAAATAGG
OR792211_Blood_gapA               CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq2_Blood_gapA                   CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq3_Blood_gapA                   CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
OR792212_NasalSwab_gapA           CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq5_NasalSwab_gapA               CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq6_NasalSwab_gapA               CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
OR792215_SynovialFluid_gapA       CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq14_SynovialFluid_gapA          CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq15_SynovialFluid_gapA          CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
OR792213_Milk_gapA                CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq8_Milk_gapA                    CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq9_Milk_gapA                    CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
OR792214_Ocularswab_gapA          CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq11_Ocularswab_gapA             CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
Seq12_Ocularswab_gapA             CCTGTTGTTAAGGTGCTAGTTGACAACTTTGGTATTGAAAATGGTTTTAT
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Seq2_NasalSwab_uvrC               AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq3_NasalSwab_uvrC               AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
OR784598_NasalSwab_uvrC           AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq9_Blood_uvrC                   AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq8_Blood_uvrC                   AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
OR784600_Blood_uvrC               AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
OR784602_OcularSwab_uvrC          AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq14_OcularSwab_uvrC             AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq15_Ocularswab_uvrC             AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
OR784599_Milk_uvrC                AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq5_Milk_uvrC                    AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq6_Milk_uvrC                    AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
OR784601_SynovialFluid_uvrC       AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq11_SynovialFluid_uvrC          AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
Seq12_SynovialFlud_uvrC           AACTAATAAAAAAGTTTTAGACCTTGCTATTTTGAATCTAAATGATTACT
OR792211_Blood_gapA               GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq2_Blood_gapA                   GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq3_Blood_gapA                   GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
OR792212_NasalSwab_gapA           GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq5_NasalSwab_gapA               GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq6_NasalSwab_gapA               GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
OR792215_SynovialFluid_gapA       GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq14_SynovialFluid_gapA          GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq15_SynovialFluid_gapA          GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
OR792213_Milk_gapA                GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq8_Milk_gapA                    GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq9_Milk_gapA                    GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
OR792214_Ocularswab_gapA          GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq11_Ocularswab_gapA             GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
Seq12_Ocularswab_gapA             GACTACTGTTCACTCATTTACTGGTGATCAAATGCTTCAAGATGGTCCAC
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Seq2_NasalSwab_uvrC               ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq3_NasalSwab_uvrC               ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
OR784598_NasalSwab_uvrC           ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq9_Blood_uvrC                   ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
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Seq8_Blood_uvrC                 ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
OR784600_Blood_uvrC             ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
OR784602_OcularSwab_uvrC        ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq14_OcularSwab_uvrC           ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq15_Ocularswab_uvrC           ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
OR784599_Milk_uvrC              ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq5_Milk_uvrC                  ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq6_Milk_uvrC                  ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
OR784601_SynovialFluid_uvrC     ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq11_SynovialFluid_uvrC        ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
Seq12_SynovialFlud_uvrC         ATGAAAAAGAACATTTA-GTAATAAAAAATCAGTTAGACAAAGC-TAGTA
OR792211_Blood_gapA              ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq2_Blood_gapA                  ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq3_Blood_gapA                  ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
OR792212_NasalSwab_gapA          ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq5_NasalSwab_gapA              ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq6_NasalSwab_gapA              ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
OR792215_SynovialFluid_gapA      ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq14_SynovialFluid_gapA         ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq15_SynovialFluid_gapA         ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
OR792213_Milk_gapA               ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq8_Milk_gapA                   ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq9_Milk_gapA                   ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
OR792214_Ocularswab_gapA         ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq11_Ocularswab_gapA            ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
Seq12_Ocularswab_gapA            ATCGTAAAGGCGATTTGCGTCGTGCTCGTGCTGCTGGTCATAACATTGTG
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Seq2_NasalSwab_uvrC             ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq3_NasalSwab_uvrC             ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
OR784598_NasalSwab_uvrC         ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq9_Blood_uvrC                 ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq8_Blood_uvrC                 ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
OR784600_Blood_uvrC             ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
OR784602_OcularSwab_uvrC        ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq14_OcularSwab_uvrC           ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq15_Ocularswab_uvrC           ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
OR784599_Milk_uvrC              ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq5_Milk_uvrC                  ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq6_Milk_uvrC                  ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
OR784601_SynovialFluid_uvrC     ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq11_SynovialFluid_uvrC        ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
Seq12_SynovialFlud_uvrC         ATATGCTTGATTCCT-TAAATAAATATTTAAATTTGCCAAAATTAAAAAA
OR792211_Blood_gapA              CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
Seq2_Blood_gapA                  CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
Seq3_Blood_gapA                  CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
OR792212_NasalSwab_gapA          CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
Seq5_NasalSwab_gapA              CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
Seq6_NasalSwab_gapA              CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
OR792215_SynovialFluid_gapA      CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
Seq14_SynovialFluid_gapA         CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
Seq15_SynovialFluid_gapA         CCTTCATCTACTGGTGCTGCTAAAGCTATTGGATTGGTTGTGCCTGAAGC
OR792213_Milk_gapA               CCTTCATCTACTGGTGCTGCTAAGGCTATTGGATTGGTTGTGCCTGAAGC
Seq8_Milk_gapA                   CCTTCATCTACTGGTGCTGCTAAGGCTATTGGATTGGTTGTGCCTGAAGC
Seq9_Milk_gapA                   CCTTCATCTACTGGTGCTGCTAAGGCTATTGGATTGGTTGTGCCTGAAGC
OR792214_Ocularswab_gapA         CCTTCATCTACTGGTGCTGCTAAGGCTATTGGATTGGTTGTGCCTGAAGC
Seq11_Ocularswab_gapA            CCTTCATCTACTGGTGCTGCTAAGGCTATTGGATTGGTTGTGCCTGAAGC
Seq12_Ocularswab_gapA            CCTTCATCTACTGGTGCTGCTAAGGCTATTGGATTGGTTGTGCCTGAAGC
                                    *  *  * *  *     ***   * *    ***          **

Seq2_NasalSwab_uvrC             CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
Seq3_NasalSwab_uvrC             CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
OR784598_NasalSwab_uvrC         CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
Seq9_Blood_uvrC                 CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
Seq8_Blood_uvrC                 CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
OR784600_Blood_uvrC             CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
OR784602_OcularSwab_uvrC        CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
Seq14_OcularSwab_uvrC           CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
Seq15_Ocularswab_uvrC           CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTGGGAG
OR784599_Milk_uvrC              CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTCGGAG
Seq5_Milk_uvrC                  CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTCGGAG
Seq6_Milk_uvrC                  CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTCGGAG
OR784601_SynovialFluid_uvrC     CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTCGGAG
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Seq11_SynovialFluid_uvrC          CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTCGGAG
Seq12_SynovialFlud_uvrC           CATTGTTGTTTTTGACAACTCAAATATTAATAACATTAATCCTGTCGGAG
OR792211_Blood_gapA                TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq2_Blood_gapA                    TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq3_Blood_gapA                    TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
OR792212_NasalSwab_gapA            TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq5_NasalSwab_gapA                TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq6_NasalSwab_gapA                TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
OR792215_SynovialFluid_gapA        TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq14_SynovialFluid_gapA           TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq15_SynovialFluid_gapA           TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
OR792213_Milk_gapA                 TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq8_Milk_gapA                     TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq9_Milk_gapA                     TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
OR792214_Ocularswab_gapA           TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq11_Ocularswab_gapA              TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
Seq12_Ocularswab_gapA              TAATGGAAAGCTTGATGGCTTTGCACTTCGTGTGCCTA---CAATAACAG
                                    * **      ****   **      **  *     **   *  *   **

Seq2_NasalSwab_uvrC               TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq3_NasalSwab_uvrC               TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
OR784598_NasalSwab_uvrC           TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq9_Blood_uvrC                   TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq8_Blood_uvrC                   TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
OR784600_Blood_uvrC               TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
OR784602_OcularSwab_uvrC          TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq14_OcularSwab_uvrC             TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq15_Ocularswab_uvrC             TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
OR784599_Milk_uvrC                TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq5_Milk_uvrC                    TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq6_Milk_uvrC                    TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
OR784601_SynovialFluid_uvrC       TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq11_SynovialFluid_uvrC          TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
Seq12_SynovialFlud_uvrC           TTGCAATTGTTTA--TACTAATGGAATTAAAAACAAAAGCTTATATAGAA
OR792211_Blood_gapA                GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
Seq2_Blood_gapA                    GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
Seq3_Blood_gapA                    GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
OR792212_NasalSwab_gapA            GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
Seq5_NasalSwab_gapA                GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
Seq6_NasalSwab_gapA                GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
OR792215_SynovialFluid_gapA        GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
Seq14_SynovialFluid_gapA           GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
Seq15_SynovialFluid_gapA           GTTCATTTGTTGACTTATCAGTTCAGTTAACAAGACAACCAACTGTTGAA
OR792213_Milk_gapA                 GTTCATTTGTTGACTTATCAGTTCAGTTAACAAAACAACCAACTGTTGAA
Seq8_Milk_gapA                     GTTCATTTGTTGACTTATCAGTTCAGTTAACAAAACAACCAACTGTTGAA
Seq9_Milk_gapA                     GTTCATTTGTTGACTTATCAGTTCAGTTAACAAAACAACCAACTGTTGAA
OR792214_Ocularswab_gapA           GTTCATTTGTTGACTTATCAGTTCAGTTAACAAAACAACCAACTGTTGAA
Seq11_Ocularswab_gapA              GTTCATTTGTTGACTTATCAGTTCAGTTAACAAAACAACCAACTGTTGAA
Seq12_Ocularswab_gapA              GTTCATTTGTTGACTTATCAGTTCAGTTAACAAAACAACCAACTGTTGAA
                                    * ** ***** *  **  * *  * **** ** * ** *   * * ***

Seq2_NasalSwab_uvrC               -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq3_NasalSwab_uvrC               -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
OR784598_NasalSwab_uvrC           -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq9_Blood_uvrC                   -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq8_Blood_uvrC                   -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
OR784600_Blood_uvrC               -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
OR784602_OcularSwab_uvrC          -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq14_OcularSwab_uvrC             -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq15_Ocularswab_uvrC             -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
OR784599_Milk_uvrC                -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq5_Milk_uvrC                    -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq6_Milk_uvrC                    -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
OR784601_SynovialFluid_uvrC       -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq11_SynovialFluid_uvrC          -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
Seq12_SynovialFlud_uvrC           -AATTTAATTTAGAAGCTTTAAATGAGCGCAGTGCTGATGTTGAATATAT
OR792211_Blood_gapA                GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
Seq2_Blood_gapA                    GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
Seq3_Blood_gapA                    GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
OR792212_NasalSwab_gapA            GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
Seq5_NasalSwab_gapA                GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
Seq6_NasalSwab_gapA                GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
OR792215_SynovialFluid_gapA        GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
Seq14_SynovialFluid_gapA           GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
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Seq15_SynovialFluid_gapA          GAAGTTAATGCAGCTTTTGAAAAATCAGCTAGCTTAGCACTTAAATATGA
OR792213_Milk_gapA                GAAGTTAATGCAGCTTTTGAAAAATCAACTAGCTTAGCACTTAAATATGA
Seq8_Milk_gapA                    GAAGTTAATGCAGCTTTTGAAAAATCAACTAGCTTAGCACTTAAATATGA
Seq9_Milk_gapA                    GAAGTTAATGCAGCTTTTGAAAAATCAACTAGCTTAGCACTTAAATATGA
OR792214_Ocularswab_gapA          GAAGTTAATGCAGCTTTTGAAAAATCAACTAGCTTAGCACTTAAATATGA
Seq11_Ocularswab_gapA             GAAGTTAATGCAGCTTTTGAAAAATCAACTAGCTTAGCACTTAAATATGA
Seq12_Ocularswab_gapA             GAAGTTAATGCAGCTTTTGAAAAATCAACTAGCTTAGCACTTAAATATGA
                                   ** *****  **    *  ***       **    *   ** *****

Seq2_NasalSwab_uvrC               TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq3_NasalSwab_uvrC               TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
OR784598_NasalSwab_uvrC           TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq9_Blood_uvrC                   TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq8_Blood_uvrC                   TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
OR784600_Blood_uvrC               TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
OR784602_OcularSwab_uvrC          TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq14_OcularSwab_uvrC             TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq15_Ocularswab_uvrC             TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
OR784599_Milk_uvrC                TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq5_Milk_uvrC                    TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq6_Milk_uvrC                    TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
OR784601_SynovialFluid_uvrC       TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq11_SynovialFluid_uvrC          TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
Seq12_SynovialFlud_uvrC           TAAGCAATCAAT---TTCTAAATTTTTTAGTAGCAACAAAAACACTAAAG
OR792211_Blood_gapA               AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq2_Blood_gapA                   AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq3_Blood_gapA                   AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
OR792212_NasalSwab_gapA           AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq5_NasalSwab_gapA               AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq6_NasalSwab_gapA               AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
OR792215_SynovialFluid_gapA       AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq14_SynovialFluid_gapA          AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq15_SynovialFluid_gapA          AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
OR792213_Milk_gapA                AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq8_Milk_gapA                    AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq9_Milk_gapA                    AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
OR792214_Ocularswab_gapA          AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq11_Ocularswab_gapA             AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
Seq12_Ocularswab_gapA             AACAGACCCTATCGTTTCTTCAGACGTTATTGGTTCATATTATGGTTCAA
                                   *   *  * **   ****  *    *** * *     *  *   *  *

Seq2_NasalSwab_uvrC               ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq3_NasalSwab_uvrC               ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
OR784598_NasalSwab_uvrC           ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq9_Blood_uvrC                   ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq8_Blood_uvrC                   ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
OR784600_Blood_uvrC               ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
OR784602_OcularSwab_uvrC          ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq14_OcularSwab_uvrC             ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq15_Ocularswab_uvrC             ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
OR784599_Milk_uvrC                ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq5_Milk_uvrC                    ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq6_Milk_uvrC                    ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
OR784601_SynovialFluid_uvrC       ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq11_SynovialFluid_uvrC          ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
Seq12_SynovialFlud_uvrC           ATTATGACTTAGTTATAGCTGATGGCGGTATACAACAAGTTAATGAAGCT
OR792211_Blood_gapA               CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
Seq2_Blood_gapA                   CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
Seq3_Blood_gapA                   CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
OR792212_NasalSwab_gapA           CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
Seq5_NasalSwab_gapA               CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
Seq6_NasalSwab_gapA               CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
OR792215_SynovialFluid_gapA       CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
Seq14_SynovialFluid_gapA          CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
Seq15_SynovialFluid_gapA          CATTTGATTCGACATTAACTAAAATCAAAGAATCAAATGGCCACAGAATA
OR792213_Milk_gapA                CATTTGATTCGACATTAACTAAAATCAAAGAATCGAATGGCCACAGAATA
Seq8_Milk_gapA                    CATTTGATTCGACATTAACTAAAATCAAAGAATCGAATGGCCACAGAATA
Seq9_Milk_gapA                    CATTTGATTCGACATTAACTAAAATCAAAGAATCGAATGGCCACAGAATA
OR792214_Ocularswab_gapA          CATTTGATTCGACATTAACTAAAATCAAAGAATCGAATGGCCACAGAATA
Seq11_Ocularswab_gapA             CATTTGATTCGACATTAACTAAAATCAAAGAATCGAATGGCCACAGAATA
Seq12_Ocularswab_gapA             CATTTGATTCGACATTAACTAAAATCAAAGAATCGAATGGCCACAGAATA
                                    * *** *      ** ** *   *     *    * *   *   *

Seq2_NasalSwab_uvrC               AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
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Seq3_NasalSwab_uvrC              AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
OR784598_NasalSwab_uvrC          AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq9_Blood_uvrC                  AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq8_Blood_uvrC                  AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
OR784600_Blood_uvrC              AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
OR784602_OcularSwab_uvrC         AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq14_OcularSwab_uvrC            AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq15_Ocularswab_uvrC            AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
OR784599_Milk_uvrC               AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq5_Milk_uvrC                   AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq6_Milk_uvrC                   AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
OR784601_SynovialFluid_uvrC      AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq11_SynovialFluid_uvrC         AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
Seq12_SynovialFlud_uvrC          AAAAAAACGCTTAAAACGCTTAATATAAACATCCCTGTTATTGGATTAGT
OR792211_Blood_gapA               TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
Seq2_Blood_gapA                   TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
Seq3_Blood_gapA                   TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
OR792212_NasalSwab_gapA           TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
Seq5_NasalSwab_gapA               TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
Seq6_NasalSwab_gapA               TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
OR792215_SynovialFluid_gapA       TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
Seq14_SynovialFluid_gapA          TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
Seq15_SynovialFluid_gapA          TACAAAATATTTGCCTGGTATGATAATGAAATGTCTTATACTGCACAATT
OR792213_Milk_gapA                TACAAAATATTTGCCTGATATGATAATGAAATGTCTTATACTGCACAATT
Seq8_Milk_gapA                    TACAAAATATTTGCCTGATATGATAATGAAATGTCTTATACTGCACAATT
Seq9_Milk_gapA                    TACAAAATATTTGCCTGATATGATAATGAAATGTCTTATACTGCACAATT
OR792214_Ocularswab_gapA          TACAAAATATTTGCCTGATATGATAATGAAATGTCTTATACTGCACAATT
Seq11_Ocularswab_gapA             TACAAAATATTTGCCTGATATGATAATGAAATGTCTTATACTGCACAATT
Seq12_Ocularswab_gapA             TACAAAATATTTGCCTGATATGATAATGAAATGTCTTATACTGCACAATT
                                   * ****   **        * ***   * **  **  ** ** *  * *

Seq2_NasalSwab_uvrC              AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq3_NasalSwab_uvrC              AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
OR784598_NasalSwab_uvrC          AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq9_Blood_uvrC                  AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq8_Blood_uvrC                  AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
OR784600_Blood_uvrC              AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
OR784602_OcularSwab_uvrC         AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq14_OcularSwab_uvrC            AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq15_Ocularswab_uvrC            AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
OR784599_Milk_uvrC               AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq5_Milk_uvrC                   AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq6_Milk_uvrC                   AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
OR784601_SynovialFluid_uvrC      AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq11_SynovialFluid_uvrC         AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
Seq12_SynovialFlud_uvrC          AAAAAATGAGTTTCACAAAACCAAAGCCTTAATTGACCTAGATATGAATG
OR792211_Blood_gapA               AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
Seq2_Blood_gapA                   AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
Seq3_Blood_gapA                   AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
OR792212_NasalSwab_gapA           AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
Seq5_NasalSwab_gapA               AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
Seq6_NasalSwab_gapA               AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
OR792215_SynovialFluid_gapA       AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
Seq14_SynovialFluid_gapA          AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
Seq15_SynovialFluid_gapA          AATTAGAACATTAACATACTTTGCTAAACTAACTAAGTAAA---------
OR792213_Milk_gapA                AA----------------------------------------------
Seq8_Milk_gapA                    AA----------------------------------------------
Seq9_Milk_gapA                    AA----------------------------------------------
OR792214_Ocularswab_gapA          AA----------------------------------------------
Seq11_Ocularswab_gapA             AA----------------------------------------------
Seq12_Ocularswab_gapA             AA----------------------------------------------
                                  **

Seq2_NasalSwab_uvrC              AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
Seq3_NasalSwab_uvrC              AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
OR784598_NasalSwab_uvrC          AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
Seq9_Blood_uvrC                  AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
Seq8_Blood_uvrC                  AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
OR784600_Blood_uvrC              AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
OR784602_OcularSwab_uvrC         AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
Seq14_OcularSwab_uvrC            AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
Seq15_Ocularswab_uvrC            AAATTCATATTAATGACTTAGAACTATACAACTACTTAGTT
OR784599_Milk_uvrC               AAATTCATATTAATGACTTAGAACTAT--------------
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Seq5_Milk_uvrC                  AAATTCATATTAATGACTTAGAACTAT--------------
Seq6_Milk_uvrC                  AAATTCATATTAATGACTTAGAACTAT--------------
OR784601_SynovialFluid_uvrC     AAATTCATATTAATGACTTAGAACTAT--------------
Seq11_SynovialFluid_uvrC        AAATTCATATTAATGACTTAGAACTAT--------------
Seq12_SynovialFlud_uvrC         AAATTCATATTAATGACTTAGAACTAT--------------
OR792211_Blood_gapA              ----------------------------------------
Seq2_Blood_gapA                  ----------------------------------------
Seq3_Blood_gapA                  ----------------------------------------
OR792212_NasalSwab_gapA          ----------------------------------------
Seq5_NasalSwab_gapA              ----------------------------------------
Seq6_NasalSwab_gapA              ----------------------------------------
OR792215_SynovialFluid_gapA      ----------------------------------------
Seq14_SynovialFluid_gapA         ----------------------------------------
Seq15_SynovialFluid_gapA         ----------------------------------------
OR792213_Milk_gapA               ----------------------------------------
Seq8_Milk_gapA                   ----------------------------------------
Seq9_Milk_gapA                   ----------------------------------------
OR792214_Ocularswab_gapA         ----------------------------------------
Seq11_Ocularswab_gapA            ----------------------------------------
Seq12_Ocularswab_gapA            ----------------------------------------
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Appendix 3: Local obtained sequences of *uvrC* gene of *Mycoplasma bovis* and the sequences for the same bacteria in the GenBank database

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>OR784598_Nineveh_Iraq
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GCTTGTTAATAAAAAACAAAGACTATAACTTTTGGATTAATCAGTTTAATAAAATTAAAGAA
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CAATAATATGCAATTTGAACTTGCATTATTTTTGCGTGATGGCTTAACATATTTAAAAAAGT
TAAAAGAAAGTCAAATTATAGAGCTAAGTCAATATAAAAATATTGACGTATTTGCTTATAAA
ACAGACGAAAAATTAATTTTTGCTACAGTTTTGTTCTATCGCTATGGAATATTAATCAACAA
GGTTAATTTAACAATTCCACTAGGTTTAAGTGTTGATGAATCACTTAGAGTTTTCTTTGAAC
AATTCTATGAGGATAAAATACTGCCAGATAATTTAATTGTACAGGAAGAACTTTTAAACTTT
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Appendix 4: Local obtained sequences of gapA gene of *Mycoplasma bovis* and the sequences for the same bacteria in the GenBank database

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المستخلص

تهدف الدراسة الحالية ما يلي: تحديد مدى انتشار المتفطرات البقرية في الابقار في محافظة نينوى، العراق، باستخدام طرائق تشخيصية مختلفة: طريقة الزرع الجرثومي، واختبار الممتز المناعي المرتبط بالأنزيم غير المباشر وتقنية تفاعل البلمرة المتسلسل؛ مع تقييم كفاءة الطرائق المختبرية المستخدمة؛ والتحقق من بعض عوامل الخطورة المرتبطة بانتشار الجرثومة؛ وتسجيل العلامات السريرية الظاهرة على الحيوانات المصابة؛ مع التحقيق من تحليلات النشوء الجيني للمفطورات البقرية المشخص في هذه الدراسة. شملت الدراسة 352 من الابقار مختلفة الاعمار والاجناس والمنشاء حجم القطيع والحالة الصحية وحالة الحمل، ومناطق جمع العينات، والمواسم. ومن ضمنها 15 من الابقار السليمة سريريا والسالبة مختبريا للمتفطرات البقرية والتي عدت كمجموعة سيطرة. من خلال الفترة الممتدة من أذار 2022 إلى شباط 2023، استُخدم نموذج استمارة فحص سريري خاصة لتسجيل العلامات السريرية وغيرها من المعلومات الوبائية. وجمعت عينات مختلفة من 352 من الابقار، شملت عينات دم (352) ومسحات الانف (352) ومسحات من العين (40) والسائل المفصلي (65) وعينات الحليب (30)، تم الحصول عليها من مناطق مختلفة في محافظة نينوى. استُخدمت المسحات الانفية لكل من طريقة الزرع الجرثومي وتقنية تفاعل البلمرة المتسلسل التقليدي اعتمادا على الجين 16S rRNA لجنس المفطورات، كما استخدمت عينات الانف والعينات المذكورة اعلاه في تقنية تفاعل البلمرة المتسلسل المتعدد اعتمادا على الجين *uvrC* والجين *gap*A للمفطورات البقرية. فضلا عن، استخدام عينات مصل الدم لاختبار الممتز المناعي المرتبط بالأنزيم غير المباشر للكشف عن الاجسام المضادة للمفطورات البقرية. كما تم تقييم التوافق بين الطرائق التشخيصية المختلفة على أساس قيمة كابا. فضلا عن، حساب الحساسية والخصوصية والدقة لتقنية تفاعل البلمرة المتسلسل المتعدد واختبار الممتز المناعي المرتبط بالأنزيم غير المباشر مقارنة مع طريقة الزرع الجرثومي. مما مجموعه 30 ناتج موجب لتفاعل البلمرة المتسلسل للحامض النووي منقوص الاوكسجين للمفطورات البقرية للجين *uvrC* (15) والجين *gap*A (15) من العينات الابقار المختلفة (عينات الدم ومسحات الأنف ومسحات العين والسائل المفصلي وعينات الحليب)، تم إرسال ثلاثة نواتج لكل نوع من العينات على التوالي، الى شركة تجارية للتنقية والحصول على التسلسل الجيني. كما تم التحليل الجيني وانشاء شجرة النشوء الجيني للتسلسلات المحلية للمفطورات البقرية باستخدام برامج مختلفة على متصفح ويب. في الدراسة الحالية، بلغت نسبة الانتشار الكلي للمفطورات البقرية في الابقار في محافظة نينوى 23.57% و47.15% و30.68% باستخدام طريقة الزرع الجرثومي واختبار الممتز المناعي المرتبط

بالأنزيم غير المباشر وتقنية تفاعل البلمرة المتسلسل المتعدد على التوالي. واعتمادا على قيمة كابا (0.807) لوحظ وجود توافق تام بين تقنية تفاعل البلمرة المتسلسل المتعدد وطريقة الزرع الجرثومي، مع نسبة حساسية 98.79% وخصوصية 90.33% وبدقة 92.32%، لتقنية تفاعل البلمرة المتسلسل المتعدد. في حين اعتمادا على قيمة كابا (0.444) لوحظ وجود توافق معتدل بين اختبار الممتز المناعي غير المباشر وطريقة الزرع الجرثومي، مع نسبة حساسية 89.15%، وخصوصية 65.79%، وبدقة 71.30%، لاختبار الممتز المناعي المرتبط بالأنزيم غير المباشر. اعتمادا على تقنية تفاعل البلمرة المتسلسل التقليدي، لم يكن هناك فرق معنوي في نسبة انتشار المفطورات البقرية في مختلف أنواع العينات: عينات الدم ومسحات الأنف ومسحات العين والسائل المفصلي وعينات الحليب، والتي بلغت 27.55% و30.68% و22.50% و27.69% و23.33% على التوالي. فضلا عن انه كانت هناك عوامل خطر معنوية مرتبطة بارتفاع نسبة انتشار المفطورات البقرية في سجلت العجول التي اعمارها أكبر من 6 أشهر إلى سنتين وذكور الأبقار والحيوانات المستوردة والأبقار الحوامل والقطعان الكبير الحجم والأبقار التي تتغذى داخل الحضائر والمناطق الشرقية من محافظة نينوى وفصلي الخريف والشتاء. كما لوحظ من خلال الفحص السريري للأبقار المصابة أنها عانت من الحمى وفقدان الشهية والخمول والهزال واضطراب التنفسية وعلامات على العينين وعلامات على المفاصل، وعلامات على الضرع. حدثت زيادة ملحوظة في درجة حرارة الجسم ومعدل ترداد التنفس ومعدل ضربات القلب في الأبقار المصابة سريرياً مقارنة مع مجموعة السيطرة. اظهرت نتائج تحليل 5 تسلسلات جينية محلية الجين *uvrC* للمفطورات البقرية والتي تم تسجيلها في بنك الجينات وبأرقام تسلسلية (OR792211.1 - OR792215.1)، انها مرتبطة وبشكل كبير (99.81% - 100٪ نسبة تشابه) بالتسلسلات الاخرى المسجلة في بنك الجينات من بلدان مختلفة شملت كندا وإيران وبولندا وسويسرا ومصر. كما واظهرت نتائج تحليل 5 تسلسلات جينية محلية الجين gapA للمفطورات البقرية والتي تم تسجيلها في بنك الجينات وبأرقام تسلسلية (OR784598.1 - OR784602.1)، انها مرتبطة وبشكل كبير (99.13% - 100% نسبة تشابه) بالتسلسلات الاخرى المسجلة في بنك الجينات من بلدان مختلفة شملت كندا ومصر. استنتج من الدراسة، الانتشار الواسع للمفطورات البقرية في الأبقار في محافظة نينوى، العراق؛ تعد تقنية تفاعل البلمرة المتسلسل المتعدد أكثر كفاءة من اختبار الممتز المناعي غير المباشر في تشخيص المفطورات البقرية؛ هناك العديد من عوامل الخطر المرتبطة بارتفاع نسبة انتشار المفطورة البقرية؛ عانت الابقار المصابة بالمفطورات البقرية وبشكل رئيسي من اضطرابات تنفسية وعلامات على العين والمفاصل والضرع؛ تساهم الابقار المصابة سريريًا وتحت سريري، في انتشار المفطورة البقرية. فضلا عن التحليل النشوئي الجيني

للتسلسلات الجينية المحلية التي تم تسجيلها ولأول مرة في محافظة نينوى، تعد مهمة للسيطرة الاستراتيجية على المفطورات البقرية في مناطق الدراسة.

دراسة جزيئية ووبائية للتمفطرات البقرية في الابقار في محافظة نينوى، العراق

أطروحة تقدم بها

خضر أحمد محمود العزو

إلى

مجلس كلية الطب البيطري في جامعة الموصل

وهي جزء من متطلبات نيل شهادة الدكتوراه فلسفة

في اختصاص الطب البيطري / الطب الباطني والوقائي البيطري

بإشراف

الأستاذ الدكتور	**الأستاذ الدكتور**
قيس طالب شكر	**محمد علي حمد**

2024م □ **1446هـ**

جامعة الموصل
كلية الطب البيطري

دراسة جزيئية ووبائية للتمفطرات البقرية في الابقار في محافظة نينوى، العراق

خضر أحمد محمود العزو

أطروحة دكتوراه

□ الطب البيطري / الطب الباطني والوقائي البيطري

□

بإشراف

الأستاذ الدكتور
قيس طالب شكر

الأستاذ الدكتور
محمد علي حمد

2024م □ 1446□هـ